WELCOME
6TH WORLD CONGRESS ON MELANOMA

We'd like to take this opportunity to welcome you to the World Congress on Melanoma. This is a major event world-wide among scientists and physicians dealing with cutaneous melanoma. We're confident that your participation in this, the 6th World Congress, will provide you with the same great educational experience as previous meetings.

Held every four years since the first congress in 1985, this is the meeting where clinicians and researchers focus on the state of the art in prevention, treatment and new lines of basic scientific investigation. The program committee has prepared an innovative and informative program that will provide you with an excellent scientific program, complimented by networking and social opportunities designed to enhance your stay in this incomparable Canadian city.

The 6th Congress program is very exciting and includes a wide variety of posters and exhibits.

We truly hope that you will find that the meeting provides fresh insight as we move forward together to achieve the goals of prevention and effective treatment of melanoma.

Natale Cascinelli & Richard Gallagher
Congress Co-Presidents

CONGRESS COMMITTEE

Natale Cascinelli	Congress Co-President
Richard Gallagher	Congress Co-President
Jason K. Rivers	Congress Secretary-General
David I. McLean	Local Organizing Committee

SCIENTIFIC COMMITTEE

Charles M. Balch	USA
Raymond L. Barnhill	USA
Marianne Berwick	USA
Natale Cascinelli	Italy
Jean-Pierre Cesarini	France
Jean François Doré	France
Alexander Eggermont	The Netherlands
Richard Gallagher	Canada
Claus Garbe	Germany
Meenhard Herlyn	USA
Kowichi Jimbow	Japan
Rona MacKie Scotland,	UK
William H. McCarthy	Australia
David I. McLean	Canada
Martin C. Mihm, Jr	USA
Hubert Pehamberger	Austria
Ulrik Ringborg	Sweden
Jason K. Rivers	Canada
Merrick I. Ross	USA
Mario Santinami	Italy
Arthur J. Sober	USA
John F. Thompson	Australia

REGISTRATION AND INFORMATION DESK

A Registration and Information Desk will be set-up in the Convention Lobby of the Vancouver Convention & Exhibition Centre (VCEC) during the following days and times:

Tuesday, September 6	07:30 - 19:00
Wednesday, September 7	07:30 - 17:30
Thursday, September 8	08:00 - 17:30
Friday, September 9	08:00 - 16:00
Saturday, September 10	08:00 - 12:00

Any inquiries about the Congress, social functions, sightseeing tours, etc. may be answered by approaching any of the Secretariat staff at the registration desk. Delegates may pick up their Congress documents, badges and social tickets at the registration desk.

FULL REGISTRATION PACKAGE INCLUDES:

- All Congress Scientific Sessions
- One copy of the Final Program/ Abstract Book
- Opening Ceremony & Welcome Reception
- Lunches & Refreshment Breaks
- *Gala Congress Banquet

ACCOMPANYING PERSON PACKAGE INCLUDES:

- Opening Ceremony & Welcome Reception
- International Fair
- *Gala Congress Banquet

*Please note that tickets for the Gala Congress Banquet will be included in your registration materials. In order to assist with anticipated attendance at the Banquet, kindly let the registration staff know if you are unable to attend at the time you pick up your registration package.

**STUDENT REGISTRATION PACKAGE INCLUDES:

- All Congress Scientific Sessions
- One copy of the Final Program/ Abstract Book
- Opening Ceremony & Welcome Reception
- Lunches & Refreshment Breaks

**Student Registration does not include the Congress Gala Banquet. Tickets for this event can be purchased for an additional fee of $120.00 Cdn.*

SPEAKER READY ROOM

The Speaker Ready Room is located in Meeting Room 4 on the Meeting Room Level.

MEDIA CENTRE

A Media Room is located on the Meeting Room Level of the VCEC in Meeting Room 6 to handle local and international inquiries and to support journalists at the congress.

POSTER SESSION SET-UP AND TAKE-DOWN SCHEDULE

The Poster Sessions take place in Exhibit Hall A. The schedule for poster set-up and take-down is as follows:

Wednesday, September 7

Poster set-up	08:00 - 11:00
Poster Session I	12:00 - 13:30
Poster take-down	13:30 - 15:30

Thursday, September 8

Poster set-up	08:00 - 11:00
Poster Session II	12:00 - 13:30
Poster take-down	13:30 - 15:30

Friday, September 9

Poster set-up	08:00 - 11:00
Poster Session III	12:00 - 13:30
Poster take-down	13:30 - 15:30

Poster Presenters must remove their posters as per the schedule in order to allow ample time for set up of the next session.

EXHIBITION LOCATION AND HOURS

The Exhibit is located in Exhibition Hall A and is open on the following dates and time

Wednesday, September 7	09:30 - 16:00
Thursday, September 8	09:30 - 16:00
Friday, September 9	09:30 - 16:00

MEALS AND BREAKS

Morning and afternoon coffee breaks and lunches will be served daily for all Conference delegates in Exhibit Hall A.

NAME BADGES AND TICKETS

Please wear your Congress name badge at all times to ensure automatic entry to the sessions and social functions for which you have registered.

SOCIAL PROGRAMME

The following events are included in the full conference registration fee and accompanying person's package. Additional Tickets can be purchased at the registration desk.

Opening Ceremony
Tuesday, September 6 18:30 - Ballrooms B/C

Welcome Reception
Tuesday, September 6 19:30 - Parkview Terrace

Congress Gala Banquet
Thursday, September 8 19:00 - Ballrooms A/B/C

SMOKING PROHIBITED

Smoking is not permitted in the VCEC, restaurants, food service establishments, casinos, hotels, public buildings and bars (except on a patio or in a smoking room).

OFFICIAL LANGUAGE

The official language of the Congress is English.

PARKING

Limited parking (on a pay and display basis) is available directly underneath the VCEC and is labeled Canada Place parking.

CURRENCY EXCHANGE

The monetary system in Canada is based on Canadian dollars and cents. International currency exchange services are available at the Vancouver International Airport, downtown banks and currency exchange outlets located throughout the city. Most hotels will also offer currency exchange services.

PUBLIC TRANSPORT

A modern public transit system reaches every point in the city. Buses serve Vancouver and its suburbs and the SkyTrain (a light rapid transit train) connect the downtown area to the outlying areas. The SeaBus, an extension of the SkyTrain, travels across the harbour between downtown and the North Shore. All-day passes allowing unlimited travel may be purchased. Tickets and transfers are dispensed by drivers on buses (correct change only), by machines at SkyTrain stations and SeaBus terminals, and at corner store ticket outlets located throughout the city. Daily or weekly transit passes may be purchased. Additional transit information will be available at the conference registration and information desk

TAXIS

Taxis can be hailed, caught at designated taxi pickup areas or reserved by telephone. The drivers are courteous and helpful. Credit cards are accepted by most. Details of local taxi companies are available at the Registration Desk in the Convention Lobby of the VCEC.

RESTAURANTS

Restaurants in Vancouver offer a wide range of ethnic diversity. Details of local restaurants are available from the Registration Desk in the Convention Lobby of the VCEC.

TIPPING

Customary gratuity should be calculated at 15%.

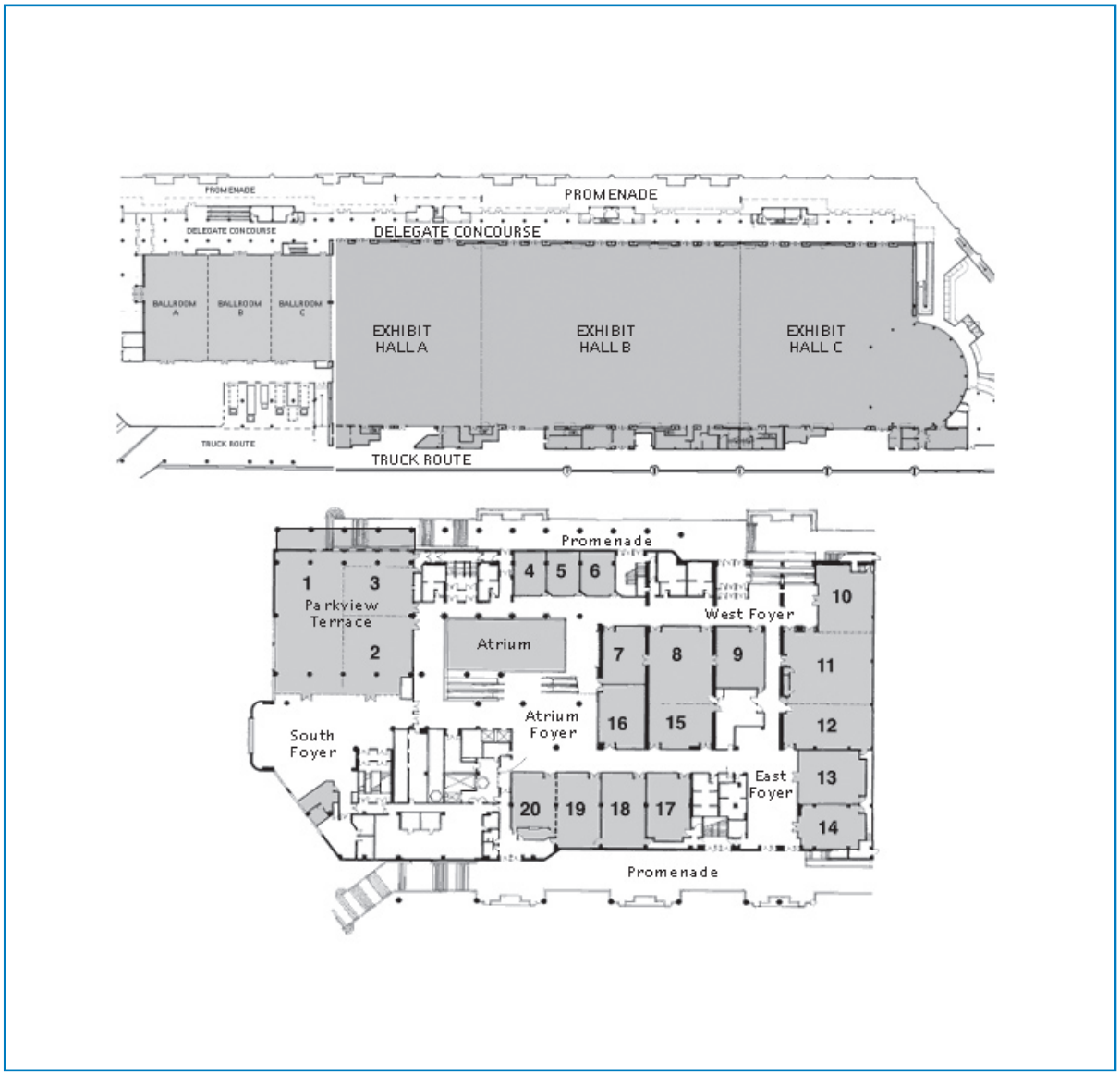
TAXES

British Columbia has both a Provincial Sales Tax (PST) of 7.5% and a Federal Goods and Services Tax (GST) of 7%. These taxes will apply to items purchased, meals in restaurants, services rendered, tourist activities, etc. Visitors residing outside of Canada are eligible for a rebate of GST paid while they were in Canada. Full details and rebate forms are available at government agencies (border crossings, post office). The rebate does not apply to all items - details are described in the rebate form.

ACCREDITATION:

UBC The Division of CONTINUING MEDICAL EDUCATION

The University of British Columbia, Faculty of Medicine, Division of Continuing Medical Education is fully accredited by the Committee on Accreditation of Canadian Medical Schools (CACMS) and by reciprocity through the Accreditation Council for Continuing Medical Education (ACCME) of the United States to sponsor continuing medical education for physicians. The UBC Division of Continuing Medical Education designates this educational program as meeting the accreditation criteria for a maximum of 20.5 Category 1 credits toward the American Medical Association Physician's Recognition Award. This program is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of the Royal College of Physicians and Surgeons of Canada. This program has been reviewed and approved by UBC Division of Continuing Medical Education. Each physician should claim only those credits he/she actually spent in the activity.



VANCOUVER CONVENTION & EXHIBITION CENTRE

TIME	P #	TITLE	PRESENTER	MEETING ROOM
PRE-CONGRESS EVENT - MONDAY 5TH SEPTEMBER				
12:00 - 1400		Public Screening		Plaza
18:00 - 20:00		Toyota Public Forum		M.R. 8/15
DAY 1: TUESDAY 6TH SEPTEMBER				
07:30 - 19:00		Registration		VCEC Foyer
08:30 - 17:00		Satellite Symposium: Pathology	*Chairs: Martin Mihm, Raymond Barnhill*	M.R. 8
08:30 - 08:55	001	Borderline lesions: Do they exist?	*Stanley McCarthy*	
08:55 - 09:20	002	Standardized reporting of melanoma	*Martin Trotter*	
09:20 - 09:45	003	Vertical growth phase and prognostic factors	*Martin Mihm*	
09:45 - 10:10	004	Mucosal melanomas: An update	*Klaus Busam*	
10:10 - 10:40		Coffee Break		
10:40 - 11:05	005	Comparative genomic hybridization and melanocytic lesions: An update	*Boris Bastian*	
11:05 - 11:30	006	Atypical melanocytic nevi from "special sites"	*Richard Scolyer*	
11:30 - 11:55	007	Regression in melanoma: State of the Art	*Lynn From*	
12:00		Lunch		
13:00 - 13:25	008	Histologic dysplasia of melanocytic nevi and melanoma risk	*Michael Piepkorn*	
13:25 - 13:50	009	Melastatin and melanoma	*Lyn Duncan*	
13:50 - 14:15	010	BRAF Mutations and melanoma	*Claudio Clemente*	
14:15 - 14:40	011	A Melanoma Registry on the Internet: the Brazilian experience Childhood Melanoma	*Gilles Landman*	
14:40 - 15:10		Coffee Break		
15:10 - 15:35	012	Pathological Aspects	*Raymond Barnhill*	
15:35 - 16:00	013	The Microenvironment as Regulator of Melanoma Biomarker Expression and Function	*Meenhard Herlyn*	
16:00 - 16:25	014	Melanin Synthesis in Melanoma Clinical implications	*Andrzej Slominski*	
16:25 - 16:50	015	Vaccine Strategies and their Pathology	*Martin Mihm*	
13:00 - 16:00		Satellite Symposium: Specials on Dermoscopy (Sponsored by TeachScreen, Germany)	*Chair: Andreas Blum*	M.R. 7
		Introduction	*Andreas Blum*	
	016	A typical melanocytic tumors	*Guiseppe Argenziano*	
	017	A typical non-melanocytic tumors	*Ashfaq A. Marghoob*	
	018	Facial tumors	*Wilhelm Stolz*	
	019	Tumors of nails	*Luc Thomas*	
	020	Tumors of the palms and soles	*Peter H. Soyer*	
	021	Vessels of tumors	*Andreas Blum*	
	022	Follow-up of melanocytic lesion: the Australian approach	*Scott W. Menzies*	
	023	Follow-up of melanocytic lesion: the German approach	*Claus Garbe*	
	024	Follow-up of melanocytic lesion: the American approach	*Alfred W. Kopf*	
		Discussion and closing remarks	*Andreas Blum*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
16:00 - 18:00		Satellite Symposium: Advancing the Treatment of Melanoma (Sponsored by Schering-Plough Corporation)	Chair: *Ian Quirt*	Ballroom A
		Welcome and Introduction	*Ian Quirt*	
		AJCC Staging Guidelines	*Jeffrey Gershenwald*	
		Neoadjuvant Treatment	*John M. Kirkwood*	
		Patient Management	*Helen Gogas*	
		Approaches to Improve Adjuvant Treatment	*Peter Mohr*	
		The Role of Surgery in the Management of Limited Distant Metastatic Disease	*Richard Essner*	
		Management of In Transit Metastases: Regional and Systemic Options	*Alexander Eggermont*	
		Closing Remarks	*Ian Quirt*	
18:30 - 19:30		Opening Ceremony		Ballrooms B/C
		Official Opening of Conference: *Lieutenant Governor of BC, the Right Honourable Iona Campagnolo*		
		Presidents' Welcome: *Dr. Natale Cascinelli, Chair, WHO Melanoma Programme and Chair, Local Organizing Committee*		
		Special Presentation "The Personal Impact of Melanoma": *Ms. Karen Graham, President and CEO, The William S. Graham Foundation for Melanoma Research*		
19:30 - 21:00		Welcome Reception		Parkview Terrace

DAY 2 WEDNESDAY 7TH SEPTEMBER

07:30 - 17:30		Registration		VCEC Foyer
08:00 - 18:00		Press Room		M.R. 6
08:00 - 18:00		Speaker Ready Room		M.R. 4
09:30 - 16:00		Exhibits		Exhibit Hall A
08:30 - 09:00		Opening Plenary Session	*Natale Cascinelli*	Ballrooms A/B/C
09:00 - 09:45		Grand Review	Chairs: *Charles Balch, Natale Cascinelli*	Ballrooms A/B/C
	025	"Tom Fitzpatrick Memorial Lecture" Introduction by Arthur Sober		
		Diagnosis and treatment of cutaneous melanoma - 2005	*Claus Garbe*	
09:45 - 10:30		Grand Review - Basic and Applied Sciences	Chair: *Meenhard Herlyn*	Ballrooms A/B/C
	026	Science and cutaneous melanoma - 2005	*Hensin Tsao*	
10:30 - 11:00		Coffee Break		Exhibit Hall A

TIME	P #	TITLE	PRESENTER	MEETING ROOM
11:00 - 12:00		**Surgical Margins: Are we recommending reverting to greater clearance?**	*Chairs: Natale Cascinelli; Mario Santinami*	Ballrooms A/B/C
	027	Results of randomized trial comparing wide vs. narrrow excision for melanoma thicker than 2mm	*J Meirion Thomas*	
	028	Randomized trial of a resection margin of 2 versus 4 cm for cutaneous malignant melanoma with a tumour thickness of more than 2 mm	*Ulrik Ringborg*	
	029	Excision margins in cutaneous melanoma: meta-analysis of randomised controlled trials	*Marko Lens*	
12:00 - 13:30		**Lunch and Poster Session I** *(Supported through an unrestricted educational grant from 3M Pharmaceuticals)*	*Chair: Harvey Lui*	Exhibit Hall A
13:30 - 15:00		**Concurrent Symposia**		
		a) Pathology Symposium	*Chairs: David Elder, Lynn From*	Ballroom A
	030	Desmoplastic and desmoplastic neurotropic melanoma	*Stanley McCarthy*	
	031	Dysplastic nevi	*David Elder*	
	032	Spitz tumors and atypical variants	*Raymond Barnhill*	
	033	Blue nevi and related conditions: an update.	*Artur Zembowicz*	
		b) Palliative care in malignant melanoma	*Chairs: Bill McCarthy, Romayne Gallagher*	Ballroom B
	034	Existential support and palliative care	*Andrew Kneier*	
	035	Anorexia-cachexia - what can we do about it?	*Neil MacDonald*	
	036	Approaching pain relief for patients with melanoma	*Norelle Lickiss*	
		c) Epidemiology	*Chairs: Marianne Berwick, Loraine Marrett*	Ballroom C
	037	Sun exposure, vitamin D and survival with melanoma	*Marianne Berwick*	
	038	Non UV risk factors for melanoma in agricultural health workers	*Leslie Dennis*	
	039	Melanoma incidence in the west of Scotland over 25 years	*Rona Mackie*	
	040	Is lentigo maligna a separate epidemiological type of melanoma?	*Jean-Jacques Grob*	
		d) Basic Biology	*Chairs: Margaret Kripke, Gang Li*	M.R. 11/12
	041	A new mouse model of BRAF-induced tumorigenesis	*Martin McMahon*	
	042	MDA7/IL-24: Novel melanocyte-derived cytokine, exhibiting immune and tumor suppressor properties	*Elizabeth Grimm*	
	043	ING family members promote nucleotide excision repair of UV-damaged DNA via enhancing chromatin relaxation	*Gang Li*	
	044	Selective killing of melanoma cells by exploiting differential effects of proteasome inhibition on noxa and anti-apoptotic Bcl-2 proteins	*Maria Soengas*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
13:30 - 15:00		**Concurrent Symposia *con't***		
		e) Dermoscopy	*Chairs: Al Kopf, Peter Soyer*	M.R. 8/15
	045	Dermoscopy update	*Wilhelm Stolz*	
	046	Why dermoscopy is beneficial!	*Ashfaq Marghoob*	
	047	When dermoscopy did change my mind	*Guiseppe Argenziano*	
	048	Benefits and caveats of digital monitoring	*Scott Menzies*	
15:00 - 15:30		**Coffee Break**		Exhibit Hall A
15:30 - 17:30		**Concurrent Proffered Paper Sessions**		Ballroom A
		a) Proffered Paper Session I - Clinical	*Chair: Arthur Sober*	
	CL1-1	Impact of primary melanoma risk factors and occult metastasis in sentinel node(s) on survival: results from a multi center sentinel lymph node working group	*Stanley Leong*	
	CL1-2	Heterogeneity of microscopic stage III melanoma in the SLN Era: Implications for AJCC/UICC staging and future clinical trial design	*Jeffrey Gershenwald*	
	CL1-3	Lymphatic invasion identified by D2-40 and younger age are predictors of sentinel node involvement in malignant melanoma.	*Lynn From*	
	CL1-4	Primary melanoma may not always metastasize to the most radioactive sentinel lymph nodes in the regional nodal basin	*Stanley Leong*	
	CL1-5	Melanoma metastases in lymph nodes identified by proton magnetic resonance spectroscopy of fine-needle biopsies	*Jonathan Stretch*	
	CL1-6	LYVE-1 immunostaining as a substitute for sentinel node status	*Katharine Acland*	
	CL1-7	Metallothionein: overexpression indicates poor prognosis - Long-term follow up of the Innsbruck melanoma cohort	*Georg Weinlich*	
	CL1-8	Clinical correlates of BRAF/NRAS mutation in melanoma	*Nancy Thomas*	
		b) Proffered Paper Session I - Behavioral Sciences	*Chair: Rona Mackie*	Ballroom B
	BE1-1	Epidemiology and search for background factors in malignant melanoma in children and adolescents.	*Peter Berg*	
	BE1-2	Are sunbed induced melanoma any different in histopathology?	*Anna Måsbäck*	
	BE1-3	Etiologic and other factors predicting nevus-associated cutaneous malignant melanoma	*Mark Purdue*	
	BE1-4	Do HMG-CoA reductase inhibitors lower risk of melanoma?	*Donald Miller*	
	BE1-5	Site-specific incidence of melanoma: A comparison of two populations with contrasting levels of sunlight	*David Whiteman*	
	BE1-6	Naevus counts are strongly associated with sunburns, sunbed and sun exposure: An adult twin study	*Veronique Bataille*	
	BE1-7	Nodular melanomas differ from superficial spreading melanomas epidemiologically - possible implications for causation	*Wendy Liu*	
	BE1-8	Sun exposure and nodular melanoma incidence	*Mathieu Boniol*	
		c) Proffered Paper Session I - Basic Sciences	*Chair: Yutaka Kawakami*	Ballroom C
	BA1-1	Dysregulation of FGFR2 signaling through mutations and alternate splicing is common in melanoma	*Pamela Pollock*	
	BA1-2	Impact of Retinoblastoma Binding Protein 2-Homolog 1(RBP2-H1) deficiency on Retinoblastoma Protein (pRb) phosphorylation in human malignant melanomas	*Alexander Roesch*	
	BA1-3	Distinct regulatory mechanisms regulate the expression of procathepsin L gene in human tumor cells	*Raymond Frade*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
15:30 - 17:30		**Concurrent Proffered Paper Sessions *con't***		
		c) Proffered Paper Session I - Basic Sciences		
	BA1-4	The role of the adhesion molecule L1 in melanoma development and progression	*Friedegund Meier*	
	BA1-5	Mechanisms and consequences of Claudin-1 overexpression in melanoma	*Poloko Leotlela*	
	BA1-6	Use of human tissue to assess the oncogenic activity of melanoma-associated mutations	*Yakov Chudnovsky*	
	BA1-7	Multiple metastatic melanoma induced by neonatal ultraviolet radiation treatment of Cdk4R24C/R24C/TPras mice	*Elke Hacker*	
	BA1-8	Could immunohistochemical staining of human telomerase reverse transcriptase be useful in diagnosis of melanocytic lesions?	*Alastair MacKenzie Ross*	
		d) Special Symposium on Advanced Therapeutics	*Chair: Sanjiv Agarwala*	M.R. 8/15
	SS1-1	Interferon-alpha As Adjuvant Therapy For Melanoma: A Meta-Analysis Of The Randomised Trials	*K Wheatley*	
	SS1-2	Long term survival benefit after adjuvant treatment of high risk cutaneous melanoma with dacarbazine and low dose natural interferon alpha: a controlled, randomised, multicentre trial	*Rudolf Stadler*	
	SS1-3	Evaluation of High Dose DNA/MVA Heterologous PrimeBoost Immunotherapy in Stage III/IV Metastatic Melanoma Patients	*Adam Dangoor*	
	SS1-4	MEDI-522, a humanized monoclonal antibody directed against the human alpha v beta 3 (avb3) integrin, +/- dacarbazine (DTIC) in patients with metastatic melanoma (MM) appears to prolong survival in a randomized, multicenter Phase II study	*Peter Hersey*	
	SS1-5	Beneficial impact of addition of interleukin-2 (IL-2) to systemic therapy on the long-term survival of patients with metastatic melanoma (MM)	*Agop Bedikian*	
	SS1-6	Vaccination with autologous amplified naked tumor-mRNA in patients with metastatic melanoma - Analysis of safety and immune responses in a Phase I/II clinical trial	*Benjamin Weide*	
	SS1-7	Clinical experience targeting the cancer testis antigen NY-ESO-1 in malignant melanoma	*Jonathan Cebon*	
	SS1-8	High resolution in vivo multiphoton tomography melanoma	*Karsten König*	
17:00 - 18:30		**Satellite Symposium: Critical Questions in Melanoma Vaccine Development**		M.R. 11/12
		(Sponsored by Serono Symposia International)	*Chair: Alexander Eggermont*	
		Introduction	*Alexander Eggermont*	
		Autologous vaccines	*Bernard Fox*	
		Dendritic cell-based vaccines	*Jim Mulé*	
		Allogeneic vaccines	*Peter Hersey*	
		Panel discussion		
19:30 - 21:00		**WHO Melanoma Program Meeting**		M.R. 8/15

TIME	P #	TITLE	PRESENTER	MEETING ROOM
DAY 3 THURSDAY 8TH SEPTEMBER				
06:30 - 08:15		Satellite Symposium: The Utility of IL-2 in Stage IV Melanoma (Sponsored by Chiron)		M.R.11/12
06:30 - 07:00		Buffet Breakfast		M.R. 11
07:00 - 07:10		Welcome and Introduction: Introduction of need for therapeutic options in Stage IV disease, review of highlights from Canadian Melanoma meeting (Banff 2004) discussions re IL-2, sub groups	*Michael Smylie*	M.R. 12
07:10 - 07:40:		Clinical Update 2005: The role of IL-2 in Melanoma in 2005 - Treatment Considerations State of the art review of published scientific literature and expert opinion on the role of IL-2 in treating Stage IV melanoma	*Sanjiv Agarwala*	
		Audience Q&A		
07:45 - 08:10		Patient selection / referral options for Canadian patients: Review of indications for referral and identification of suitable patients for treatment with HD IL-2	*Michael Smylie*	
		Audience Q&A		
08:10 - 08:15		Wrap-up & announcements - Reminder of Canadian Melanoma Meeting, Banff 2006, Adjournment	*Michael Smylie*	
07:00 - 0815		Satellite Symposium: Breaking Barriers in Tumor Immunology (Sponsored by Pfizer Inc.)	*Chair: Antoni Ribas*	M.R. 8/15
		Introduction,	*Antoni Ribas*	
		Assessing Methods to Enhance Cellular Immune Responses in Humans	*Pedro Romero*	
		Modulating Infiltrating T Cells in Human Metastatic Melanoma	*Manuelle Viguier*	
		Ticilimumab, an Anti-CTLA4 Monoclonal Antibody, in Patients with Advanced Melanoma	*Antoni Ribas*	
		Q&A and Conclusions	*Antoni Ribas*	
08:00 - 17:30		Registration		VCEC Foyer
08:00 - 18:00		Press Room		M.R. 6
08:00 - 18:00		Speaker Ready Room		M.R. 4
09:30 - 16:00		Exhibits		Exhibit Hall A
08:30 - 09:30		Plenary on Metastatic Disease:		Ballrooms A/B/C
		Recent Advances in Treatment of Metastatic Disease	*Chairs: Alexander Eggermont, Richard Klasa*	
	049	Phase II trials of BAY 43-9006 alone and in combination with chemotherapy in metastatic melanoma	*Keith Flaherty*	
	050	Randomized phase II trial of melanoma peptides with Montanide ISA 51 and different doses of IL-12 with alum for resected stages IIC/III and IV melanoma	*Jeffrey Weber*	
	051	Pegylated interferon in metastatic melanoma in the adjuvant and therapeutic setting.	*Alexander Eggermont*	

TIME	P #	TITLE		PRESENTER	MEETING ROOM
09:30 - 10:30		**Plenary on Vaccine Therapy: New Trends in Vaccine Therapy**	*Chairs:*	*Merrick Ross, Giorgio Parmiani*	Ballrooms A/B/C
	052	Postoperative canvaxin therapy for high-risk melanoma		*Donald Morton*	
	053	Adoptive cell transfer therapy following lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma		*Daniel Powell*	
	054	Direct immune monitoring, a precision measurement approach to the rapid optimization of cancer vaccines in their early clinical trial phase		*Pedro Romero*	
10:30 - 11:00		**Coffee Break**			Exhibit Hall A
11:00 - 12:00		**CURRENT STATUS PLENARY: Is there more than one biologic pathway to melanoma?**			Ballrooms A/B/C
			Chairs:	*Adele Green, Jason Rivers*	
	056	Epidemiologic evidence for multiple causal pathways to cutaneous melanoma		*David Whiteman*	
	055	Implications of a dual pathway for our public health messages		*Martin Weinstock*	
	057	BRAF mutations and sun-exposure patterns in primary melanoma		*Janet Maldonado*	
12:00 - 13:30		**Lunch and Poster Session II**	*Chair:*	*Vincent Ho*	Exhibit Hall A
13:30 - 15:00		**Concurrent Symposia**			
		a) New clinical tools for diagnosis of pigmented lesions	*Chairs:*	*Hubert Pehamberger, Haishan Zeng*	Ballroom A
	058	Computerized decision support for the diagnosis of pigmented skin lesions		*Michael Binder*	
	059	Polarized light imaging of pigmented lesions		*Steven Jacques*	
	060	Optical spectroscopy for skin pigmentation assessment and pigmented lesion diagnosis		*Nikiforos Kollias*	
	061	Ultrahigh resolution optical coherence tomography of human skin		*Wolfgang Drexler*	
	062	In vivo Confocal Microscopy for Pigmented Skin Lesion Diagnosis		*Salvador Gonzalez*	
		b) New melanoma risk markers and prognostics	*Chairs:*	*David Hogg, Mary Hendrix*	Ballroom B
	063	Functional identification of novel targets of p16INK4A		*David Hogg*	
	064	The Epigenetic Reprogramming of Melanoma Cells by the Microenvironment		*Mary Hendrix*	
	065	Stem Cells as Biomarkers for Melanoma		*Meenhard Herlyn*	
		c) Sunbeds: hazard or not?	*Chairs:*	*Philippe Autier, Richard Gallagher*	Ballroom C
	066	Sunbeds: hazard or not?		*Tim Lee*	
	067	Investigating tanning equipment use and risk of multiple vs. single primary cutaneous melanoma in Ontario, Canada		*Maria Chiu*	
	068	The Norwegian-Swedish cohort study on solarium use and cutaneous malignant melanoma in women		*Marit Bragelien Veierød*	
	069	Is there a need for regulating the "tanning industry"		*Philippe Autier*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
13:30 - 15:00		Concurrent Symposia *con't*		
		d) Familial melanoma	Chairs: *Julia Newton-Bishop, Wilma Bergman*	M.R. 11/12
	070	Mapping low penetrance melanoma genes and progress on identifying the 1p22 predisposition gene	*Nicholas Hayward*	
	071	Pancreatic cancer and neural system tumors (NSTs) in melanoma-prone families with CDKN2A mutations	*Alisa Goldstein*	
	072	Prevalence and penetrance of CDKN2A mutations in familial and non-familial melanoma	*Graham Mann*	
	073	The management of patients with a family history of melanoma	*Julia Newton-Bishop*	
		e) Primary prevention – are we making progress?	Chairs: *Karen Glanz, Rudiger Greinert*	M.R. 8/15
	074	Primary prevention of melanoma in Australia	*Dallas English*	
	075	Primary Prevention of Skin Caner in the United States	*Allan Halpern*	
	076	Primary Prevention of Skin Cancer Are we making progress in Europe?	*Rüdiger Greinert*	
	077	What primary prevention strategies work? The Community Guide evidence review	*Karen Glanz*	
15:00 - 15:30		Coffee Break		Exhibit Hall A
15:30 - 17:30		Concurrent Symposia and Profferred Paper Sessions		
		1) Special Pathology Symposium: Sentinel Lymph Nodes	Chairs: *Martin Mihm, Alistair Cochran*	Ballroom A
	078	The amount and location of sentinel node melanoma predict non-sentinel node tumor status and clinical outcome.	*Alistair Cochran*	
	079	How much histopathological sectioning is necessary to adequately evaluate sentinel nodes from melanoma patients?	*Richard Scolyer*	
	080	The use of molecular techniques in parallel with histology to evaluate the sentinel lymph nodes	*Hans Starz*	
	081	Molecular dynamics and trafficking of dendritic cells and lymphocytes to and within the sentinel node.	*Martin Mihm*	
		2) Proffered Paper Session II - Clinical	Chair: *Darrell Rigel*	Ballroom B
	CL2-1	Comparative performance of four dermoscopic algorithms for the diagnosis of melanocytic lesions in the hands of non-experts	*John Kelly*	
	CL2-2	Dermoscopy False Negative Melanomas	*Josep Malvehy*	
	CL2-3	Saving Lives Through the Early Detection of Small Melanomas (MM): The ABCDs Revisted	*Robert Friedman*	
	CL2-4	Melanoma Diagnosis by Fluorescence In Situ Hybridization	*Larry Morrison*	
	CL2-5	A narrow excision of melanomas of the back less than 2 mm in thickness increases regional recurrences and mortality	*Hakan Hallberg*	
	CL2-6	Evidence-based follow-up schedules for patients with melanoma	*Anne Brecht Francken*	
	CL2-7	The intraoperative identification of the sentinel lymph node in melanoma patients using 20% radioactive threshold	*A Romanini*	
	CL2-8	Radical dissectione of groin basin after positive SNB: overtreatment or standard surgery?	*Elisabetta Pennacchioli*	
	CL2-9	Evaluation of the ratio of invasive to in situ lesions in malignant melanoma	*Darrell Rigel*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
15:30 - 17:30		**Concurrent Symposia and Profferred Paper Sessions *con't***		
		3) Proffered Paper Session II - Basic Sciences	*Chair: Jean-Pierre Cesarini*	**Ballroom C**
	BA2-1	Absence of BRAF mutations in melanocytic nevi which develop in utero implicates UV-light as a causative agent for BRAF mutations	*Jürgen Bauer*	
	BA2-2	BRAF and Skp2 are targets for development of new treatment for melanoma	*Yutaka Kawakami*	
	BA2-3	PIK3CA mutations are rare while activation of Akt and Erk is frequent in cutaneous melanoma	*Johan Hansson*	
	BA2-4	Novel potential downstream effectors of the PTEN tumour suppressor gene in melanoma	*Leisl Packer*	
	BA2-5	Mutations of PIK3CA are infrequent in primary melanoma	*John Curtin*	
	BA2-6	Establishment and characterization of a cell line derived from the radial growth phase acral melanoma	*Hiroshi Murata*	
	BA2-7	Co-opertation between mutant V599EB-Raf and Akt3 promote melanocyte transformation	*Gavin Robertson*	
		4) Proffered Paper Session II - Behavioral Sciences	*Chair: Cheryl Rosen*	**M.R.'s 11/12**
	BE2-1	Epidemiological evidence supporting the role of UVA in melanoma carcinogenesis	*Edward Gorham*	
	BE2-2	CDKN2A germline mutations in individuals with sporadic cutaneous malignant melanoma	*Irene Orlow*	
	BE2-3	Association of ASIP genotype and melanoma risk with respect to MC1R genotype	*Peter Kanetsky*	
	BE2-4	Polymorphisms of the Vitamin D receptor gene and risk of melanoma: a case-control analysis	*Qingyi Wei*	
	BE2-5	The BRAF (T1799A) mutation is associated with distinct clinical characteristics in invasive primary melanoma	*Wendy Liu*	
	BE2-6	KIR 2DS4 genotypes in sporadic and familial melanoma from Italy	*Maria Teresa Landi*	
	BE2-7	Trends for in-situ and invasive melanoma in Queensland, Australia, 1982 to 2002	*Mark Smithers*	
	BE2-8	Feasibility of a population-based registration of phenotypic, anatomic and familial data for melanoma	*Jean-Luc Bulliard*	
19:00 - 23:00		**Gala Congress Banquet**		**Ballrooms A/B/C**

DAY 4 FRIDAY 9TH SEPTEMBER

08:00 - 16:00		**Registration**		**VCEC Foyer**
08:00 - 18:00		**Press Room**		**Meeting Room 6**
08:00 - 18:00		**Speaker Ready Room**		**Meeting Room 4**
09:30 - 16:00		**Exhibits**		**Exhibit Hall A**
08:30 - 09:30		**Keynote Lectures on Basic biology: Recent basic science discoveries which will open new treatment avenues**	*Chairs: Kowichi Jimbow, Menashe Bar-Eli*	**Ballrooms A/B/C**
	082	Transciptional pathways in melanocytes and melanoma	*David Fisher*	
	083	Gene Regulation in Melanoma Progression	*Menashe Bar-Eli*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
09:30 - 10:30		**Sunscreen Symposium (Sponsored by L'Oreal): Update on photobiology and photoprotection research**	*Chairs: Henry Lim, Jason Rivers*	Ballrooms A/B/C
	084	The behavioural attitude of humans toward sun exposure and protection	*Henry Lim*	
	085	Sunscreens and melanoma: what is the evidence?	*Adele Green*	
	086	An overview of sub-erythemal UVR effects on human skin	*Anny Fourtanier*	
	087	Need for a broad spectrum sunscreen in the protection of UVA induced photodermatosis	*André Rougier*	
10:30 - 11:00		**Coffee Break**		Exhibit Hall A
11:00 - 12:00		**Plenary Session on Current Status: Managing recurrent disease**	*Chairs: John Thompson, Ulrik Ringborg*	Ballrooms A/B/C
	088	Surgical metastasectomy: The first option for stage IV melanoma patients	*David Ollila*	
	089	Isolated regional chemotherapy for metastatic melanoma	*Hans de Wilt*	
	090	Role of radiation therapy in recurrent and metastatic melanoma	*Graham Stevens*	
12:00 - 13:30		**Lunch and Poster Session III**	*Chair: Richard Gallagher*	Exhibit Hall A
13:30 - 15:00		**Concurrent Symposia**		
		a) Surgery – Difficult sites and difficult cases	Chairs: Mario Santinami, Charles Balch	Ballroom A
	091	Obstructing metastases of the ileum with retroperitoneal metastases	*John Thompson*	
	092	Bulky regional recurrance of the axilla after irradiation (or groin)	*Daniel Coit*	
	093	Desmoplastic melanomas of the head and neck	*Samuel Fisher*	
	094	Large ALM of the thumb (or heel)	*Merrick Ross*	
		b) Mucosal melanoma (oral, anal, genital)	*Chairs: Richard Crawford, Dirk Schadendorf*	Ballroom B
	095	Mucosal melanoma	*Ronal Rapini*	
	096	Treatment of ocular melanoma	*Axel Hauschild*	
	097	Genital malignant melanoma	*Boer Ragnarsson-Olding*	
	098	Anorectal melanoma - epidemiology, prognostic features, staging, and treatment	*Craig Slingluff*	
		c) Management of congenital nevi, dysplastic and atypical nevi	*Chairs: Allan Halpern, David McLean*	Ballroom C
	099	Congenital melanocytic nevi update	*Ashfaq Marghoob*	
	100	A typical melanocytic nevi - epidemiology and clinical significance	*Claus Garbe*	
	101	Clinical management of dysplastic nevi	*Allan Halpern*	
		Panel discussion	*David McLean, Claus Garbe, David Elder, Ashfaq Marghoob, Allan Halpern*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
13:30 - 15:00		**Concurrent Symposia**		
		d) UVA vs UVB in the genesis of melanoma	*Chairs: Edward De Fabo, Anne Kricker*	M.R. 11/12
	102	Ultraviolet B radiation initiates melanoma whereas ultraviolet A does not	*Edward De Fabo*	
	103	UV light from 290 to 325 nm, but not broad-band UVA or visible light, augments the formation of melanocytic nevi in a guinea-pig model for human nevi	*Scott Menzies*	
	104	Data supporting an important role for wavelengths greater than UVB in inducing melanomas	*Richard Setlow, Marianne Berwick*	
	105	How can we examine whether UVA causes melanoma in humans?	*Anne Kricker*	
		e) Should we offer genetic testing for melanoma, and for which markers?	*Chairs: Rick Kefford, Margaret Tucker*	M.R.'s 8/15
	106	Susceptibility genes for melanoma in relation to other cancer risks and genetic testing	*Nelleke Gruis*	
	107	How sunlight, moles and genes interact in melanoma susceptibility?	*Florence Demenais*	
	108	Pros and cons of genetic testing	*Sancy Leachman*	
	109	Genetic counseling and testing in familial melanoma patients: the case of Liguria	*Giovanna Bianci Scarra*	
15:00 -15:30		**Coffee Break**		Exhibit Hall A
15:30 - 17:30		**Concurrent Symposia 08 and Proffered Paper Sessions**		
		1) Special Pathology Symposium: Prognostic factors and new techniques		Ballroom A
			Chairs: Raymond Barnhill, Stanley McCarthy	
	110	A European approach to sentinel lymph node evaluation	*Martin Cook*	
	111	Prognostic factors in primary melanoma	*Alain Spatz*	
	112	Gene expression profiling of primary cutaneous melanoma	*Joost van den Oord*	
	113	Angiotropic melanoma and extravascular migratory metastasis	*Raymond Barnhill*	
		2) Proffered Paper Session III - Clinical	*Chair: Jean-Jacques Grob*	Ballroom B
	CL3-1	Routine use of FDG-PET scans in melanoma patients with positive sentinel node biopsy	*Jørgen Lock-Andersen*	
	CL3-2	Superficial ultrasonography using simple diagnostic criteria is more sensitive and specific than palpation for the detection of regional lymph-node melanoma metastases	*Philippe Saiag*	
	CL3-3	Targeting melanoma vascularization: a new therapeutic concept	*Ferdy Lejeune*	
	CL3-4	Detection of circulating melanoma cells predict treatment response to neoadjuvant biochemotherapy in stage III melanoma patients	*Kazuo Koyanagi*	
	CL3-5	Duration of remission and survival following isolated limb perfusion: long term follow up	*Amira Sanki*	
	CL3-6	Stereotactic radiosurgery (SRS) as therapy for melanoma brain metastases: Impact of added surgical resection and whole brain radiotherapy	*Wolfram Samlowski*	
	CL3-7	Low efficacy of short course 5% imiquimod cream in lentigo maligna as assessed by complete surgical excision	*Martin Haskett*	
	CL3-8	Surgical management of tumor-positive sentinel lymph nodes in the groin: role of completion dissection	*Richard Essner*	

TIME	P #	TITLE	PRESENTER	MEETING ROOM
15:30 - 17:30		Concurrent Symposia 08 and Proffered Paper Sessions *con't*		
		3) Proffered Paper Session III - Basic Sciences	*Chair: Qingyi Wei*	Ballroom C
	BA2-1	Identification of a molecular signature for metastatic malignant melanoma in laser-microdissected tumor tissue	*Manfred Kunz*	
	BA2-2	Novel immunohistochemical prognostic markers in cutaneous malignant melanoma	*Joost van den Oord*	
	BA2-3	Culture of melanoma cells in three-dimensional architectures results in impaired immunorecognition by cytotoxic T lymphocytes specific for Melan-A/MART-1 tumor associated antigen	*Anca Reschner*	
	BA2-4	Dysplastic nevus cells and melanoma cells show similar disturbances in melanin metabolism and redox balance.	*Stan Pavel*	
	BA2-5	Combined determination of plasma L-DOPA/L-TYROSINE ratio and lactate deshydrogenase in melanoma: a comparison with S100B and MIA	*Konstantin Stoitchkov*	
	BA2-6	Ubc9 is highly expressed in melanoma-infiltrated lymph nodes and its depletion induces apoptosis in melanoma cell lines	*Stergios Moschos*	
	BA2-7	Overexpression of tyrosinase in melanoma cells increases bioreductive potential: reversal by the dietary polyphenol quercetin	*R Burd*	
	BA2-8	Human endogenous Retrovirus HERV-K is expressed in human melanoma and antibodies to HERV-K can be found in sera of melanoma patients	*Uwe Trefzer*	
		4) Proffered Paper Session III - Behavioral Sciences	*Chair: Jean Shoveller*	M.R. 11/12
	BE3-1	The changing incidence of cutaneous melanoma in the west of Scotland and Queensland 1982-2001, an intercontinental comparison	*Caroline Bray*	
	BE3-2	Seasonal variation in melanoma incidence in New South Wales reveals the efficacy of early detection and educational campaigns	*Mathieu Boniol*	
	BE3-3	Melanoma prevention: evaluation of an educational campaign using a new video-game in primary school children	*Philippe Saiag*	
	BE3-4	Feasibility of behavioral intervention with melanoma survivors	*Jennifer Hay*	
	BE3-5	Tanning attitudes and beliefs among sorority and fraternity students	*Leslie Dennis*	
	BE3-6	Validating self-reported sun habits of beachgoers	*David O'Riordan*	
	BE3-7	Predicting absolute risk of melanoma: a management model for use by primary care providers	*Thomas Fears*	
	BE3-8	The study of nevi in children (SONIC): baseline findings	*Susan Oliveria*	
16:00 - 22:00		Exhibit Move-out		Exhibit Hall A

DAY 5 SATURDAY 10TH SEPTEMBER

08:00 - 12:00	Registration	VCEC Foyer
08:00 - 12:00	Press Room	M.R. 6
08:00 - 12:00	Speaker Ready Room	M.R. 4

TIME	P #	TITLE	PRESENTER	MEETING ROOM
08:30 - 09:30		**Plenary Session on Point-Counterpoint:**		
		Are we overdoing the emphasis on sun protection?		Ballrooms A/B/C
			Chairs: Bruce Armstrong, Martin Weinstock	
	114	Sun protection and early detection: do we have the balance right?	*Martin Weinstock*	
	115	Is there a safe level of sun exposure?	*Bruce Armstrong*	
09:30 - 10:30		**Concurrent Plenary Sessions on Emerging Issues:**		
		1) New genetic and molecular markers for melanoma: what is their value?		Ballrooms A/B/C
			Chairs: Nick Hayward, Tim Rebbeck	
	116	Classifying melanomas by somatic genetic alterations	*Boris Bastian*	
	117	Systems medicine in melanoma therapeutics	*Jeffrey Trent*	
		2) Plenary Sunscreen Symposium: New developments in high-protection sunscreens		M.R. 11/12
			Chairs: Jean-Francois Doré, Brian Diffey	
	118	Can modern sunscreens prevent immunosuppression?	*Anny Fourtanier*	
	119	Can modern high-protection sunscreens provide broad-spectrum protection ?	*Michael Brown*	
	120	Are modern high-protection sunscreens safe?	*Jay Nash*	
	121	Should we be surprised that sunscreens appear not to be protective in melanoma?	*Brian Diffey*	
10:30 - 11:00		**Coffee Break**		Delagate Concourse
11:00 - 12:00		**Plenary on Current Status: Sentinel lymph node biopsy: does it prolong survival?**		Ballrooms A/B/C
			Chairs: Merrick Ross, Bill McCarthy	
	122	Prognostic impact of sentinel lymphadenectomy in early-stage melanoma: results of MSLT-I, a phase III international trial	*Donald Morton*	
	123	3000 sentinel node biopsies - procedures in a single Australian Centre - What have we learned?	*John Thompson*	
12:00 - 13:30		**Closing Ceremonies**		Ballrooms A/B/C

3Gen, LLC — Booth: 7

23801 Salvador Bay
Dana Point, CA 92429 USA
Tel: 949-481-6384
Fax 949-240-7492
Contact: John Bottjer

Experience the beauty of 3Gen innovation in advanced no-oil dermoscopy and simple-to-use digital epiluminescence microscopy with DermLite DL 100. DermLite Platinum, DermLite Pro, DLII Pro, DL II Multispectral, and DermLite FOTO.

Astron Clinica Ltd. — Booths: 37 & 38

The Mount, High Street
Toft, Cambridge CB3 7RL UK
Tel: +44 1223 265 000
Fax +44 1223 264 413
Contact: Suzanne Moss

Astron Clinica has developed a unique product suite that enables doctors and skincare specialists to look beneath the surface of the skin. DERMETRICSTM and SIAscopyTM, Astron Clinica's patented technology, examine the main components of the skin – blood, melanin, dermal melanin and collagen – and provide a detailed set of images that add up to a powerful tool in diagnosis and monitoring. Originally developed to address the growing problem of malignant melanoma, SIAscopyTM and DERMETRICSTM have a range of applications across skin conditions, wound management and cosmetics development.

Bayer Pharmaceuticals Corporation/Onyx Pharmaceuticals, Inc. — Booth: 35

400 Morgan Lane
West Haven, CT 06516 USA
Tel: 203-812-6160
Fax 203-812-6733
Contact: Sunny Yang

Please join us and be part of the innovative information programs, and to learn about the clinical communications and scientific services Bayer Pharmaceuticals Corporation and Onyx Pharmaceuticals, Inc. offer in Oncology.

Bristol-Myers Squibb/Medarex — Booth: 9

P.O. Box 4500
Princeton, NJ 08543 USA
Tel: 609-897-2000
Fax 609-897-6722
Contact: Sheree Budrecki

Bristol-Myers Squibb and Medarex welcome you to Vancouver! We invite you to visit our exhibit and welcome the opportunity to discuss the products and services we have to offer.

CanAg Diagnostics AB — Booth: 36

Majnabbe Terminal
SE - 414 55 Göteborg, Sweden
Tel: +46 31 85 70 30
Fax +46 31 85 70 40
Contact: Jenny Brinkeby

CanAg Diagnostics is a Swedish biotechnology company specialized in the development, manufacturing, and sales of immunological reagents and laboratory tests for the diagnosis and management of patients with cancer and brain diseases.

Canfield Imaging Systems — Booth: 1

253 Passaic Avenue
Fairfield, NJ 07004 USA
Tel: 973-276-0300
Fax 973-276-0339
Contact: Amber Regeling

Canfield Imaging Systems is a leading supplier and developer of medical imaging products, including DermaGraphix® Body Mapping for patients at risk for melanoma, Mirror® medical imaging software, VISIA® Complexion Analysis, OMNIA Imaging System, VECTRATM 3D and other medical imaging products.

Celgene Corporation — Booths: 5 & 6

7 Powder Horn Drive
Warren, NJ 07059 USA
Tel: 732-271-1001

Chiron BioPharmaceuticals — Booth:19

4560 Horton Street
Emeryville, CA 94608 USA
Tel: 800-524-4766
Fax 510-655-9910
Contact: Richard Faillace

Chiron Corporation, headquartered in Emeryville, California is a leading biotechnology company that participates in three global healthcare markets: biopharmaceuticals, blood testing and vaccines. Chiron BioPharmaceuticals markets Proleukin®, which is FDA approved for the treatment of metastatic melanoma and metastatic renal cell carcinoma. For more information about Proleukin®, visit the web site at HYPERLINK "http://www.proleukin.com" http://www.proleukin.com.

Derma Medical Systems — Booth: 21

Wiedner Hauptstrasse 140
A-1050 Vienna, Austria
Tel: +43 1 318 69 90 19
Fax +43 1 318 69 90 9
Contact: Mr. Josef Noesterer

Derma Medical Systems is a leading technology and solution provider for Digital Epiluminiscence Microscopy (DELM) and Skin Imaging. Product and Software development, manufacturing and know-how is based and consolidated at our headquarters in Vienna.

DigitalDerm, Inc. — Booth: 4

1334 Sumter Street
Columbia, SC 29201 USA
Tel: 803-231-2002
Fax 803-978-7456
Contact: Sam Chesnutt

The MoleMap CDTM is a photographic display system on CD-ROM, designed to serve as an adjunct to the physical examination when following patients who are at high risk for developing Cutaneous Melanoma.

Electro-Optical Sciences, Inc.

Booth: 8

One Bridge Street, Suite 15
Livingston, NY 10533 USA
Tel: 914-591-3783
Fax 914-591-3785
Contact: Jon Klippel

MelaFind®, developed by Electro-Optical Sciences, Inc., is intended to aid physicians in evaluating lesions and is currently under clinical investigation. Designed to detect melanoma with high sensitivity and specificity, MelaFind®'s preliminary tests suggest it may be useful to physicians in deciding which suspicious pigmented lesions of the skin require biopsy to rule out melamoma.

Elsevier Canada

Booth: 39

1 Goldthorne Avenue
Toronto, ON M8Z 5S7 Canada
Tel: 416-253-3640
Fax 888-359-9534
Contact: Jan Stainer-White

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FotoFinder Systems, Inc.

Booth: 25

10015 Old Columbia Road, Suite B 215
Columbia, MD 21046 USA
Tel: 410-312-7635
Fax 410-312-7632
Contact: Richard Engle

FotoFinder Systems, Inc. is remanufacturer and vendor of FotoFinderdermoscope II, the world-leading system for mole mapping and skin cancer screening. The integrated software modules Bodyscan for the detection of new moles and Dynamole, the diagnostic aid for the early detection of malignant melanoma are a great support for dermatologists' daily work.

Genta Incorporated

Booth: 28

Two Connell Drive
Berkeley Heights, NJ 07922 USA
Tel: 908-286-3966
Fax 908-464-1705
Contact: Janet Pignio

Genta Incorporated is a biopharmaceutical company focused on the identification, development and commercialization of drugs for the treatment of patients with cancer. The Company's research platform is anchored by two major programs that center on RNA and DNA-based medicines, such as Genasense® (oblimersen sodium) Injection, which is currently undergoing late-stage Phase 3 clinical testing, and Small Molecules, such as Ganite® , which is approved for the treatment of cancer-related hypercalcemia that is resistant to hydration.

Inovio Biomedical Corporation

Booth: 31

11494 Sorrento Valley Road
San Diego, CA 92121-1318 USA
Tel: 858-597-6006
Fax 858-410-3396
Contact: Bob Goodenow

Inovio Biomedical Corporation develops and markets minimal invasive solutions for cancer therapy based upon proprietary electroporation technology. Inovio's tumor ablation system selectively destroys cancer lesions while preserving healthy tissue and organ function, improves QOL, and reduces costs versus surgery. Inovio is advancing its MedPulser® platform through pre-marketing studies for head and neck cancer and skin cancers in Europe, a U.S. Phase III pivotal study for recurrent head and neck cancer, and a Phase I pancreatic cancer trial.

Lippincott Williams & Wilkins

Booth: 26

4816 139th Place SE
Snohomish, WA 98296 USA
Tel/Fax: 425-379-9354
Contact: Jay Oestriech

Lippincott Williams & Wilkins (LWW) is a leading international publisher of professional health information for physicians, nurses, specialized clinicians and students. LWW provides essential information for healthcare professionals in print and electronic formats, including textbooks, journals, CD-ROM, and via Intranets and the Internet.

Lucid Incorporated

Booths: 29 & 30

2320 Brighton Henrietta Townline Road
Rochester, NY 14623 USA
Tel: 585-239-9800
Fax 585-239-9806
Contact: Karen Loos

Lucid, Inc. is the world leader in real-time in–vivo cellular imaging with confocal microscopes for research and medical applications. Lucid's VivaScopes® have allowed dermatologists, clinical research centers and hospitals the capability to image skin and other tissues - in–vivo – with unprecedented resolution. Lucid's products have generated over 60 articles in refereed research and medical journals for applications ranging from basic skin research through skin cancer surgery to imaging of malignant and non-malignant skin disease.

L'Oreal Canada

Booth: 11

1500 University Street, Suite 600
Montreal, QC H3A 357 Canada
Tel: 514-287-4900
Fax 514-289-9008
Contact: Sandrine Michard

A leader in the Canadian cosmetics market, L'Oréal Canada offers its customers a wide range of choices with its diverse portfolio of brands and encompasses all aspects of beauty. Cosmetological and dermatological research is the focal point of L'Oréal's development strategy, with about 3% of its turnover devoted to research. More than 3,000 new formulas emerge from their laboratories every year. L'Oréal is the world leader among companies applying for patents in the cosmetics field, filing a patent application just about every day! One major patent is Mexoryl SX, a new, photostable molecule that filters short UVA rays. Today, this filter is included in the majority of L'Oréal's sunscreen products.

Melanoma International Foundation — Booth: 2

250 Mapleflower Road
Glenmoore, PA 19343 USA
Tel/Fax: 610-942-3432
Family/Patient Helpline: 866-463-6663
Web: www.melanomainternational.org
Contact: Catherine Poole

The Melanoma International Foundation, MIF, intends to save lives NOW. Through an aggressive campaign to teach skin self-examination and offer free dermatology screenings, we are finding melanoma early, when it is 90% curable. MIF also empowers patients/families to understand diagnostic and treatment issues through the hotline and international email response system.

Pfizer Inc. — Booths: 44 & 45

235 E. 42nd Street
New York, NY 10017 USA
Tel: 860-441-0858
Contact: Jon Sloss

Pfizer Oncology is focused & nbsp;expanding the clinical applicability of Aromasin and Campto(sar) and developing new medicines for cancers with high unmet need. Oncology is a major area of R&D investment and the Pfizer Oncology pipeline is one of the most robust in the industry, with 13 new agents in clinical testing.

Schering-Plough Corporation — Booth: 20

2000 Galloping Hill Road, K-5-2 B5
Kenilworth, NJ 07033 USA
Tel: 908-298-4877
Fax 908-298-7136
Contact: Marceline Phelps

Schering-Plough is a global science-based health care company with leading prescription, consumer and animal health products. Schering-Plough discovers, develops, manufactures and markets advanced drug therapies to meet important medical needs. Visit the Schering booth to learn more about melanoma.

Serono International S.A. — Booths: 22, 23, & 24

15bis, Chemin Des Mines
CH 1211 Geneva 20, Switzerland
Tel: +41 22 739 3000
Fax +41 22 731 2179
Contact: Jean-Pierre Malkowski

Serono is the world's third largest biotechnology company. Serono has strong market positions in neurology, reproductive health, metabolism and growth and has recently entered the psoriasis area with Raptiva®. Serono has a growing R&D program in oncology and together with its' partner CancerVax Inc. is developing a specific active immunotherapy, Canvaxin, for Stage 3 melanoma.

The William S. Graham Foundation for Melanoma Research, Inc. Booth:12

The "Billy Foundation"
26203 Production Avenue, Suite 12-A
Hayward, CA 94545 USA
Tel: 888-88-BILLY
Fax 510-264-9079
Contact: Karen L. Graham

The primary activities of this nonprofit foundation are to raise funds to support research for finding the cure of the deadly disease, Malignant Melanoma; to educate the public regarding the cause and prevention; and through public awareness programs, assist in the early detection of this insidious cancer.

Viragen - Biotechnology for Life Booth: 42

865 SW 78th Avenue, Suite 100
Plantation, FL 33324 USA
Tel: 954-233-8746
Fax 954-233-1414
Contact: Sandy Boyett

Viragen is a biotechnology company and manufacturer of Multiferon®, a natural leukocyte derived multi-subtype interferon alpha. Viragen is currently seeking international approval for Multiferon® for the first line adjuvant treatment of high risk malignant melanoma.

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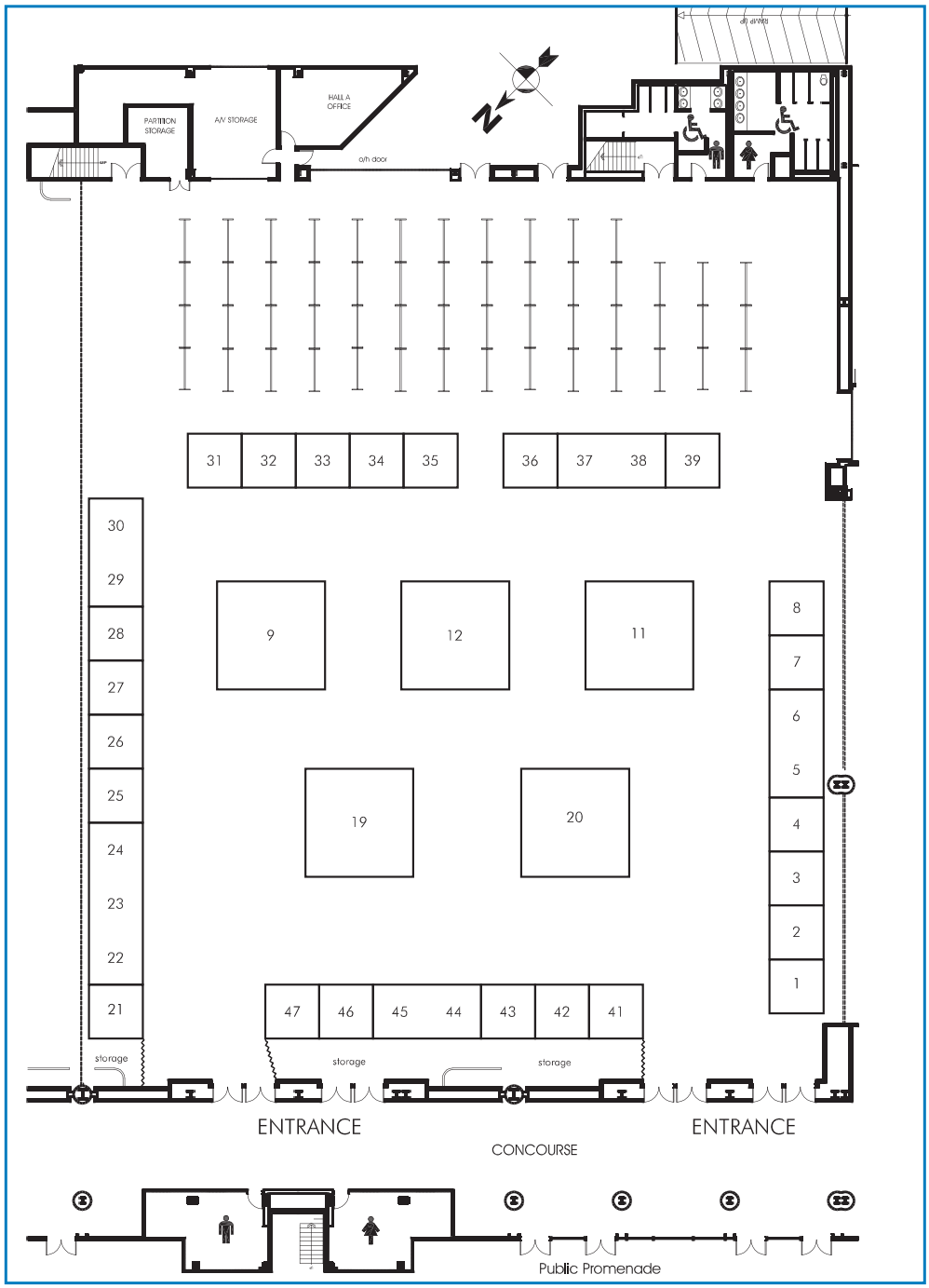

6th World Congress on Melanoma

POSTER LISTINGS

Poster Session I - Basic Sciences

P-001	MC1R variants as risk modifiers in CDKN2A mutation carriers of southern Swedish melanoma families	*Katja Backenhorn*
P-002	A novel melanoma-predisposing mutation, CDKN2A E27X, with a founder effect in Northern Italy	*Giovanna Bianchi Scarra*
P-003	An exonic tiling CGH microarray to interrogate 1p22: high-resolution analysis of familial melanoma	*Kevin Brown*
P-004	Frequency of COX-2 and C-KIT in malognant melanoma	*Melek Erkisi*
P-005	Quantitative trait loci underlying hereditary cutaneous melanoma in the MeLiM Swine model	*Claudine Geffrotin*
P-006	Association of the 609 C/T NAD(P)H: quinone oxidoreductase (NQO1) polymorphism with development of cutaneous malignant melanoma	*Nikolas Hodges*
P-007	Malignant melanoma in Isfahan, Iran	*Fariba Iraji*
P-008	Mapping of a novel ocular and cutaneous malignant melanoma susceptibility locus to chromosome 9q21.32	*Goran Jönsson*
P-009	Genomic profiling of malignant melanoma cell lines using high-resolution BACarray Comparative Genomic Hybridization	*Goran Jönsson*
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6th World Congress on Melanoma

A B S T R A C T S

001 BORDERLINE LESIONS: DO THEY EXIST?

S. McCarthy (Dept of Anatomical Pathology, Royal Prince Alfred Hospital, Sydney, Australia)*

Histopathologists are often confronted with otherwise banal naevoid lesions showing one or more atypical features. Mitoses are the main problem. Frequent mitoses (>2/mm2), deep mitoses and abnormal mitoses should normally be regarded as being of uncertain malignant potential, especially in adults. In some cases (young age ,pregnancy, recent trauma, exposure to strong sunlight and possibly hormonal medications) there may be increased normal-looking mitoses which should be assessed carefully. Pagetoid epidermal invasion is often used as an indicator of malignancy but may also be found in acral naevi,genital naevi, regenerating naevi and 'irritated' naevi . Irritation includes rubbing, topical agents and strong sunlight. Lymphovascular invasion is a strong indicator of malignancy but perineural invasion may be found in most benign and malignant melanocytic lesions. The most common borderline lesions have the morphology of Spitz tumours, compound dysplastic naevi, balloon cell tumours and variants of blue naevi including cellular blue naevus, deep penetrating naevus and pigmented epithelioid melanocytoma. Naevoid melanomas are often borderline as they frequently lack epidermal invasion and have a minimal junctional component. They may also have small cells and large areas devoid of mitoses. For medico-legal reasons and optimal patient management a second opinion from a melanopathologist is always advisable for borderline lesions

002 STANDARDIZED PATHOLOGY REPORTING OF PRIMARY CUTANEOUS MELANOMA

M. Trotter (University of Calgary, Canada)*

Essential elements for inclusion in a melanoma pathology report should be those required for accurate staging. Other prognostic factors, some of which are necessary for application of survival models, non-staging-related clinical decisions, or research, should also be included, but may be reported within a Comment section of the report. Essential elements in the Diagnosis field are: (1) Breslow thickness (mm); (2) Clark level; (3) ulceration (present or absent); (4) satellites and/or in-transit metastases (present or absent); and (5) status of margins. The American Joint Committee on Cancer (AJCC) staging system presents several challenges for the surgical pathologist. Problems frequently arise in interpretation of T-classification criteria. For example, factors complicating Breslow thickness measurement include tumor ulceration, epidermal hyperplasia, adnexal involvement by melanoma, microsatellites, and nevoid melanoma. The distinction between Clark level III (melanoma fills papillary dermis) and Clark level IV (invasion of reticular dermis) can be very problematic. The definition of ulceration requires refinement, and determining the presence or absence of ulceration is not always straightforward. Clinicians should be aware of problems faced by pathologists in the interpretation of these T-classification histologic criteria.

003 THE VERTICAL GROWTH PHASE AND PROGNOSTIC FACTORS IN MELANOMA

M. Mihm (Massachusetts General Hospital, USA), A. Piris (Massachusetts General Hospital, USA)*

The incidence of malignant melanoma is rising rapidly. In 1980, 1 in 250 Americans were diagnosed with melanoma. By contrast, in 2005, 1 in 75 Americans will be diagnosed with this disease. There has been extensive work toward the understanding and prevention of melanoma which has helped to increase early detection. However, work continues now on attempting to better understand 'prognosis' in melanoma. The most basic prognostic factor is the determination of whether there is radial or vertical growth phase. These two aspects of tumor biology will be discussed in detail. The next most basic factor in determining prognosis is the thickness of the primary malignant tumor as measured by the Breslow method. In 1970, Breslow established the criteria for diagnosis of malignant melanoma. Many studies have consequently confirmed the usefulness of this variable. Breslow's thickness represents one of the primary determinants for the T classification in the new AJCC staging system. As far as the overall prognostic variables are concerned, the most useful model for prognosis is that of Clark published in the Journal of the National Cancer Institute in 1989. These variables include Breslow thickness, number of mitoses, age and sex of the patient, presence of regression, and primary site of the tumor. In this communication we will discuss Breslow's and Clark's parameters highlighting those that are included in the new AJCC staging system. We also cover the recently validated prognostic indicators such as ulceration, microscopic satellites, and tumor infiltrating lymphocytes. A new approach to the evaluation of these infiltrating cells will be presented. Likewise, variables in melanoma vertical growth phase such as nevoid vertical growth phase and desmoplastic melanoma will be presented. The latest molecular markers of prognosis will also be reviewed.

NOTES:

004 MUCOSAL MELANOMAS

K. Busam (Memorial Sloan-Kettering Cancer Center, USA)*

Mucosal melanomas include primary tumors arising at a mucosal site as well as metastatic tumor deposits. Primary mucosal melanomas are rare. The incidences vary depending on gender and ethnicity. Primary melanomas may affect the mucosal epithelium of the respiratory, digestive, genito-urinary tract as well as the conjunctiva. Most primary melanomas tend to be large at the time of diagnosis and are followed by an aggressive clinical course. This review addresses issues related to the diagnosis, staging and management of mucosal melnanomas from a pathologist's perspective.

006 ATYPICAL MELANOCYTIC NAEVI FROM "SPECIAL SITES"

R. Scolyer (Department of Anatomical Pathology, Sydney Melanoma Unit & the Melanoma and Skin Cancer Research Institute, Royal Prince Alfred Hospital, Sydney, Australia, Australia)*

The diagnosis of melanocytic lesions can be extremely difficult. Part of the difficulty undoubtedly stems from the fact that both benign and malignant melanocytic lesions often share clinical and pathological characteristics. Pathological diagnosis of naevi and melanomas requires careful pathological assessment, knowledge of diagnostic criteria and awareness of potential pitfalls, as well as correlation with clinical data. In recent times it has been recognized that naevi occurring in "special sites" may display features that, if detected at another site, would be evidence for melanoma. These special sites include acral regions, the genitalia, perineum, umbilicus, conjunctiva, flexural surfaces, pinna and other sites. The atypical features include both architectural and cytological features as well as features of the host response. The difficulties associated with the diagnosis of atypical melanocytic lesions, including those occurring in special sites, are compounded when examining incomplete biopsy specimens such as shave or punch biopsies. Of all the naevi occurring in special sites, those on the acra are most easily confused with melanoma by virtue of their increased cellularity, lentiginous growth and suprabasilar scatter of melanocytes. A subset of acral naevi may also show poor lateral circumscription, elongation of rete ridges and cytological atypia causing particular difficulty in distinguishing them from melanoma. Naevi involving the genitalia are also prone to cause diagnostic problems. Some occurring in the vulval region, particularly in young women, may simulate aspects of melanoma. Naevi occurring in association with lichen sclerosis may show features in common with regenerating naevi and can be easily confused with melanoma. Naevi can also occur in other unusual sites at which they may cause difficulty in distinguishing from melanoma particularly if they show atypical features. These include atypical naevus cell rests within lymph nodes and naevi with atypical features occurring in mucosal sites.

007 WHY BOTHER REPORTING ON REGRESSION IN MELANOMA?

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Regression is seen in 10-20% of melanomas, on clinical and/or pathological examination. It has been reported for nearly forty years. Yet, the significance of regression has always been controversial with some studies suggesting a survival benefit and others finding no benefit. In multivariate analyses, regression has rarely been a significant prognostic factor. Part of the problem is the pathological definition. Is only end stage regression reported or does every dense lymphocytic infiltrate qualify as regression? A more rigid definition of regression, characterized by widening of the papillary dermis, neovascularization, melanophages, and a marked decrease or absence of melanoma cells might be helpful for future studies. The presence of regression may influence clinical management in the individual patient. A surgeon should be more diligent in planning the re-excision if regression has been identified. A clinician should consider that the prognosis based on Breslow's thickness might not be accurate when regression is present.

NOTES:

008 A CASE CONTROL STUDY OF HISTOLOGICAL DYSPLASIA IN MELANOCYTIC NEVI

M. Piepkorn (University of Washington, USA)*

Although clinically atypical nevi are associated with increased stochastic risk for melanoma, histologically dysplastic nevi have not been quantitatively assessed as melanoma risk factors by case-control study design. We conducted a case-control analysis at the University of Washington, enrolling 80 newly incident cases of melanoma and their respective spouses as controls. All subjects underwent skin examination and completed a questionnaire directed at melanoma risk factors. The clinically most atypical macular nevus from each case and control was removed for biopsy examination. A 13-member panel of dermatopathologists from the North American Melanoma Pathology Study Group independently evaluated each sample, blinded as to the clinical status. Histological dysplasia was accordingly assigned for each sample on a subjective scale, with 0 = no dysplasia, 1 = mild dysplasia, 2 = moderate dysplasia, and 3 = severe dysplasia. All data were evaluated by univariate and multivariate analyses. In the assessment of histological dysplasia, those subjects with panel average ratings > 1 had an increased relative risk of melanoma (odds ratio 2.60, 95% confidence interval 0.99-6.86) by univariate analysis. This effect persisted after adjustment for confounders (OR 3.99, 95% CI 1.02-15.71). The kappa statistic was 0.28 for the panel histological diagnoses, indicating poor interobserver reproducibility. In sum, histological dysplasia does appear to constitute an independent risk for melanoma; however, the association is weak and thus does not lend itself well as a reliable risk indicator for melanoma.

009 MELASTATIN (MLSN/TRPM1)IN MALIGNANT MELANOMA

S. Zand (Harvard Medical School, USA), E. Buzney (Harvard Department of Dermatology, USA), L. Duncan (MGH Harvard Medical School, USA)*

The melanocyte specific gene melastatin (MLSN/TRPM1) was identified using differential mRNA analysis of murine B16 melanoma cell lines with varying capacity to metastasize in vivo. MLSN/TRPM1 mRNA is expressed at high levels in human benign nevi and melanomas in situ; MLSN/TRPM1 expression is variable in invasive melanoma and shows loss of expression in all melanoma metastases. In patients with localized primary cutaneous melanoma, downregulation of MLSN/TRPM1 mRNA in the primary tumor is associated with a reduced disease-free survival. While MLSN/TRPM1 may be a prognostic marker for patients with melanoma, little is known about the regulation of MLSN/TRPM1 in vivo. MITF, an essential melanocyte transcription factor, directly regulates expression of MLSN/TRPM1 in human melanoma cell lines. MITF has also been shown to regulate the melanoma markers MART1/MLANA (melan-A) and SILV/PMEL17/GP100 (HMB-45). In the melanocyte lineage, MITF appears to play dual roles of survival and differentiation. When large melanoma metastases in lymph nodes are examined histologically, all display a significantly large region without detectable MLSN/TRPM1 mRNA expression. However, many of these metastatic tumors also display a subpopulation of melanoma cells with strong MLSN/TRPM1 mRNA signal. While primary cutaneous melanoma is known to be morphologically and molecularly heterogeneous, heterogeneity in lymph node metastases is not well characterized. The expression of MLSN/TRPM1 mRNA by a subset of melanoma cells in lymph node metastasis may be consistent with upregulation of MLSN/TRPM1 mRNA in the tumor cells after they have metastasized to a specific location. This upregulation is of particular interest given that loss of melanoma expression is associated with tumoral metastasis. In studies of human melanoma metastases MLSN/TRPM1 mRNA expression does not correlate in a simple fashion with the expression of S-100, HMB-45, MART-1, or NK1/C3. These findings support the concept that multiple pathways of MLSN/TRPM1 are in play in melanocytic tumorigenesis.

010 BRAF V599E MUTATION AND AP2A EXPRESSION IN SPITZ AND REED NEVI AND IN RADIAL AND VERTICAL GROWTH PHASE MELANOMA

C. Clemente (Anatomia Patologica e Citopatologia, Italy), C. La Porta (Department of Biomolecular Scinece, University of Milano, Italy)*

Spitz/Reed nevi which occur typically in childhood and adolescence showing histopatologic characteristic features resembling melanoma, are usually considered a benign lesion. Herein we have analysed in Spitz/Reed nevi, in RGP and in VGP the possible presence of V599E BRAF mutation. All the bioptic samples of Spitz/Reed nevi and VGP melanoma showed such a mutation, while in RGP V599E mutation seems to be extremely rare. Furthermore, in the same biopsy samples and in lymph node metastasis the expression and localization of the transcription factor AP2 and of wnt-5 was also considered. In fact, AP2 was demonstrated to be crucial in the development of malignant melanoma, demonstrating a high level of expression in RGP and a low in VGP melanoma, but no data have been yet reported in the literature for AP2 expression in Spitz and Reed nevi. In contrast, wnt-5 was demonstrated to be expressed at high levels in VGP melanoma but at low level in RGP melanoma. Our findings show a strong nuclear signal in RGP only for AP2. In fact, both Spitz/Reed nevi, VGP and metastasis are negative for such a factor. Taken together, the screening for neither V599E BRAF mutation nor for AP2 is useful for discriminate Spitz/Reed nevi from melanoma. However, from these findings, an open question arises: are Spitz/reed nevi really a benign lesion? In fact, they show the expression of the same factors of VGP melanoma.

011 BRAZILIAN INTERNET BASED MELANOMA REGISTRY: A PILOT WITH 465 PRIMARY CUTANEOUS MELANOMA FOR A NATIONWIDE DATABASE

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Mean melanoma incidente in Brazil is 5.2 per 100.000. However, in each region, population has a wide ethnic variability; therefore one must consider the geographic area when evaluating melanoma. Very few standardized protocols have been established to compare data from different geographical regions. OBJECTIVES: we aimed to develop a nationwide standardized melanoma registry, through internet (www.gbm.org.br). PATIENTS AND METHODS: In a 4 years period, the registry collected information on patients clinical data, risk factors, histopathology, staging, sentinel lymph node (SLN), adjuvant therapy and follow-up. Four institutions enroled in a pilot study. Statistics was performed using SPSS 10.0. RESULTS: 465 patients (275 female and 190 males) had 77 (16.6%) in situ melanoma and 388 (83.4%) invasive primary cutaneous melanoma. Mean age at diagnosis was 52.7 years-old. The majority were caucasians (n=429 – 92.2%). Most lesions occured in the limbs (48.4%) and trunk (35.9%). Twenty (4.3%) patients had family history of melanoma; 132 (28,4%) / 165 (35,5%) light hair and eyes; 35 (7.5%) atypical nevus, 40 (8,6%) small congenital nevus; and 128 (27,5%) multiple nevi. Most invasive melanoma were ≤ 1.0 mm in depth (187 patients - 40.2%). Superficial spreading melanoma prevailed (257 - 66.2%), followed by nodular (49 -12.7%), acral-lentiginous (44 - 11.3%), lentigo maligna melanoma (16 - 4.2%), and other subtypes (22 - 5.2%). Ulceration was present in 95 (20.4%) lesions and regression in 69 (14.8%). SLN were evaluated in 212 (45.6%) patients. Micrometastatic melanoma was found in 45 (21.9%) patients, a rate of 1.15 metastases per base. CONCLUSION: This registry has proven its efficacy and is a very important tool to obtain valuable and reliable data in a large country like Brazil. We expect in the near future to have at least one institution per state enroled in this registry.

012 CHILDHOOD MELANOMA: PATHOLOGICAL ASPECTS

R. Barnhill (University of Miami, USA)*

The diagnosis of malignant melanoma in childhood and adolescence remains a difficult problem for the pathologist. The reasons for this have largely remained the same for many years. Melanoma in prepubertal individuals is extremely rare, and physicians are reluctant to diagnose melanoma in such young patients without substantial evidence that they are correct. In addition, there continues to be considerable difficulty in distinguishing true melanoma from atypical Spitz tumors, atypical melanocytic nevi, and atypical melanocytic proliferations developing in congenital melanocytic nevi. Recognized variants of childhood melanoma include: melanomas resembling conventional adult melanomas, melanomas arising in congenital nevi, small cell melanomas, and so-called spitzoid melanomas. The author urges caution in rendering a diagnosis of melanoma in childhood without adhering to stringent criteria, since many such lesions are not true biological melanoma. Borderline lesions not meeting strict criteria for melanoma should be considered biologically indeterminate and patients managed with re-excision and close follow-up examinations.

NOTES:

013 THE MICROENVIRONMENT AS REGULATOR OF MELANOMA BIOMARKER EXPRESSION AND FUNCTION

M. Herlyn (The Wistar Institute, USA)*

When normal human melanocytes are cultured, they express approximately 80% of all melanoma antigens on their surface, whereas melanocytes in normal human skin do not. When the melanocytes are co-cultured with keratinocytes, they loose melanoma antigen expression within 3 to 4 days. The keratinocytes, thus, control whether and which markers are expressed on the surface of the melanocytes. They also control melanocyte growth. Melanocytes and keratinocytes closely adhere to each other through E-cadherin, desmoglein 1 and likely other cadherins. Melanoma cells, on the other hand, are refractory to regulation by keratinocytes. Forced expression of E-cadherin in melanoma cells re-establishes the control of keratinocytes over the melanoma cells, i.e, they are no longer invasive and tumorigenic. The mechanisms for the down-regulation of invasion-related molecules on melanoma cells by keratinocytes are not known. Re-establishing these mechanisms may lead to new therapeutic opportunities and may also have diagnostic consequences. The cell surface molecules that are regulated by keratinocytes include the migration and invasion-related integrin avß3 and the cell-cell adhesion receptor of the Ig supergene family Mel-CAM (Cd146), which are both strongest expressed by metastatic cells. When melanoma cells of the radial growth phase that lack both ß3 and Mel-CAM are transduced with the respective cDNA for overexpression, there are few changes as long as the cells are cultured in conventional culture flasks. However, the same transduced cells show highly invasive growth patterns when placed in a three-dimensional matrix. Skin organotypic cultures consist of a 'dermis' of collagen with embedded fibroblasts and an 'epidermis' of multilayered keratinocytes with melanocytes. This model system is superbly suited to investigate the functional significance of genes associated with melanoma progression. A novel series of melanoma biomarkers can also be developed from experimentally induced lesions that arise in human skin grafted to immunodeficient mice and treated with adenoviral vectors for the growth factors bFGF, SCF, and ET-3 and with concomitant UV B irradiation. These lesions are benign and require continuous stimulation by growth factors unless the melanocytes have been genetically modified to express oncogenes or suppress tumor suppressor genes. Global gene expression analyses of experimentally induced lesions reveal activation of specific sets of genes that are similar to those found in patients lesions. Biomarkers also help us to trace the origins of melanomas, i.e., whether tumors arose from precursor versus mature melanocytes. We are learning to identify subpopulations of cells in melanoma lesions that characterize stem cells, i.e., small subpopulations that can self-renew and differentiate and that are responsible for progression and tumor fate.

014 MELANIN SYNTHESIS IN MELANOMA: CLINICAL IMPLICATIONS

A. Slominski (University of Tennessee HSC, USA), A. Carlson (Albany Medical College, USA), J. Wortsman (University of Southern Illinois, USA), M. Mihm (Massachusetts General Hospital, USA)*

The presence of melanin provides an important diagnostic tool for melanomas. In addition, melanin synthesis may either play a role in pathogenesis of melanoma or affect its clinical course. Melanin pigment is a result of transformation of L-tyrosine through series of oxidoreduction reactions catalyzed by enzymes. There are intrinsic properties of melanogenesis, which affect the behavior of melanoma cells themselves or, the surrounding environment. Melanogenesis can modify cellular metabolism and generate an oxidative environment; some of its intermediates are in fact highly reactive and show genotoxic and mutagenic properties. This can potentially lead to genetic instability with generation of more aggressive cell populations. Furthermore, melanogenesis intermediates, of which an example is L-DOPA, can directly inhibit activity of T and B lymphocytes to generate an immunosuppressive environment. The combined mutagenic and immunosuppressive effects generated by active melanogenesis could, within a tumoral environment, destabilize tumor cells and their stroma to result in melanoma progression. From the therapeutic point of view, structural or enzymatic proteins of the melanogenic apparatus can serve as antigens in melanoma therapy, but active melanogenesis could counteract immunotherapy directed at melanoma antigens. Lastly, the intrinsic biophysical properties of melanin could attenuate the effect of radiotherapy, phototherapy, or chemotherapy, since melanin can bind (chelate) chemotherapeutic and cellular toxins, thus decreasing their efficiency in chemotherapy. Melanin can also produce a hypoxic environment that may lead to radioresistance. It also absorbs light (photofilter) that can decrease the efficiency of photodynamic therapy. Thus, melanogenesis can affect the outcome of melanoma by independent actions at multiple steps of the tumor course. In this context, our previously proposed strategy of melanogenesis inhibition as adjuvant strategy in therapy of melanoma is becoming more robust concept that should probably be moved from merely theoretical to the experimental and perhaps clinical grounds.

NOTES:

016 ATYPICAL MELANOCYTIC TUMORS

G. Argenziano (University of Naples, Italy)*

The use of dermoscopy has definitely changed the practice of dermatologists for several reasons. The first and most important one is that dermoscopy forces physicians to dedicate more time and care for an individual with a pigmented skin lesion. This fact alone might be the reason why patients like dermoscopy and even ask physicians to perform this technique, at least in Europe. Second, using dermoscopy to differentiate suspicious lesions from benign ones is helping dermatologists make better, more selective decisions about biopsy excisions. This non-invasive diagnostic technique uses optic magnification to make the lesion's morphologic features more visible than by eyesight alone, thus increasing the clinician's confidence in differentiating banal from suspicious lesions. By using dermoscopy, doctors can decrease the number of benign lesions excised unnecessarily by approximately one-third from the number excised solely on the basis of standard clinical examination. Third, it is in our trust that dermoscopy increases early detection of melanoma. Prior to using dermoscopy, a lesion should be evaluated clinically using visual information. Whether or not such a clinical examination raises the suspicion of melanoma, all lesions should again be checked using dermoscopy. Most often dermoscopy just confirms the diagnosis already performed by the naked eye, but sometimes it opens the mind to new possibilities.

017 DERMOSCOPY OF NON-MELANOCYTIC TUMORS

A. Marghoob (Memorial Sloan-Kettering Cancer Center, USA)*

Dermoscopy is helpful in the evaluation of both pigmented and non-pigmented non-melanocytic lesions. However, there also exist primarily non-melanocytic lesions that can on occasion have associated melanocytes. Two such lesions include basal cell carcinoma (BCC) and metastatic breast cancer. The dermoscopic appearance of these tumors reveals structures and features that can be confused with melanoma. Aside from these rare tumors dermoscopy has shown utility in the evaluation of non-melanocytic lesions lacking pigment. The presence of blood vessels, which may represent neoangiogenesis, has helped dermoscopist to correctly identify tumors including non-pigmented BCC, merkel cell carcinoma, malignant fibrous histiocytoma, sebaceous carcinoma, and squamous cell carcinoma. Furthermore, there exist a group of lesions that are termed 'collision' tumors. These clinically suspect growths consist of two benign lesions occurring, by coincidence, juxtaposed to one another (i.e., seborrheic keratosis and hemangioma). Dermoscopy has greatly helped in the evaluation of these collision tumors. In conclusion, dermoscopy can increase a clinician's index of suspicion for some non-pigmented cutaneous malignancies and can lower the index of suspicion for some clinically confusing lesions such as collision tumors.

018 FACIAL TUMORS

W. Stolz (Dept. of Dermatology, Hospital Munich Schwabing, Germany)*

Histological examination of adult facial skin elucidates that the rete ridges are much flatter. Thus a regular pigment network does not develop. Instead dermatoscopic examination reveals a distinct pigment pattern which is known as a pseudonetwork. This feature is seen in both melanocytic and non-melanocytic lesions. The distinction between a flat seborrheic keratosis (lentigo senilis) and a lentigo maligna (or even lentigo maligna melanoma) is of special importance on the face. A multivariate analysis showed that asymmetrical pigmented follicular openings, blue-gray dots and globules, as well as dark brown and black annular streaks (the annular-granular pattern) strongly suggest a lentigo maligna. Horn pseudocysts, finger print-like pattern, moth-eaten border and jelly sign suggest areas indicate a flat seborrheic keratosis. Both a pigmented actinic keratosis and a benign lichenoid keratosis can mimic a lentigo maligna.

NOTES:

019 DERMOSCOPIC EXAMINATION OF NAIL PIGMENTATION

L. Thomas (Department of Dermatology, Hotel Dieu, France)*

Diagnosis of longitudinal melanonychia is usually difficult and neither single clinical criterion nor combination of symptoms are efficient enough to clearly distinguish malignant from benign band-like pigmented nail lesions. Biopsy is painful and often leaves definitive dystrophic scars. Dermoscopy offers new semiological elements to more accurately decide whether or not pathological examination should be performed. In doubtful cases pathlogy remains the gold standard. Seven semiological patterns have been described : 1) Blood spots (purple-blue (recent lesions) or a brown-black coloration (Older lesions)); are in favor of sub-ungual hemorrhages but only in the absence of other symptoms. 2) Brown background: is associated with melanocytic hyperplasia (nevus, melanoma). 3) Brown longitudinal parallel lines with regular coloration, spacing and thickness and absence of parallelism disruption are in favor of benignity; their association with a brown background coloration is in favor of a nail apparatus nevus. 4) Longitudinal brown-to-black lines with irregular thickness, spacing or coloration and parallelism disruption is strongly indicative of melanoma. 5) Homogeneous grayish lines with gray pigmentation of the background are found in cases of epithelial hyperpigmentation without marked melanocytic hyperplasia and are therefore observed in ungual lentigo, ethnic pigmentation, Laugier-Hunziker syndrome, post-inflamatory (trauma-induced) pigmentation and drug-induced pigmentation. 6) 'Micro-Hutchinson's sign', is only observed in melanoma, but is rare and its specificity is not assessable. 7) Microscopic longitudinal grooves are unspecific and observed in several conditions and associated with any type of ungual discoloration. We believe that dermoscopical examination of the nail plate in case of longitudinal melanonychia provides useful information that could help clinicians to more accurately decide if a nail apparatus biopsy should be performed. S Ronger et al. Arch dermatol (2002), 138, 1327-33

020 TUMORS OF THE PALMS AND SOLES

HP. Soyer (Department of Dermatology, Medical University Graz, Austria)*

Dermoscopy has been demonstrated to significantly improve the accuracy in diagnosing melanocytic lesions and melanoma on acral sites. And more specific identification of benign dermoscopic patterns avoids unnecessary surgery on palms and soles. The following dermosocpic patterns can be found on acral skin: 1) parallel furrow 2) lattice-like 3) fibrillar 4) homogeneous 5) globular 6) acral reticular 7) nontypical. At this anatomic site, the surface skin markings are arranged in a parallel fashion. The eccrine pores are indicated by small open circles, which help us to distinguish the ridges of the surface skin markings from the sulci. The major dermoscopic patterns observed in melanocytic nevi on acral skin are the parallel furrow pattern, the lattice-like pattern, and the fibrillar pattern. In the parallel furrow pattern, parallel linear pigmentation along the sulci of the skin markings are observed. And subtle variations on the theme are frequently recognized in this pattern. The latticelike pattern shows linear pigmentation following and crossing the surface sulci. The fibrillar pattern exhibits numerous fine fibrillar lines running in a slanting direction to the skin markings. In melanoma, including melanoma in situ, the most characteristic dermoscopic pattern is the parallel ridge pattern, which is characterized by a band-like pigmentation on the ridges of the surface skin markings. Another dermoscopic feature of melanoma affecting acral skin is diffuse pigmentation with variable shades from tan to black. Other dermoscopic features frequently detected in melanoma on volar skin are irregular dots/globules and an abrupt sharp demarcation at the edge of the lesions. Other relevant pigmented lesions on acral sites are the so-called black heel or intracorneal hematoma as this lesion occasionally poses problems in the clinical differentiation from melanoma.

021 VESSELS OF TUMORS - SPECIALS ON DERMOSCOPY

A. Blum (Private and Teaching Practice of Dermatology, Germany)*

In dermoscopy the pattern of the vessels of tumors can give excellent hints for the pathology of skin tumors. Hereby the kind of examination is the first step which has to be considered, followed by the knowledge about the different normal vessel typs of locations at the body. Benign and malignant skin tumors can present different pattern of vessels: Lacunas, arborizing vessels, crown vessels, comma vessels, pinpoint and hairpin vessel. Finally skin tumors can also present a polymorphia of vessels. All these vssel pattern are presented with different benign and malignant epithelial or melanocytic skin tumors.

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022 FOLLOW-UP OF MELANOCYTIC LESINS: THE AUSTRALIAN APPROACH

SW. Menzies (Sydney Melanoma Diagnostic Centre, Sydney Cancer Centre & University of Sydney, Australia)*

Digital monitoring of melanocytic lesions can be divided into long term or short term monitoring. The Sydney Melanoma Unit described the first study using short term monitoring of suspicious melanocytic lesions (Menzies SW et al. Arch Dermatol. 2001;137:1583-89). Here, melanocytic lesions without dermoscopy features of melanoma but are moderately atypical without a patient history of change or mildly atypical with a history of change are monitored over a 3 month period. Any change warrants excision. The specificity of the technique is 83% and the theoretical sensitivity for the diagnosis of melanoma is 100%. The monitoring protocol allows the detection of dermoscopy featureless melanomas while allowing a reduction of the benign:malignant excision ratio to detect these from 44:1 (without monitoring) to 8:1 (with monitoring). Our experience with the technique over an 8 year period will be described.

023 FOLLOW-UP OF ATYPICAL MELANOCYTIC NEVI - THE GERMAN APPROACH

C. . Garbe (Division of Dermatologic Oncology, Department of Dermatology, University of Tuebingen, Germany)*

Nevus screening should be regularly performed in persons with elevated risk for cutaneous melanoma (CM) development, particularly in persons with atypical nevus syndrome (> 50 melanocytic nevi, > 5 atypical melanocytic nevi), in persons with multiple common nevi (> 100 melanocytic nevi) and in CM patients. In our experience screening examinations once a year are sufficient for the early detection of CM. The combination of macroscopic and microscopic pictures seems to be the most appropriate approach. About half of all CM develop de novo and can be recognized exclusively on macroscopic pictures. The other half develops on pre-existing melanocytic nevi. By clinical and dermoscopic means it is impossible to predict on which nevus CM will develop. Unfortunately, we are presently unable to identify the precursor lesions of CM. Atypical melanocytic nevi have to be regarded as markers of melanoma risk, but not as true precursors. Our approach to nevus screening is based on digital dermoscopy and digital storage of macroscopic pictures. Body sites with many or atypical melanocytic nevi are documented with macroscopic pictures and these are stored in the computer. Atypical melanocytic nevi are highlighted and enumerated on the macroscopic pictures. Then, additionally, dermoscopic pictures of the highlighted lesions with a 20fold magnification are stored in the computer. Thus, a follow-up of these lesions is enabled. As a rule, also atypical melanocytic nevi remain very stable in adulthood with a tendency towards regression. Indication for excision depends on growth of these lesions with asymmetric or CM-like features. In conclusion, computer-stored macroscopic and microscopic pictures of melanocytic lesions enable an exact follow-up of pigmented lesions and can be performed in a short time (15 minutes or less). Thus, this procedure is safe and satisfactory for the patient and the physician.

024 FOLLOW-UP OF MELANOCYTIC LESIONS: THE AMERICAN APPROACH

A. Kopf (New York University School of Medicine, USA)*

This lecture will concentrate on the management and follow-up of patients with atypical (dysplastic) nevi. The key points presented are as follows: 1) Clinically, dysplastic nevi (DN) share some or all of the ABCDE features of malignant melanoma (MM). 2) Dermoscopically, DN present as a broad spectrum of patterns ranging from 'benign' to 'indeterminate.' 3) On dermoscopy, the vast majority of DN can be characterized as 'benign.' 4) 'Indeterminate' DN are lesions that share dermoscopic features also seen in MM. 5) Follow-up using baseline total cutaneous photography coupled with periodic total cutaneous examination and self-examination of the skin has proved eminently successful in avoiding fatal MM. 6) Sequential digital imaging; 'machine vision'; and teledermoscopy are evolving technologies that show promise in following DN. 7) In sum, dysplastic nevi are atypical moles that clinically and dermoscopically share features with melanoma. Patients with dysplastic nevi require life-long follow-up. Substantial challenges remain in the diagnosis and management of DN with 'indeterminate' features shared by DN and MM. Promising technologies are being developed which may lead to improved sensitivity, specificity, predictive values, and diagnostic accuracy.

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025 DIAGNOSIS AND TREATMENT OF CUTANEOUS MELANOMA - 2005

C. Garbe (Division of Dermatologic Oncology, Department of Dermatology, University of Tuebingen, Germany)*

An overview on the current status, contemporary needs and probable future developments of diagnosis and treatment of cutaneous melanoma (CM) is given. Progress achieved during the last years and further progress expected in the near future are figured out. The following topics shall be addressed: (a) Early diagnosis of CM has been improved by digital dermoscopy. (b) Histopathologic diagnosis of melanoma is facilitated by comparative genomic hybridization. (c) Detection of micrometastases by RT-PCR based techniques did not have a breakthrough, but CM tumor markers were established. (d) New imaging techniques improve early diagnosis of metastasis and facilitate surgical metastectomy. (e) Uniform recommendations have been established for safety margins in primary CM. (f) Sentinel lymph node biopsy has become a standard procedure and there is growing evidence of its clinical benefit. (g) Adjuvant interferon treatment is well accepted, the question of how much and how long remains unanswered. (h) No progress has been achieved in the systemic medical treatment of metastatic melanoma over decades. (i) Patient selection for systemic medical treatments and for trials needs to be better developed. (j) Promising new treatment strategies are developed as targeted therapies. (k) The impact of scheduled follow-up examination has been widely neglected, but patients seem to significantly benefit from such procedures. Most important, the development of more effective medical treatments for metastatic melanoma is an urgent demand. Furthermore, there is a need for the development of more elaborated surveillance strategies in the follow-up of melanoma patients.

027 SURGICAL MARGINS - ARE WE RECOMMENDING REVERTING TO GREATER CLEARANCE?

*M. Thomas * (Royal Marsden Hospital , UK)*

Melanoma usually spreads by the lymphatic route and wide margins of excision wereadvised in the hope that the incidence of loco-regional recurrence would be reduced. Previous trials failed to show benefit for wide excision, but these investigated patients either with exclusively or predominantly thin melanomas. The Intergroup Melanoma Surgical Trial (Ann Surg Oncol 2001; 8:101-108) tested 2 cm versus 4 cm excision margins for melanomas 1-4 mm in thickness (with a secondary randomisation to elective lymph node dissection). No overall difference in outcome was found but the mean tumour thickness was 1.96 mm and only 207 patients (44%) had melanomas greater than 2 mm in thickness. Therefore, this Trial may not have the authority to provide guidance on excision margins for deep melanomas. Nevertheless, the conclusions of this Trial have influenced treatment guidelines which generally state that no melanoma requires a greater than 2 cm excision margin. The British MSG/BAPS Study (NEJM 2004;350:757-766) tested 1 cm versus 3 cm excision margins for melanomas 2 mm or greather in thickness. Sentinel node biopsy was not allowed. A 1cm excision margin was associated with a significantly greater risk of loco-regional recurrence but with a similar overall survival rate. An overview of three margin trials (MSG/BAPS, Intergroup and Swedish Melanoma Group Trial) suggested a significantly increased risk of death from melanoma associated with a narrow margin of excision. The MSG/BAPS results suggest that in a small number of patients, the melanoma cells that remain after a 1 cm excision margin will prove fatal. The Study provides no data to support the preferred use of 2 cm or 3 cm margins and it would seem reasonable pending further data, for patients to make a choice after an informed discussion of the surgical options.

028 RANDOMIZED TRIAL OF A RESECTION MARGIN OF 2 VERSUS 4 CM FOR CUTANEOUS MALIGNANT MELANOMA WITH A TUMOUR THICKNESS OF MORE THAN 2 MM

U. Ringborg (Dept of Oncology-Pathology, Karolinska Institute, Sweden), E. Månsson-Brahme (Dept of Oncology-Pathology, Karolinska Institute, Sweden), K. Drzewiecki (Rigshospitalet, Denmark), H-P. Gullestad (Norwegian Radium Hospital, Norway), M. Niin (Estonian Cancer Centre, Estonia)*

In 1991 a prospective randomized Scandinavian trial was initiated comparing 2 and 4 cm surgical margins in patients with localized, histologically proven cutaneous melanoma with a thickness greater than 2 mm. Patients were recruited from 9 medical centres in Denmark, Norway, Estonia and Sweden. Patients were randomly allocated to the two treatment arms and stratification was done according to geographical region and tumour thickness. Clinical data, histopathologic information and follow-up data were prospectively registered regionally and data were validated against the National Cancer registries and Cause-of-Death registries. A total of 936 patients were included. Follow-up time ranged from 6 months to 13 years. Overall survival, recurrence free survival and type of recurrences were calculated for the two randomized groups and the results will be presented.

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029 EXCISION MARGINS IN CUTANEOUS MELANOMA: META-ANALYSIS OF RANDOMISED CONTROLLED TRIALS

M. Lens (Imperial College, London, United Kingdom)*

The optimal excision margin for primary cutaneous melanoma remains controversial although several clinical studies have suggested that wide local excision is unnecessary. We performed a meta-analysis of randomised controlled trials evaluating excision margins for cutaneous melanoma with an aim to describe the published evidence and determine the effectiveness of wide surgical margins compared to narrow surgical margins. All randomised controlled trials published by March 2005 were included in the analysis. Five randomized controlled trials comprising 3313 participants were retrieved and analyzed. All included trials failed to demonstrate statistically significant differences in overall survival (OS) and disease-free survival (DFS) when comparing wide versus narrow excision. Pooled odds ratios for OS and DFS indicated a statistically non-significant improvement with wide excision. In this analysis, there was no statistically significant difference in the occurrence of local recurrence among patients treated with wide surgical margins when compared with patients treated with narrow excision margins. In the random effects model the pooled OR was 0.95 with 95% CI (0.52,1.72) and the test for overall effect of p=0.85. The c2 test for heterogeneity did not detect statistically significant heterogeneity among trials (p=0.37). Although our analysis did not show any statistically significant difference between the two groups of patients treated with narrow or wide excision margins with regard to local recurrence and OS and DFS, current evidence is not sufficient to address the optimal surgical margins for all melanomas. Further research is required to establish the appropriate local treatment for different types of primary melanoma and subgroups of patients.

030 DESMOPLASTIC AND DESMOPLASTIC NEUROTROPIC MELANOMA

S. McCarthy (Royal Prince Alfred Hospital, Sydney, Australia)*

Desmoplastic melanoma (DM) is not infrequently misdiagnosed clinically and histologically at its initial presentation. Subtle increase in bland or mildly atypical dermal spindle cells associated with foci of lymphocytes are readily diagnosed as chronic inflammation and reactive fibroblasts. Many DMs lack melanin pigment. Interpretation is more difficult in punch, curette and shave biopsies which lack atypical junctional melanocytes and any neural involvement. The latter may be perineural, intraneural/endoneural or neural transforming. In Sydney Melanoma Unit (SMU) cases only those DMs exceeding 1.5mm in thickness showed neurotropism. Mitoses may be sparse but at least some elongated enlarged spindle cell nuclei are present. Associated actinic keratosis with basal epidermal atypia and marked dermal elastosis may also hinder interpretation. Easier examples of DM have bundles of elongated spindle cells or a storiform pattern. HMB45 and Melan A (MART-1) are usually negative unless non-desmoplastic foci are present. Metastases are usually late and may be composed of spindle and/or epithelioid cells. The differential diagnosis includes scar tissue (some cells may be S-100 positive), blue naevus (poorly pigmented dendritic or sclerosing cellular types), desmoplastic naevus, dermatofibroma, fibromatosis, spindle cell sarcomas and recurrent or metastatic melanoma. At the SMU the presence of any desmoplastic or neurotropic component in a melanoma will result in more extensive local surgery

031 DYSPLASTIC NEVI

D. Elder (University of Pennsylvania, USA)*

Dysplastic nevi were first recognized as large clinically atypical nevi in familial melanoma patients, and were later recognized in nonfamilial melanoma patients and in random community members. Histologically, they are characterized, like dysplastic lesions in other sites, by architectural disorder and cytologic atypia. Like nevi in general, their significance is mainly in relation to melanoma. They are important as potential precursors of some melanomas, as markers of individuals at increased risk of melanoma, and as simulants of melanoma. The risk of progression of individual lesions is low, and only about one third of melanomas arise in association with a nevus, so that wholesale excision of nevi is not indicated as a means of preventing melanomas. Case control and cohort studies have demonstrated that dysplastic nevi are the single most important phenotypic risk marker for melanoma. In the largest case-control study (Tucker et al, JAMA 277:1439, 1997), the risk of melanoma (adjusted for other risk factors such as total number of nevi, age, sex, sunburns, freckles, sun damage, scars, excisions, personal and/or family history of melanoma) was elevated 2 fold in individuals with a single dysplastic nevus, and 12-fold in persons with 10 or more of these lesions. Melanoma risk is also associated with histologic atypia. In a study by Arumi-Uria et al (Mod Pathol 16:764, 2003), a personal history of prior melanoma was present in 5.7% of 2,504 patients with mild, 8.1% of 1657 with moderate, and 19.7% of 320 with severe atypia, indicating that risk of melanoma is greater for persons who tend to make nevi with high grade histological atypia. Dysplastic nevi are also important as simulants of melanoma and, although there is overlap at each end of a spectrum with nondysplastic nevi and with melanomas respectively, they can be diagnosed reproducibly, both clinically and histologically.

032 SPITZ TUMORS AND ATYPICAL VARIANTS

R. Barnhill (University of Miami, USA)*

Spitz tumors and their relationship to and distinction from malignant melanoma continue to provoke considerable discussion and controversy. Since a subset of Spitzoid lesions defy categorization as being either clearly benign or malignant, one approach to this problem advocated by this author and others has been to subject lesions to standardized scoring with a battery of criteria to facilitate risk stratification and management of patients. Such criteria include conventional histopathological criteria, clinical criteria, and other techniques as they prove applicable to this problem. However at present there are no definitive data involving sufficient numbers of patients that have been well characterized and with long-term follow-up to indicate the most important predictive factors for such lesions. There is preliminary information from a number of sources that some of the important criteria include: large size of such lesions, i.e., diameter and Breslow thickness; asymmetry; irregularity of the epidermis; ulceration; significant cellular density; diminished to absent maturation; significant mitotic rate, e.g., > 6/mm2; absence of Kamino bodies; increasing age of patients beyond 10 to 20 years; increased labeling with Ki-67, e.g., beyond 5 to 10%; increased expression of HMB45 and other such markers in the dermal component; and increasing cytogenetic aberrations, as shown by comparative genomic hybridization, etc. After assessing these various criteria, lesions can be placed into one of three categories: Spitz tumors without significant atypia; Spitz tumors with atypical features, including biologically indeterminate lesions; and melanoma. This methodology for risk stratification of Spitz tumors at present remains subjective and is dependant on significant experience in evaluating such lesions. In summary, the author describes a practical method for examining Spitzoid lesions and their biological potential.

033 BLUE NEVI AND RELATED CONDITIONS: AN UPDATE.

A. Zembowicz (Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA), M. Mihm (Massachusetts General Hosptial and Harvard Medical School, Boston, MA, USA)*

Dermal dendritic melanocytic proliferations, a broad group of congential and acquired melanocytic lesions characterized by the presence of dermal spindled and dendritic cells resembling melanocytes migrating from the neural crest to the epidermis. They are lesions containing variable proportions of spindled and dendritic melanocytes many of which resemble embryonal melanocytes migrating from the neural crest to the skin during embryonic development. Immunophenotypically, the tumors usually express HMB-45, in addition to S-100 and Mart-1 (melan A). Clinically, they share a bluish-gray coloration. For decades, dermal dendritic melanocytic proliferations have been classified into 3 broad categories, hamartomatous congenital dermal melanocytoses (Mongolian spot, nevus of Ota and Ito), benign classic and cellular blue nevi, and melanoma arising in blue nevus (malignant blue nevus). Recently, a number of histological variants of blue nevi or related tumors such as amelanotic/hypomelanotic blue nevus, amelanotic cellular blue nevus, epithelioid blue nevus, compound blue nevus, atypical blue nevus, pilar neurocristic hamartoma, blue nevus-like metastatic melanoma, pigmented epithelioid melanocytoma and cutaneous neurocristic hamartoma/malignant neurocristic tumor have been introduced to the pathological literature. Most of these entities, although said to be new, can be allocated into traditional categories of dermal dendritic melanocytic proliferations. However, the nosological standing of pigmented epithelioid melanocytoma and cutaneous neurocristic hamartoma is controversial as the original descriptions of these lesions suggested an intermediate malignant potential between a benign blue nevus and a common melanoma. This raises a question if these should be separated as a separate category of borderline melanocytic tumors. We review the current literature on dermal dendritic melanocytic proliferations with emphasis on atypical variants of cellular blue nevus and newly described entities.

034 EXISTENTIAL SUPPORT AND PALLIATIVE CARE

A. Kneier (UCSF Melanoma Center, USA)*

One aspect of palliative care for melanoma patients involves existential and spiritual support in coming to terms with death. This is especially true for patients who are relatively young. Such patients have voiced their desire for this kind of support over the course of dealing with Stage 4 disease, long before they progress to end-stage. This presentation will report qualitative data on the question: What thoughts, emotions, beliefs, and experiences help young Stage 4 patients in their quest for meaning, peace, or acceptance as they face the prospect of an early death? This data, derived from semi-structured interviews with such patients, covers a variety of themes, most notably: 1) gratitude for the life the person has enjoyed; 2)a sense of pride in one's accomplishments and/or in one's positive traits as a person; 3) taking stock of one's legacy (e.g., in their children or in how they will be remembered); and 4) the role of religious faith, especially in accepting God's plan. Transcribed portions of the interviews will be read to illustrate these and other themes. This data – on finding meaning and coming to terms with death – is being utilized in a support group intervention with other young Stage 4 patients. The intervention aims to elicit discussion, sharing, and exploration of the issues and themes in this data, with the hope of helping patients in the process of facing the psychologtical, existential, and spiritual issues that come in facing death at an early age. The intervention is being evaluated in this regard. The results to date will be presented. This research aims to show that patients benefit from this type of support, which is often neglected due to the focus on maintaining hope and a positive attitude) and can be an important aspect of palliative care.

035 ANOREXIA-CACHEXIA - WHAT CAN WE DO ABOUT IT?

N. MacDonald (McGill University, Canada)*

A dichotomy exists between the devastating effects of weight and functional loss in advanced cancer, and research on this topic. Acute symptoms such as pain catch our attention, and the management of cancer pain has been revolutionized in recent years. A slowly developing, albeit inexorable symptom complex such as anorexia-cachexia, unlike pain, has in the past been regarded by clinicians as an inevitable cost of dying from cancer. Consequently, the current management remains problematic. This presentation will emphasize that, similar to other problems in medicine, early diagnosis of weight and functional loss is critically important. Simple assessment techniques will be described. While the pathophysiology remains complex, sufficient information is now emerging from basic studies to allow us to develop a platform for treatment, and the launching of clinical trials. The presentation will discuss current management, with an emphasis on the importance of integrating nutritional care of advanced cancer patients with other approaches to tumour management. Specific programs which illustrate this point will be described.

036 APPROACHING PAIN RELIEF FOR PATIENTS WITH MELANOMA

N. Lickiss (Sydney Istitute of Palliative Medicine, Australia)*

Pain experienced by patients with melanoma may be due to the disease, its treatment or various proceedures; pain is subjective, personal, and its recognition and relief requires close attention. Ratio of benefit to cost is very high if existing knowledge is applied. The approach to relieving pain may be considered to have four steps : 1. Reduce noxious stimulus (which implies precise diagnosis on the basis of clinical history, clinical examination, necessary investigations) by locally acting appropriate therapeutic measures such as radiotherapy,surgery,analgesic drugs such as NSAIDS,paracetamol,low dose corticosteroids; 2. Raise the patient's threshhold if a lowered threshhold is apparent - by support, counselling,occasionally anxiolytics or antidepressants;3.Consider precise use of opioids(avoiding sedation, and preventing constipation),recogni zing that morphine(usually oral or SCI) is still the gold standard, but other choices may be appropriate in certain circumstances;4.If neurogenic pain continues to be a major problem despite the above measures, consider the addition of antidepressants, anticonvulsant or corticosteroids, despite the potential side effects of all of these drugs. Patients illustrating some of these matters will be discussed.

037 SUN EXPOSURE, VITAMIN D AND SURVIVAL WITH MELANOMA

M. Berwick (University of New Mexico, USA)*

Vitamin D synthesis is a critical component of cellular networks that inhibit cellular proliferation and encourage apoptosis. A question has been raised as to whether vitamin D synthesis is higher at lower latitude and whether solar exposure is protective for the development of numerous cancers. It would seem that melanoma should not be one of those tumors. Excessive intermittent sun exposure is clearly causal for the development of melanoma. However, data from Western Australia and Connecticut show that a marker for high levels of sun exposure, solar elastosis, is associated with improved survival in melanoma. Several investigators have noted that survival with melanoma increases with increasing melanoma incidence and have suggested that melanoma occurring in association with high ambient sun exposure might be biologically more benign. One explanation for the association between high levels of solar exposure prior to development of melanoma and improved survival is that the vitamin D synthesis pathway among these individuals may be aberrant. Evidence from the literature based on serum levels of vitamin D among melanoma patients, or those at high risk for melanoma, is conflicting. Highly sun protected individuals, such as Xeroderma pigmentosum patients, have been found to have normal levels of vitamin D. However, other individuals have been found to have very low serum levels of vitamin D. One explanation could be that one or more genetic elements of the vitamin D pathway within individuals who have poorer survival are aberrant. If this were true, then those individuals may not be able to synthesize vitamin D well enough for damaged cells to go into apoptosis. So, in those individuals, low levels of solar exposure may lead to reduced survival with melanoma.

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038 NON UV RISK FACTORS FOR MELANOMA IN AGRICULTURAL HEALTH WORKERS

L. Dennis (College of Public Health, University of Iowa, USA), C. Lynch (College of Public Health, University of Iowa, USA), J. Lowe (College of Public Health, University of Iowa, USA), M. Alavanja (Division of Cancer Epidemiology and Genetics, National Cancer Institute, USA)*

OBJECTIVE: Melanoma is a growing problem as incidence rates continue to increase. Few risk factors other than UV exposure have been identified. Standardized incidence ratios (SIRs) for the Agricultural Health Study from enrollment 1994-97 through the end of 1999 suggest non-UV factors. Based on increased outdoor activity, we may expect to see a higher SIR in the applicators (farmers) but none was seen (SIR=0.82). Among the spouses the SIR was 1.45 for melanoma cases. Potential factors that may account for differences seen between applicators and their spouses are examined including vitamin supplements, cooking habits, self-reported prior diagnoses, and reproductive factors. Among applicators pesticides may be important. MATERIALS & METHODS: The Agricultural Health Study is a cohort of 52,395 pesticide applicators and 32,347 spouses in Iowa and North Carolina. Detailed information was collected on occupational and environmental pesticide exposures, lifestyle characteristics and personal and family health history (www.aghealth.org). Among the applicators 2.6% are female, whereas, less than 1% of recruited spouses are male. Crude relative risks and 95 percent confidence intervals were calculated. Logistic regression was used to estimate adjusted relative risks. RESULTS: Sun sensitivity was examined as a potential confounding factor (blond/red hair and tendency to burn). Among females, associations with most reproductive factors disappeared after adjustment for age. Having an incident or experience that resulted in an unusually high pesticide exposure had a crude RR for melanoma of 2.0 (1.1-3.8). Even higher risks were seen for frequent use of arsenic herbicide use, and for chemicals from exposure during the applicator's longest non-farm job. CONCLUSION: The increased risk of melanoma seen with arsenic exposure supports a prior study in Iowa that examined arsenic content in toenails. Further study of chemicals and trace elements as related to melanoma is needed. Non-UV risk factors may provide additional avenues for melanoma prevention.

039 MELANOMA INCIDENCE IN THE WEST OF SCOTLAND OVER 25 YEARS

R. MacKie (Glasgow University, UK)*

In countries with the highest incidence of melanoma worldwide such as New Zealand and Australia, the incidence of melanoma appears to have plateaued in younger age groups. In contrast, in Scotland (54 degrees N) the rising trend continues, and not all the increase is seen in good prognosis primary tumours <1mm at diagnosis. Between 1979 and 2003, 5926 new invasive melanomas were diagnosed. The overall incidence of cutaneous melanoma of all histological types trebled. The increase in males was x4, and in females x2.4. Throughout the 25 year period the proportion of melanomas on the legs in women remained constant at 50%. The proportion of melanomas <1.0mm at diagnosis in males remained at less than 50% throughout the 25 year period, but in women the proportion of lesions <1.0mm rose from 33% to over 50% throughout the time period. Older males continue to have a high proportion of thick poor prognosis melanomas, particularly on the trunk and foot. These data indicate that despite greater public awareness of melanoma, the incidence is continuing to rise. The contrast in these trends between Scotland and high incidence southern hemisphere countries indicates that apparently successful models of public awareness and prevention in these countires should be carefully studied and applied to countries such as Scotland with appropriate modification for climatic differences.

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040 S LENTIGO MALIGNA A SEPARATE EPIDEMIOLOGICAL TYPE OF MELANOMA?

JJ. Grob (CHU Marseille, France)*

Hutchinson and Dubreuilh identified lentigo maligna (LM) as a slowly enlarging lentigo, which secondarily becomes a real tumor. LM is now considered as a melanoma (MM), specific by its histological pattern, its age, and its particular epidemiological link to cumulative sun exposure, in opposition to other types of MM. However, we have to be conscious that the definition chosen for LM, i.e. a lentiginous type of MM, associated with chronic dermal lesions of sun damage, selects, by itself, a particular group of MMs: 1/ mainly located on face in the elderly, since lentiginous MMs occurring in younger people or out of the face would not be classified as LMs, for lack of intense contiguous sun damage; 2/ associated with a high cumulative exposure, but possibly because all elderly people have received a high cumulative amount of sun exposure, and there is no clear data that LMs have different relation to intense and intermittent sun exposure than other MMs; 3/ with a slow and long-lasting horizontal growth phase, which may be the translation of the lentiginous pattern required by LM definition, and which could be also true for other lentiginous MMs, such ALMs. 4/ with a typical clinical profile of a slowly enlarging senile lentigo, which may also be the consequence of comment 1 and 2. One can doubt whether LMs are really an epidemiologically different MMs linked to cumulative exposure, or only an age-related and topographic subtype of usual MMs. This can be discussed in the light of the literature, and of a recent study comparing 76 LMs, 76 other MMs in old people (oMMOs) and in 152 controls. We found that significant risk factors common to all MMs over 65, were clear skintype (hair, skin), frequency of sunburns all along the life, daily life in the youth in low-sun areas, and more sun induced lesions removed. Risk factors which were found to be stronger in LM than in oMMOs, were freckles at the age of 20, skin cancers treated in the last 20 years, but neither occupational sun exposure and cumulative exposure, nor wrinkles and keratoses. The only significant risk factors for oMMOs and not LMs were the number of nevi on forearms. Finally, there is no evidence that LM has different epidemiological factors than oMMOs. Furthermore, these risk factors seem quite similar MMs in younger people, although the occurrence over 65 suggests a "higher resistance" to induction of MM by intermittent exposure. The only specificity of LM is thus the lack of link to nevi (1), which suggests a MM with a weak genetic susceptibility of the melanocytic system. Two hypotheses can be discussed: 1/ a classical hypothesis of LM being an epidemiologically different MM linked to cumulative sun exposure, which might in fact result from its definition (old, face); 2/ another hypothesis of LM being an epidemiologically common MM, linked to intermittent sun exposure in sun-sensitive individuals, with an artificial definition which selects the summit of the MM susceptibility pyramid, i.e. the individuals with the "highest resistance to MM induction" and the "lowest melanocytic genetic predisposition", among those who will eventually develop a MM.

041 A NEW MOUSE MODEL OF BRAF-INDUCED TUMORIGENESIS

D. Dankort (UCSF Comprehensive Cancer Center, USA), M. McMahon (UCSF Comprehensive Cancer Center, USA)*

The RAF-MEK-ERK signaling pathway is a key regulator of cancer cell cycle and apoptosis and is the target of intense investigation to understand the aberrant proliferation of cancer cells. BRAF is mutationally activated in ~7% of human tumors suggesting that BRAF is a bona fide human oncogene. The most common mutation found in BRAF replaces valine 600 with glutamic acid (BRAF-VE) and this mutation is detected with a striking prevalence in melanoma. However despite the prevalence of mutated BRAF in human cancer, there is no animal model system to explore the role of BRAF in cancer initiation and progression. By manipulation of ES cells, we have generated mice carrying a Cre-activated BRAF allele (BRAF-CA) that allows us to explore the ability of BRAF-VE to promote tumorigenesis. This allele expresses normal BRAF until subjected to genomic rearrangement by Cre recombinase at which time BRAF-VE is expressed. This approach is ideal since BRAF-VE is expressed at physiological levels under the control of its own promoter, subject to normal patterns of splicing and in a manner temporally regulated by expression of Cre. To test this approach we isolated primary mouse embryo fibroblasts from BRAF-CA and control mice and initiated expression of BRAF-VE by infection with an Adenovirus expressing Cre (Ad-Cre). Data describing the consequences of BRAF-VE expression in MEFs will be presented. Additionally, to determine the function of BRAF-CA in vivo, and because BRAF-VE is detected in lung cancer, we administered Ad-Cre intra-nasally to activate BRAF-VE expression in lung epithelial cells of mice. Data describing a role for BRAF-VE in lung carcinogenesis will be presented. We believe that the BRAF-CA mouse will serve as a platform to assess the role of BRAF-VE, either alone or in cooperation with other alterations, in the initiation and progression of a variety of malignancies especially melanoma.

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042 MDA7/IL-24: NOVEL MELANOCYTE-DERIVED CYTOKINE, EXHIBITING IMMUNE AND TUMOR SUPPRESSOR PROPERTIES

E. Grimm (Univ of Texas, MDACC, USA), S. Chada (Introgen Therapeutics, USA), S. Ekmekcioglu (Univ of Texas, MDACC, USA), J. Mumm (Univ of Texas, MDACC, USA), N. Poindexter (Univ of Texas, MDACC, USA), J. Ellerhorst (Univ of Texas, MDACC, USA), K. Kim (Univ of Texas, MDACC, USA)*

Interleukin-24 is now known as the protein product of Melanoma-Associated Differentiation-Associated Gene-7 (MDA7), and appears as a unique link between melanocytes and the immune system. The mda7/IL-24 gene was originally isolated by subtractive hybridization of growth-arrested melanoma, and identified as a tumor suppressor when transfection of mda7 gene into metastatic melanoma lines led to growth arrest. We have developed MDA7 protein detection methods, and documented presence in the cytoplasm of melanocytes and nevi, but not in metastatic melanoma; the loss of protein expression has been proposed as a hallmark of melanoma invasion. Chromosomal localization studies indicated that mda-7 is encoded in the IL-10 locus in 1q31-32, and its gene sequence indicated that it contained secretory and other cytokine sequence characteristics. We found that IL-24 is constitutively expressed in melanocytes and some smooth muscle cells, and can be induced in PBMC by immune stimulation, and is part of a TH1 cytokine cascade. Similar to what has been previously reported for mda7 gene transfer, co-culture of the IL-24 protein with melanoma can lead to melanoma growth arrest. In Phase I clinical studies, it was found that the IL-24 protein is expressed in situ at cellular locations correlating with areas of tumor apoptosis; currently, a phase II clinical trial of intralesional gene therapy using an adenoviral construct of mda7 is in progress. Evidence for protein production as a result of gene transfer in clinical trials has been used to support the hypothesis that the IL-24 protein is responsible for bystander melanoma cell and pancreatic growth control. This mda7/IL-24 cytokine data not only suggests that melanocytes are active members of the innate immune system; but the gene construct and purified protein provide unique tools for melanoma therapy, combining tumor suppressor and immune potentiation in the same molecule.

043 ING FAMILY MEMBERS PROMOTE NUCLEOTIDE EXCISION REPAIR OF UV-DAMAGED DNA VIA ENHANCING CHROMATIN RELAXATION

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The mammalian ING1 (inhibitor of growth 1) gene encodes a protein that has been implicated in apoptosis, senescence, cell cycle checkpoint control, transcriptional regulation and DNA repair. ING family proteins (ING1-5) associate with a spectrum of histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes that target various substrates including the p53 tumor suppressor and histones. We have demonstrated that ING1b enhances the repair of ultraviolet (UV) light-damaged reporter plasmid in a host-cell-reactivation assay to restore gene expression. We showed that in an immunofluorecent study that ING1b mobilizes globally within the nucleus upon localized UV-light infliction. Deletion of the highly conserved plant homeodomain (PHD) abrogates the elevated DNA repair capacity mediated by ING1b. However, elevated level of global histone acetylation by treatment with HDAC inhibitor, trichostatin A, bypasses the requirement for the PHD domain in ING1b. Micrococcal nuclease digestion of inter-nucleosomal DNA confirmed that ING1b could induce a global chromatin relaxation after UV-induced genomic injury. We also show that all the ING family members can enhance DNA repair in both brain and skin cancer cells. Therefore, we proposed that ING family members associate with HAT activity to alleviate chromatin condensation for DNA repair. In addition, immunoprecipitation of ING1b lacking the PHD domain fails to pull down a significant amount of core histones compared to the wild type counterpart. We believe that the PHD domain is responsible for tethering the ING-HAT complexes to nucleosomes to permit chromatin remodeling. Elucidating these mechanisms will contribute to the knowledge of cellular maintenance of genomic integrity. They may also help to develop novel therapeutic targets for cancer treatments.

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044 SELECTIVE KILLING OF MELANOMA CELLS BY EXPLOITING DIFFERENTIAL EFFECTS OF PROTEASOME INHIBITION ON NOXA AND ANTI-APOPTOTIC BCL-2 PROTEINS

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Melanoma incidence and mortality are rising and patients with advanced disease have a dismal prognosis. Using Bortezomib as a prototypic proteasome inhibitor we identified a new and critical role of the proteasome in the malignant phenotype of melanoma cells that could have direct translational implications. Proteasome inhibition in otherwise chemoresistant melanoma cells promoted a dramatic (>50-80 fold) induction of Noxa, a BH3-only protein of the Bcl-2 family. Kinetic and biochemical analyses of cellular events elicited by Noxa revealed an effective activation of intrinsic and extrinsic apoptotic pathways. Importantly, at concentrations of Bortezomib leading to a massive melanoma cell death, normal melanocytes remained viable and maintained undetectable expression of Noxa (p=0.05). RNA interference (RNAi) proved that Noxa is critical for the therapeutic effect of Bortezomib. Thus, our results identified for the first time Noxa as the long sought-after mediator of the tumor-selective effect of this proteasome inhibitor. Notably, the ability to induce Noxa was unique to Bortezomib as it could not be recapitulated by conventional anti-cancer drugs such as Adriamycin or Cispatin, less effective and selective towards melanoma cells. Intriguingly, the impact of Bortezomib on apoptotic modulators was found to be highly specific. Thus, potent anti-apopotic proteins such as Bcl-2 or Bcl-xL were not affected by Bortezomib treatment. In fact, we found that both Bcl-2 and Bcl-xL act as fail-safe mechanism to counteract proteasome inhibition. This hypothesis was confirmed in vitro and in vivo by blocking RNAi and small molecule inhibitors. In summary, this study identifies Noxa as a new biomarker to assess the efficacy of proteasome inhibitors and provides the basis for the rational design of improved therapies to reactivate a dormant apoptotic machinery in melanoma cells and overcome chemoresistance.

046 WHY DERMOSCOPY IS BENEFICIAL!

A. Marghoob (Memorial Sloan-Kettering Cancer Center, USA)*

Slowly but surely, dermoscopy is winning acceptance as a diagnostic tool in North America. Approximately 25 percent of dermatologists currently use dermoscopy at least sometimes and this figure represents an increase over a few years ago. Dermoscopy has multiple benefits, at minimum, it forces the clinician to concentrate on skin lesions. This alone has tremendous benefit for the clinician's confidence in his/her clinical differential diagnosis as well as reassuring patients that a thorough evaluation is being performed. There are multiple benefits for the use of dermoscopy. Among these include: 1. Allows observer to focus on the lesion and formulate a logical differential diagnosis. 2.Helps differentiate melanocytic from non-melanocytic lesions. 3.Helps differentiate benign from malignant lesions. 4.Increases the clinician's diagnostic accuracy. 5.Increases the confidence in the observer's clinical diagnosis. 6.Confirms the diagnosis made by 'naked eye' examination (It is a warning sign if the clinical-dermoscopic correlation is not congruent!). 7.Improves the malignant to benign biopsy ratio (avoids unnecessary biopsies). 8.Helps isolate suspicious foci within larger lesions that can be marked, thereby directing the pathologist to step section through these areas. (Clinical-dermoscopy-pathology correlation). 9.Helps to more precisely define the borders of some lesions during pre-surgical margin planning. 10.Helps in the surveillance of patients with many nevi/dysplastic nevi. 11.Helps to reassure patients (patient acknowledge & appreciate the attention).

047 WHEN DERMOSCOPY DID CHANGE MY MIND

G. Argenziano (Dept Dermatol II Univ Naples, Italy)*

The use of dermoscopy has changed the practice of dermatologists for several reasons. The first and most important one is that dermoscopy forces physicians to dedicate more time and care for an individual with a pigmented skin lesion. For these reasons, dermatologists (albeit not all) and patients (basically all) love dermoscopy. Second, using dermoscopy to differentiate suspicious lesions from benign ones is helping dermatologists make better, more selective decisions about biopsy excisions. This non-invasive diagnostic technique uses optic magnification to make the lesion's morphologic features more visible than by eyesight alone, thus increasing the clinician's confidence in differentiating banal from suspicious lesions. The most common clinical mistake is to judge a lesion as a possible melanoma when it is, indeed, a benign nevus. By using dermoscopy, doctors can decrease the number of benign lesions excised unnecessarily by approximately one-third from the number excised solely on the basis of standard clinical examination. Third, it is in our trust that dermoscopy increases early detection of melanoma. Prior to using dermoscopy, a lesion should be evaluated clinically using visual information. Whether or not such a clinical examination raises the suspicion of melanoma, all lesions should again be checked using dermoscopy. Most often dermoscopy just confirms the diagnosis already performed by the naked eye, but sometimes it opens the mind to new possibilities. One has to look for a good clinical-dermoscopic correlation: a clinically benign lesion should exhibit benign features dermoscopically. But sometimes a clinically banal lesion may exhibit some of the melanoma criteria dermoscopically, thus a red flag should be raised in order not to miss a melanoma.

048 BENEFITS AND CAVEATS OF DIGITAL MONITORING

S. Menzies (University of Sydney and Sydney Melanoma Diagnostic Centre, Australia)*

Digital monitoring of melanocytic lesions can be divided into two approaches. Long term monitoring of dysplastic nevi, usually in the setting of Dysplastic (Atypical) Nevus Syndrome, occurs over the standard surveillance period (usually 12 months). Certain significant changes requiring excision of the lesion are contrasted with those found in changing benign lesions. Short term digital monitoring of suspicious lesions occurs over the shorter interval of 3 months. Here any change requires excision. Both techniques allow detection of dermoscopically featureless melanoma. However, the efficiency of the procedures differ with respect to total monitored lesion:melanoma detection ratios, which is much greater in the setting of long term monitoring. Issues of compliance and diagnostic accuracy will be discussed with both approaches.

049 PHASE II TRIALS OF BAY 43-9006 ALONE AND IN COMBINATION WITH CHEMOTHERAPY IN METASTATIC MELANOMA

K. Flaherty (University of Pennsylvania, USA)*

BAY 43-9006 (BAY) is an inhibitor of proliferation and angiogenesis through blockade of the RAF/MEK/ERK pathway at the level of RAF kinase and the receptor tyrosine kinases VEGFR-2 and PDGFR- . BAY inhibits BRAF (wild type/V599E mutant) and inhibits growth of melanoma xenografts. BAY enhances the cytotoxicity of taxanes, platinum analogues and agents from other classes in several xenograft models. Four phase II trials have been conducted with BAY in melanoma: two administering single-agent BAY, one in combination with carboplatin (AUC 6) and paclitaxel (225 mg/m2), and one in combination with DTIC (1000 mg/m2). In one of the single-agent phase II trials, tumor samples were obtained pretreatment and during treatment in order to corroborate the inhibition of RAF and the effects on gene expression of pro-apoptotic proteins (N=10). DCE-MRI was performed at baseline and after 4 weeks to investigate the effects of BAY on tumor perfusion (N=20). Sixty-one patients were enrolled on the two single-agent phase II trials. One patient had a confirmed partial response (PR) (1.6%), and 19 patients with stable disease (SD) (31%). Fifty-four patients were accrued to the phase I/II trial of BAY in combination with carboplatin and paclitaxel. There was one complete response, 19 PR (ORR 37%), and 26 SD (48%). Sixty-three percent of patients are free of progression for more than 6 months. A phase I/II trial of BAY in combination with DTIC is ongoing. Preliminary response data are not yet available. BAY 43-9006 is well tolerated as a single-agent and is associated with disease stabilization in a subset of patients with melanoma. Compared to historical experience with carboplatin and paclitaxel, BAY appears to enhance to efficacy of these agents in metastatic melanoma. E2603, a double-blind, placebo-controlled, randomized phase III trial will compare the efficacy of BAY, carboplatin and paclitaxel to carboplatin and paclitaxel alone.

050 RANDOMIZED PHASE II TRIAL OF MELANOMA PEPTIDES WITH MONTANIDE ISA 51 AND DIFFERENT DOSES OF IL-12 WITH ALUM FOR RESECTED STAGES IIC/III AND IV MELANOMA

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We wished to augment immunity to defined melanoma antigens with IL-12 and/or GM-CSF added to a multi-peptide vaccine (Lee et al, J Clin Oncol 2001). Sixty patients with resected stages IIC/III and IV melanoma randomly received peptides gp100 209-217 (210M), MART-1 26-35 (27L), and tyrosinase 368-376 (370D) with Montanide ISA 51 and: IL-12 at 30 ng/kg/alum (group A) IL-12 at 100 ng/kg/alum (group B), or IL-12 at 30 ng/kg with 250 mcg of GM-CSF (group C). Vaccinations were given subcutaneously every other week four times, every four weeks three times, eight weeks later, then again six months later for nine injections. Median age was 50; 35 males and 25 females; 3 stage IIC, 50 stage III and 7 stage IV. One patient had grade III colitis and discontinued therapy with resolution of symptoms; one patient had grade III headache that resolved after stopping IL-12 but continued the peptides. Other toxicities were grades I/II and resolved rapidly. Fifty-nine patients had ELISPOT assays of fresh peripheral blood. Higher immunity to gp100 and MART-1 was observed in group B (17/19) versus groups A (8/19) or C (1/21) (p=0.005 for gp100 and 0.02 for MART-1). Mean amplitude of response to gp100 and MART-1 was higher in group B compared with groups A or C. With 17 months of median follow-up, 6 patients of 60 have died, four in the two low dose IL-12 groups, and two in the high dose IL-12 group. Twenty have relapsed; 10 in group C and 6 in A (the low dose IL-12 groups), and only 4 in the high dose IL-12 group. Immunity to MART-1 correlated with time to relapse(p=0.03) and marginally for gp100 (p=0.06). Higher doses of IL-12 are well-tolerated, are effective vaccine adjuvants, and augment an immune response to melanoma antigens that correlates with clinical benefit.

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051 PEGYLATED INTERFERON IN METASTATIC MELANOMA IN THE ADJUVANT AND THERAPEUTIC SETTING.

A. Eggermont (ErasmusMC, Netherlands)*

Pegylated interferons, peginterferon alfa-2a (PEG-IFN±-2a, PEGASYS®), and peginterferon alfa-2b (PEG-INTRON®)have the potential for improved tumor response and survival with lower toxicity than IFN±. Peg-IFNalfa-2a in Stage IV With PEG-IFN±-2a, (PEGASYS®)a large randomized, phase II study in stage IV melanoma patients evaluated the safety, tolerability and efficacy of subcutaneous PEG-IFN±-2a adminstered at 180 1/4g (n=48), 360 1/4g (n=53) or 450 1/4g (n=49) once weekly for 24 weeks, with maintenance therapy for responders. The major response rate (CR or PR) was 12% in the 450 1/4g group (CR 6%, PR 6%), 8% in the 360 1/4g group (CR 2%, PR 6%) and 6% in the 180 1/4g group (CR 2%, PR 4%) without marked differences between the groups in time to achieve a major response, duration of major response, disease progression or 12-month survival. There were few withdrawals due to adverse events (4%, 11% and 12% in the 180 1/4g, 360 1/4g and 450 1/4g groups, respectively). The most common adverse events were fatigue, pyrexia, rigors, nausea and headache. Adjuvant PEG-IFNalfa-2b Stage III: EORTC 18991 The EORTC Melanoma Group has conducted an adjuvant trial in 1256 resected stage III melanoma patients. Patients are randomized to Longterm treatment with PEG-Intron or to Observation. Peg-Intron treatment consisted of 6.0 µg/kg, sc, weekly for 8 weeks followed by 3.0 µg/kg, sc, weekly for 5 yrs (- 8 wks) or untill distant relapse. Dose reductions are foreseen to 2.0 and 1.0 µg/kg to keep patients at performance status of 0% or better. Longterm maintenance treatment is hypothesized to have anti-angiogenic effects to maintain an impact on DFS that persists and will translate into a survival benefit. Primary endpoint is Distant Mestastasis Free Survival (DMFS) 571 events required for analysis. Demographic and toxicity data will be presented. Final efficacy analysis is expected late 2005/early 2006.

0052 POSTOPERATIVE CANVAXIN THERAPY FOR HIGH-RISK MELANOMA

D. Morton (John Wayne Cancer Institute, U.S.A.)*

Extensive phase II trials of melanoma patients matched by AJCC prognostic factors have indicated a significant overall survival (OS) benefit for postoperative adjuvant immunotherapy with CanvaxinTM (CancerVax Corp., Carlsbad, CA). In stage III melanoma, 739 pairs of Canvaxin and non-Canvaxin patients had respective OS rates of 49% and 37% at 5 years, and 42% and 31% at 10 years (P = .0001). In stage II melanoma, 315 pairs of Canvaxin and non-Canvaxin patients had corresponding OS rates of 76% and 70% at 5 years, and 65% and 58% at 10 years (P = .03). Canvaxin also has been used to treat measurable disease: in 54 patients with in-transit melanoma, it produced 9 (17%) objective responses and 7 (13%) complete remissions that had a median duration exceeding 22 months. Canvaxin's clinical effect is correlated with specific humoral and delayed-type hypersensitivity (DTH) responses. In stage II patients, 5-year OS and disease-free survival rates were 94% and 89%, respectively, when maximal anti-TA90 IgM titer was at least 1:800, compared to only 52% and 17%, respectively, for lower titers (P = .0001). These data represent the largest phase II experience for any cancer vaccine and indicate the clinical efficacy of stimulating the endogenous immune response to melanoma. Underway at 85 centers around the world is a phase III randomized trial of Canvaxin after complete resection of stage III melanoma. Patients receive either vaccine plus bacille Calmette-Guerin (BCG) or placebo plus BCG. Results will indicate whether the survival benefit observed in phase II studies can be replicated in a multicenter placebo-controlled study. Supported by NCI grant CA12582.

0053 ADOPTIVE CELL TRANSFER THERAPY FOLLOWING LYMPHODEPLETING CHEMOTHERAPY FOR THE TREATMENT OF PATIENTS WITH REFRACTORY METASTATIC MELANOMA

D. Powell Jr. (National Cancer Institute, USA), S. Rosenberg (National Cancer Institute, USA)*

Adoptive immunotherapy can mediate cancer regression in metastatic melanoma. We previously reported objective clinical responses in six of 13 patients (47%) with IL-2 refractory metastatic melanoma treated with nonmyeloablative conditioning followed by adoptive transfer of autologous tumor reactive lymphocytes. This approach resulted in the persistent clonal repopulation of T cells in those cancer patients, with transferred cells proliferating in vivo, displaying functional activity, and trafficking to tumor sites. At present, 18 of 35 treated patients (51%) have experienced an objective clinical response (four complete responses and 14 partial responses). Some patients also exhibited symptoms of autoimmune melanocyte destruction including vitiligo and uveitis. Further analysis revealed a significant correlation between clinical response and persistence of individual transferred T cells in vivo. Strategies designed to improve this therapy are currently investigation, including the adoptive transfer of peptide-stimulated or T cell receptor modified peripheral blood lymphocytes, selective cell subset depletions and application of a more complete lymphoconditioning regimen.

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0054 DIRECT IMMUNE MONITORING, A PRECISION MEASUREMENT APPROACH TO THE RAPID OPTIMIZATION OF CANCER VACCINES IN THEIR EARLY CLINICAL TRIAL PHASE

P. Romero (Division of Clinical Onco-Immunology, Ludwig Institute for Cancer Research, Lausanne branch and NCCR Molecular Oncology, Lausanne, Switzerland)*

Vaccines aimed at eliciting potent specific T cell responses are regarded as highly promising agents for the treatment of cancer. Strategies for the delivery of cancer vaccines are diverse and include naked DNA, recombinant viral and bacterial vectors, recombinant proteins, synthetic peptides and antigen loaded or transduced dendritic cells. Clinical experience with melanoma therapeutic vaccines pose a paradox: while specific CTL responses of various strengths can be demonstrated in variable proportions of vaccinees, clinical efficacy is apparent only in few patients. The reasons underlying this dissociation between specific immune response and clinical outcome may include weak immunogenicity of the vaccine formulation, triggering of immune regulatory mechanisms or tumor associated factors that either dampen the immune response or contribute to the ability of tumors to evade its effector mechanisms. To address these crucial issues of vaccine development, early clinical trial testing requires efficient immunological read outs. Fortunately, tremendous progress has been made recently in this area. To date, two major assays have gained consensus for use as first line monitoring of specific CTL mediated immunity in patients. One type relies on the enumeration of cytokine, generally IFN-g, releasing cells in response to a short period of stimulation with antigen. Such assays include ELISPOT and cytokine flow cytometry (CF). The other type is based on the preparation of fluorescent multimers of soluble MHC class I/antigen peptide complexes that can bind stably to their specific TCR on the surface of CTL. We have standardized both types of assays using as model the response to the immunodominant HLA-A2 restricted CTL response to the Melan-A/MART-126 35 peptide. I will discuss our recent results of peptide based vaccine optimisation based on quantitative direct immunomonitoring and the implications of these observations for further vaccine development in cancer.

055 IMPLICATIONS OF A DUAL PATHWAY FOR OUR PUBLIC HEALTH MESSAGES

M. Weinstock (Brown University, USA)*

Evidence has been presented that there is more than one pathway for the development of melanoma. Certain individuals, group I, may develop melanoma earlier, with less ultraviolet radiation exposure, and may be most susceptible to Intermittent exposure patterns, and are most likely to develop melanomas on the trunk. Others, group C, may develop melanoma only after a threshold of Cumulative exposure has been reached; these melanomas may be most likely to occur on the head and neck. Much regarding these distinct pathways to melanoma remains to be discovered and verified. New or changed messages must be carefully evaluated prior to widespread use. The primary prevention message regarding sun protection continues to apply to both groups, although one might speculate that the focus in group I would be childhood exposure, which should be quite strict in susceptible individuals to maximize effectiveness. On the other hand, the focus in group C might be on protection against intense exposures throughout life, without much concern regarding modest exposures. Prior to public campaigns along these lines, however, reliable criteria would have to be described for distinguishing the groups and confirming the appropriateness of the tailored messages. It is doubtful, however, that the early detection messages will need substantial alteration. The message regarding new or changing skin lesions is appropriate for both. Nodular or desmoplastic melanomas appear to occur in both groups, so the ABCD criteria, which describes only a subset of each group of melanomas, should not be the primary message for early detection in either group.

056 EPIDEMIOLOGIC EVIDENCE FOR MULTIPLE CAUSAL PATHWAYS TO CUTANEOUS MELANOMA

D. Whiteman (Queensland Institute of Medical Research, Australia)*

For the most part, epidemiologic studies of cutaneous melanoma have implicitly assumed that such tumours arise through similar causal pathways. While sun exposure is clearly a prime determinant of the rate at which melanocytes are transformed towards malignancy, there are likely to be anatomical and constitutional differences in the susceptibility of melanocytes to neoplasia. We have recently put forward a model for the development of cutaneous melanoma that accounts for the relative contributions of these complementary causal factors (environmental, anatomic and genetic) in the development of melanoma. The model proposes that people with low nevus counts (assumed to have an inherently low propensity for melanocytic proliferation) require chronic sun exposure to induce melanoma, and will tend to develop melanomas at habitually sun-exposed sites. In contrast, people with high nevus counts require smaller doses of sun exposure to initiate melanoma development, and will tend to develop melanomas at sites with large melanocyte populations. We recently tested the 'divergent pathway' hypothesis for the development of melanoma in a population-based epidemiological study. We found that patients with head and neck melanomas were significantly less likely than patients with melanomas of the trunk to have more than 60 nevi (OR = 0.34, 95% CI = 0.15 to 0.79) but were significantly more likely to have more than 20 solar keratoses (OR = 3.61, 95% CI = 1.42 to 9.17). Our more recent analyses demonstrate different patterns of sun exposure between patients with melanomas of the head and neck and the trunk. These findings, together with recent reports from molecular and epidemiologic studies, suggest that the null hypothesis (that all cutaneous melanomas arise though the same causal pathway) can be rejected. Elucidating the various pathways to melanoma should be the focus of continued research.

057 BRAF MUTATIONS AND SUN-EXPOSURE PATTERNS IN PRIMARY MELANOMA

J. Maldonado (Department of Dermatology, University of California, San Francisco , USA)*

The RAS/mitogen-activated protein kinase pathway sends external growth promoting signals to the nucleus. The critical serine/threonine kinase BRAF is frequently activated by somatic mutation in melanoma. Using a cohort of 126 primary invasive melanomas, we showed that mutations in either BRAF or NRAS are significantly more common in melanomas occurring on skin subject to intermittent sun-exposure. By contrast, BRAF or NRAS mutations in melanomas on chronically sun damaged skin and melanomas on skin relatively or completely unexposed to sun, such as palms, soles, subungual sites, and mucosal membranes are rare. Using array CGH, we found the mutated BRAF allele was frequently found at elevated copy number, implicating BRAF as one of the factors driving selection for the frequent copy number increases of chromosome 7q in melanoma. Based on the unique features of melanomas that develop on intermittently sun-exposed skin, we hypothesize that they are a reflection of a particular susceptibility of melanocytes that are either more likely to acquire BRAF mutations or respond differently to these mutations. Individuals with these melanocytes develop melanomas at low exposure levels because they are more susceptible to UV exposure than other individuals, and their susceptibility leads to the development of benign and malignant tumors with mutations in BRAF. By contrast, resistant individuals develop melanomas only after sufficient exposure to cause chronic sun damage to the skin. These tumors do not have BRAF mutations and are restricted to anatomic sites uncovered by clothing most of the time. This hypothesis motivates a search for the cause of the melanocyte susceptibility and suggests that therapies effective in one type of melanoma may not be effective in the other.

058 COMPUTERIZED DECISION SUPPORT FOR THE DIAGNOSIS OF PIGMENTED SKIN LESIONS

M. Binder (Dept. of Dermatology, Austria)*

Clinical decision support systems are on the verge of becoming routine software tools in numerous clinical settings. Especially for morphologic discrimination between benign and malignant, automated systems appear to be suitable. Several studies describe the development of computerized systems aiming to support dermatologists in the early diagnosis of melanoma. In some studies the performances of those systems exceed those of dermatologists. We analyzed the discriminatory power of k-nearest neighbors, logistic regression, artificial neural networks (ANNs), decision tress, and support vector machines (SVMs) on the task of classifying pigmented skin lesions as common nevi, dysplastic nevi, or melanoma. Three different classification tasks were used as benchmarks: the dichotomous problem of distinguishing common nevi from dysplastic nevi and melanoma, the dichotomous problem of distinguishing melanoma from common and dysplastic nevi, and the trichotomous problem of correctly distinguishing all three classes. Using ROC analysis to measure the discriminatory power of the methods showed that excellent results for specific classification problems in the domain of pigmented skin lesions can be achieved with machine-learning methods. On both dichotomous and trichotomous tasks, logistic regression, ANNs, and SVMs performed on about the same level, with k-nearest neighbors and decision trees performing worse.

059 POLARIZED LIGHT IMAGING OF PIGMENTED LESIONS

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Polarized light imaging offers a means of selecting only photons that have been scattered by superficial layers of the skin. Hence, the image contrast is based on the few photons that are scattered by the superficial layers and avoids the blinding effect of the preponderance of multi-scattered photons. Photons scatter off of melanin granules and present a brighter region relative to non-pigmented regions of skin. METHOD: The skin is illuminated with a linearly polarized light source, and a second linear polarizer is placed in front of a CCD camera that observes the skin. Two images are acquired, one with the camera viewing light polarized parallel to the illumination (called PAR) and one viewing light polarized perpendicular to the illumination (called PER). About 90% of the light penetrates the skin deeply and is multiply scattered, and contributes equally to both images. Photons that scatter from superficial skin layers retain the orientation of polarization of the illumination and are collected only by the PAR image and rejected by the PER image. The difference image, PAR - PER, creates an image based only on superficially scattered photons and rejects the multiply scattered light. RESULTS: Studies on human skin illustrate the ability to image pigment lesions, including melanoma, with enhanced contrast. Studies on a mouse melanoma model, in which melanoma originates in the dermis and invades the epidermis, illustrate the ability to discriminate superficial from deep melanoma. Deeper melanoma lesions present dark regions in PER images due to absorption of multiply scattered light but slightly brighter regions in PAR-PER images due to reflectance of polarized photons by the superficial skin layers. Polarized light images allow rapid surveillance of the entire mouse's dorsal skin area to locate melanoma lesions and discriminate superficial versus deeper lesions.

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060 OPTICAL SPECTROSCOPY FOR SKIN PIGMENTATION ASSESSMENT AND PIGMENTED LESION DIAGNOSIS

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Melanin pigmentation is assumed to be easily recognizable in human skin because of its distinct visual appearance - it appears brown-black - and it is the only material in skin that bears this color. It has been shown that in mixed pigment-vascular reactions the concentration of melanin pigmentation may be overestimated. Furthermore the appearance of pigmented lesions may vary depending on the means used to document them e.g. digital photography, video microscopy with or without optical coupling. Optical spectroscopy may be used to characterize human pigmentation based on its absorption spectral characteristics or the vibrational states of its molecular constituents - as in Raman spectroscopy. Optical spectroscopy may be coupled with imaging to yield distribution maps of absorbers classified by their absorption properties not only in the visible spectrum (range of sensitivity of the eye) but in the UVA-deep blue and the near infrared. Spectroscopic investigations may yield simultaneously information on the type and form of melanin that is present and on the other skin chromophores and allow estimation of the optical thickness of the absorbers. Specific and distinct spectroscopic features of melanin pigmentation include its absorption in the UVA-deep blue, in the red part of the visible spectrum, in the near IR, fluorescence excited in the near IR and Raman signals from high melanin concentrations as in hair. These features may be used to characterize pigmented lesions in a multidimensional matrix going beyond visual perception. Recent advances in optical technology such as Confocal Reflectance microscopy and Optical Coherence tomography have enabled in vivo documentation of the distribution of melanin granules in pigmented lesions both at the macro and the microscopic level with impressive clarity. Finally, optical spectroscopy may yield information on the UV induced changes in melanin which may be responsible for photosensitization reactions.

061 ULTRAHIGH RESOLUTION OPTICAL COHERENCE TOMOGRAPHY OF HUMAN SKIN

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Ultrahigh resolution OCT (UHR OCT) was performed on normal and pathologic human skin biopsies using an ultrabroad-bandwidth (260 nm) Titanium:sapphire laser, enabling sub-micrometer (0.9 µm) axial UHR OCT resolution. Penetration, image contrast as well as resolution capabilities achieved are analyzed and compared to histopathology for correct interpretation of UHR OCT tomograms and optimum UHR OCT in vivo performance. With the achieved resolution and the penetration depth, the transition between the dermis and the epidermis is clearly visible by UHR OCT, and also the anomalies of pathologies. In vivo three dimensional UHR OCT of normal skin is demonstrated with less than 3 µm axial resolution at video-rate with up to 50 B-scans/second, each tomo¬gram consisting of 512x1024 pixels, resulting in 25 Megavoxels/second.

062 IN VIVO CONFOCAL MICROSCOPY FOR PIGMENTED SKIN LESION DIAGNOSIS

*S. Gonzalez * (Memorial Sloan-Kettering Cancer Center, USA)*

Early diagnosis of malignant melanoma remains a challenge to the clinician and is of paramount importance for the prognosis. To date, early detection relies on conventional clinical evaluation, dermatoscopic assessment and routine histology. High-resolution non-invasive imaging techniques have been explored in recent years to enhance the accuracy of melanoma diagnostic. Reflectance-mode confocal microscopy (RCM) is a novel technique that enables non-invasive imaging of human skin in vivo at resolutions comparable to routine histology. Visualization of cellular detail is based on the presence of endogenous contrast, such as melanin, making melanocytic lesions particularly amenable to confocal microscopy. In this paper, the fundamentals of confocal microscopy will be presented and the characteristic features of benign and malignant melanocytic lesions as well as its potential clinical applications will be discussed.

063 FUNCTIONAL IDENTIFICATION OF NOVEL TARGETS OF P16INK4A

D. Hogg (University of Toronto, Canada), R. Agatep (University of Toronto, Canada), A. Lowrance (University of Toronto, Canada), M. Shennan (University of Toronto, Canada), A. Hao (University of Toronto, Canada), L. Liu (University of Toronto, Canada)*

Germline mutations in the tumour suppressor gene CDKN2A can predispose carriers to melanoma. Most of the corresponding mutant p16INK4A proteins possess a decreased affinity for the G1 kinases CDK4 and CDK6, and the resultant overactivity of these kinases permits cell cycle progression at the restriction point R. However, this model does not explain the tumorigenic nature of the 1_24 dup24 CDKN2A mutant, a variant that encodes a p16INK4A bearing an N-terminal 8 amino acid duplication. The corresponding protein, designated p16INK4A(+24), binds CDK4/6 with wild-type affinity and can inhibit cellular proliferation, yet strongly predisposes germline carriers to melanoma. We hypothesized that p16INK4A possesses additional tumor suppressor functions in addition to CDK4/6 inhibition. To address this possibility, we developed a novel genetic screen in yeast capable of identifying interacting proteins that bind to wild type but not the p16INK4A(+24) variant. We report that p16INK4A-interacting proteins identified in this screen participate in cellular activities that may be relevant to tumor progression.

064 THE EPIGENETIC REPROGRAMMING OF MELANOMA CELLS BY THE MICROENVIRONMENT

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A dynamic, complex relationship exists between tumor cells and their microenvironment, which plays a pivotal role in cancer progression, yet remains poorly understood. Particularly perplexing is the finding that aggressive melanoma cells express genes associates with multiple cellular phenotypes, in addition to their ability to form vasculogenic-like networks in three-dimensional (3-D) matrix called vasculogenic mimicry. Key to identifying the molecular mechanisms underlying tumor cell transdifferentiation and vasculogenic mimicry is understanding the unique role of the microenvironment in this process. Our work addresses the epigenetic influence of the microenvironment of aggressive melanoma cells. We utilized a novel cellular and molecular strategic approach to determine an epigenetic induction of a transdifferentiated phenotype in poorly aggressive melanoma cells, and melanocytes, exposed to the microenvironment of aggressive melanoma cells, including the acquisition of a plastic and invasive phenotype. The data reveal profound changes in the global gene expression of poorly aggressive melanoma cells and melanocytes exposed to 3-D matrices preconditioned by aggressive melanoma cells, including the acquisition of a vasculogenic cell phenotype, upregulation of ECM remodeling genes, and increased migratory/invasion potential indicative of an epigenetic, microenvironment-induced transdifferentiation. These findings offer an unique perspective of the inductive properties associated with an aggressive melanoma microenvironment that might provide new insights into the regulation of tumor cell plasticity and differentiation, as well as mechanisms that could be targeted for novel therapeutic strategies. Furthermore, the implications of these results in developmental systems are worthy of experimental scrutiny.

065 STEM CELLS AS BIOMARKERS FOR MELANOMA

M. Herlyn (Wistar Institute, USA), D. Fang (Wistar Institute, USA)*

Cancer stem cells are rare cells with indefinite potential for self-renewal and differentiation into diverse progenies. In this study, we identified a stem cell fraction in approximately 20% of specimens of both fresh metastatic melanomas and established cell lines using a growth medium suitable for human embryonic stem cells. This cell population, when propagated as non-adherent spheres, could differentiate under appropriate conditions into multiple lineages, including melanocytic cells and mesenchymal osteoblast, chondrocyte and adipocyte differentiation. Spheroidal melanoma cells grafted to mice were more tumorigenic than the adherent melanoma cell population isolated from the same specimens. Multipotent spheroid cells persisted after serial cloning in vitro and transplantation in vivo, indicating their self-renewal capacity. We then identified a small subpopulation of spheroidal cells expressing both melanoma markers and the B-cell marker CD20. This population is likely responsible for self-renewal, multi-lineage differentiation, and thus, the malignant phenotype in vivo. Identification of stem-like populations in melanoma may provide the most important target yet identified for the development of new anticancer therapies.

066 SUNBEDS: HAZARD OR NOT?

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Objective: The usage of sunlamps and sunbeds which emitting artificial ultraviolet radiation has been increasing rapidly in the Western countries, especially among young people. There are concerns that these tanning devices may cause cutaneous malignant melanoma (CMM) because ultraviolet radiation from solar exposure causes CMM and the strongest association is for the exposure in early life. However, in spite of the best effort from a number of epidemiologic studies, the causal relationship between CMM and tanning devices could not be determined conclusively. The main challenge was the lacking of study power, resulting unstable risk estimates for CMM with wide 95% confidence intervals. In order to evaluate risk and exposure, we conducted a systematic review and meta-analysis on the tanning device studies. Materials & Methods: We reviewed all literatures reported by MEDLINE between Jan 1, 1984 to April 2004. After applying straight exclusion/inclusion criteria, we analysed the measures 'ever vs never exposed', 'first exposure as a young adult', and 'longest duration or highest frequency of use' for 9 case-control studies and 1 cohort study. The summary odd ratios (OR) and 95% confidence intervals (CI) were calculated. The heterogeneity of the original study estimates was assessed by Q statistics. Results: A positive association was found between exposure and risk for 'ever vs never exposed' (Summary OR =1.25; 95%CI= 1.05-1.49). Significant heterogeneity between studies was present. Evaluation of the measures 'first exposed as a young adult' (5 studies) and 'longest duration or highest frequency of exposure' (6 studies) also yielded significantly elevated risk estimates (Summary OR=1.69; 95%CI= 1.32-2.18, and 1.61; 95%CI=1.21-2.12, respectively, with no heterogeneity in either analysis). Conclusion: Results suggest that there is a significant association between risk of CMM and tanning devices emitting ultraviolet radiation.

067 INVESTIGATING TANNING EQUIPMENT USE AND RISK OF MULTIPLE VS. SINGLE PRIMARY CUTANEOUS MELANOMA IN ONTARIO, CANADA

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OBJECTIVE: To examine the association between tanning equipment (TE) use and risk of multiple primary melanoma in Caucasian adults, adjusting for important covariates, such as sun exposure, constitutional factors, and family history of melanoma. MATERIALS & METHODS: Subjects were participants in the Ontario, Canada portion of the Genes, Environment and Melanoma (GEM) study and were ascertained from the population-based Ontario Cancer Registry. They had either an invasive single primary melanoma (SPM) diagnosed in January-August 2000, or an in situ or invasive higher order melanoma (MPM) diagnosed between January 2000 and July 2003. Data were collected using reliability-tested questionnaires. TE exposure in SPM and MPM is compared, and multivariable logistic regression is used to analyze the effects of sun exposure (total weekday, total weekend, lifetime, and average annual hours); host characteristics (skin, eye, and hair colour, sun sensitivity, childhood freckling, and moles on the upper back); as well as family history of melanoma (1+ first-degree relatives) on relative risk estimates. RESULTS: 531 subjects (125 MPM/406 SPM) participated, of which 152 (28.6%) reported TE use. MPM were significantly older than SPM, with mean ages, 63.8 and 54.1 years, respectively, and were more likely to be male (63% versus 48%, respectively). Adjusted for age and sex, TE use was associated with a significant increase in MPM risk, OR=1.68 [1.02,2.77]. Subsequent control for host factors revealed slightly higher odds ratios. ORs adjusted for family history, and sun exposure will also be estimated. CONCLUSION: Although past studies have suggested that artificial ultraviolet radiation increases the risk of melanoma, many studies have not adequately controlled for natural exposure, thus making it difficult to determine whether the association is causal or due to confounding. A major strength of the current study is the availability of high quality data on both pattern and amount of sun exposure.

068 THE NORWEGIAN-SWEDISH COHORT STUDY ON SOLARIUM USE AND CUTANEOUS MALIGNANT MELANOMA IN WOMEN

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OBJECTIVE: To study solarium use and risk of malignant melanoma in a large cohort study, which also includes investigation of reproducibility and recall bias. MATERIALS & METHODS: The Norwegian-Swedish Women's Lifestyle and Health Cohort Study first included 106 379 women aged 30-50 years in 1991/92. In Norway the cohort was expanded by inclusion of 44 000 women aged 45-69 in 1996/97. Exposure was collected at inclusion through a self-administered questionnaire. Linkages to national registries ensure complete follow-up. A second questionnaire recorded exposure after 5 year of follow-up, and reproducibility of this questionnaire is studied in a subsample of 1474 Norwegian women. Recall bias will be studied in a cases-control study conducted within the Norwegian cohort. Poisson regression was used to estimate relative risks (RRs). Reproducibility is examined by weighted kappa (o). RESULTS: Analysis of 106 379 women followed through December 31, 1999, included 187 melanoma cases. Solarium use at ages 20-29 years, adjusted for sunburns and sunbathing vacations, was significantly associated with melanoma risk (RR=2.58 for e1 time/month versus never, 95% CI (1.48, 4.50)). Moreover, women who used a solarium e1 time/month during the 10-39 age period had a significantly higher risk than women who never/rarely used a solarium in this age period (adjusted RR=1.55, 95% CI (1.04, 2.32)). Through December 31, 2001, the expanded cohort of 150 000 women included 360 melanoma cases, and a new analysis is planned. Reproducibility was good for the 5 year follow-up question on solarium use, o = 0.70 (95% CI (0.67, 0.73)), but RR estimates comparing extreme categories will be attenuated and this will be illustrated for selected scenarios of true solarium-melanoma associations. CONCLUSION: Solarium use was associated with significantly increased risk of melanoma. The study has great potential to explore this further and study important methodological issues.

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069 IS THERE A NEED FOR REGULATING THE "TANNING INDUSTRY"

Ph. AUTIER (Jules Bordet Institute, Belgium)*

The 'tanning industry' can be defined as commercial activities developed around the behaviours of intentional sun exposure, for tan acquisition or for search of well being. The main marketing concept developed by the tanning industry is the 'safe tan acquisition', that is the acquisition of a tan without incurring (or with incurring less) detrimental effects of UV exposure, mainly sunburns, skin cancers, and skin ageing. Public health efforts try to discourage sunbed use. However, for the majority of people, information and advertisings disseminated by the cosmetic and the tanning industry are the main source of information regarding tan acquisition and sun protection. Behavioural studies show that people know about skin cancer and the damaging affect of sunbathing, and about possible dangers associated with sunbed use, but that knowledge does not alter their tanning behaviours in general. Regulations of sunbed installation, operation and use are now common in industrialized nations, but their enforcement remains inadequate. In contrast, the existence of regulations is presented by many tanning salon operators as a guarantee that sunbed use is safe. There is thus an emerging need for actions aiming at controlling the information disseminated by the 'tanning industry' on suppositions that sunbed use would be safer than sun exposure, and on hypothetical health benefits of tanning. The step forward should be the control of advertisings and information disseminated by the tanning industry to the general public. The sunbed manufacturers and operators should no longer be able to claim health benefits of any sort attributable to sunbed use, and to other forms of intentional sun exposure. Indeed, this strategy would concern other segments of the tanning industry, such as sunscreen companies that base their marketing strategy on the possibility to acquire a healthy and safe tan thanks to the use of their product.

070 MAPPING LOW PENETRANCE MELANOMA GENES AND PROGRESS ON IDENTIFYING THE 1P22 PREDISPOSITION GENE

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Susceptibility to melanoma is determined in part through interaction between the pigmentary system and UV-light, with lighter skin tones, blue eyes and red or blond hair significantly increasing the risk of melanoma . To identify genes responsible for some of these melanoma risk phenotypes we carried out a genome-wide scan in a large sample of twins and their families, for which we have collected comprehensive melanoma risk factor data, including full-body nevus counts and pigmentation phenotypes. We mapped the principal eye colour gene to chromosome 15q and identified that locus as the OCA2 gene. Similarly, we have identified several other regions that show suggestive linkage to either hair, eye or skin colour, although the causative genes have yet to be determined. Additionally, we have mapped novel loci for nevus count to chromosomes 2p, 4p and 17p. Work is ongoing to fine-map the loci under these nevus linkage peaks and to determine whether some of the candidate genes from these regions are involved in melanocytic neoplasia by screening for mutations in melanoma cell lines. We recently localized a novel melanoma susceptibility locus to chromosome 1p22. Recombinants in linked families localized the gene to a 10 Mb region between D1S430 and D1S2664. To more finely map the locus we assessed allelic loss across the region in melanomas from 1p22-linked families, sporadic melanomas and melanoma cell lines. 80% of familial melanomas show loss of heterozygosity (LOH) in the region, with a smallest region of overlapping deletions (SRO) of 9 Mb between D1S207 and D1S435. This high frequency of LOH suggests the susceptibility locus is a tumour suppressor. Several candidate genes have been screened in 1p22-linked families but no mutations have been found. Array-comparative genomic hybridization is being used to screen for homozygous deletions within this region in melanoma cell lines with LOH.

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071 PANCREATIC CANCER AND NEURAL SYSTEM TUMORS (NSTS) IN MELANOMA-PRONE FAMILIES WITH CDKN2A MUTATIONS

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Two high-risk melanoma susceptibility genes have been identified to date. CDKN2A is the major known melanoma susceptibility gene with germline mutations observed in approximately 20% of melanoma-prone families from around the world. CDKN2A encodes two distinct proteins translated, in alternate reading frames (ARFs), from alternatively spliced transcripts. The alpha transcript encodes the p16 protein; the beta transcript encodes p14ARF. In contrast, few families with germline mutations in CDK4 have been identified. Several studies have demonstrated an increased risk of pancreatic cancer (PC) among CDKN2A melanoma-prone families, although the precise risks of PC in CDKN2A mutation carriers are unclear. Several additional factors are associated with frequency of detected CDKN2A mutations: increased numbers of melanoma patients in a family, occurrence of multiple melanoma tumors (MPM) and early median age at melanoma diagnosis in a family. Sample size requirements have precluded examination of these four factors simultaneously. NSTs have also been reported to be associated with mutations that affect p14ARF and not p16, but these studies are based on small numbers. GenoMel, comprising major familial melanoma research groups from North America, Europe, and Australasia, has created the largest sample yet available to evaluate the relationship between PC, NSTs, and melanoma-prone families with mutations in melanoma susceptibility genes. Seventeen GenoMel centers participated. Families with >=3 melanoma patients were eligible for study. Variables included numbers of melanoma patients/family, numbers of melanoma patients with MPM, age at first melanoma diagnosis, numbers of melanoma patients and their first-degree relatives with PC or NSTs, and absence/presence/type of CDKN2A, p14ARF (exon 1b) or CDK4 mutation. GenoMel collected data on 439 families (2035 melanoma patients). There were 178 CDKN2A mutations (41%), 4 p14ARF mutations (2%=4/236), 5 CDK4 mutations (2%=5/231), and 3 whole gene deletions (2%=3/147). Results from the evaluation of PC and NSTs in these 439 families will be presented.

072 PREVALENCE AND PENETRANCE OF CDKN2A MUTATIONS IN FAMILIAL AND NON-FAMILIAL MELANOMA.

G. Mann (Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead, NSW, Australia)*

Certain variants (mutations) affecting the p16INK4A and/or p14ARF products of the CDKN2A locus are firmly established as causing a large increase in individual risk of melanoma. These so-called high penetrance alleles are observed in individuals with a family history of melanoma, albeit at widely varying frequency, and in occasional cases without a family history of melanoma. The Melanoma Genetics Consortium (GenoMel) has collated data from members of multiple case melanoma kindreds in countries in Northern and Mediterranean Europe, North America and Australia, and from several population-based series of cases of melanoma in which a family history was not a reason for ascertainment. The probability of finding a CDKN2A mutation in familial melanoma is inversely related to the underlying population incidence, i.e. melanoma clusters in high-incidence regions are considerably less likely to be due to CDKN2A mutation. In other familial cancers, the risk associated with mutations in major susceptibility genes is affected by family context. In melanoma as well, such mutations observed in the absence of a strong family history of melanoma may have lower penetrance than when they are observed in dense clusters of affected relatives. These complexities, and others relating to the unreliability of unverified reports of melanoma family history, place severe limits on the utility of CDKN2A predictive genetic testing.

073 THE MANAGEMENT OF PATIENTS WITH A FAMILY HISTORY OF MELANOMA

J. Newton Bishop (Genetic Epdemiology Division, CR-UK Clinical Centre at Leeds, UK), M. Harland (Genetic Epdemiology Division, CR-UK Clinical Centre at Leeds, UK), L. Whitaker (Genetic Epdemiology Division, CR-UK Clinical Centre at Leeds, UK), J. Randerson-Moor (Genetic Epdemiology Division, CR-UK Clinical Centre at Leeds, UK), T. Bishop (Genetic Epdemiology Division, CR-UK Clinical Centre at Leeds, UK)*

Individuals with a family history of melanoma are at increased risk and should therefore be counselled by a specialist dermatologist or clinical geneticist. Management in clinic is directed first towards an estimation of risk based upon pedigree analysis: the number of cases of melanoma in the family, the presence of other cancers and the degree of relatedness of the cases. Personal risk is also affected by the presence of the atypical mole syndrome and the presence of multiple primaries. Having estimated risk then the imperative is to counsel the patient about primary prevention and secondary prevention. The role of genetic testing remains as yet unclear and this will be addressed.

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074 PRIMARY PREVENTION OF MELANOMA IN AUSTRALIA

D. English (The Cancer Council Victoria, Australia)*

Early efforts to prevent melanoma in Australia began in Queensland in the 1970s following recognition that this state had the highest rates of melanoma in the world. Slip, Slop Slap began in 1980 and in 1988, the Anti-Cancer Council of Victoria established SunSmart, which was a comprehensive program that included regular evaluation. Ultimately, the success of primary prevention efforts is judged by their impact on melanoma incidence and mortality rates. In the past twenty years, incidence rates of invasive melanoma in Australia have been stable for those less than 45 years of age and increasing in older people. The incidence of thick melanomas (> 3 mm) has been stable, or even showed small declines, in the younger age groups, but increased in the older age groups. In-situ melanomas and thin melanomas have increased in all age groups. Interpreting trends in these rates is complicated by changes in case detection over time, especially in early detection, and because there is likely to be a long lag between the initiation of prevention campaigns and their effect on incidence. Nevertheless, these trends are consistent with a beneficial effect on incidence of thick melanomas in younger age groups. Monitoring changes in the population's relevant exposure to sunlight can be useful intermediate outcomes. The proportion of the population reporting weekend sunburn dropped substantially from the introduction of SunSmart through the 1990s. At the same time, the proportion reporting that they preferred not to tan increased from around 40% to over 60% (although there has been a slight fall in this preference recently). Favourable changes such as these are consistent with a beneficial effect of SunSmart, but given the complexity of the relationship between sun exposure and risk of melanoma, these can be difficult to interpret in terms of the likely effect on incidence.

075 PRIMARY PREVENTION OF SKIN CANER IN THE UNITED STATES

A. Halpern (Memorial Sloan Kettering Cancer Center, USA)*

In this session we will discuss the evolution of primary prevention efforts in the US. We will discuss the global challenges to impacting prevention behaviors, factors that are somewhat unique to the United States programs, and current areas of focus in US prevention efforts. As of the late 1970s there were no formal programs for skin cancer education in the United States. In the 1980s programs initiated by the Skin Cancer Foundation (SCF) set the stage for additional programs from the American Academy of Dermatology, Environmental Protection Agency, and American Cancer Society. Over the course of the 1990s there was a proliferation of skin cancer related private foundations in the U.S., leading to increased but poorly coordinated efforts in public education. In 1998, the National Council on Skin Cancer Prevention was established with funding from the Centers for Disease Control (CDC) to help set a national agenda and promote coordinated efforts in skin cancer prevention. Challenges to these efforts include the lack of government funding, absence of a centralized school system/curriculum, and very limited outcomes data. Over the past two decades there has been a significant increase in skin cancer knowledge and awareness in the US population. While there were initial encouraging trends in change of sun protective behaviors in the early 1990's, recent data suggest that improved knowledge has had marginal impact on the sun protective behaviors of the most important target populations; children and adolescents. This has led to a growing awareness in the field of the need to complement public education efforts with efforts to change legislative policies and to impact the social environment. There is also a growing appreciation of the need for a scientifically sound public health campaign that incorporates the coordinated promotion of sun protection, exercise, and vitamin D sufficiency.

076 PRIMARY PREVENTION OF SKIN CANCER ARE WE MAKING PROGRESS IN EUROPE ?

R. Greinert (Center of Dermatology Buxtehude, Germany)*

Primary Prevention of Skin Cancer Are we making progress in Europe ? R. Greinert Secretary General, EUROSKIN Dermatology Center Buxtehude, Germany Skin cancer incidence is increasing worldwide. Proper registration of all skin cancers, malignant melanom (MM), basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), show that incidences are doubling every 10-15 years. This rate of increase is higher than for any other cancer and makes skin cancer, in the sum of MM and nonmelanocytic skin cancers (NMSC), the most frequent cancer. This trends hold equally for Europe, and therefore a number of strategies, campaigns, interventions and programmes in primary prevention have been started and are still continued throughout Europe in order to counteract the increase in skin cancer incidence. European efforts in primary prevention of skin cancer will be summarized, underscoring the importance of 'periods of life' programmes, which inform specific age groups successively in the field of public education. To be successful this has to be done in a harmonized European way for which the European Society of Skin Cancer Prevention, EUROSKIN, gives certain recommendations how to behave safe in the sun and under artificial UV-radiation. This kind of risk communication and it's possibilities to reduce the burden of skin cancer by means of alleviating human and economic costs wil be discussed, especially for the case of malignant melanoma of the skin.

077 WHAT PRIMARY PREVENTION STRATEGIES WORK? THE COMMUNITY GUIDE EVIDENCE REVIEW

K. Glanz (Emory University, USA)*

This presentation describes an evidence review of skin cancer prevention interventions that was conducted for the Guide to Community Preventive Services (n=85 studies), and summarize knowledge about the effectiveness of interventions to reduce UV radiation exposure. A series of systematic evidence examined behavioral, educational, policy and environmental strategies for changing behaviors in order to reduce skin cancer risk and improve health. The evidence reviews covered nine different categories of interventions. Six reviews focused on distinct settings: health care settings and health care providers; occupational settings; recreation and tourism settings; secondary schools and colleges; primary schools; and child care centers. Three other reviews focused on a target population, children's parents and caregivers and broad types of interventions, media campaigns, and communitywide multicomponent interventions. The focus was strictly on prevention, not on detection or patient education related to cancer treatment. The Task Force recommended two interventions to improve sun-avoidance or covering-up behaviors: educational and policy interventions in primary schools, and programs for adults in outdoor recreational settings. These recommendations represent tested interventions that promote decreased UV exposure at the community level. They can be used for planning interventions to promote UV protection or to evaluate existing programs. The other interventions that were reviewed, but for which evidence was insufficient to determine effectiveness, may also prove useful in providing a broader taxonomy of interventions that might be tried in communities and in promoting additional testing and evaluation. The reviews on which the recommendations are based also provide a starting point for improving the quality and usefulness of existing research. As might have been predicted in this emerging area of research, many questions about these interventions remain to be answered. We hope that the documentation of evidence gaps in these reviews will help to improve the next generation of research.

078 THE AMOUNT AND LOCATION OF SENTINEL NODE MELANOMA PREDICT NON-SENTINEL NODE TUMOR STATUS AND CLINICAL OUTCOME.

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Most patients with melanoma spread to the sentinel node (SN) (67%) do NOT have tumor in the non-sentinel nodes (NSN). If melanoma has extended to the NSN it usually involves a limited number of nodes and the amount of tumor present is small and generally less than the tumor in the SN. Nonetheless the presence of tumor in NSN often has grave prognostic significance. In studies undertaken prior to our development of the SN approach (Morton et al., Arch Surg. 1992) we showed that assessment of tumor area or micrometer-measured tumor diameter (both relative to nodal area or diameter)were predictive of outcome (Cochran et al., AJ Surg Path.1989). Starz et al. (Cancer. 2001) have very successfully applied the micrometer approach to SN and we have shown that the area of SN tumor accurately predicts NSN status, likelihood of recurrence and death from melanoma (Cochran et al., Mod Pathol,2004). Cook et al., (J Pathol.2004) have reported that the location of tumor in the SN (subcapsular sinus versus lymphatic parenchyma is predictive of outcome. In the presence of small tumor volume, (quantitative) PCR and reduction of dendritic cell density and frequency (the latter an index of nodal immune suppression) provide clinically useful information on likely outcome. Strengths and weaknesses of these different approaches and their interactions will be reported and discussed. Supported by CA29605-John Wayne Cancer Institute Subcontract.

079 HOW MUCH HISTOPATHOLOGICAL SECTIONING IS NECESSARY TO ADEQUATELY EVALUATE SENTINEL NODES FROM MELANOMA PATIENTS?

R. Scolyer (Department of Anatomical Pathology, Sydney Melanoma Unit & the Melanoma and Skin Cancer Research Institute, Royal Prince Alfred Hospital, Sydney, Australia, Australia)*

The sentinel node (SN) biopsy technique is a highly accurate method of staging cutaneous melanoma and, furthermore, the tumour-harbouring status of the SN is the most important prognostic factor for melanoma patients. Although the formal reporting of the results of randomized trials must be awaited to determine whether the SN biopsy technique, with full regional node dissection if a positive SN is found, is of any therapeutic value, recent preliminary interim results from the MSLT I randomized clinical trial reported in abstract form, suggest that this is the case. The assessment of SNs requires a team approach involving surgeons, nuclear medicine physicians and pathologists. For a SN to provide accurate prognostic information it is essential that true SNs are removed and examined thoroughly. Technical failures may occur as a result of errors in lymphatic mapping and sentinel lymphadenectomy or because of erroneous histopathologic evaluation. Numerous studies have shown that the pathological examination of multiple sections and the use of imunohistochemistry increase the detection rate of micrometastatic disease in SNs. The challenge is to identify the protocol that optimally balances the accuracy of the result against the labour and costs involved. It is clearly impractical to perform complete serial sectioning and pathologic examination of every SN (which would involve the examination of more than 600 sections per SN). Other issues that must be considered in determining the optimal sectioning protocol are: 1. the biological significance and clinical relevance of metastatic deposits detected by more thorough pathological examination, 2. the use of techniques (such as carbon dye or antimony analysis) to better localize the site of metastases within the SN and 3. the role of non-histopathological methods of SN evaluation such as molecular staging and magnetic resonance spectroscopy as either replacement of, or as an adjunct to, histopathological examination.

080 THE USE OF MOLECULAR TECHNIQUES IN PARALLEL WITH HISTOLOGY TO EVALUATE THE SENTINEL LYMPH NODES

H. Starz (Klinikum Augsburg, Germany), C. Haas (Klinikum Augsburg, Germany)*

Introduction: Evaluation of sentinel lymph nodes (SLNs) means looking for micrometastases that may be present in an only very small proportion of the entire node volume. This implies a potential sampling error even for laborious techniques of screening the SLNs with the microscope. Some authors therefore claimed that the RT-PCR detection of tyrosinase mRNA is a practical and more sensitive way to diagnose even 'submicroscopic' melanoma metastases. But they did not provide sufficient evidence for the diagnostic specificity of this method. Method: We have established a combined technique for the RT-PCR analysis of paraffin sections adjacent to further sections that are used for histology including immunohistochemistry with anti-S100 and HMB45. 520 SLNs of 250 melanoma patients were cut into 1 mm slices each of which provided the mentioned series of paraffin sections. Results: In 53/107 patients with positive RT-PCR results, tyrosinase mRNA was detected in the same SLNs where melanoma involvement was confirmed by histology. In 42/107 patients, however, positive RT-PCR results were explained only by the presence of benign capsulotrabecular nevus cells in the respective SLNs. No micromorphologcal correlate was found in 12/107 patients. Negative RT-PCR results were obtained in 15/68 patients despite the presence of melanoma cells in SLNs. In 14 of these 15 cases the melanoma cells were confined to the subcapsular zone (classifications SI or SII). In cases of melanoma-negative SLN histology, the recurrence-free survival was not different by log-rank-test between patients with (n=54) and without (n=128) tyrosinase mRNA (average follow up 19 months). Conclusions: Tyrosinase RT-PCR is not an alternative, but only an optional supplement to histology and immunohistochemistry for the evaluation of SLNs in melanoma patients. Upcoming molecular multimarker assays must still be tested in correlation with this gold standard, e.g. using our combined technique.

081 MOLECULAR DYNAMICS AND TRAFFICKING OF DENDRITIC CELLS AND LYMPHOCYTES TO AND WITHIN THE SENTINEL NODE.

*M. Mihm * (Mass. General Hospital , USA), A . Piris (Mass. General Hospital , USA)*

Dendritic cells and naive T cells in the skin cannot home to draining regional lymph nodes without the expression of the chemokine receptor, CCR7. The ligands for this receptor are found on the high endothelial venules that lie in close proximity to the T cell rich areas of the paracortex. The ligands originate in stromal cells; they rest on the luminal surface of the venules. Once the dendritic cells arrive in the paracortical area their successful presentation of antigen to the naive T cells apparently depends upon the cytokine milieu of the node. An increase in substances such as IL-10 or IDO can impede this process. We will review the basics of the trafficking of these cells, the conditions for successful antigen presentation and attempt to propose therapeutic strategies based on these findings.

082 TRANSCIPTIONAL PATHWAYS IN MELANOCYTES AND MELANOMA

D. Fisher* (Harvard Medical School, USA)

Melanocyte development provides a useful means to gain insights into the biology of melanoma. An important regulator of melanocytic development is the MITF transcription factor, a protein which is essential for the development of all melanocytes as well as for survival of melanoma cells. MITF was found to structurally mimick many biochemical features of the Myc oncoprotein, yet it transcriptionally regulates expression of the pigmentation pathway in melanocytes. MITF is a major transcriptional mediator of the Melanocyte Stimulating Hormone pathway in melanocytes. Recent studies have indicated that dysregulated expression of MITF causes malignant transformation. This has been identified in melanoma (where MITF was found to be amplified) as well as in several other solid tumors in which closely related MITF family members are targets of translocation/fusion events. Through identification this shared transcriptional dysregulation these tumors have been recognizably linked to the biology of melanoma. Ongoing studies are relating the vital activity of MITF to signaling pathways of importance in melanoma (eg BRAF) as well as downstream target genes which might serve as drug targets.

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083 GENE REGULATION IN MELANOMA PROGRESSION

M. Bar-Eli (M.D. Anderson Cancer Center, USA)*

Gene Regulation in Melanoma Progression Menashe Bar-Eli, Ph.D. University of Texas M. D. Anderson Cancer Center Department of Cancer Biology, Houston, Texas 77030 The molecular changes associated with the transmission of melanoma cells from radial growth phase (RGP) to vertical growth phase (VGP, metastatic phenotype) are not yet well defined. We have demonstrated that the progression of human melanoma is associated with loss of expression of the transcription factor AP-2. In metastatic melanoma cells, this loss resulted in overexpression of MCAM/MUC18 and MMP-2 and lack of c-KIT expression. In addition, inactivation of AP-2 in primary cutaneous melanoma cells by dominant-negative AP-2 (AP-2B) augmented their tumorigenicity in nude mice. We have also recently demonstrated that loss of AP-2 expression in metastatic melanoma cells resulted in over production of the thrombin receptor, PAR-1, which in turn, contributes to the metastatic phenotype of melanoma by upregulating the expression of adhesion molecules, proteases and angiogenic factors. Additionally, the transition of melanoma cells from RGP to VGP is associated with overexpression of the transcription factors CREB and ATF-1, both of which may act as survival factors for human melanoma cells. Inactivation of CREB/ATF-1 activities in metastatic melanoma cells by dominant-negative CREB or by anti-ATF-1 single chain antibody fragment (ScFv), resulted in deregulation of MMP-2 and MCAM/MUC18, increased the sensitivity of melanoma cells to apoptosis, and inhibition of their tumorigenicity and metastatic potential in vivo. The notion that the balance between AP-2 and CREB/ATF-1 expression determines the progression of melanoma cells towards the metastatic phenotype will be discussed.

084 THE BEHAVIOURAL ATTITUDE OF HUMANS TOWARD SUN EXPOSURE AND PROTECTION

H. Lim (Dermatology, Henry Ford Hospital, USA)*

Several studies have reported the behavioral pattern of the US public on sun exposure and photoprotection. A 1994 study in Minnesota showed that 34% of high school students had used indoor tanning saloons (Oliphant, JA, Am J Public Health 1994; 84:476). Two 2002 studies of US adolescents demonstrated a positive association between indoor tanning and being female, increasing age, favorable attitudes about tanning, and a parent/guardian who tans indoor (Geller AC, Pediatrics 2002; 109:1009. Cokkinides VE, Pediatrics 2002; 109:1124). A US nationwide survey of over 6900 non-Hispanic white adolescents revealed that 37% of girls and 11% of boys had used an indoor tanning facility (Demko CA, Arch Pediatr Adolesc Med 2003; 157:854). A survey of 489 college students showed that 47% had used tanning lamp in the preceding 12 months (Knight JM, Arch Dermatol 2002; 138:1311). A 1997 telephone survey of over 500 households reported that 13% of children, and 9% of adults had sunburn, and tan was viewed as being healthy (Robinson JK, JAAD 2000; 42:746). A 1998 survey of 1000 public schools in the US showed only 3% had sun protection policy for their students (Buller DB, Arch Dermatol 2002; 138:771). Recently, it was reported that UV photographs, photoaging information, and the use of sunless tanning lotion were associated with an improvement in the photoprotective behaviors (Mahler HIM, Arch Dermatol 2005; 141:373). Taken together, despite public education effort, American public, especially teenagers and young adults, continue to seek exposure to both natural and artificial UV radiation (Lim HW, J Am Acad Dermatol, May 2005).

085 SUNSCREENS AND MELANOMA: WHAT IS THE EVIDENCE?

A. Green (Queensland Institute for Medical Research, Australia)*

Currently no high level evidence is available from randomised controlled trials to answer the question of the relationship between sunscreen use and the incidence of cutaneous melanoma. Existing evidence from the results of observational, predominantly case-control, studies is known to be severely limited by potential confounding, for example, since sun-sensitive people prone to melanoma are those most prone to use sunscreen. Such confounding is difficult to control and may produce spurious positive associations or dilute negative (protective) associations. In addition recall biases affecting assessment of pre-disease sun exposure and sunscreen use are also likely. While rigorous systematic reviews of existing evidence suggest there is no positive association, that is, sunscreen use is unlikely to cause melanoma, it is not yet possible to properly address the most important question of whether proper use of high-protection sunscreens can prevent melanoma development.

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086 AN OVERVIEW OF SUB-ERYTHEMAL UVR EFFECTS ON HUMAN SKIN

AF. Fourtanier (L'Oreal Recherche, France)*

The effects of acute or repeated sub-erythemal solar UVR exposure on human skin have been poorly investigated. Such exposure almost certainly has important long-term consequences that include skin ageing and skin cancer. The correct use of a sunscreen should result in a sub-erythemal exposure, depending of course of the sun protection factor (SPF) and absorption spectrum, the level of UVR exposure and the sensitivity of the user. Sunscreens are often applied at an application density (<2mg/cm2) that will not achieve optimal SPF, even if erythema is prevented. However, the presence or absence of erythema is an inadequate indicator of cutaneous damage, e.g. immunosuppression can occur with substantially less than 1 minimal erythema dose (MED). This presentation will review the published data on sub-erythemal exposure and summarize recent unpublished results, using a wide range of endpoints, obtained in our laboratories. The effects of UVA (320-400 nm), UVB (290-320 nm) and total solar UVR (290-400 nm) will be presented and compared. We will demonstrate that sunburn avoidance does not prevent the majority of cutaneous biological damage. This review will also help to determine the level of photoprotection that is needed in sunscreens and daily used products

087 NEED FOR A BROAD SPECTRUM SUNSCREEN IN THE PROTECTION OF UVA INDUCED PHOTODERMATOSIS

A. Rougier (La Roche-Posay Pharmaceutical Laboratories, France)*

Since Ultraviolet B have 1000 times the energy of ultraviolet A they have long been considered to be responsible for the development of most human photodermatoses . However, the proportion of UVA rays emitted by the sun and reaching the surface of the earth is twenty times that of UVB rays. Moreover, unlike UVB rays, UVA rays are not attenuated by ozone layer surronding our planet. They pass through clouds and glass, ans are emitted at a constant rate throughout the day from sunrise to sunset . Finally, while 90% of UVB radiation is blocked by the stratum corneum, over 50% of the UVA received is capable of penetrating deep into the skin as far as the papillary and reticular dermis. It has long been thought that the majority of human photolesions were due to UVB rays. It is today well established that UVA plays a determinant role in different photo-pathologies such as photoaging, , DNA damages and skin cancers including melanoma, response of the cutaneous immune system, different photodermatoses, exogeneous photosensitizations etc&.. Concerning this last point, some examples of the use of broadspectrum sunscreens equally efficient in the UVB and UVA wavelenghts in the prevention of different photodermatoses will be presented.

088 SURGICAL METASTASECTOMY: THE FIRST OPTION FOR STAGE IV MELANOMA PATIENTS

D. Ollila (University of North Carolina, USA)*

Recently, patients with stage IV metastatic melanoma have been treated with a variety of toxic systemic agents including dacarbazine, interleukin-2 and combination biochemotherapy. Unfortunately, the five-year disease-free survival remains ~5% and consideration of surgical resection occurs late, if at all. Complete surgical metastasectomy offers a patient with limited stage IV disease a 15-41% five-year survival depending on the site(s) of disease. Patients with completely resected skin and soft tissue metastases have a 15-33% five-year survival, pulmonary 19-27%, gastrointestinal 28-41%, and hepatic 20-29%. For patients with incomplete surgical metastasectomies or palliative procedures, the median survival, ~6 months, mirrors the median survival for patients managed without surgical intervention. The median survival for patients with cerebral metastases remains poor, <6 months, even if complete metastasectomy is performed. In conclusion, until more effective, lower toxicity agents systemic therapies are available, complete surgical metastasectomy should be considered the first option in patients with limited stage IV disease. A partial metastasectomy should only be considered for palliative reasons.

089 ISOLATED REGIONAL CHEMOTHERAPY FOR METASTATIC MELANOMA

JHW. de Wilt (Erasmus MC- Daniel den Hoed, The Netherlands)*

For melanoma patients with bulky recurrent disease or multiple in-transit metastases, not easily treated by simple local therapies, regional chemotherapy with vascular isolation is generally accepted as the treatment of choice, and sometimes provides the only alternative to amputation. Isolated limb perfusion (ILP) is the most commonly employed regional chemotherapy modality, but an isolated limb infusion (ILI) technique developed at the Sydney Melanoma Unit produces similar results. Numerous single center and multicenter ILP studies have been reported in the literature. The median complete response rate for all reported ILP and ILI studies with melphalan is approximately 50%, and the overall response (OR) rate approximately 85%. Addition of TNF is generally safe and seems to improve the CR rate after ILP to 75% and the OR rate to 95%. Five-years overall survival after TNF-based ILP is approximately 30%. Both response rate and survival are influenced by stage of disease. Even in stage IV melanoma patients excellent responses and thus limb salvage, have been reported. To establish the value and effective dose of TNF in ILP for melanoma patients randomized studies are needed. New agents or other combinations of drugs are neede to achieve limb salvage for patients who fail the current treatment modalities.

090 ROLE OF RADIATION THERAPY IN RECURRENT AND METASTATIC MELANOMA

G. Stevens (Melanoma Foundation of NZ, NZ)*

Many patients with recurrent and metastatic melanoma may have improved survival or reduction of disabling symptoms through the appropriate use of radiation treatment. The previous nihilism regarding the value of radiation in melanoma is being reversed by timely referral and the innovative use of different modalities and scheduling. These issues will be explored in relation to different clinical scenarios.

091 OBSTRUCTING METASTASES OF THE ILEUM WITH RETROPERITONEAL METASTASES

J. Thompson (Sydney Melanoma Unit, Australia), R. Martin (Sydney Melanoma Unit, Australia)*

The treatment of obstructing small bowel (SB) melanoma metastases is most unlikely to be curative when retroperitoneal metastases are also present. Even when there is known metastatic disease at other systemic sites, however, active intervention can not only provide immediate relief of very distressing symptoms but will often postpone death by many months or even years. Although heroic resections with wide clearance margins are clearly inappropriate, easily resectable mesenteric and retroperitoneal disease is best dealt with at the same time as the SB obstruction is relieved, to prevent symptoms from developing in the future. Relief of the SB obstruction will usually involve limited SB resection with end-to-end anastomosis, but occasionally a side-to-side anastomosis is required, leaving in place a tumour mass that cannot be removed with safety (e.g. because it encases the superior mesenteric vessels). The situation most commonly encountered is one in which there is obstruction due to intussusception of a mucosally-based SB tumour, with a mass in the mesentery representing metastatic disease in mesenteric nodes. With care, such mesenteric masses can usually be enucleated without damage to adjacent mesenteric vessels, and to do so is generally worthwhile. SB metastases are often multiple, so it is important to check the rest of the SB for the presence of other deposits that can be resected without undue difficulty, to avoid further episodes of obstruction in the short to medium term. Symptoms can be palliated in 80-97% of patients (with reported mortality rates of 1.4 - 15% and morbidity rates of 8.8 - 20%). The median survival reported for patients undergoing complete resection of gastrointestinal tract metastases is 10 - 49 months, with 5 year survival 28 - 41% The median survival reported after palliative resections is 1 - 5.4 months.

093 DESMOPLASTIC MELANOMAS OF THE HEAD AND NECK

S. Fisher (Duke University, U.S.A.)*

Desmoplastic melanoma is a rare variant of malignant melanoma occurring in less than one percent of all melanomas. Seventy-five percent of these occur in the head and neck. Difficulties in diagnoses have improved with immunohistiochemical staining; however, many have and will remain to be misdiagnosed in the future. The propensity for neurtropism has lead to a high rate of recurrence ranging from 25-85%. Treatment remains wide surgical excision; however, given certain anatomic locations in the head and neck, adequate surgical margins are sometimes difficult to obtain. Difficult case presentations will be presented and discussed regarding the diagnosis, surgical treatment, reconstructive techniques, and failures with emphasis on surgical resection. Adjunctive therapies will also be discussed including the role of chemotherapy, immunotherapy, and radiation therapy.

095 MUCOSAL MELANOMA

R. Rapini (Univ TX MD Anderson Cancer Center, USA)*

Objective: Review the problem of mucosal melanomas. Materials and methods: The speaker published a review of 177 cases of melanoma of the mouth based upon a retrospective literature review. The presentation will mainly focus upon the difficulty of lesions of the mouth and further discuss mucosal lesions of other locations. Results: Melanomas of the mouth are most common on the upper jaw of patients older than 50 years. Melanomas of mucosal sites often have a poor prognosis because there is a delay in diagnosis, resulting in deeper lesions. Conclusion: Histological subclassification is problematic. Treatment of mucosal melanoma is mostly surgical, but radiation therapy sometimes produces a good response. Early detection is important. Reference: Rapini RP: Seminars in Cutan Med Surg 1997;16:320-322.

NOTES:

096 TREATMENT OF OCULAR MELANOMA

A. Hauschild (Dep. of Dermatology, University of Kiel, Germany)*

Malignant melanoma is the most common primary intraocular malignancy and a significant cause of mortality. The frequency is approximately 10% that of cutaneous melanomas. Tumor thickness is the most important prognostic factor. 20 to 25% of ocular melanoma patients will die due to metastatic spread. Thirty years ago, eyes with suspected ocular melanoma were enucleated routinely. Later on, large prospective studies evaluated that radiation, given as an alternative to enucleation, has similar survival rates. Therefore, in most of the cases a brachytherapy with Ruthenium applicators is used today. Once ocular melanoma has metastasized, the liver is affected in approximately 90% of all cases. Despite therapy, the median survival of patients is only 6 months. Responses to systemic chemotherapy are very rare. However, some small phase II trials have demonstrated promising response rates with Treosulfan und Gemcitabine when using an ATP-based in-vitro tumor chemosensitivity assay. Despite encouraging results with combined Interleukin 2, interferon a2b and histamindehydrochloride in a small patient series, immunotherapy is obviously not effective in stage IV ocular melanoma patients. Therefore, due to the unique metastatic pattern to the liver an intraarterial liver perfusion with or without chemoembolization is considered as a standard of care. Response rates up to 40% have been reported when using Cisplatine-based regimens or Fotemustine in the liver. Recently, a German multicenter evaluation of intrahepatic Fotemustine application elaborated a response rate of 18.2% and a high number of stabilized patients (48.5%) in a series of 33 uveal melanoma patients. However, until now it is unclear whether these remissions are durable enough to affect the overall survival time significantly. In conclusion, in patients with metastatic ocular melanoma confined to the liver clinical trials are highly warranted. Outside of clinical trials liver perfusion with cytotoxics as Fotemustine seems to be the most effective treatment available yet.

097 GENITAL MALIGNANT MELANOMA

B. Ragnarsson-Olding (Dept. of Oncology, (Radiumhemmet), Karolinska University Hospital, Stockholm, Sweden)*

Primary malignant melanomas of the mucous membranes have attracted growing interest among researchers. The lingering, poor prognosis of patients afflicted with those melanomas, despite numerous therapeutical (mainly surgical) strategies, as well as evidence that factors other than UV radiation cause melanoma underlie this new attention. The latter issue has gained importance because vulvar melanomas in Sweden, although rare in absolute number, have a density (number of tumors per square unit) higher than the average density of cutaneous melanomas. In this paper, genital melanomas of the vulva, vagina, and penis are reviewed in terms of epidemiological, clinical, histopathological, molecular genetic, therapeutic, and prognostic parameters. The main focus is melanomas of the vulva, which are the most frequently found genital melanomas in Sweden and in most other countries. Records documenting the spectrum of patients with melanomas in Sweden during the 40 years from 1960 to 1999 cited 37099 cutaneous melanomas (females only, 19176) versus 3062 ocular, 358 vulvar, 253 ano-rectal, 152 sino-nasal, 61 oral, 56 vaginal, and 33 penile melanomas. Unlike the increase in cutaneous melanomas, the incidence of vulvar melanomas was stable or decreased. Vulvar and cutaneous melanomas differ in several striking ways, e.g., tumor pigmentation, histogenetic phenotypes, average tumor thickness, Nras and BRAF (but not TP53) mutations. We have also found significant biological differences between melanomas in the two compartments of the vulva - the hairy and the glabrous (i.e., mucosal) skin, even though both occupy the same sun-shielded area. This diversity, along with genetical investigations by others support the hypothesis that the genesis of melanomas may be dependent on anatomical location and/or local tissue factors unrelated to sun-bathing habits and genetical/familial factors, the traditionally accepted causes of cutaneous melanomas. In conclusion, genital melanomas may be useful in modeling the genesis of non-UV light- associated melanomas.

098 ANORECTAL MELANOMA - EPIDEMIOLOGY, PROGNOSTIC FEATURES, STAGING, AND TREATMENT

C. Slingluff (University of Virginia, USA)*

Primary anorectal melanoma accounts for approximately 1% of primary melanomas and typically arises near the dentate line. These mucosal melanomas, unlike cutaneous and ocular melanomas, have a similar incidence in Caucasians and African-Americans. The most common presentation is rectal bleeding. It is often nonpigmented and often is diagnosed when thick. Prognosis is dismal. It is usually lethal. Regional metastases to inguinal and pelvic nodes are common. Even with abdomino-perineal resection, distant metastases are common and prevent control of disease. Medical therapy for this disease has not been effective. Surgical therapy has a role in removal of the primary lesion, staging of regional metastases, in palliation of rectal bleeding. Wide local excision is often feasible. Abdomino-perineal resection may also be performed for these cancers. The optimal surgical therapy is debated. APR is not associated with better survival than WLE, but may decrease pelvic recurrences. WLE and APR each have a role in this disease, and each should be discussed with patients preoperatively. In some cases of locally advanced tumors, APR may be the most appropriate choice. In the case of smaller lesions, WLE is medically acceptable. Radiation therapy has not been formally evaluated in the management of this clinical entity, but may have a role. Since adults over age 50 commonly now undergo colonoscopic surveillance, it may be possible to increase early diagnosis. Because patients with this cancer have a dismal prognosis, they should be included in clinical trials of immunotherapy and of new targeted molecular therapies.

099 CONGENITAL MELANOCYTIC NEVI UPDATE

A. Marghoob (Memorial Sloan-Kettering Cancer Center, USA)*

Congenital melanocytic nevi (CMN) are tissue malformations of the neuroectoderm, which are comprised of melanocytes and occasionally neural elements. Analysis of clinical data in registries enrolling patients with CMN has improved our understanding of risk factors for melanoma and neurocutaneous melanocytosis (NCM). Furthermore, research into the etiology of CMN and NCM has allowed us to recognize the risk of melanoma, risk of other cancers such as rhabdomyosarcoma, and NCM from a molecular perspective. These insights may open the door to more effective screening methods (i.e., physical examination including inspection and palpation, dermoscopic evaluation, neurological examination, etc.), follow-up testing (i.e., MRI, PET, etc.), potential new methods for preventing cancer and NCM progression (i.e., chemoprevention), and potential new methods for treating patients with CMN that develop melanoma or NCM (i.e., temozolomide, growth factor inhibitors, etc.). Although the future looks promising, currently patients presenting with CMN need individualized treatment based upon nevus size, thickness, location, known risk factors for developing melanoma and psychological characteristics of the patient and family. It is important to remember that the only absolute indication for surgery is the presence of malignancy. All other indications for therapy (i.e., prophylactic therapy or treatment for improved cosmesis) are considered relative. Risks and benefits of no treatment versus treatment should always be discussed including surgical options, such as excision, chemical peels, dermabrasion, curettage and laser therapy. The main focus of treatment, in all cases, is to address the concern for developing malignancy while at the same time optimizing the aesthetic and functional outcomes.

100 ATYPICAL MELANOCYTIC NEVI - EPIDEMIOLOGY AND CLINICAL SIGNIFICANCE

C. Garbe (Division of Dermatologic Oncology, Department of Dermatology, University of Tuebingen, Germany)*

Atypical melanocytic nevi are defined by clinical criteria including the size of the lesion, the border, variations in the color and the presence of a macular component. The criteria have been used rather uniformly by different investigators. On the contrary, the term dysplastic nevus defined by histopathology is much more controversial because no uniform criteria have been established. There are only few data on the epidemiology and the melanoma risk of histopathologically defined dysplastic nevi. Atypical melanocytic nevi occur in 2 - 10% of adult persons in white populations. As a rule, they are associated with the presence of high numbers of melanocytic nevi on the entire integument. It is well established that they are an independent marker of the melanoma risk. The most important risk factor for melanoma development is the total number of melanocytic nevi distinguishing between a risk rate of 1 to 10. The additional presence of atypical melanocytic nevi discriminates a risk rate between 1 to 6 - 8. Both risk factors are related to each other in multiplicative fashion. Thus, atypical melanocytic nevi are risk markers for melanoma development, but they are only very seldom precursors of melanoma. They require surveillance, but not prophylactic excision. In conclusion, the clinical significance of atypical melanocytic nevi is the recognition of persons with high risk for melanoma development. The simultaneous presence of 5 and more atypical melanocytic nevi defines the atypical nevus syndrome. These persons require a regular surveillance.

101 CLINICAL MANAGEMENT OF DYSPLASTIC NEVI

A. Halpern (Memorial Sloan Kettering Cancer Center, USA)*

In this session we will discuss the identification of individuals affected with dysplastic nevi and aspects of clinical management including initial clinical assessment, routine professional follow up, instruction in patient self-examination, and use of imaging modalities as aids to melanoma detection. Dysplastic nevi are important markers of melanoma risk. The degree of risk varies greatly depending on nevus phenotype and family/personal history of melanoma. Accordingly, emphasis will be placed on the importance of a validated family history of melanoma and clinical assessment of nevus phenotype to determine melanoma risk and customize patient care. The contribution of histology to risk assessment and patient management will be discussed. Strategies for counseling patients in primary prevention as well as the routine performance of self-examination will be covered. Growing evidence for the utility of dermoscopy for the assessment of individual lesions and whole body photography for the recognition of new and changed lesions during routine follow up will be addressed with specific attention to practical approaches to implementing these modalities in clinical practice. Factors that should influence the frequency of professional examinations will be discussed along with the importance of screening family members and the current very limited role of genetic testing.

NOTES:

102 ULTRAVIOLET B RADIATION INITIATES MELANOMA WHEREAS ULTRAVIOLET A DOES NOT.

E. De Fabo (Environmental & Occupational Health, SPHHS, The George Washington University, Washington, D.C., USA)*

WHO estimates 132 000 cases of malignant melanoma (MM) annually. In Norway and Sweden, the annual incidence rate has tripled in the past 45 years. In the USA, the rate has doubled in the past 25 years. Sunlight is strongly implicated in the etiology of MM and the UV portion of sunlight is considered responsible. Data are, however, conflicting on the roles of ultraviolet B [UVB; 280–320 nanometers (nm)] and ultraviolet A (UVA; 320–400 nm), which differ in their ability to initiate DNA damage, cell signaling pathways and immune alterations. We have used specialized sources, emitting isolated or combined UVB or UVA wavebands or solar simulating radiation, together with our hepatocyte growth factor/scatter factor-transgenic mouse model of UV-induced melanoma that recapitulates human disease. Results: Only UVB-containing sources initiated melanoma: the isolated UVB waveband (>96% 280–320 nm), the unfiltered F40 sunlamp (250–800 nm) and the solar simulator (290–800 nm). Kaplan-Meier survival indicated the isolated UVB was more effective ($P < 0.02$) than the F40 sunlamp or the solar simulator ($P = 0.38$). The latter two sources showed similar effectiveness. In contrast, transgenic mice irradiated with the isolated UVA waveband (>99.9% 320–400 nm, 150 kJ/m2), or an F40 sunlamp filtered to remove > 96% of the UVB responded like unirradiated animals. Conclusion: within the constraints of this animal model, UVB is responsible for the induction of mammalian MM whereas UVA is ineffective even at doses physiologically relevant. These findings may have key implications with respect to risk assessment and development of protection strategies. In this light continuing loss of stratospheric ozone with associated increased UVB, and the uncertainty in its recovery, argues for prompt attention to a potentially worsening environmental health problem. For at-risk melanoma-sensitive populations, minimal exposure to UVB radiation is strongly recommended.

103 UV LIGHT FROM 290 TO 325 NM, BUT NOT BROAD-BAND UVA OR VISIBLE LIGHT, AUGMENTS THE FORMATION OF MELANOCYTIC NEVI IN A GUINEA-PIG MODEL FOR HUMAN NEVI.

S. Menzies (University of Sydney and Sydney Melanoma Diagnostic Centre, Australia), G. Greenoak (University of Sydney, Australia), C. Abeywardana (University of Sydney, Australia), M. O'Neill (University of Sydney, Australia), K. Crotty (Sydney Melanoma Diagnostic Centre, Australia)*

We have previously described a guinea-pig model where pigmented nevi similar to human nevi can be produced by application of low-dose topical 7,12-dimethylbenzanthracene (DMBA) followed by solar-simulated light (Cancer Res. 1998;58(23):5361-6). In these experiments five groups of guinea-pigs were used to test the effect of various spectral bands of solar-simulated light on low-dose DMBA-induced melanocytic nevi. Animals were irradiated with either UVB to near UVA2 (290-325 nm), UVA, visible light, full solar spectrum or no irradiation three times per wk for 12 mo to determine the broad-band effect of nevi-inducing irradiation. There was a significant increase in nevi/animal in the UVB-treated group (mean 1.53) compared with all groups (versus UVA 0.3, $p<0.001$; versus visible light 0.24, $p<0.001$; versus full spectrum (UVB+UVA+visible) 0.68, $p=0.02$; versus control (nil irradiation) 0.37, $p=0.01$). Hence we present a report of the active waveband of melanocytic nevi induction; where UVB to near UVA2 is the likely responsible waveband. J Invest Dermatol. 2004;123:354-60

104 DATA SUPPORTING AN IMPORTANT ROLE FOR WAVELENGTHS GREATER THAN UVB IN INDUCING MELANOMAS

R. Setlow (Brookhaven National Laboratory, USA)*

DNA is one of the more UVB-absorbing cellular molecules. Its absorbance falls to negligible values at ~365nm. Photoproducts resulting from direct absorption, or from photosensitivation by other absorbers, result in mutations and may initiate cancers such as squamous cell cancer (SCC) or cutaneous malignant melanoma (CMM). The data implicating specific wavelength regions are in epidemiology, animal models and photobiology of cells. Epidemiology is the most important, not only because it applies directly to humans, but because there are extensive data, from many countries, on the incidence of SCC and CMM at different average sunlight levels of UVB, UVA and longer wavelengths. Incidence decreases with increasing latitudes and decreases are greater for UVB than for UVA. The ratio SCC/CMM decreases with latitude. Since SCC is associated with the formation of dipyrimidine photoproducts, formed by radiation (UVB) absorbed by DNA, these data imply that CMM is associated with wavelengths greater than UVB, i.e. with UVA and visible. Exposed "albino" blacks suffer from actinic damages and high levels of SCC, but negligible levels of CMM, indicating that melanin acts a photosensitizer for DNA damage and CMM induction. Melanin absorbs UVA and visible light and its photosensitizing action is indicated by the greater ratio of SCC/CMM among whites compared to blacks in the USA. Both types of cancer arise from photochemical damage to DNA as indicated by the ~1000-fold higher levels for both in individuals defective in nucleotide excision repair. Animal models have given conflicting results. The action spectrum for a fish model implies that ~90% of CMM could arise from UVA+visible. UVA exposure of an opossum model induces melanocytic hyperplasia. However, in a mouse model, melanomas are induced by UVB, but not by UVA. I interpret the differences among the models as indicating different levels of photosensitization, perhaps different cellular locations, of melanin. Brookhaven National Laboratory is operated by Brookhaven Science Associates under contract with the US-DOE.

105 HOW CAN WE EXAMINE WHETHER UVA CAUSES MELANOMA IN HUMANS?

A. Kricker (School of Public Health, University of Sydney, Australia)*

There are only limited approaches available to study the effect of UVA on risk of melanoma. One approach is to examine artificial sources of UVA used by people to gain a tan, that is sunbeds and solaria, as these have changed over time from predominantly UVB to UVA emission. We have systematically reviewed the research literature on use of sunbeds and solaria to determine whether it is possible to demonstrate that risk, if any, has changed with the change in lamps from UVB to UVA. The results of this review will be presented and gaps in research identified whereby further research may help clarify the issue. In the absence of detailed information, we will speculate that past and current behaviour patterns around tanning will continue into the future and so studies up to some point in time will be informative.

106 SUSCEPTIBILITY GENES FOR MELANOMA IN RELATION TO OTHER CANCER RISKS AND GENETIC TESTING

N. Gruis (LUMC, Netherlands), F. . de Snoo (LUMC, Netherlands), S. Riedijk (LUMC, Netherlands), T. Bishop (ICRF, United Kingdom), J. ter Huurne (LUMC, Netherlands), I. van Leeuwen (NFDHT, Netherlands), C. van der Drift (NFDHT, Netherlands), A. Tibben (LUMC, Netherlands), R. Willemze (LUMC, Netherlands), W. Bergman (LUMC, Netherlands)*

The CDKN2A gene, a tumour suppressor gene that restrains the cell cycle, is the most important melanoma susceptibility gene identified so far. By using different first exons, CDKN2A encodes for 2 proteins: p16 and p14ARF. In the Netherlands we have a unique founder population of melanoma families, displaying the same 19 basepair inactivating mutation in CDKN2A (p16-Leiden). This unique material both allows us to study genetic and clinical aspects of familial melanoma. Our clinical studies have determined the risk estimates for cancers other than melanoma in p16-Leiden carriers prospectively. Especially for pancreatic cancer, we observed an extremely increased relative risk of 37 for pancreatic cancer in CDKN2A mutation carriers. Accordingly, based on well-defined risk characteristics we have started offering predictive genetic testing in the p16-Leiden families. Given the disease characteristics of p16-Leiden melanoma families, decision-making is thought to be difficult and therefore we aimed to evaluate uptake, motivation and psychological implications of genetic testing. Variables significantly predictive for counselling uptake were being a parent, higher prior risk and older age. Age was the only significant predictor for test acceptation. Furthermore, counselees reported lower distress levels after the first counselling session than those reported in other oncogenetic testing settings, despite being informed about pancreatic cancer. We report a relatively high uptake rate for p16-Leiden testing and no clinically worrisome levels of distress after the first counselling session, concluding that there are no adverse effects for offering genetic testing for p16-Leiden.

107 HOW SUNLIGHT, MOLES AND GENES INTERACT IN MELANOMA SUSCEPTIBILITY?

F. Demenais (INSERM-Université d'Evry, France)*

The etiology of cutaneous malignant melanoma is heterogeneous and complex, involving both genetic and environmental factors. We have examined the joint effects of genes, pigmentary traits (skin, eye and hair color), nevus phenotypes (high number of nevi and presence of dysplastic nevi), sun exposure and skin reactions to sunlight on melanoma risk in two French family samples: 295 families unselected by family history and 53 melanoma-prone pedigrees (20 of which having co-segregating mutations in CDKN2A gene). We modeled melanoma risk using a logistic regressive model incorporating the effect of a melanoma-predisposing gene, familial dependence and covariates. Together a major gene (identified as CDKN2A in melanoma-prone families), number of nevi and /or dysplastic nevi and sun-related covariates were found to influence significantly melanoma risk in the two family samples. The potential modifying effect of MC1R gene (which plays a key role in human pigmentation) on CDKN2A penetrance was then investigated in the 20 melanoma-prone families. The factors increasing significantly CDKN2A penetrance were dysplastic nevi (OR = 2.94) and sunburn (OR=3.90) in addition to three MC1R variants, R160W (OR=3.39), D294H (OR=4.22) and R163Q (OR=5.53). In CDKN2A mutation carriers, the cumulative risk of melanoma was 0.58 by age 80 years and was higher than 0.92 when adding either one of the modifying factors. This study emphasizes the complexity of melanoma pathogenesis which is likely to involve several pathways. Examination of the joint effects of MC1R variants and other risk factors on CDKN2A penetrance from families all around the world, as can be made possible by the Melanoma Genetics Consortium (Genomel), would be of interest. Such studies may have important consequences to improve the prediction of melanoma risk in different familial settings.

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108 PROS AND CONS OF GENETIC TESTING

S. Leachman (University of Utah, USA), E. Dola (University of Utah, USA), M. Eliason (University of Utah, USA), M. Hart (University of Utah, USA), S. Florell (University of Utah, USA)*

Genetic testing has tremendous potential to improve patient care when combined with prevention and early detection in carefully selected, high-risk patients. Appropriate transition of genetic testing from the research arena into clinical care is challenging, and no national or international governing body is responsible for making this decision. An international consortium of leaders in the field of melanoma genetics (Genomel) has published recommendations advising that, at the present time, genetic testing should only be conducted as part of a research protocol. ASCO has also suggested criteria by which genetic testing may be evaluated with regard to clinical usefulness. Despite these recommendations, the availability and marketing of the test has resulted in migration into the clinical setting. As a result, practitioners are ultimately responsible for assessing the clinical utility and value of p16 genetic testing for their patients. A data-based evaluation of the potential risks and benefits of clinical melanoma genetic testing will provide the best foundation for appropriate implementation of the test, now or later. We suggest that genetic testing for p16 mutations is not only valuable as part of a research protocol but may also be of value in a clinical setting when attention is paid to patient selection, patient and family education and counseling needs, valid test interpretation, and alteration of medical management in appropriate individuals. The challenge remains to proceed with caution without losing site of the enormous potential this test may have in the prevention and early detection of an otherwise deadly disease.

109 GENETIC COUNSELING AND TESTING IN FAMILIAL MELANOMA PATIENTS: THE CASE OF LIGURIA

G. Bianchi-Scarra' (Dept. of Oncology Biology and Genetics, University of Genova, Italy)*

This study attempted to establish the clinical utility of follow-up for melanoma patients and families who undergo genetic counseling and testing for mutations in CDKN2A at our Genetics Service. The International Melanoma Genetics Consortium recommends that susceptibility testing only be performed in research settings, but recognizes that in areas with low incidence, prevalent founder mutations and few or no phenocopies, testing may improve prevention and surveillance. One such area is Liguria and its surrounding provinces, where the age-standardized incidence rate of melanoma per 100,000 people is 7.2 for males, 6.1 for females, the known CDKN2A G101W and the newly identified E27X founder mutations are prevalent, CDKN2A mutations are found in 38% of familial cases, and the two suspected phenocopies previously found were not confirmed. Seventy-three self-referred familial melanoma patients (with at least 2 confirmed cases in the family) underwent counseling and testing at our Service in 1994-2003, and 30 were found to be CDKN2A-positive. Nineteen clinically sporadic CDKN2A-positive patients, recruited through a hospital-based research project, underwent the same counseling since their relatives agreed to participate as well. The observation period of all the families (n = 92) lasted 1-10 years, until 2004. In general, fewer relatives of mutation-negative than of mutation-positive probands responded and came to our Service. Moreover, fewer families of mutation-negative cases were willing or able to provide accurate, up-to-date clinical information. Twelve lesions (11/12 thinner than 0.75mm) were diagnosed during the observation period, 9 in mutation-positive and 3 in mutation-negative families. In particular, 3 were diagnosed in the families of the 19 CDKN2A-positive clinically sporadic cases. Comparison of the thickness of all the melanomas diagnosed before and after genetic counseling was 1.22 vs 0.33 mm. These results confirm that genetic counseling and testing in areas with founder mutations may improve early diagnosis through increased awareness.

110 AN EUROPEAN APPROACH TO SENTINEL LYMPH NODE EVALUATION

M. Cook (University of Surrey, UK)*

Although sentinel lymph node (SLN) status is widely regarded as an important prognostic criterion there remain several pathological points for further evaluation. These include the value of extended sampling, the possibility of refining prognostic accuracy by additional assessments of the SLN metastasis, the clinical significance of extra metastases discovered by extended sampling or molecular techniques and the role of molecular biology in evaluation of SLN. Our view is that 1. Extended sampling of the SLN does increase the detection rate of the metastases up to 34% from less than 20% 1. 2. The extra metastases discovered are not all sub-capsular but are usually small. 3. Metastases confined to a subcapsular site are not associated with non SLN involvement2. 4. The prognostic value of SLN assessment can also be improved by estimating the relative tumour volume3. the maximum depth of metastases from the capsule4, or a combination of other pathological features5. 5. The evidence that extra metastases found by extended evaluation of SLN may have limited clinical significance6. is counterbalanced by the finding by some that histologically negative SLN can be upstaged by multiple marker RT-PCR 7.8. In Guildford we are working on a multi marker RT-PCR based test as an adjunct to our intensive sentinel lymph node assessment regimen. We expect that this combination of studies will resolve many of the problems related to SLN assessment and act as a basis for further advances Ref. 1.Cook et al J Pathol. 200.314-9 2003 2.Dewar et al J Clin Oncol 22 3345-9 2004 3.Cochran et al Mod Pathol 17 747-55 2004 4.Starz et al Cancer 91 2110-20 2001 5.Scolyer et al Am J Clin Pathol 122 532 2004 6 Scolyer et al Cancer 101 2141-2 2004 7.Tukeuchi et al J Clin Oncol 22 2671-80 2004 8.Kammula et al J Clin Oncol 22 3989-96 2004

112 GENE EXPRESSION PROFILING OF PRIMARY CUTANEOUS MELANOMA

J.J. van den Oord (Lab. Morphology and Molecular Pathology, Dept. Pathology, University Hospitals, Katholieke Universiteit Leuven, Belgium)*

Gene expression data on primary cutaneous melanomas (PCM) with long-term clinical follow-up are still lacking as melanoma-specimens are usually entirely fixed for routine microscopy. Since the early 80's, we have frozen representative parts of pigment-cell lesions and have now used a series of 83 PCM's with vertical growth phase or >1mm. thickness to correlate gene expression profiles with clinical follow-up and histology. For hybridization, Agilent's two-channel oligonucleotide micro-arrays carrying 41.675 probes, were used. Unsupervised analysis revealed 2 clusters of patients that differed significantly in outcome. Supervised analysis and multiple random validation strategy identified a 'refined' signature of 60 genes with high probability to predict 4-yrs. distant metastasis free survival (DMFS). This signature was validated on an independent population of 17 patients, and by immunohistochemistry on tissue micro-arrays (TMA's). Few genes were overexpressed in non-metastasizing (M-) patients, e.g. PTGDS and CST5. Most genes were underexpressed in M- patients and were involved in chromosomal positioning, stabilization and replication, DNA-synthesis, RNA-processing, cell-cycle and transport. On TMA's, the expression of 8 gene-products was significantly associated with DMFS or overall survival (OS), incl. various mcm-proteins, kpna2 and kallikrein 7. Additional supervised analyses revealed gene profiles, associated with ulceration, high mitotic activity, brisk TILS, spindle cell morphology and thickness. Clusterization of the latter genes into different functional groups identified distinctive trends in gene expression throughout tumor progression in PCM. Comparing gene expression between nevi and PCM revealed 590 significantly differentially expressed genes, whereas PCM did not differ substantially from their metastases. In conclusion, this micro-array study allowed to identify genes with predictive power in PCM, resulting in new immunohistochemical prognostic markers. Use of these data will contribute to prognostication. As several of the DMFS-related genes are targets of experimental or established therapies, our data may have impact on improved survival in advanced stages of tumor progression.

113 ANGIOTROPIC MELANOMA AND EXTRAVASCULAR MIGRATORY METASTASIS

R. Barnhill (University of Miami, USA)*

Our studies using electron microscopy and immunohistochemistry have demonstrated, in both human melanoma and experimental melanoma models, a tumor-endothelial cell interaction which we have termed the angio-tumoral complex. Angiotropism in human melanoma, the histopathological counterpart of the angio-tumoral complex, suggests that melanoma cells may migrate along the external surfaces of vessels, a mechanism termed "extravascular migratory metastasis" (EVMM), as distinct from intravascular dissemination. In addition, we have shown that angiotropism in human melanoma could be a prognostic factor predicting risk for metastasis. In-transit melanoma metastases are defined as metastases localized between the primary tumor and the regional lymph nodes. Such "in-transit" metastases are compatible with a step-by-step tumor cell dissemination, i.e., EVMM. In the present study, we have studied over time the growth and dissemination of melanoma cells stably expressing Green Fluorescent Protein (GFP) in a new preparation of the chicken chorioallantoic membrane (CAM). The results have shown a clear progression over time of angiotropic melanoma cells spreading several centimeters along the abluminal surfaces of vessels on the CAM, where they have occupied a pericytic location. Using double immunostaining, angiotropism of melanoma cells on the CAM has been compared with the histopathology of human cutaneous in-transit melanoma metastases. The results have demonstrated morphological similarities between these two tumor systems, i.e., the presence of a pericytic angiotropism of tumor cells at the invasive front of the tumor or at some distance from the tumor mass. These data suggest that this CAM assay is a relevant model for studying tumor cell dissemination, and support the concept of EVMM as a mechanism by which some melanoma cells spread to nearby and even distant organs.

114 SUN PROTECTION AND EARLY DETECTION: DO WE HAVE THE BALANCE RIGHT?

MA . Weinstock (V A Medical Center, R I Hosp and Brown University, USA)*

A common formulation of the public health approach to melanoma has been that the first priority is to prevent it in the first place, but if that fails, it should be detected early so the damage from that cancer can be minimized. Hence, although early detection issues have not been ignored in the public sphere, the focus of most groups has been protection from ultraviolet radiation for primary prevention, whether the campaigns have been based on Slip!Slop!Slap!, Avoid.Cover.Screen., or similar slogans. This presentation argues that we do not have the appropriate balance. It has been 50 years since the ecologic evidence has pointed to the sun as a cause of melanoma, and more than 20 years since analytic epidemiologic evidence has strongly supported that conclusion. However, our experience in the intervening years is that despite the campaigns we have failed in our efforts to substantially reduce melanoma incidence, and we have also uncovered a variety of potential adverse consequences of the efforts to reduce exposure to ultraviolet radiation. On the other hand, our efforts at early detection have achieved considerable success. Furthermore, there is tremendous potential for further improvement in these efforts, and these efforts result in mortality reductions much more quickly than a possible successful sun protection campaign. We need to recalibrate the balance, with a heavier weight placed on early detection.

115 IS THERE A SAFE LEVEL OF SUN EXPOSURE?

B. Armstrong (The University of Sydney, Australia)*

A safe level of sun exposure implies either that sun exposure has no adverse effects below this level or that beneficial effects of sun exposure are sufficient up to it to counterbalance the adverse effects. While the possibility of a threshold exposure below which sun exposure does not cause melanoma and, perhaps, other skin cancers cannot be rejected it seems implausible, at least in people with fair skins. Beneficial effects of sun exposure through production of provitamin D in the skin have been known for many years but it has generally been assumed that even in strict sun protection regimens residual cutaneous sun exposure can maintain sufficiency in vitamin D in populations in which skin cancer is at all common. There is evidence now that this is not so, even in a high exposure population such as in Australia. There is growing evidence also of beneficial effects of sun exposure that may not be due to vitamin Ds effects on calcium metabolism or, perhaps, to vitamin D at all. They include possibilities of protection against multiple sclerosis, prostate cancer and non-Hodgkin lymphoma and improvement in survival from prostate cancer and melanoma. Thus it is increasingly likely that there is a safe level of sun exposure, that is, a non-zero level below which benefits of sun exposure outweigh its hazards, and, more importantly perhaps, a non-zero optimal level at which the net benefits of sun exposure are both positive and greatest. These concepts present challenges in public communication about sun exposure and sun protection. Recommending vitamin D supplementation might be an attractive but perhaps simplistic approach because there is no certainty that all known, much less yet to be known, beneficial effects of sun exposure are mediated through vitamin D production. Thus we cannot easily escape engaging the challenges.

116 DISTINCT SETS OF GENETIC ALTERATIONS IN MELANOMA

B. Bastian (University of California, San Francisco, USA)*

Background: UV exposure is a major etiologic factor for melanoma. However, the relationship to sun exposure is complex. We hypothesized that this can be explained by the existence of biologically and genetically distinct types of melanoma with different etiologic susceptibility to UV-light. Methods: We compared genome-wide DNA copy number alterations and mutational status in BRAF and RAS genes of 126 primary melanomas arising in four groups with different UV exposure. Results: We found significant differences in the frequencies of regional DNA copy number changes and mutation frequencies in BRAF between the four groups. Based on the copy number changes alone 70% of samples could be correctly classified into the four groups. Whereas 81% of melanomas on skin without chronic sun damage had mutations in either BRAF or NRAS, the majority of melanomas in the other groups had mutations in neither gene. In melanomas wild-type for BRAF or RAS we found frequent copy number increases targeting down-stream components of the RAS-BRAF pathway such as CDK4 and CCND1. Conclusions: The divergent patterns of genetic alterations in melanomas of different anatomic sites and sun exposure patterns indicate distinct genetic pathways in the development of melanoma. The demonstration of the strong complementarity of genetic alterations in BRAF, NRAS, CDK4 and CCND1 implicates CDK4 and CCND1 as independent oncogenes in melanomas that are without mutations in BRAF or NRAS. Our demonstration of genetically distinct types of melanoma is relevant for the design of future clinical, experimental and therapeutic studies.

117 SYSTEMS MEDICINE IN MELANOMA THERAPEUTICS

J. Trent (TGen, US)*

Systems Medicine in Melanoma Therapeutics. Jeffrey Trent,1 David Azorsa,1 David Evans, 1 Jeff Kiefer,1 Kevin Brown,1 Tom McCarty,1 Hong Wang,2,1 Haiyong Han,2 Olli Kallioniemi,1 Michael Bittner,1 Daniel Von Hoff,1,2 Spyro Mousses,1. Translational Genomics Research Institute (TGen), 1Phoenix, Arizona, 2Arizona Cancer Center, University of Arizona Tucson. The molecular and genetic context of a cell defines a particular state of a cell and also determines the relative dependency on certain genes that are essential for growth and survival. To gain a deeper understanding on how specific cancer associated perturbations (including for melanoma) can alter the relative dependency on specific genes and pathways for survival, we are performing multidimensional analysis using a host of genomic, genetic, and functional tools against a range of cancer cell models to identify genes selectively and differentially required for growth, survival and vulnerability to anticancer drugs. The integration of data across multiple cancers using multiple approaches (including hi-throughput RNAi profiles, gene expression, single copy-single/single-exon gene copy CGH) is beginning to shed light on not only on etiologically relevant dependencies, but also providing a view for the first time into contextually relevant alterations. Information will be presented from analysis of isogenic cancer cell lines, as well as models of cancer drug perturbation focused on revealing vulnerabilities recognizable in the cellular context of drug response. We are advancing this 'systems medicine' approach with the expectation that it will ultimately advance drug development of anticancer agents for patients with defined genetic alterations in their melanomas.

118 CAN MODERN SUNSCREENS PREVENT IMMUNOSUPPRESSION ?

A. Fourtanier (L'Oreal Recherche, France)*

Sunscreens were initially designed to protect against sunburn/erythema. Their efficacy against this acute reaction is labelled by the sun protection factor (SPF). As a lot of other biological damage, particularly those induced by the long UV wavelengths have been described these last 25 years, it became important to improve sunscreen formulation and efficacy. Today with the introduction of new UV filters and the knowledge of the best way to combine them to obtain good absorption spectra these goals have been achieved. However, some published studies have raised doubts about the sunscreen ability to prevent UVR induced immunosuppression or to offer comparable protection against erythema and immunosuppression. Five recent (2001-2004) published human studies, followed by an international expert meeting and a consensus paper (in press in the J. Invest. Dermatol.) addressed this point. These studies have compared the capacity of sunscreens, with different levels of UVB and UVA protection, to inhibit UV induced suppression of either the induction arm or the elicitation arm of the contact hypersensitivity (CHS) or the delayed type hypersensitivity (DTH) responses. All demonstrated that, to offer a good protection against immunosuppression, at least equal to the erythema protection a sunscreen must provide a high UVA filtering capacity.

119 CAN MODERN HIGH-PROTECTION SUNSCREENS PROVIDE BROAD-SPECTRUM PROTECTION ?

M. Brown (The Boots Company PLC, UK)*

Sunscreen manufacturers have produced preparations to protect the skin from the burning effects of the sun for many decades. But the true effectiveness of sunscreen products was not made visible until the 1970s with the 'invention' of the 'SPF'. Even so, products from this era still provided predominantly UVB protection with any UVA protection being small, or often completely absent. Such products were formulated according to the scientific and medical doctrine of the time; that UVB was hazardous whilst UVA was 'safe' and could be utilised in the popular pursuit of a tanned skin. As acceptance of the harmful potential of the UVA component of the sun grew, manufacturers began introducing UVA absorbing actives into their products. However for many years, the ability to formulate sunscreen products, which were effective UVA protectors as well as UVB, was hampered by legislation, filter availability, formulation expertise and aesthetics (eg skin whitening effects). Consequently, it was not until the 1990s (or even later in some territories) that even modest UVA protection was offered in sunscreen products, despite there having been awareness of the need for UVA protection since the 1980s. In the UK, the early 1990s saw the introduction of a system for labelling UVA protection on sun products, which initiated a race across Europe and other territories, to provide better and better protection from UVA. The demand for improved UVA protection drove the development of new materials, capable of absorbing UVA in addition to UVB. Several of these new filters can already be found in modern sunscreen products whilst other new and exciting molecules are currently in the development pipeline. By selectively combining UV-filters and carefully choosing the solvents which maximise absorbance in selected wavebands, modern products can be made to deliver effective broadspectrum protection throughout the UVB and the UVA.

120 ARE MODERN HIGH-PROTECTION SUNSCREENS SAFE?

J. Nash (The Procter & Gamble Company, USA)*

Daily use of sunscreens is part of a 'safe sun strategy' advocated by healthcare professionals throughout the world. The protection afforded by sunscreen products has increased steadily. For example, the primary index of UV protection, the Sun Protection Factor (SPF), has grown from SPF 15 during the 1980s to SPF 60 and beyond. Given this change, it is logical to ask "Are Modern High-Protection Sunscreens Safe?" This broad question can be divided into two parts: first, "Are there any human health concerns associated with the exposure to UV filters present in high SPF sunscreen products?" In general, currently marketed high SPF sunscreen products are safe. Many if not all UV filters have undergone thorough toxicological evaluations. This is particularly true for newer UV filters such as those introduced in the past 5 years in Europe. In the US, the UV filters used are Category I, safe and effective, according to the FDA sunscreen monograph. Sunscreen products are formulated to minimize the dermal penetration and systemic exposure of UV filters to maximize efficacy. The human safety of final product formulation is supported by testing conducted by the manufacturer. The second part of this safety question is: "Does the regular application of sunscreens block or ameliorate any beneficial effects of solar light on skin?" In general, the benefit of sun safety including the use of SPF 15 sunscreen is greater than any known or suspected health risk. For example using the most conservative assumptions, the daily use of an SPF 15 sunscreen would allow for production of vitamin D in skin. Likewise, intermittent use of higher SPF products would be expected to have little effect on vitamin D synthesis. Thus, broadly speaking, modern sunscreens are safe and effective agents as part of a strategy to reduce damage produced by solar UV.

121 SHOULD WE BE SURPRISED THAT SUNSCREENS APPEAR NOT TO BE PROTECTIVE IN MELANOMA?

B. Diffey (Newcastle General Hospital, UK)*

Meta-analyses of case-control studies have demonstrated no protective benefit of sunscreens and the development of malignant melanoma. Given the period during which the case-control studies were conducted, the sunscreens prevalent at that time, and how sunscreen is used and applied in practice, it is perhaps not surprising that these studies failed to find any association between their use and the risk of melanoma. However, it is reasonable to suppose that the improvement in performance provided by modern high SPF, broad spectrum sunscreens will lead to a worthwhile benefit of current sunscreens as a preventative agent against melanoma, although these benefits may not be seen for several decades.

122 PROGNOSTIC IMPACT OF SENTINEL LYMPHADENECTOMY IN EARLY-STAGE MELANOMA: RESULTS OF MSLT-I, A PHASE III INTERNATIONAL TRIAL

D. Morton (John Wayne Cancer Institute, USA), J. Thompson (Multicenter Selective Lymphadenectomy Trial Group, Australia), A. Cochran (Multicenter Selective Lymphadenectomy Trial Group, USA), R. Elashoff (Multicenter Selective Lymphadenectomy Trial Group, USA), R. Essner (Multicenter Selective Lymphadenectomy Trial Group, USA), E. Glass (Multicenter Selective Lymphadenectomy Trial Group, USA), . Multicenter Selective Lymphadenectomy Trial Group (, USA)*

Elective complete lymphadenectomy (CLND) in early-stage melanoma remains controversial because it cannot benefit the 80% of patients whose regional nodes are tumor-free. We developed lymphatic mapping and sentinel lymphadenectomy (LM/SL) as a minimally invasive technique to identify patients with clinically occult regional metastases. The international Multicenter Selective Lymphadenectomy Trial (MSLT) was initiated to evaluate the diagnostic and prognostic accuracy of LM/SL. This 17-center trial randomized 2001 patients with melanoma > 1.0 mm thick to two treatment arms: wide excision plus LM/SL, with immediate CLND for sentinel node (SN) micrometastasis (60%); or wide excision plus postoperative nodal observation (WEO), with delayed CLND for nodal recurrence (40%). The intraoperative incidence of SN micrometastases in the LM/SL group was compared to the postoperative incidence of nodal recurrence in the WEO group. Patients were stratified by site and Breslow thickness. Statistical analysis used log rank and Cox regression. After a median follow-up of 54 months, the 5-year melanoma-related survival of LM/SL patients dropped from 88% to 71% when the SN contained micrometastases (p = .0001). The false-negative rate of LM/SL was 5.2% (25/483) after 50 procedures. The incidence of SN metastases (LM/SL) vs. postoperative nodal recurrence (WEO) was 15% vs. 16.1% when Breslow was 1.2-3.5 mm, and 33.0% vs. 32.8% when Breslow exceeded 3.5 mm. SN involvement is the most important prognostic factor for disease-free and melanoma-specific survival; SN micrometastases will lead to nodal recurrence if not removed with the primary melanoma. The accuracy of LM/SL increases with surgical volume and reaches 95%. These findings indicate that LM/SL should become standard care for primary melanoma.

123 3000 SENTINEL NODE BIOPSIES - PROCEDURES IN A SINGLE AUSTRALIAN CENTRE - WHAT HAVE WE LEARNED?

J. Thompson (Sydney Melanoma Unit, Australia)*

Soon after the sentinel node (SN) biopsy technique was described by Drs Morton and Cochran and their associates, a validation study involving 118 patients was undertaken at the Sydney Melanoma Unit (SMU). The results were remarkably similar to the results reported from the John Wayne Cancer Institute, and confirmed that SN status accurately reflects the status of the entire node field in patients with melanoma. It soon became apparent, however, that although simple in concept, the SN biopsy procedure was often technically challenging. The great value of pre-operative lymphoscintigraphy quickly became clear, and routine pre-operative lymphoscintigrams using the small particle colloid available in Australia (antimony sulphide colloid) allowed lymphatic channels to be delineated with great accuracy, and revealed previously unexpected lymphatic drainage pathways to SNs in sites that had not previously been suspected. These included drainage from the back to SNs in the triangular intermuscular space (lateral to the scapula), drainage to retroperitoneal and para-aortic lymph nodes from primary sites on the lower back, and direct drainage to supraclavicular nodes from melanoma sites on the hand and forearm. Drainage from primary sites in the head and neck region was found to be particularly unpredictable. With over ten years of follow up in many patients who have undergone SN biopsy, the value of the technique for accurate staging and as a guide to prognosis can now be assessed. The overall 5 year survival for patients with a negative SN at the SMU is 90% and patients with a positive SN 56%. The morbidity of the SN biopsy procedure has been found to be low, and the incidence of in transit metastasis has been no greater than in patients treated by wide excision only. However, some patients reported to be the SMU have later developed metastatic disease in the node field (false negative rate 13.3%). Analysis of reasons for false negative results suggests surgical failure in about 1/3 of the cases, pathological false in another 1/3, and nuclear medicine failure in the remaining 1/3. Knowledge of SN status allows more accurate staging and provides a more reliable estimate of prognosis. These benefits are important not only for patients themselves, but also for stratification in adjuvant therapy trials. As well, data are now available suggesting that immediate node field dissection in SN positive patients impairs survival outcome. The next challenges are to develop minimally invasive and non-invasive methods SN assessment, and to find reliable methods of predicting and detecting involvement of non-sentinel nodes in SN positive patients (so that the great majority of SN positive patients can be spared a complete regional lymph node dissection). Magnetic resonance spectroscopy, high resolution ultrasound and new methods of histological assessment of SNs may allow these challenges to be met.

PROFFERED PAPER PRESENTATIONS

BA1-1 DYSREGULATION OF FGFR2 SIGNALING THROUGH MUTATIONS AND ALTERNATE SPLICING IS COMMON IN MELANOMA

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Background: FGF signaling has been implicated in melanoma progression for many years through evidence of an autocrine loop involving FGF2 and FGFR1. Although FGF2 has been shown to bind to all four FGFRs, the only FGFR that has been extensively studied regarding its role in melanoma progression is FGFR1. In other cancers FGFRs have been demonstrated to act as both an oncogene (eg FGFR3 in myeloma) or as a TSG (eg FGFR2 in prostate cancer). Objective: To investigate the family of FGFRs as possible oncogenes/TSGs involved in melanoma progression. Results: All four FGFRs were sequenced in an initial panel of 47 melanoma cell lines and mutations were identified in FGFR2 but not FGFR1. This mutation analysis has now been expanded, and over 20 novel FGFR2 mutations have been identified in 14/113 (12%) cell lines and 3/26 (12%) metastatic tumors. In addition, approximately 80% of the FGFR2 coding sequence has been assessed in a panel of 83 primary tumors and to date, mutations have been identified in 7/83 (8%) tumors. These mutations occur predominantly in the IgII, IgIII and kinase domains of FGFR2. Functional analysis of several FGFR2 mutants suggests that while some mutations in the extracellular immunoglobulin domains appear to result in constitutive activation of the receptor via ligand-independent receptor dimerization, other mutations in the kinase domain appear to be functionally inactive. RTPCR has revealed that ~40% of the melanoma samples have undergone a C-terminal splicing switch from the C1 to the C3 isoform. We are currently investigating the role of the C3 isoform in melanoma progression, as it has been shown to be more transforming in other tumor types. Conclusion: We have identified FGFR2 as a novel gene involved in melanocytic transformation and demonstrated that dysregulation of FGFR signaling appears to be play an important role in melanoma progression.

BA1-2 IMPACT OF RETINOBLASTOMA BINDING PROTEIN 2-HOMOLOG 1 (RBP2-H1) DEFICIENCY ON RETINOBLASTOMA PROTEIN (PRB) PHOSPHORYLATION IN HUMAN MALIGNANT MELANOMAS

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In malignant melanomas (MM), the loss of cell cycle control is thought to be due to a lack of retinoblastoma protein (pRb)-activity. Members of the previously described family of retinoblastoma binding proteins (RBPs) are supposed to act as pRb-modulating factors. Based on RNA-fingerprinting of UVB-irradiated human melanocytes, we previously described a new family member with high sequence homology to the retinoblastoma binding protein-2 (RBP-2), termed RBP2-Homolog 1 (RBP2-H1). However, a direct impact of RBP2-H1 on pRb-function has not been demonstrated and the role of RBP2-H1 in MM has not been defined, yet. In this study, we show by real time RT-PCR and immunohistochemistry (tissue microarrays and conventional sections) that RBP2-H1 expression is progressively downregulated in advanced and metastatic MM in vivo. By co-immunoprecipitation in constitutively RBP2-H1-expressing cell lines, we provide the first evidence that a subfraction of total RBP2-H1 can bind to pRb which makes this protein a true pRb-interacting factor. In lentivrus-transduced MM cells (A375-SM), re-expressing the non-T/E1A-pRb binding domain of RBP2-H1 which is critically involved in the pRb/RBP2-H1-interaction, we show that hypophosphorylation at pRb Ser795 is stabilized in contrast to GFP-transduced control cells. Furthermore, the re-expression of the non-T/E1A-pRb binding domain resulted in a significant growth reduction in this highly aggressive MM cell line. Subsequent microarray analysis of non-T/E1A-pRb binding domain-transduced A375-SM cells revealed significant changes in gene expression patterns of MM relevant genes, such as the E2F-regulated bone morphogenetic protein 2, follistatin, transforming growth factor alpha, but also other non-E2F-regulated factors, such as endothelin 1, transcription factor 4, microphthalmia transcription factor and hepatocyte growth factor. We conclude that RBP2-H1 is a functional co-factor of pRb that suppresses pRb-hyperphosphorylation, mainly at Ser795, and therefore could stabilize pRb function. In MM, the lack of RBP2-H1 could be one further key mechanism that contributes to the escape from growth control.

BA1-3 DISTINCT REGULATORY MECHANISMS REGULATE THE EXPRESSION OF PROCATHEPSIN L GENE IN HUMAN TUMOR CELLS

D. Jean (INSERM, France), N. Rousselet (INSERM, France), R. Frade (INSERM, France)*

We previously demonstrated that procathepsin L, a cysteine proteinase, which cleaves human C3 (the third component of complement) is overexpressed and consequently oversecreted in human melanoma cells. These two molecular events confere a high tumorigenic and metastatic phenotype to human melanoma cells. Recently, we demonstrated that inhibition of procathepsin L secretion by stable expression of an anti-cathepsin L ScFv (Single chain Fragment variable) strongly inhibited tumorigenic and metastatic phenotype of melanoma cells (Rousselet et al., Cancer Research, 2004). We herein analyzed the mechanisms which could be involved in overexpression of human procathepsin L in human tumor cells. First, we analyzed transcriptional regulation of cathepsin L gene and identified a regulatory region crucial for cathepsin L promoter activity. This region contains one CCAAT motif and two GC boxes on which NF-Y and Sp1/Sp3 transcription factors bind, respectively. However, these three transcription factors, despite being essential for basal cathepsin L transcription, are not directly involved in its overexpression. Thus, other mechanims which may be involved in up-regulation of human cathepsin L gene in tumor cells, were analyzed. For this purpose, we analyzed gene amplification, methylation of genomic DNA, transcriptional regulation and mRNA stability. We herein demonstrated that depending on the cell type, regulation of cathepsin L transcription depended on complex and heterogenous mechanims, and could not be restricted to only one mechanism.

BA1-4 THE ROLE OF THE ADHESION MOLECULE L1 IN MELANOMA DEVELOPMENT AND PROGRESSION

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The L1 adhesion molecule is expressed in human primary melanomas and cutaneous metastases but is not detectable on melanocytes and keratinocytes. L1 expression in human melanoma appears to be correlated with av-integrin expression. L1 supports homophilic L1-L1 binding and heterophilic binding to integrins including avb3 integrin. It has been reported that L1 can be cleaved from the cell surface and can trigger cell migration by binding to av-integrins. The objective of this study was to further investigate the potential role of the adhesion molecule L1 in melanoma development and progression. We determined immunohistochemically L1 expression in melanocytes of reconstructed human skin, in reconstructed human early radial growth phase melanomas, advanced vertical growth phase melanomas and melanoma metastases. We found pronounced expression of L1 in advanced vertical growth phase melanomas and melanoma metastases but not in melanocytes and early radial growth phase melanomas suggesting that L1 is involved in melanoma progression. De novo expression of L1 in human melanocytes using an adenoviral vector did not affect their biological behavior in human skin reconstructs. To address the role of L1 in the complex process of melanoma progression from the radial growth phase without metastatic potential to the vertical growth phase with metastatic potential, we examined the biological consequences of overexpressing L1 in early radial growth phase melanoma cells. Overexpression of L1 in radial growth phase melanoma cells initiated invasive growth of tumor cell strands from the epidermis into the dermis in human skin reconstructs indicating that L1 is decisively involved in melanoma progression.

BA1-5 MECHANISMS AND CONSEQUENCES OF CLAUDIN-1 OVEREXPRESSION IN MELANOMA

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Tight junction proteins regulate the intracellular transport of molecules in and out of cells. Serial analysis of gene expression followed by pathway analysis has implicated the tight junction protein claudin1 in melanoma progression. Linear discriminant analysis of these SAGE libraries also demonstrated that claudin 1 was not preferentially expressed in libraries made from other cancers where other claudins play a role, such as ovarian cancer, and this was supported by published data. To further investigate the role of claudin 1 in melanoma progression we stained a tissue microarray for this protein, and found that claudin 1 was not only overexpressed in melanoma as compared to nevi, but was also aberrantly expressed in the cytoplasm of these malignant cells, implying that claudin 1 might be playing a role other than transport. Indeed, melanoma cells in culture demonstrate no tight junction function, as measured by a trans-epithelial resistance assay. However, we observed that our most motile cells had high levels of cytoplasmic claudin1, and that the expression of claudin1 seemed to correlate to the levels of activated PKC in these cells. To determine if PKC could affect the expression of claudin 1, cells that did not express claudin1 were treated with 200nM TPA (phorbol ester) for up to 12 hours. PKC activation by TPA caused an increase in claudin 1 transcription in as little as 30 minutes, and in claudin 1 protein by 12 hours. In addition, zymograms demonstrate that increases in claudin expression can also cause increases in matrix metalloproteinase activity. We are currently using siRNA against claudin1 to determine if claudin1 can directly contribute to the invasive potential of melanoma, via regulation of MMPs, and if this loop is a necessary component of PKC-induced motility in melanoma cells.

BA1-6 USE OF HUMAN TISSUE TO ASSESS THE ONCOGENIC ACTIVITY OF MELANOMA-ASSOCIATED MUTATIONS

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Melanoma development is associated with alterations in Ras effector pathways, particularly Raf-MEK-ERK and PI3K-Akt, as well as the p16INK4a/CDK4,6/Rb and p14ARF/HDM2/p53 tumor suppressor cascades. Activating Ras mutations are found in 10-15% of melanomas, while BRAF mutations occur in 50-60% of melanomas and about 80% of benign nevi. Akt activation is also observed in about 60% of melanomas. The p16INK4a-Rb and p14ARF-p53 pathways are often inactivated via alterations at the CDKN2A locus, which encodes both p16 and p14. Rb may also be inhibited via activating CDK4 mutations, while p53 is mutated in 10-25% of melanomas. Other genetic alterations, notably telomerase activation, are also implicated in melanoma progression. To directly ascertain the tumorigenic roles of melanoma-associated pathways, we generated a human tissue melanoma model using defined genetic elements. Primary human melanocytes were engineered to express one or more genes of interest and combined with normal keratinocytes on human dermis to regenerate human skin on immunodeficient mice. Co-expression of CDK4[R24C] (constitutively-active CDK4), p53[R248W] (dominant-negative p53), and hTERT (the catalytic subunit of telomerase) did not induce melanocytic neoplasia, leading to a normal skin phenotype. Ras[G12V] (oncogenic Ras), alone or co-expressed with hTERT, induced junctional melanocytic hyperplasia without dermal invasion. However, co-expression of Ras[G12V], CDK4[R24C], p53[R248W], and hTERT induced invasive melanocytic neoplasia displaying major features of malignant melanoma. Furthermore, inhibition of either Rb (via CDK4[R24C]) or p53 (via p53[R248W]) was sufficient, in cooperation with Ras[G12V] and hTERT, to induce invasive neoplasia. hTERT proved dispensable for development of junctional hyperplasia but essential for further progression to invasive cancer. Surprisingly, the activated B-Raf[V599E] mutant only induced junctional hyperplasia, whereas constitutively active PI3K could replace Ras[G12V] to induce invasive melanocytic neoplasia. These studies define a minimal set of pathways sufficient for the development of human melanocytic neoplasia and present a potentially useful model for evaluation of novel therapeutics.

BA1-7 MULTIPLE METASTATIC MELANOMA INDUCED BY NEONATAL ULTRAVIOLET RADIATION TREATMENT OF CDK4R24C/R24C/TPRAS MICE

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OBJECTIVES: To explore whether a single neonatal ultraviolet radiation (UVR) treatment is sufficient to induce melanoma in pigmented mice and to assess co-operation between the Ras/Raf/MAPK pathway and UVR in the development of malignant melanoma (MM). We also sought to compare neonatal UVR-induced melanomas from mice carrying a melanocyte-specific mutant Hras (G12V) transgene (TPras) to those also with an oncogenic mutation (R24C) in Cdk4. MATERIALS & METHODS: Brown TPras or TPras/Cdk4R24C/R24C mice (mixed C3H/Sv129 background) were treated with a similar neonatal UVR regimen to that used by Noonan et al. (2001). Pups (2-3 days old) were exposed to a dose of 8.15 kJm (UVA 320-400 nm, 2.36 kJm-2, UVB 280-320 nm, 5.77 kJm-2, UVC 250-280, 0.02 kJm-2). The UVR-treated cohorts of TPras (n=14) and TPras/Cdk4R24C/R24C (n=14) mice were studied for MM development over a period of 12 months. RESULTS: It has previously been shown that adult TPras mice do not develop MM when treated with chronic doses of UVR (5.6-8.06kJ/m2 biweekly for 28 weeks). However, after a single neonatal UVR treatment we found that the MM incidence increased to 57% at 1 year. All TPras lesions were small in situ cutaneous melanomas. Cdk4R24C/R24C/TPras mice developed MM spontaneously with a penetrance of 12.5%, which rose to 80% after neonatal UVR. All mice of this genotype developed multiple MMs and two mice had metastatic tumours. We are exploring the molecular differences between the MMs from the two genotypes via real-time PCR analysis of Cdk6, Cdkn2a, Cdc25a and Myc, and microarray expression studies. CONCLUSION: These findings demonstrate that neonatal UVR treatment is effective at inducing MM in pigmented mouse strains. In this model Ras activation alone is sufficient to predispose melanocytes to UVR-induced transformation, with the mutant Cdk4 more important for tumour progression, producing larger more aggressive, metastatic MMs.

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BA1-8 COULD IMMUNOHISTOCHEMICAL STAINING OF HUMAN TELOMERASE REVERSE TRANSCRIPTASE BE USEFUL IN DIAGNOSIS OF MELANOCYTIC LESIONS?

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Objective Telomerase plays a key role in avoiding crisis, the exposing of chromosome ends after extended proliferation, resulting in cessation of DNA replication and cell division. Its activity, assessed using a telomeric repeat amplication protocol (TRAP), has been reported in melanocytic naevi and melanoma, but studies conflict as to whether activity differs significantly between these. It has also been suggested as a marker of malignancy, in melanoma and other cancers. We wanted to see if the presence of telomerase, as detected by the more routinely performed technique of immunohistochemistry, varied in melanocytic lesions. Methods Antibodies to human telomerase reverse transcriptase (hTERT, limiting subunit for activity) were used for automated immunohistochemical staining of archived formalin-fixed, paraffin-embedded melanocytic lesions, namely benign compound naevi (number of specimens 10), benign intradermal naevi (10), dysplastic naevi (10), melanocytic intraepidermal neoplasia [MIN] (10), MIN with microinvasion (10) [invasive in radial growth phase], and vertical growth phase melanoma (15). Staining was assessed by proportion and intensity within the lesion. Results Staining was nearly universally nuclear. There was considerable intra-category variability in proportion and intensity, which is being quantitated. Preliminarily there was no clear difference in staining between lesion categories. Staining was not found in normal melanocytes, neutrophils and some other normal tissues. Normal lymphocytes and some basal cells of the epidermis, hair follicles, sebaceous and sweat glands did stain. We are currently examining whether there are any spatial relationships between lesional hTERT and markers of cell senescence such as p16. Conclusion hTERT immunohistochemistry seems unlikely to be a useful diagnostic marker in melanocytic lesions. hTERT is present in normal proliferating cells of the skin. It is nonetheless interesting and unexpected that substantial hTERT expression is common among benign naevi, thought to be senescent lesions, since hTERT overexpression can overcome senescence in some cultured cell types.

BA2-1 ABSENCE OF BRAF MUTATIONS IN MELANOCYTIC NEVI WHICH DEVELOP IN UTERO IMPLICATES UV-LIGHT AS A CAUSATIVE AGENT FOR BRAF MUTATIONS.

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OBJECTIVE: Most melanomas and melanocytic nevi develop on sun-exposed skin and commonly harbor BRAF mutations. Because mutations lack a UV-signature, it has been difficult to establish a causative link between UV-light and mutations in BRAF. Occasionally, nevi are present at birth, and therefore must have developed independently of UV-light. We hypothesized that if BRAF mutations were induced by UV-light, they would be expected to be absent in nevi that develop in utero. MATERIALS & METHODS: To test this hypothesis, we sequenced BRAF exon 15 from DNAs extracted from micro-dissected tumor bearing tissue of 34 melanocytic nevi that were present at birth. Additionally we analyzed nine cases of atypical nodular proliferations developing within nevi presenting at birth. These lesions present as rapidly growing nodules in the neonatal period and are often suspicious for melanoma clinically and microscopically. DNAs from archival tissue of melanomas from intermittently sun-exposed skin, known to harbor frequent BRAF mutations, were used as positive controls. RESULTS: All 34 melanocytic nevi present at birth (100%), as well as the nine atypical nodular proliferations showed wild-type sequences for BRAF exon 15. CONCLUSION: BRAF mutations frequently found in acquired melanocytic nevi and cutaneous melanomas are absent in melanocytic neoplasms developing in utero. These results are in striking contrast with previous studies, reporting high frequencies of BRAF mutations in "congenital" melanocytic nevi. The diagnosis of "congenital" melanocytic nevus is frequently made based on histopathological features such as periadnexal and perivascular distribution of melanocytes rather than definite history. Our findings suggest that these features are inadequate predictors of a congenital origin of melanocytic nevi. Together with reports that BRAF mutations are absent in melanomas from mucosa-lined body cavities these data strongly suggest that UV-exposure is required for BRAF mutations. Future studies are required to delineate the exact mechanism how UV-light causes these mutations.

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BA2-2 BRAF AND SKP2 ARE TARGETS FOR DEVELOPMENT OF NEW TREATMENT FOR MELANOMA

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OBJECTIVE: BRAF and Skp2 were evaluated whether they can be targets for new melanoma treatment, including immunotherapy, gene therapy and molecular target therapy. MATERIALS AND METHODS: Gene amplification and mutations of BRAF were evaluated for 40 melanoma cell lines with CGH analysis and DNA sequencing. Expression of BRAF and Skp2 in various tissues and cancers were evaluated with GeneChip, RT-PCR or Western blot analysis. Recognition of recombinant bacterial BRAF/Skp2 proteins by serum IgG Ab from melanoma patients were evaluated with Western blot analysis. Roles of BRAF and Skp2 in proliferation and invasion ability of melanoma were evaluated with infection of the specific short hairpin type siRNA lentivirus using in vitro cell proliferation assay, Matrigel invasion assays, and in vivo melanoma growth in NOD/SCID mice. RESULTS: Mutations (V599E or V599D) or increased expression of BRAF were frequently detected in 40 melanoma cell lines tested. Increase of the Skp2 protein was observed in 3 of 8melanoma cell lines. Serum IgG Ab was detected for BRAF in 6 of 37melanoma patients, but not for Skp2. Infection of melanoma cell lines with the siRNA lentivirus specific for BRAF (V599E) or Skp2 inhibited in vitro cell proliferation and Matrigel invasive ability of melanoma cell lines accompanied with increase of the p27Kip1 protein, and infection of BRAF siRNA lentivirus inhibited in vivo melanoma growth in NOD/SCID mice. Simultaneous suppression with the tandem BRAF and Skp2 siRNA lentivirus inhibited melanoma growth and Matrigel invasion more effectively with more p27Kip1 increase than the single siRNA lentivirus. CONCLUSION: BRAF is an immunogenic tumor antigen in melanoma patients. Inhibition of BRAF or Skp2, particularly simultaneous inhibition, suppresses growth and invasive ability of melanoma. Therefore, BRAF and Skp2 are good candidates as targets for development of new melanoma treatment, including immunotherapy, gene therapy and molecular target therapy.

BA2-3 PIK3CA MUTATIONS ARE RARE WHILE ACTIVATION OF AKT AND ERK IS FREQUENT IN CUTANEOUS MELANOMA

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Between 20 to 30% of cutaneous melanomas harbour activating NRAS codon 61 mutations, and another 55 - 60% have mutations in the BRAF gene, most commonly a BRAF V600E mutation. As a result of these mutations the Raf-Mek-Erk pathway is activated in a majority of melanomas. Another effector molecule of Ras is the p110 catalytic subunit of phosphatidylinositol 3-kinases (PI3Ks). The primary substrate of PI3Ks is the 3position of phosphatidylinositol 4,5-bisphosphate, phosphorylation of which results in increased levels of phosphatidylinositol 3,4,5-trisphosphate. This molecule functions to recruit the serine/threonine kinase Akt to the plasma membrane. Once at the plasma membrane, Akt is activated through phoshorylation at critical residues (threonine 308 and serine 473). Recently it was shown that the gene that encodes the p110a catalytic subunit of class 1A PI3Ks, PIK3CA, is mutated at a high frequency in colorectal cancers as well as in some other tumour types. Here we report that PIK3CA is also mutated in cutaneous melanomas. Using PCR-single strand conformation polymorphism analysis and nucleotide sequencing of aberrant bands, we analysed a series of melanoma metastases and cell lines with known NRAS and BRAF mutation status for PIK3CA exons 1, 9 and 20 mutations. PIK3CA missense mutations were identified in 3 of 101 metastases (3%), and occurred only in lesions that did not carry a NRAS mutation. Using phospho-specific antibodies, we also assessed the expression of activated Erk and Akt in the same sample series. These investigations showed that both Erk and Akt are activated in the majority of melanoma metastases and cell lines, irrespective of NRAS or BRAF mutation status. Taken together, these results demonstrate that both the Raf-Mek-Erk and PI3K-Akt signalling pathways are frequently involved in melanoma tumourigensis and that these pathways can be activated through multiple mechanisms.

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BA2-4 NOVEL POTENTIAL DOWNSTREAM EFFECTORS OF THE PTEN TUMOUR SUPPRESSOR GENE IN MELANOMA.

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The tumour suppressor PTEN encodes a dual-specifity protein phosphatase which functions in cell cycle arrest and apoptosis. Somatic mutations and deletions in PTEN have been found in a variety of cancer types. PTEN antagonizes phosphoinositide 3-kinase (PI3K), which is believed to be responsible for the growth-suppressive effects. Although loss of PTEN has been shown to assist tumorigenesis, the precise contribution of PTEN mutations to melanoma development is yet to be elucidated. This study aimed to identify novel participants in the PTEN pathway. Sixty melanoma cell lines were sequenced for PTEN to identify mutations and deletions. RNA from these cell lines was hybridized to cDNA microarrays containing 19,008 ESTs from the Ontario Cancer Institute. The cell lines were grouped according to their PTEN mutation/deletion status and statistical analyses were used to identify genes whose expression correlated with these groups. We found 632 clones differentially expressed between wild-type PTEN samples and samples with PTEN mutations/deletions (P-value<0.05, Mann-Whitney U-test). While this is not significantly different to the 647 clones expected to be differentially expressed by chance, many of the genes are known to be involved in the PI3K pathway and other signaling pathways influenced by PTEN. We thus reasoned that some of the genes on this list may still be regulated by PTEN activity. The expression of a number of interesting candidate genes that were potential downstream effectors of PTEN was validated by qRT-PCR in the original 60 cell lines and in a second independent set of 20 melanoma cell lines with known PTEN mutation status. Additionally, siRNA was used to knock-down PTEN levels in two wild-type samples and the resulting expression of candidate genes was examined. To determine whether these candidate genes function downstream of the PI3K pathway, four wild-type cell lines and four deleted/mutated lines were treated with the PI3K inhibitor LY294002 and the transcript levels of these genes were measured by qRT-PCR. Through these experiments we have identified a number of potential downstream effectors of PTEN which may help to explain how PTEN inactivation contributes to melanoma pathogenesis.

BA2-5 MUTATIONS OF PIK3CA ARE INFREQUENT IN PRIMARY MELANOMA

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OBJECTIVE: Recent reports indicate that PIK3CA, which encodes the p110 subunit of Phosphatidylinositol 3-kinase (PI3K), is frequently mutated in breast, colorectal, gastric and hepatocellular carcinoma. PI3K is a direct target of activated RAS and is negatively regulated by PTEN. In melanoma the PI3K pathway is frequently targeted by deletions or mutations of PTEN locus or mutation of NRAS. We screened two mutation hotspots in PIK3CA for somatic mutations in melanoma. MATERIAL AND METHODS: We sequenced exons 9 and 20 in five types of primary melanoma samples: skin with (n=26) and without (n=28) marked solar elastosis as a measure of chronic sun damage; palms, soles and subungual (acral) sites (n=34); mucosa (n=17); and desmoplastic melanomas (n=13). RESULTS: Sequencing of 118 primary melanoma samples revealed only one mutation of PIK3CA in primary melanoma. Interestingly the mutation was a CT transition which can result from UV exposure due to pyrimidine dimer formation and the mutated sample was from the group with chronic sun exposure. The C1773T (Genbank: NM_006218) transition resulted in a P539L substitution. Normal tissue from this sample lacked this mutation ruling out the possibility of germ line mutation. This is the first report of a P539L substitution in PIK3CA, although a P539R substitution has previously been reported. This amino acid substitution results in a heterocyclic to an alkyl amino acid substitution, however both amino acids are neutral. The functional significance of the P539L substitution is currently unknown. CONCLUSION: PIK3CA mutations, as assessed by sequencing the most frequently mutated regions, are infrequent in primary melanoma. We are currently investigating other components of the PI3K pathway that could be targeted in samples and could account for activation of this pathway in samples that are wildtype for NRAS and PTEN.

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BA2-6 ESTABLISHMENT AND CHARACTERIZATION OF A CELL LINE DERIVED FROM THE RADIAL GROWTH PHASE ACRAL MELANOMA

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BACKGROUND: Recent studies showed that acral melanomas may have unique pathogenesis characterized by frequent gene amplifications from the early stage of development and low frequency of NRAS/BRAF mutations. We have established a cell line (SMYM-PRGP) from a radial growth phase lesion of a 69-year old acral melanoma patient. Neoplatsic melanocytes were dispersed from the epidermis with trypsin / EDTA and were grown in MGM2 medium supplemented with 5% fetal bovine serum (FBS). SMYM-PRGP is now maintained on MCDB153 medium containing 5% FBS, bFGF, EGF, SCF, endothelin-1, GRO-alpha, insulin, transferrin, and TNF-alpha. RESULTS: This cell line requires supplementation of the above growth factors to grow. Mutation analyses showed that SMYM-PRGP had wild-type NRAS/ BRAF. Western blotting did not detect phosphorylated ERK1/2 proteins, suggesting the absence of constitutive activation of the MAPK pathway. Genetic profiling by the use of Mutiplex Ligation-dependent Probe Amplification (MLPA) method showed that SMYM-PRGP had copy number losses of CDKN2A and CDKN2B genes and prominent amplification of cyclin D1, EMS1 and FGF3, all located on chromosome 11q13. The amplification of the cyclin D1 gene was confirmed by FISH. CONCLUTIONS: SMYM-PRGP may well represent molecular and biological characteristics of the neoplastic melanocytes in the radial growth phase of acral melanoma. This cell line can be used to further elucidate the molecular pathway leading to the development of acral melanoma.

BA2-7 CO-OPERTATION BETWEEN MUTANT V599EB-RAF AND AKT3 PROMOTE MELANOCYTE TRANSFORMATION

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B-RAF is the most mutated gene in melanoma with ~90% of mutations occurring as a single base substitution converting a valine to glutamic acid at codon 599 (V599E) in the kinase domain of the protein. This mutation leads to constitutive activation of B-Raf and the downstream MAP kinase signaling cascade. While V599EB-Raf regulates cell proliferation and vascular development in established melanoma tumors, its role in melanocyte transformation remains uncertain. Specifically, up to 82% of nevi contain V599EB-Raf but relatively few ever progress into melanomas. Furthermore, ectopic expression of V599EB-Raf in melanocytes tends to be toxic and surviving cells express low levels of protein. This has led to speculation that additional co-operative oncogenic events are needed for complete transformation of melanocytes containing V599EB-Raf protein. Recently, Akt3 activation (either through PTEN loss or Akt3 overexpression) has been reported to occur in up to 70% of melanomas with B-Raf mutation occurring in many of the same cells as active Akt3. In this study, we provide evidence that V599EB-Raf and Akt3 co-operate to promote melanocyte transformation. Comparison of the kinetics of activation of B-Raf and Akt in melanocytes showed that both were transiently turned-on while in melanoma cells both were constitutively active. Ectopic expression of active Akt3 in early stage melanoma cells containing V599EB-Raf protein enhanced anchorage independent growth. Furthermore, expression of both V599EB-Raf and active Akt3 in melanocytes synergistically promoted anchorage independent growth. Pharmacological or siRNA mediated inhibition of V599EB-Raf and Akt3 in melanoma cell lines, led to a synergistic reduction in anchorage independent growth and decreased chemoresistance. Mechanistically, Akt3 directly interacts with V599EB-Raf in melanoma cells to phosphorylate and lower activity of the mutant protein to levels that promote rather than inhibit cell proliferation. Collectively, these results suggest that both B-Raf mutation and Akt3 activation are necessary co-operative events for melanocyte transformation.

BA3-1 IDENTIFICATION OF A MOLECULAR SIGNATURE FOR METASTATIC MALIGNANT MELANOMA IN LASER-MICRODISSECTED TUMOR TISSUE

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Distant metastasis is a central event in malignant melanoma progression. At this late stage only few therapeutic options exist with little impact on the patients overall survival. In order to better understand the process of melanoma progression and identify putative markers for tumor metastasis genomewide gene expression profiling was performed for 39 primary melanomas and melanoma metastases using oligonucleotide microarray technique. Melanoma cells were excised by laser-capture microdissection from tumor tissues to focus on melanoma cell-specific gene expression. By this means 195 genes were identified which were significantly differentially expressed between primary melanomas and metastases. A majority of these genes belonged to the functional groups ectoderm development, cellular proliferation, and cell adhesion ($p<10\text{-}5$ Fisher's exact test). A predictive diagnostic model (support vector machine) was trained and reached a performance of more than 85% for correct classifications using cross validation. Further functional studies on new target genes underscored the functional relevance of these findings. Even melanoma subtypes (nodular versus superficial spreading type) could be identified based on gene expression profiles. Taken together, this is the first large-scale gene expression study of malignant melanoma (analysing more than 22.000 transcripts) after laser-capture microdissection of tumors. A molecular signature for metastasis was identified which not only provides deeper insights into the pathogenesis of melanoma progression but may also be used in future disease monitoring studies.

BA3-2 NOVEL IMMUNOHISTOCHEMICAL PROGNOSTIC MARKERS IN CUTANEOUS MALIGNANT MELANOMA

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Global gene expression in 83 primary cutaneous melanomas (CM) using 44K oligonucleotide micro-arrays revealed a "refined" set of 60 genes with high probability to predict 4-yrs distant metastasis free survival (DMFS) as well as gene-lists, correlated with certain histological/cytological features. For validation, we applied antibodies directed to DMFS-associated genes, or associated with ulceration or Breslow thickness on tissue micro-arrays (TMA's) from 62 melanomas, 8 metastases and 14 nevi. The degree of immunoreactivity of 7/23 antibodies was significantly associated with 4 yrs-DMFS at $p<0.05$, i.e. those directed against nme1, kallikrein (klk)7, mcm3, mcm6, karyopherin-alpha2, geminin and coagulation factor X. The expression of antibodies to klk7, mcm4, karyopherin-alpha2 and factor X was significantly associated with overall survival (OS). Use of these 8 antibodies in routine practice may contribute to prognostication and treatment stratification. Listed in the "refined" 60-gene set was Pituitary Tumor Transforming Gene (PTTG), a proto-oncogene encoding "securin", involved in sister chromatid separation. PTTG was significantly overexpressed in metastasizing CM as compared to non-metastasizing CM; in 29 matched pairs of nodular melanoma (NM) and superficial spreading melanoma, securing was significantly overexpressed in NM (paired t-test, $p=0.018$) but not significantly associated with thickness. Securin expression was particularly found in anaplastic and multinucleated melanoma cells. Forced overexpression of PTTG has been shown to promote formation of poorly differentiated tumors; on a cellular level, PTTG induces proliferation, aneuploidy and angiogenesis via induction of bFGF, VEGF and IL-8. Conclusions. Based on gene expression analysis, we have identified 8 immunohistochemical markers, the expression of which predicts 4-yrs DMFS and/or OS. However, validation of these results on a large cohort is needed to confirm our findings. In addition, our data show overexpression of the proto-oncogene PTTG in nodular melanoma, which adds to the already existing evidence that different pathogenetic mechanisms are involved in different types of CM.

BA3-3 CULTURE OF MELANOMA CELLS IN THREE-DIMENSIONAL ARCHITECTURES RESULTS IN IMPAIRED IMMUNORECOGNITION BY CYTOTOXIC T LYMPHOCYTES SPECIFIC FOR MELAN-A/MART-1 TUMOR ASSOCIATED ANTIGEN.

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Objective: To assess the effects of the culture of melanoma cells in 3D architectures on their immunorecognition by cytotoxic T lymphocytes (CTL) specific for tumor associated antigens Methods: Culture of HBL melanoma cells expressing Melan-A/Mart-1 tumor associated antigen (TAA) and HLA-A0201 on poly-2-hydroxyethyl methacrylate (polyHema) coated plates resulted in the generation of aggregates of 400-500 1/4 diameters containing on average 30.000 cells and characterized by slower proliferation, as compared to monolayer (2D) cultures. HLA-A0201 restricted Melan-A/Mart-127-35 specific CTL clones were used to evaluate tumor cell immunorecognition measured as specific IFN-g production. Comparative gene and protein expression in 2D and 3D cultures were studied by real-time PCR and flow-cytometry, respectively. Overall differences in gene expression profiles between 2D and 3D cultures were evaluated by high density oligonucleotide array hybridization. Results: HLA-A0201 restricted Melan-A/Mart-127-35 specific CTL clones produced high amounts of IFN-3 upon short term (4-24 hours) co-incubation with HBL cells cultured in 2D but not in 3D, thus suggesting altered antigen recognition. Indeed, Melan-A/Mart-1 expression, at both gene and protein level was significantly decreased in 3D as compared to 2D cultures. Concomitantly, a parallel decrease of HLA class I molecules expression was also observed. Differential gene profiling studies on HBL cells showed an increased expression of genes encoding molecules involved in inter-cellular adhesion such as junctional adhesion molecule 2 and cadherin-like 1 (>20 and 8 fold upregulated, respectively) in 3D as compared to 2D cultures. Conclusions: Taken together our data suggest that mere growth of melanoma cells in 3D architectures, in the absence of immunoselective pressure, may result in defective recognition by TAA specific CTL.

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BA3-4 DYSPLASTIC NEVUS CELLS AND MELANOMA CELLS SHOW SIMILAR DISTURBANCES IN MELANIN METABOLISM AND REDOX BALANCE.

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OBJECTIVE: It is generally recognized that the occurrence of pigmented nevi is connected with the degree of skin pigmentation. Individuals with less pigmented skin are more likely to develop the nevi. A large number of pigmented nevi is currently considered the important risk factor for cutaneous melanoma. This holds particularly good for dysplastic nevi. The objective of this study was to investigate possible differences in the melanin synthesis and the level of oxidative imbalance between the melanoma cells and dysplastic naevus cells and to compare the data with those obtained from normal melanocytes. MATERIALS & METHODS: With the use of different techniques (X-ray microanalysis, HPLC, specific molecular probes) we measured differences in melanin synthesis, in the contents of some metals and in the level of oxidative stress in melanoma cells, dysplastic nevus cells, normal skin melanocytes and dermal nevus cells. RESULTS: We present the evidence that dysplastic nevus cells and melanoma cells synthesize more pheomelanin than do normal melanocytes. We also found that melanosomes from these two types of cells bind significantly more iron and calcium than do melanosomes from normal melanocytes or dermal nevus cells. Higher iron and calcium contents are indicative of the existence of chronic oxidative stress in the both types of cells. We confirmed it by FACS analysis after incubating cultured cells with redox-sensitive molecular probes. We have also the first indications of higher contents of free iron in the nuclei of dysplastic naevus cells. Such situation can lead to the higher risk of oxidative DNA damage. CONCLUSION: We propose that the chronic increase of free iron in dysplastic nevus cells and melanoma cells is implicated in the elevated mutation rate in these cells. Our findings also support the idea of dysplastic nevi being true precursor lesions of melanoma.

BA3-5 COMBINED DETERMINATION OF PLASMA L-DOPA/L-TYROSINE RATIO AND LACTATE DESHYDROGENASE IN MELANOMA: A COMPARISON WITH S100B AND MIA

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Background: A number of molecules have been investigated for potential use as serologic tumor markers in melanoma. Unfortunately, they all have limited role in screening and early diagnosis. In metastatic disease (stage III-IV), however, some of them have demonstrated a relation with tumor burden, successful therapy, disease progression and/or prognosis. There is currently little data allowing a comparison between available serologic melanoma markers, such as the L-Dopa/L-tyrosine ratio (a marker of tyrosinase activity), S100B and MIA (melanoma antigens), and the non-specific lactate deshydrogenase (LDH). Methods: Plasma markers were measured by HPLC (L-dopa, tyrosine), immunoassays (S100B, MIA) and colorimetry (LDH) in n=155 melanoma patients (stage I-II: n=53, III: n=50, IV: n=52, AJCC staging). Blood samples were obtained during patient first inclusion and laboratory analysis were performed in a blind fashion. Results: Melanoma markers were significantly elevated in stage IV and in stage III also for the L-Dopa/L-tyrosine ratio. S100B and MIA highly correlated in stage IV (0.828, $p<0.0001$); they also correlated with LDH, especially in stage IV. The L-Dopa/L-tyrosine ratio weakly correlated (<0.50) with other markers and in stage IV only. At 80% specificity (ROC analysis), sensitivity to confirm stage III-IV reached 68.6% for the L-Dopa/L-tyrosine ratio (<51% for other makers) and 79.4% combined with LDH as a second marker. During progression to higher stages (n=13), median L-Dopa/L-tyrosine ratio increased by 27% ($p<0.05$); there was no significant change for other markers. All markers displayed a prognostic value in the sub-group of deceased patients. Conclusion: The combination of the L-Dopa/L-tyrosine ratio and LDH has the potential to suggest the presence of occult metastases and disease progression (in stage I-III patients). Its prognostic value is similar to melanoma antigens S100B and MIA. Here, it appears as an attractive approach for the biological follow-up of melanoma patients.

BA3-6 UBC9 IS HIGHLY EXPRESSED IN MELANOMA-INFILTRATED LYMPH NODES AND ITS DEPLETION INDUCES APOPTOSIS IN MELANOMA CELL LINES

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INTRODUCTION: Melanoma is curable at early stages but no treatment has shown to improve survival in stage IV disease. Therefore, new treatment approaches, taking into account the intrinsic resistance of melanoma cells to apoptosis are necessary. We studied the proteomic profile of melanoma-infiltrated lymph nodes in 11 patients with bulky regional lymphadenopathy (stage IIIB). METHODS: Protein lysates from melanoma-infiltrated lymph nodes were arrayed on commercially available antibody blots. RESULTS: Ubc9, the SUMO E2 ligase involved in the attachment of SUMO to target proteins, emerged as the most highly expressed protein across all samples. Ubc9 expression was confirmed by immunoblots on the same protein lysates and by immunohistochemistry in tissue biopsies taken from patients at different stages in the melanoma progression pathway but not in normal skin or benign nevi. To more specifically determine the biological role of Ubc9 in melanoma cells, small interfering RNA (siRNA) targeting the Ubc9 mRNA (siUbc9) was designed and its effect was tested in melanoma cell lines. siUbc9 compared to scrambled siRNA, demonstrated suppression of Ubc9 protein levels that was maximal at 48 hours whereas Ubc9 mRNA was suppressed from 8 hours through 72 hours after transfection. In two adherent melanoma cell lines, siUbc9 compared to scrambled siRNA-treated controls significantly decreased viable cell numbers at 72 and 96 hours after transfection. Flow cytometric analysis of si RNA-treated melanoma cells stained with propidium iodide revealed increased apoptosis in the siUbc9-treated cells compared to those exposed to scrambled siRNA at 72 and 96 hours after transfection. CONCLUSIONS: Ubc9 is highly expressed in metastatic melanoma and may partially explain the resistance of melanoma cells to apoptosis. Further studies to identify the mechanism of apoptosis induced by Ubc9 depletion are in progress. Treatment approaches targeting Ubc9 may be particularly effective.

BA3-7 OVEREXPRESSION OF TYROSINASE IN MELANOMA CELLS INCREASES BIOREDUCTIVE POTENTIAL: REVERSAL BY THE DIETARY POLYPHENOL QUERCETIN

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Objective: Quercetin (Qct) is an abundant dietary polyphenol that is oxidized in melanoma cells by tyrosinase. Significant blood levels of Qct can be achieved by diet and supplementation. The oxidized quinones bind to glutathione (GSH) in cells and create cytotoxic Qct-GSH adducts. The present study tested the hypothesis that quercetin will induce the formation of cytotoxic quercetin-GSH adducts and decrease cellular GSH in melanoma cells, thereby increasing sensitivity to cytotoxic therapies. Methods: DB-1 melanoma cells were transfected with pcDNA3 containing a tyrosinase cDNA. GSH levels were measured by a hydroxyethyl disulfide (HEDS) assay. Cell survival was performed using an MTS survival assay. Results: In a cell-free system consisting of GSH and Qct, the level of free GSH was almost completely diminished 5 minutes after the addition of purified tyrosinase. Formation of a Qct-GSH adduct coincided with the depletion of GSH. In melanoma cells overexpressing tyrosinase, the rate of HEDS bioreduction was higher compared to control cells, indicating that the bioreductive potential of the cells was increased. Clones tyr10 and 12, the highest overexpressors, had nearly a two-fold increase in bioreductive potential. However, the addition of at least 25 micromolar Qct to the overexpressing cells decreased the bioreductive potential and increased cell death by Qct when compared to control cells. When the expression of enzymes involved in redox cycling was investigated, glutathione S-transferase (GST) was found to be significantly increased in the tyrosinase overexpressing clones. Discussion: These findings support the hypothesis that Qct can reduce GSH levels and increase cell cytotoxicity through a tyrosinase-mediated mechanism. Upregulation of GST, which has been implicated in drug resistance, may further enhance the cytotoxicity of Qct, in that it conjugates GSH to Qct. Therefore, depletion of GSH by Qct should lead to sensitization of melanoma cells to cytotoxic therapies. (Grant support: AICR)

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BA3-8 HUMAN ENDOGENOUS RETROVIRUS HERV-K IS EXPRESSED IN HUMAN MELANOMA AND ANTIBODIES TO HERV-K CAN BE FOUND IN SERA OF MELANOMA PATIENTS.

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OBJECTIVE: In contrast to all other human endogenous retroviruses, proviruses of the human endogenous retrovirus family HERV-K have maintained open reading frames for all viral proteins. Although most proviruses are defective, structural proteins Gag and Env, the reverse transcriptase and two regulatory proteins, Rec and np9, have been described. Although the full length mRNA of HERV-K is expressed in many tissues, expression of viral proteins and particle production had only been demonstrated for teratocarcinomas and more recently for melanomas. Our aim was to extend these data. MATERIALS & METHODS: A set of seven primers was developed that allows discrimination between full-length and spliced mRNA, and mRNA from deleted and undeleted proviruses such as HERV-K, -E,-H,-R and W. Antisera specific for several HERV-K proteins were generated and used for immmunohistochemistry and sera from melanoma patients were investigated for HERV-K specific antibodies. RESULTS: Full length mRNAs of all HERVs were found in all human cells. Spliced env and rec of HERV-K were detected in 45% of the melanoma metastases (n=34) and in 44% of the melanoma cell lines (n=19). None of the control cells showed expression of spliced env or rec mRNA. Expression of HERV-K in situ was shown by immunohistochemistry. In addition, 22% of the patients sera tested showed antibodies against the HERV-K transmembrane envelope protein. CONCLUSION: For the first time we demonstrate expression of viral proteins (including the transmembrane envelope protein) in melanomas using immunohistochemistry with a newly developed HERV-K specific antiserum and the presence of HERV-K-specific antibodies in sera of patients with melanomas. Further studies are required to determine whether the expression of HERV-K can be of prognostic or diagnostic use. In addition, the retroviral proteins expressed may provide excellent targets for an anti-tumor vaccine.

BE1-1 EPIDEMIOLOGY AND SEARCH FOR BACKGROUND FACTORS IN MALIGNANT MELANOMA IN CHILDREN AND ADOLESCENTS.

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Objective: To investigate malignant melanoma in children and adolescents. Through epidemiological studies look at incidence, clinical factors and prognosis, as well as etiological factors such as phototherapy in newborns and the effect of congenital nevi on the risk of malignant melanoma. Methods: The Swedish Cancer Register, the Swedish Medical Birth Register, questionnaires, record studies, clinical investigations and matched controls. Results: The incidence of malignant melanoma in children was constant, but in adolescents a doubling was seen for the past few decades. Median survival time was three years after diagnosis, 15 % of patients dying as a result of the diagnosed condition. No specific anatomical location was over-represented as fatal. No malignant melanoma was found after review of nearly 4000 congenital nevi. Further, no clear association was found between congenital nevi and maternal illness/infection during pregnancy. Congenital nevi seemed to have had limited effects on social life, but had resulted in greater caution with regard to sun exposure. Discussion: The most common type of malignant melanoma in adolescents is the superficial spreading type, the one most likely to be sun-related. Since the anatomical location is the same in adolescents as in adults, the same etiological factors are presumably of importance; but some other factor may reduce the induction time. Phototherapy of newborns has probably been ruled out as a factor in the increased incidence of malignant melanoma: on the contrary phototherapy may well be a protective factor just as outdoor activities in childhood are. Congenital nevi are often surgically removed unnecessarily. It must be considered whether the reason for removal is the risk of malignant transformation or is merely cosmetic. Congenital nevi or their treatment seem to play no part in the increased incidence of malignant melanoma below the age of 20.

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BE1-2 ARE SUNBED INDUCED MELANOMA ANY DIFFERENT IN HISTOPATHOLOGY?

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Objective: In our prospective population based cohort in South Sweden of 40 000 invited women aged 25-65 melanoma risk was significantly increased in younger women using sunbeds more than 10 times a year. Methods: Our cohort was established in 1990-92 and matched with the Swedish Cancer Registry and vital status. Cancer incidence were followed up until Dec 2004. All melanoma were reviewed concerning type, Breslow thickness, Clarks level of invasion, ulceration, inflammatory reaction, regression, naevus component, and tumour infiltration lymphocytes (TIL), Site of primary tumour were registered. Melanomas in patients frequently using sunbeds (>10 times /year) were compared with the other melanomas. Logistic regression was used. Results: During the follow up a total of 101 melanoma had developed. In the univariate analysis melanomas in women using sunbeds more than 10 times a year had less inflammation (p = 0.01) compared to women with less frequent use of sunbeds. A tendency were also seen with Clarks invasion at a less aggressive level. and fewer cases with a naevi component. There were no tendency towards the SSM-type or deviating tumor thickness (analyzed as a continuous variable). Multivariate analysis did not show any deviating results. Conclusion: In this prospective population based cohort a the current numbers of melanoma (101) showed that women using sunbed more often seemed to have melanomas with less inflammation, furthermore there was a tendency of fewer tumours with a naevi component and a lower level of Clark invasion.

BE1-3 ETIOLOGIC AND OTHER FACTORS PREDICTING NEVUS-ASSOCIATED CUTANEOUS MALIGNANT MELANOMA

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Cutaneous malignant melanomas (CMM) with histologic evidence of an associated nevus (N+) may have a risk factor profile distinct from that seen in melanomas without it (N-). To address this question, a case-only analysis of 932 people with CMM was done to identify etiologic and other factors associated with N+ melanoma. Evidence of an associated nevus was found in 36% of melanomas. N+ melanomas were thinner (Ptrend = 0.0009) and more likely to be of the superficial spreading type than other types of melanoma. Subjects with N+ melanomas were younger (Ptrend<0.0001) and reported a higher nevus density on their skin than subjects with N- melanomas (odds ratio (OR) 3.1, 95% confidence interval (CI) 1.6-6.0 for high nevus density vs. no nevi). Indicators of high accumulated sun exposure were less prevalent among subjects with N+ melanomas (OR 0.3, 95% CI 0.2-0.4 for melanoma location on the head and neck vs. location on trunk; OR 0.2, 95% CI 0.1-0.4 for severe solar elastosis adjacent to the melanoma vs. no elastosis; OR 0.2, 95% CI 0.1-0.4 for lentigo maligna melanoma subtype vs. superficial spreading subtype). No associations with self-reported measures of sun exposure, sunburn or pigmentation phenotype were apparent. These results did not materially change upon exclusion of lentigo maligna melanomas and melanomas of thickness > 1mm. Our findings provide some support for the hypothesis of etiologically separate pathways for melanoma, with N+ melanomas appearing less likely to develop in the presence of characteristics suggesting high accumulated sun exposure than N- melanomas. We cannot rule out the possibility, though, that continuing UV exposure induces involution of nevus remnants in melanomas.

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BE1-4 DO HMG-COA REDUCTASE INHIBITORS LOWER RISK OF MELANOMA?

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Objective: Statins (HMG-CoA reductase inhibitors) are anti-hypercholesterolemic agents that may induce apoptosis and exert antitumor activity through depletion of cellular mevalonate. Previous studies found lower melanoma incidence in statin users. We evaluated the association of statin use and melanoma risk in an observational study of United States veterans receiving care in the Veterans Health Administration (VA). Materials and Methods: We studied VA patients using national pharmacy and patient care databases. We identified 1.2 million patients in 1999 with hypercholesterolemia and no evidence of prior melanoma who we followed through 2003 to identify new codes for melanoma. We compared rates in those prescribed statins to other patients and used logistic and proportional hazards regression to estimate melanoma risk associated with statin use, with control for confounding. We also conducted a nested case control study, comparing prior statin use in new melanoma cases with controls matched on sex, age, race, location, and year. Results: In this population of predominantly white (73%), older (55% over 65 years), men (95%), over 10,000 patients had new melanoma codes in the 5 years of follow-up. Melanoma rates varied little with statin use. In 1999, 34% were using statins and the adjusted odds ratio for melanoma was 0.98 (95% confidence interval: 0.96-1.04). In follow-up of those without prior statin use, 59% initiated statins and the adjusted hazards ratio for subsequent melanoma was 0.91 (0.86-0.96). Work is continuing to evaluate other potential confounding and explore variation of effects in subgroups. Conclusions: The small decrease in melanoma risk associated with statin use in this study provides weak support for the hypothesis. Additional studies are needed to substantiate the value of statins as a potential chemopreventive agent for melanoma.

BE1-5 SITE-SPECIFIC INCIDENCE OF MELANOMA: A COMPARISON OF TWO POPULATIONS WITH CONTRASTING LEVELS OF SUNLIGHT

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Background: The divergent pathway hypothesis for cutaneous melanoma posits that melanomas arising at different anatomical sites have different associations with sunlight. To further explore this hypothesis, we compared the site-specific incidence of cutaneous melanoma at different ages in two genetically-similar populations with contrasting levels of ambient sunlight. Methods: We ascertained all new cases of melanoma in the west of Scotland [WoS] and Queensland [Q] between 1982 and 2001. We calculated melanoma incidence, standardized to the European population and adjusted for surface area, for four broad anatomical regions (head and neck, trunk, upper and lower limbs). Analyses were restricted to melanomas of the superficial spreading and nodular histological subtypes; acral lentiginous and lentigo maligna melanomas were specifically excluded. Results: Overall, melanoma incidence at all ages was typically 3 to 5 fold higher at all body sites in Queensland than in Scotland. While melanoma incidence was reasonably uniform across body sites under age 40 years, the anatomical distribution of melanoma varied with age in a similar manner in both settings. Among males aged 40 to 59 years, highest rates were observed on the trunk (Q 84/100,000; WoS 15/100,000), whereas among females in the same age range, highest rates were observed on upper limbs in Queensland (71/100,000) and lower limbs in Scotland (17/100,000). After age 60, melanoma rates were highest on the head and neck in both sexes (Q males 215/100,000, females 107/100,000; WoS males 49/100,000, females 33/100,000). Conclusions: The overall higher incidence of melanoma at all ages in Queensland than Scotland confirms the role of sunlight in initiating these cancers. Despite markedly different levels of ambient sunlight in the two populations, similar anatomical distributions of melanoma were observed with advancing age. These data accord with the hypothesis that the site of melanoma reflects, at least partly, age-specific differences in susceptibility within a population.

BE1-6 NAEVUS COUNTS ARE STRONGLY ASSOCIATED WITH SUNBURNS, SUNBED AND SUN EXPOSURE: AN ADULT TWIN STUDY.

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Studies have shown that sun exposure is naevogenic. This twin study involving 752 MZ and 2034 DZ twins was set up to investigate the role of genes and environment on the expression of naevi. Mean total body naevus count was 34. Naevi were found to be under strong genetic control with greater correlation in naevus counts in MZ twins (intra-class correlation 0.86) compared to DZ twins (intra-class correlation 0.57) giving an heritability of 55% 1. The role of sun exposure and sunburns on naevi expression was also investigated. The mean number of weeks over a lifetime on holidays in hot climates was 55. Numbers of naevi were strongly associated with number of weeks on holidays even after adjusting for age ($p<0.0001$). The risk of having more than 50 naevi increased significantly with increasing number of weeks (test for trend $p<0.0001$). The mean numbers of naevi for twins having more than 35 weeks of holidays was 39 compared to 30 for those having equal or less than 35 weeks of holidays. Mean number of severe sunburns over a lifetime was 2.7. Numbers of sunburns were also strongly associated with high naevus counts even after adjusting for age ($p<0.0001$). A positive trend was found for risk of having more than 50 naevi with increasing numbers of sunburns (test for trend $p<0.001$). Naevus counts were also strongly correlated with sunbed exposure even after adjusting for age, weeks on holidays and sunburns ($p<0.0001$). Mean number of naevi in sunbed users was 40 compared to 24 in non users. Analyses of discordant twin pairs for sun exposure and sunbed exposure also confirmed the role of ultraviolet light in the induction of naevi. Linkage and association studies are currently under way as well as studies looking at gene-environment interactions.

BE1-7 NODULAR MELANOMAS DIFFER FROM SUPERFICIAL SPREADING MELANOMAS EPIDEMIOLOGICALLY - POSSIBLE IMPLICATIONS FOR CAUSATION

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Objective: Nodular melanomas (NM) account for most thick melanomas and exhibit distinct clinicopathological features. Little epidemiological evidence is available in support of NM as a separate entity that differs from other radial growth phase melanomas. The objective of this study was to explore the epidemiological associations of NM in comparison to superficial spreading melanoma (SSM). Methods: A cross-sectional study was conducted between April 2003 and September 2004, based on clinical interviews, skin examinations and pathology reviews of participants with primary melanomas. Participating centres included public hospital-based multidisciplinary melanoma referral centres and private dermatology practices in Victoria and New South Wales, Australia. Results: Logistic regression analysis was performed on 88 participants with NMs and 332 with SSMs. In comparison to SSMs, NMs more often occur on chronically sun-exposed sites and in individuals with a history of solar keratoses. NMs more often occur in those who report low level of childhood sun exposure and no history of blistering sunburns. NMs more often occur in individuals with no dysplastic naevi, fewer total body melanocytic naevi, darker complexion and no family history of melanomas. NMs are more common among males and the elderly. When all above variables (significant on univariate analysis, $P<0.05$) were entered into a multivariate model, chronic sun-exposed sites (odds ratio (OR) 4.0, 95% confidence interval (CI) 1.2-13, low level of childhood sun exposure (OR 4.1, CI 1.4-12), the absence of dysplastic naevi (OR 10, 95% CI 1.1-101), older age (>70, OR 5.4, CI 1.7-17) and male gender (OR 3.0, 95% CI 1.1-8.5) remained as independent associations with NMs. Conclusion: There appear to be important differences between NM and SSM with respect to the early childhood sun exposure, cumulative lifetime sun exposure, host melanocytic naevi pattern, gender predilection and age distribution. These associations potentially suggest differences in aetiology.

BE1-8 SUN EXPOSURE AND NODULAR MELANOMA INCIDENCE.

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BACKGROUND: Nodular melanomas represent only 10-15 % of melanomas but are important contributors to melanoma mortality and a challenge in melanoma control. We investigated the eventual role of sun exposure in nodular melanoma aetiology by searching for a gradient in incidence with decreasing latitude. METHODS: We used data from the 9 original registries (but excluding Hawaï) of the National Cancer Institute Surveillance Epidemiology and End Results (SEER) Program. We calculated the age-adjusted incidence rate (world population, ASRw) for Northern vs Southern counties, using the latitude of 40°N as a cut off between North and South. We built a Poisson regression model of the age-adjusted incidence rate in a county according to year of diagnosis, sex, and latitude of the county. RESULTS: During years 1973-2000, 72037 invasive melanomas were reported to SEER registries. ASRw was significantly higher for Southern counties than for Northern counties for superficial spreading melanoma (SSM), for both males (4.96/100000 in Southern counties vs 4.08/100000 in Northern counties $p = 0.03$) and females (4.94/100000 vs 4.12/100000, $p = 0.03$). But this was not the case for nodular melanoma, neither for males (1.06/100000 vs 1.04/100000, $p = 0.41$) nor for females (0.70/100,000 vs 0.70/100,000, $p = 0.49$). In multivariate analysis, we confirmed the increase of incidence with decreasing latitude of residence for SSM, Lentigo maligna melanoma and other forms but not for nodular melanoma. CONCLUSIONS: Nodular melanoma does not exhibit the classical gradient in incidence with decreasing latitude. Further research should aim at redefining melanoma risk factors according to histology, more especially for nodular melanoma, if melanoma mortality is to be controlled.

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BE2-1 EPIDEMIOLOGICAL EVIDENCE SUPPORTING THE ROLE OF UVA IN MELANOMA CARCINOGENESIS

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It is important to delineate the specific roles of UVA and UVB radiation in melanoma carcinogenesis because most current sunscreen formulations transmit large amounts of UVA. While UVB directly mutates DNA, UVA mutates DNA indirectly through free-radical mediated oxidative damage (1). A pertinent natural experiment began in 1922 with the discovery of UVB sunscreens, which allowed much longer exposures to UVA. Increasing sales of these products, first marketed as suntan lotions and later as sunscreens, was accompanied by a marked rise within 15-25 years in age-adjusted incidence rates of melanoma in several countries (2, 3). A recent study (4) assessed whether melanoma mortality rates are more closely related to the global distribution of UVA or UVB. Age-adjusted melanoma mortality rates were obtained for 45 countries reporting cancer data to the World Health Organization. Paradoxically, melanoma mortality rates decreased with increasing UVB in men ($r = -0.48$, $p < 0.001$), and women ($r = -0.57$, $p < 0.001$), and increased with increasing UVA in both sexes. After multiple adjustment that included controlling for skin pigmentation, UVA was positively associated with melanoma mortality rates in men ($p < 0.02$) with a suggestive but non-significant trend for women ($p = 0.12$). Further epidemiological evidence supporting a role of UVA in melanoma carcinogenesis is presented. 1. Wang SQ, Setlow R, Berwick M, Polsky D, Marghoob AA, Kopf AW, et al. Ultraviolet A and melanoma: a review. J Am Acad Dermatol 2001;44(5):837-46. 2. Garland C, Garland F, Gorham E. Could sunscreens increase melanoma risk? Am J Public Health 1992;82:614-5. 3. Garland C, Garland F, Gorham E. Rising trends in melanoma:an hypothesis concerning sunscreen effectiveness. Ann Epidemiol 1993;3:103-10. 4. Garland CF, Garland FC, Gorham ED. Epidemiologic evidence for different roles of ultraviolet A and B radiation in melanoma mortality rates. Ann Epidemiol 2003; 13(6):395-404.

BE2-2 CDKN2A GERMLINE MUTATIONS IN INDIVIDUALS WITH SPORADIC CUTANEOUS MALIGNANT MELANOMA

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Introduction: CDKN2A has been identified as the major melanoma susceptibility gene based on the presence of germline mutations in high-risk melanoma families. In the present study we sought to characterize the mutation spectrum of CDKN2A mutations in individuals with sporadic melanoma using a novel population-based study design. Methods: A total of 3723 DNA samples from 1238 individuals with multiple primary melanoma and 2485 with single primary melanoma were available for screening of CDKN2A mutations. All participants signed informed consent and completed an epidemiologic questionnaire. The identification of CDKN2A mutations consisted of an initial screening by Denaturing High Performance Liquid Chromatography (DHPLC) followed by confirmation with direct sequencing. The functional impact of specific nucleotide changes in the p16 protein was determined based on intragenic position, existing data from in vitro studies, sequence and structural analysis. Results: The initial screening by DHPLC revealed 2037 potentially positive samples. Sequencing confirmed nucleotide changes in the 5 - UTR (31 patients), in the coding region (n=301), in intron 1 (n=25), and in the 3 UTR (n=1680). The nucleotide changes found in the coding region included 4 insertions or deletions that affected p16 in 11 patients, 29 missense mutations that affected p16 and/or p14ARF in 57 patients, and the common polymorphism in codon 148, specifically Ala148Thr, in 232 Patients. We found a total of 33 relevant mutants that affected 65 melanoma patients. The prevalence of functionally relevant mutations was 3.0% in cases and 1.3% in controls. The following new point mutations were identified: Arg58Gln, Ala60Val, His83Glu, Arg99Trp, Ala102Thr and Arg124Cys. The relevant mutants will be described in detail. Conclusion: CDKN2A mutations are more common among sporadic cases of melanoma than previously thought.

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BE2-3 ASSOCIATION OF ASIP GENOTYPE AND MELANOMA RISK WITH RESPECT TO MC1R GENOTYPE

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Objective: Although polymorphisms in agouti signaling protein (ASIP) and the melanocortin-1 receptor (MC1R) genes have been associated with melanoma-risk pigmentation phenotypes, only variants in MC1R have been shown to be independently associated with melanoma risk. Because of their biologic relationship as ligand and receptor, we investigated the combined effect of ASIP and MC1R variants upon melanoma risk in a white population from the mid-Atlantic region of the United States. Methods: DNA from individuals with melanoma (n=524) and healthy controls (n=215) enrolled in an ongoing case-control study of melanoma etiology were genotyped for the ASIP g.8818A>G SNP using PCR-RFLP and for MC1R variants by direct sequencing. Stratified logistic regression models were used to examine the association of the ASIP g.8818G-allele and melanoma against different MC1R genetic backgrounds and after adjustment for age and sex. Results: There was no overall effect of the ASIP SNP upon melanoma risk [OR=0.98 (0.67, 1.5)]. Among MC1R consensus carriers, the OR for the ASIP g.8818G-allele was 1.2 (0.55, 2.5). The effect of ASIP appeared to act in opposite directions among individuals carrying only low risk MC1R variants [defined as V60L, G89R, V92M, T95M, I120T, R163Q, Y183D, V205M, P230L, G236D, N279L, N290S; OR=0.64 (0.34-1.2)] and among those carrying high risk variants [defined as carriage of g.86_87insA, R67W, S83L, A81P, S83P, D84E, G104S, g.411delC, R142H, R151C, Y152X, I155T, R160W, g.537_538ins, D294H, R306H; OR=1.5 (0.73, 3.2)]. Conclusion: Although results were not statistically significant, these data suggest a difference in the effect of the ASIP g.8818A>G polymorphism dependent upon the genotypic constitution of MC1R. This informal introductory look at a gene-gene 'interaction' of low penetrance genotypes in the etiology of melanoma indicates the important of considering such effects, and points toward the necessity for large scale, well-designed, molecular/genetic epidemiological studies of melanoma.

BE2-4 POLYMORPHISMS OF THE VITAMIN D RECEPTOR GENE AND RISK OF MELANOMA: A CASE-CONTROL ANALYSIS

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Objective: Calcitriol [1,25-(OH)2D3], the hormonal derivative of vitamin D3, is an antiproliferative and prodifferentiation factor for several cell types, including cultured melanocytes and malignant melanoma (MM) cells. Several restriction fragment length polymorphisms (RFLPs) of the vitamin D receptor (VDR) gene have been described, including a FokI RFLP in exon 2 and BsmI and TaqI RFLP in exon 9. These polymorphisms have been shown to be associated with several systemic malignancies. We hypothesize that VDR polymorphisms are associated with the risk of developing MM. Methods: A case-control study of 504 MM cases and 505 controls was conducted to assess the association between VDR polymorphisms and MM risk. The cases and controls were frequency-matched on age, sex, and ethnicity. Polymorphisms at the FokI, BsmI, and TaqI sites were determined using genomic DNA by polymerase chain reaction-RFLP methods. Results: The homozygous TaqI TT was associated with a significantly increased MM risk (adjusted odd ratio [OR] = 1.37, 95% confidence interval [CI] = 1.05-1.78) compared with the combined genotype (Tt + tt), but this association was not observed for the BsmI and FokI polymorphisms. However, when the three polymorphisms were evaluated together by the number of risk alleles (i.e., the TaqI T, BsmI b, and FokI f alleles), the combined genotype with more than four risk alleles was associated with significantly increased risk (OR = 1.57, 95% CI = 1.10-2.23) compared with other genotypes, and the trend in increasing risk associated with increasing number of risk alleles was statistically significant (Ptrend = 0.021). Discussion and Conclusion: Genetic variants in the VDR gene may contribute to the etiology of MM. Specifically, the TaqI TT homozygotes appeared to have an increased risk of MM, and the TaqI T, BsmI b, and FokI f alleles appear to have a joint role in the susceptibility to MM.

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BE2-5 THE BRAF (T1799A) MUTATION IS ASSOCIATED WITH DISTINCT CLINICAL CHARACTERISTICS IN INVASIVE PRIMARY MELANOMA

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Objective: Mutation in the BRAF gene, predominantly a single base substitution (T1799A), has been found in 66% of malignant melanomas. BRAF (T1799A) encodes an activated form of BRAF (V600E) that leads to activation of the RAS/RAF/MAPK pathway. Despite a large number of studies, the clinical and pathological associations of T1799A in primary melanoma remain poorly understood. The objective of this study was to assess the clinical correlates of primary melanomas containing the T1799A mutation. Methods: A total of 264 patients with 265 invasive primary melanomas were prospectively interviewed and examined with respect to their melanoma characteristics and risk factors. Independent pathological reviews were performed. All primary melanomas were screened with allele-specific PCR for the T1799A mutation. The allele-specific PCR technique allows for detection of 2% mutant DNA sequences in wild type DNA. Results: Of 265 tumour samples, 251(95%) samples were successfully amplified. The T1799A mutation was found in 112(45%) of the primary melanomas. Univariate analysis revealed that the T1799A mutation is significantly associated with: 1) thinner tumours; 2) less mitotically active tumours; 3) superficial spreading melanomas (SSM); 4) pigmented melanomas; 5) intermittently sun-exposed sites; 6) younger age; 7) the lack of a history of solar keratoses; 8) individuals with fewer freckles. The rate of T1799A mutation was 7%(1/15) in amelanotic melanomas occurring in individuals with a history of solar keratoses, compared to 61%(69/114) in pigmented melanomas occurring in individuals with no history of solar keratoses. When all competing associations were entered into a multivariate logistic regression model, individuals with fewer freckles, SSM, pigmented melanomas and the lack of history of solar keratoses remained as independent associations with the T1799A mutation (P<=0.05). Conclusion: In primary invasive melanomas, the T1799A mutation was associated with degrees of freckles and the lack of cumulative sun exposure in host, as well as histological subtype and tumour pigmentary characteristics.

BE2-6 KIR 2DS4 GENOTYPES IN SPORADIC AND FAMILIAL MELANOMA FROM ITALY

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OBJECTIVE. To explore the role of the natural killer cell activating receptor KIR2DS4 in sporadic and familial melanoma from northeastern Italy. METHODS: We genotyped KIR2DS4 for a common deletion variant (denoted A197) which produces a soluble KIR molecule in 635 subjects, including 258 cases and 377 controls from a case-control and a family study of melanoma. Subjects' host factors were assessed by a single dermatologist by skin examination and questionnaire. Odds ratios were estimated by logistic regression models. RESULTS. The A197 genotype was negatively associated with melanoma risk after adjustment for age and sex (OR=0.45, 95%CI=0.2-0.9, and OR=0.55, 95%CI=0.2-1.8, in the case-control and family study, respectively). The KIR2DS4 genotypes were not associated with the thickness or body location of melanoma lesions in a case-only analysis. We tested several variables for effect modification on the association between KIR2DS4 and melanoma, given their potential role in the regulation or alteration of the immune system. The A197 genotype was significantly and negatively associated with melanoma risk in subjects who had intense sun exposure within 5 months prior to the study (OR = 0.18, 95% CI =0.04- 0.8). Conversely, glucocorticoid use, parity, life-time sun exposure, and number of nevi did not significantly modify the association between KIR2DS4 and melanoma. CONCLUSIONS. The A197 genotype, in combination with the inflammatory/immune-suppressive effects of recent and intense sun exposure may play an important role in protecting against melanoma formation. KIR genes are tandem arrayed over 150 Kb, with the remarkable feature that gene content varies between haplotypes. The A197 marker may be in linkage disequilibrium with an unknown gene on the same KIR array. Further research on additional KIR genes and their role in melanoma risk is planned.

NOTES:

BE2-7 TRENDS FOR IN-SITU AND INVASIVE MELANOMA IN QUEENSLAND, AUSTRALIA, 1982 TO 2002

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Objective: In Queensland, early detection programs began in the 1960s and primary prevention in the 1980s. We wished to assess the trends for in-situ and invasive melanomas to consider the implications for early detection and primary prevention. Methods: Data from 1988 – 2002, in the Queensland Cancer Registry, was analysed using Poisson regression to estimate the annual percentage change in rates across the 21 years of incidence data for in-situ and invasive lesions, stratified by age and sex. Joinpoint analyses were used to assess whether there had been a statistically significant change in the trends. Results: In 2002 the overall incidence was 78/100,000. In-situ melanomas increased by 10.4% per year among males and 8.4% per year among females. Invasive lesions increased to a lesser degree (males 2.6%, females 1.2%). The more reliable data on thickness was available from 1991 to 2002 when thin-invasive lesions increased faster than thick-invasive lesions (eg males: thin 3.8%, thick 2.0%). There was a non-significant trend to a lower proportionate increase for the most recent years. Patients younger than 35 years had a stable incidence of invasive melanoma. There was a suggestion of a birth cohort effect from about 1958. Mortality rates were stable across all ages, although there was a non-significant trend to a decreasing rate among young women. Conclusions: There was a suggestion that the stabilisation of mortality rates may be related to earlier detection implying progress with primary prevention in Queensland. Incidence rates are stabilising in those younger than 35 years and the proportionate increase in both in-situ and invasive lesions appears to be lower for the most recent period compared with the past. However, even taking the most favourable view of these trends, primary prevention is unlikely to lead to decreases in the overall incidence rate of melanoma for at least another 20 years.

BE2-8 FEASIBILITY OF A POPULATION-BASED REGISTRATION OF PHENOTYPIC, ANATOMIC AND FAMILIAL DATA FOR MELANOMA

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A multicentric study was set up to assess the feasibility for Swiss cancer registries of actively retrieving and coding 3 additional variables of epidemiological and aetiological relevance for melanoma, and of use for the evaluation of prevention campaigns. A questionnaire was developed to seek skin type, family history of melanoma and precise anatomical site of all melanomas registered in Switzerland over 3 to 6 consecutive years, ranging from 1995 to 2002. Four regional registries participated and sent 1,645 questionnaires to the physicians who originally notified the tumours; 1,420 (86.3%) were returned. Forthe anatomical site, a cross-validation analysis was performed between the textual information collected by registries and the pictorial information coded from the questionnaires, and site-specific rates per unit of body surface area were calculated. Detailed cutaneous site and skin type were reliably obtained for more than 90% of returned questionnaires. Family history was known in 76% of instances, but this information needs further validation. Prevalence of sun-sensitive subjects and patients with melanoma affected first-degree relatives, two target groups for early detection and surveillance campaigns, were 54.1% and 3.4%, respectively. Overall, 94.6% of anatomical site codes from printed and pictorial information support concurred. Discrepancies occurred mostly for lesions on the upper, outer part of the shoulder for which the clinician's textual description was "shoulder blade". This differential misclassification suggests an under-estimation by about 10% of melanomas of the upper limbs and an over-estimation of 5% for truncal melanomas. Spatial differences in the cutaneous distribution of melanomas in Switzerland and in phenotypically different Anglo-Saxon populations, for which most of the published material referred to, are discussed. The feasibility of retrieving the skin type, the precise anatomical location and the family history of melanoma was demonstrated thanks to the collaboration of Swiss dermatologists. Recommendations to improve quality and availability of detailed body site information are devised.

NOTES:

BE3-1 THE CHANGING INCIDENCE OF CUTANEOUS MELANOMA IN THE WEST OF SCOTLAND AND QUEENSLAND 1982-2001, AN INTERCONTINENTAL COMPARISON

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We have compared the changing incidence of cutaneous melanoma in the west of Scotland and Queensland, Australia over the decades 1982-1991 and 1992-2001 by age group, sex and body site, divided into head and neck, trunk, upper limb and lower limb.We have studied superficial spreading and nodular melanomas combined. In total there were 3885 superficial spreading and nodular melanomas in the west of Scotland, and there were significant increases in both sexes and all three age groups 0-39, 40-59 and 60 and over when the decades 1982 -1991 and 1992-2001 were compared. In males aged 60 years and over there were highly significant increases of 83%in melanomas on all sites, 115% in trunk lesions, and 96% in upper limb lesions. In females the greatest increases were 94% in trunk lesions, and 99% in upper limb lesions, both in females in the 40-59 year age group. For 19,634 melanomas in Queensland there were no significant changes in incidence in females aged 0-39, or females aged 40-59, or males aged 0-39, but significant increases in males aged 40-59, and in both males and females aged 60 and over. In the 60 and over group, there were significant rises in all body sites in males, but in fenmales the rise was only significant in upper limb lesions These data suggest that public education on melanoma prevention is showing results in Queenslnd and indicate that such public education is required in Scotland.

BE3-2 SEASONAL VARIATION IN MELANOMA INCIDENCE IN NEW SOUTH WALES REVEALS THE EFFICACY OF EARLY DETECTION AND EDUCATIONAL CAMPAIGNS

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Seasonal variation in melanoma incidence with a summer peak is yet poorly understood. If it were due to a seasonal increase in awareness, then an increase in thinner melanomas and better survival from summer melanomas independent of other prognostic factors would be expected. Alternatively, if late stage promotion of melanoma were responsible for the summer peak, an increase in more aggressive melanomas in summer and a worse prognosis might be expected. During the period 1989-1998, 25,845 cases of melanoma were reported to the New South Wales Cancer Registry (10,869 females and 14,976 males). Breslow thickness was reported for 23,116 cases (mean = 1.36mm , median of 0.70, interquartile range 0.45-1.50). There was significant seasonal variation in incidence of melanoma ($p<0.0001$); summer to winter ratio was greater for women, younger people, lesions on the limbs, and SSM. Melanomas were thickest in winter (mean 1.45 mm, median 0.75, IQ 0.45-1.6) and thinnest in summer (mean 1.28 mm, median 0.70, IQ 0.45-1.3), mainly due to an increase of thick melanomas in winter ($p<0.0001$). The mean follow-up of cases was 68 months (median 63 months, interquartile range 37 - 97 months); 2,710 (10.5%) died from their melanoma. Fatality from melanoma was lower for melanomas diagnosed in summer compared to winter with a relative fatality of 0.72 (95% CI 0.65-0.81) in univariate analysis; the 5 years survival rate was 92.1% for diagnosis in summer and 89 % for diagnosis in winter. This result remained significant and of the same order after multiple adjustment (relative fatality 0.82). In Australia, the increase in melanoma incidence in summer is correlated with a significant 6% decrease of Breslow thickness and a significant 40% change on the prognosis. Hence, the seasonal diminution of melanoma thickness in summer is an insufficient explanation for the difference in prognosis by season of diagnosis.

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BE3-3 MELANOMA PREVENTION: EVALUATION OF AN EDUCATIONAL CAMPAIGN USING A NEW VIDEO-GAME IN PRIMARY SCHOOL CHILDREN

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OBJECTIVE: To evaluate the effectiveness of a professionally designed CD-ROM game to limit children's sun exposure. METHODS: Prospective, multicenter trial with before-after comparisons settled in 4 primary schools of the Paris suburb. A CD-ROM game named 'Adventures of burned head', written by dermatologists and professional video-games designers, was video-projected during a 4-week period (June 2004) to primary school children in their fourth year (N= 246, age: 9). They played the game collectively under their teacher's supervision. Standardized questionnaires were filled by the children before and immediately after playing the game and in October (after the summer break). Parents filled another questionnaire before the campaign and in October. Main outcome measures were: 1/ changes after the video-game in children's answers concerning their knowledge, attitude, and behavior toward the sun, 2/ impact of these messages on the children and parents behaviors during summer holidays. Comparisons between the children's and parents' answers before and after the campaign were made using paired chi2 tests, and analysis of variance. RESULTS: Compared with the pre-video-game answers, children improved their knowledge and behavior, which were maintained after the summer holidays (i.e., 85% claimed that a parasol provides sufficient sun protection before video-game vs. 30% post-holidays; P<0,001). Children with a fair complexion showed identical improvement in their responses. In the post-holidays questionnaires: 1/ 92% (95%CI: 87-97) of the children stated that the game helped to learn sun-protection attitudes; 2/ 66% (95%CI: 60-72) of the parents stated that the video-game had changed their child's behavior regarding sun exposure; 3/ 48% (95%CI: 41-55) of them declared that this campaign modified their own behavior. CONCLUSION: Health education campaigns using an attractive video-game may improve the knowledge, attitude, and behavior of young children and perhaps of some of their parents.

BE3-4 FEASIBILITY OF BEHAVIORAL INTERVENTION WITH MELANOMA SURVIVORS

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Survivors of melanoma are at an approximately 25% risk of developing another primary melanoma. Performance of skin self and physician examination, and vigilant sun protection, are clinically recommended to reduce the risk for new or unresectable diagnoses. To lay the groundwork for an intervention study to increase utilization of risk reduction behaviors among melanoma survivors, we assessed current behavioral adherence and preferred skin cancer information sources in recently diagnosed melanoma patients. Participants (N=35) were recruited through the National Cancer Institute-funded population-based cancer registry in the Pacific Northwest into the Cancer Genetics Network, a familial registry of cancer patients and their families. Participants were, on average, 50 years old (range 27-73 years), were exclusively Caucasian, 60% female, and had been diagnosed on average 1.6 years previously (range 0.5-3.3 years) at the time of recruitment. Adherence to sun protection and early detection strategies was not universal, with 62% wearing sunscreen SPF > 15, 47% wearing a hat, and only 48% seeking shade often/always while outdoors. Of note, 26% stated they never wore a hat with a brim wide enough to protect their ears and neck (2.5 inches). Most participants (89%) reported physician skin cancer screening annually; but only 12% reported performance of thorough, deliberate skin examination (Weinstock et al., 1999). Participants were highly receptive to physician-provided melanoma information, with 85% reporting that they talk with a physician some or a lot concerning melanoma. Almost half (44%) reported that they went to the internet for melanoma information at least some of the time; and a third (35%) reported, respectively, that they consulted magazines and consulted medical journals/articles at least some of the time for melanoma information. These results indicate opportunities to improve prevention and early detection behaviors, and concomitant receptivity to melanoma information provided via physicians and the internet among melanoma survivors.

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BE3-5 TANNING ATTITUDES AND BELIEFS AMONG SORORITY AND FRATERNITY STUDENTS

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OBJECTIVE: The objective was to describe tanning habits among fraternity and sorority students. We also examined nevi as an early marker for melanoma. MATERIALS & METHODS: We surveyed students about their attitudes towards tanning, their tanning bed use, sunless tanning products, sunscreen, sunburns, sun sensitivity, and recent sun exposure. A dermatologist conducted a skin examination including nevi counts and measured skin color using a colorimeter. Clustering techniques accounted for tendency of attitudes and behavior to cluster within Greek houses and extrapolated frequencies to all university fraternity and sorority students. The Wald log-linear chi-square test was used to test differences by sex and skin color. RESULTS: Eight Greek houses were recruited after Spring Break with 163 students age 18-23 participating. Among females, 91% were current tanning bed users compared to 49% of males. Likewise, 90% of females and 77% of males stated that tanning was somewhat or very important. The most popular reason for wanting a tan was to feel more attractive, followed by looking healthier. 57% of females used tanning beds before they know they are going to be in the sun. However, 25% of females reported using self-tanning creams because they are safer than the sun or tanning beds. Unlike medium or dark skinned individuals, fair skinned subjects stated theyused tanning beds so that they could spend more time in the sun. CONCLUSION: These findings have several potential implications to skin cancer prevention. College age females are more likely than males to intentionally tan. However, it is encouraging that some of these women use tanning creams because they are considered safer. Unfortunately, many use tanning beds prior to sun exposure or to prolong their sun exposure. Thus public health statements need to address the harmful effect of tanning bed use and prolonged sun exposure.

BE3-6 VALIDATING SELF-REPORTED SUN HABITS OF BEACHGOERS

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Objective: To examine the validity of beachgoers' self-reported sun protection and UV exposure using objective measures. Materials & Methods: Participants (n=88) completed a brief survey when they arrived at the beach, their skin was swabbed for the presence of sunscreen, while a second observer recorded participants' clothing items and presence of sunburn. Upon leaving the beach, participants completed a diary detailing their activities and sun habits while on the beach, follow-up sunscreen swabs were obtained, and sunburns were recorded. Clothing observations were made for a subgroup (n=24) of participants during their beach stay. Results: On average, participants reported spending 3.1 hours at the beach, which was consistent with researcher observations ($r=0.96$, $p<0.01$). Participants' self-report of clothing worn to the beach (from follow-up diary) had good agreement with researcher observation: Kappa's [k] ranged from 0.63 for footwear to 0.77 for headwear. Agreement between self-report and observation varied for clothing worn on the beach: lower agreement was obtained for sunglasses (k=0.11) and footwear (k=0.25) while there was good agreement for upper body clothing (k=0.79). At baseline a moderate to high level of agreement was achieved between reported use of sunscreen and sunscreen swabbing (leg: k=0.50; arm: k=0.66; face: k=0.77). Agreement between self-reported and observed sunburn was low (face: k=0.21; arms: k=0.39; legs: k=0.33) with participants reporting more sunburn upon arrival than was observed. Conclusion: Overall, self-report measures of time outside, clothing worn, and sunscreen use demonstrated good criterion validity when compared with observation and sunscreen swabbing. Sunscreen swabbing, previously tested in a controlled environment, proved an effective procedure for detecting sunscreen at a beach setting. This study improved our understanding of how to incorporate both innovative and recognized objective procedures to assess the validity of conventional data collection approaches toward sun protection measurement.

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BE3-7 PREDICTING ABSOLUTE RISK OF MELANOMA: A MANAGEMENT MODEL FOR USE BY PRIMARY CARE PROVIDERS.

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Background Routine screening for melanoma with complete skin exams is controversial at this time because of high costs and error rates. There is, however, agreement that primary care providers can play an important role by identifying individuals at high risk. Methods Data were analyzed from 718 non-Hispanic white patients with invasive cutaneous melanoma from melanoma clinics in Philadelphia and San Francisco. Matched controls were 945 patients from outpatient clinics with similar catchment areas. We identified characteristics that can be easily and quickly ascertained during a routine physical exam. Host characteristics included: skin complexion and sunburn type and presence of large/small nevi on examination of only the back. Measures of sunlight exposure and response included: blistering sunburn, degree of freckling on the back, and solar damage on the back. Results Relative risk models yielded an attributable risk of 86% for men and 95% for women using only seven of these variables. Attributable risks did not vary by age, UVB flux or hours outdoors. The models, along with incidence and mortality rates, were used to develop estimates of the absolute risk of developing melanoma within five years. Discussion We developed procedures to estimate the absolute risk of developing melanoma that are specifically to assist the primary health care provider in identifying patients at high risk. Such high risk individuals might then be given (or referred for) a complete skin examination, counseled to avoid sun exposures and scheduled or perhaps referred for regular surveillance. It is important to emphasize that these projections are not intended to identify melanoma cases.

BE3-8 THE STUDY OF NEVI IN CHILDREN (SONIC): BASELINE FINDINGS

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Objective: The purpose of this study is to document the natural history of nevi and assess associations between sun exposure, sun protective behaviors, pigmentation genes and prevalence and progression of common nevi over a four year follow-up. We report baseline data from the first US longitudinal study of children during preadolescence. Materials & Methods: We conducted a survey, examination, and imaging (overview, close up and dermoscopic images of back) in consenting 5th graders in Framingham, Massachusetts (USA) School System (n=443/691). We obtained information on demographics, phenotype, sun sensitivity, childhood sun exposure and sun protection practices. An examination of back nevi was performed (digital photography and dermoscopy) and images are being evaluated for multivariate analyses. Genetic specimens are currently being collected. Results: 62% of children were male, 70% were Caucasian, and median age was 10.7 years. Thirty four percent reported that they burn easily in the sun, while over 70% reported that they get a moderate-deep tan when outside on sunny days. Student self-reported mole count showed that 69% reported 0-5 moles on a single arm. Fifty-two percent of the students reported that they like to tan and nearly 86% reported trying to get a tan. Over 50% of students reported e 1 sunburns in the previous summer. Students reported using sunscreen often/always while at the beach or pool (>62%). Fair skin and tendency to burn were positively associated with self-reported mole count, although use of sunscreen was not a significant factor. Conclusion: Recent studies have shown childhood is a critical time for the evolution of nevi and that clinical features of nevi in this age group are dynamic. The insights into nevus etiology and evolution gleaned from this study are anticipated to have significant implications for reduction of melanoma mortality through improved risk stratification and more informed prevention/detection strategies.

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CL1-1 IMPACT OF PRIMARY MELANOMA RISK FACTORS AND OCCULT METASTASIS IN SENTINEL NODE(S) ON SURVIVAL: RESULTS FROM A MULTICENTER SENTINEL LYMPH NODE WORKING GROUP

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OBJECTIVE(S): Sentinel lymphadenectomy represents a major advance in the management of primary melanoma (PM) patients. Our objective is to determine the impact of PM risk factors and sentinel lymph node (SLN) status on disease-free and overall survival (OS) in PM patients. METHODS: The SLN Working Group was founded in 2003 for investigators to advance the treatment of cancer patients with emphasis in the SLNs. Nine centers have submitted data on 1370 PM patients with IRB approval and adherence to the HIPAA regulations. Cox proportional hazard model was used to analyze 1356 evaluable patients. RESULTS: Over a median follow-up period of 3.7 years, 16.3% (n=221) of subjects had at least one positive SLN. Tumor thickness (TT) was greater in the SLN positive group (3mm vs 2mm, p<0.0001). Ulceration was twice as prevalent in SLN positive than the negative group(p<0.0001). The incidence of positive nodes was nearly 50% for trunk melanoma and 11% for head and neck (H&N) and upper extremity melanom. Overall mortality was 6.4% with a higher proportion of deaths in the positive SLN group (p<0.0001). Multivariate analysis showed that SLN status was the most important predictor of mortality (p<0.0001). Other significant variables to predict OS were TT (p<0.0001) and ulceration (p=0.009). The H&N (p=0.03) and the upper extremity melanoma (p=0.03) had a much worse outcome as compared to that of the lower extremity. About 20% of the subjects experienced at least one recurrence with a rate about three times higher in the SLN positive than the negative group (p<0.0001). By multivariate analysis, only SLN status (p<0.0001) and TT (p<0.0001) were significant predictors of recurrence CONCLUSIONS: TT, tumor anatomical sites and SLN status have been found to be significant prognosticicators in this large multicenter analysis, which may be used as important determinants to subgroup PM patients for adjuvant therapy.

CL1-2 HETEROGENEITY OF MICROSCOPIC STAGE III MELANOMA IN THE SLN ERA: IMPLICATIONS FOR AJCC/UICC STAGING AND FUTURE CLINICAL TRIAL DESIGN

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Objective: The recent AJCC/UICC analysis for patients with stage III (nodal) melanoma demonstrated that this group of patients is heterogeneous in prognosis. Five-year survival rates ranged from 13%-69% and depend on: # of positive lymph nodes (LN), macroscopic versus microscopic disease, and presence of primary tumor ulceration. Patients with microscopic nodal involvement based on sentinel lymph node (SLN) biopsy are presumed to have a better prognosis than patients with macroscopic nodal disease, but little is known concerning survival and prognostic heterogeneity of the SLN positive population. Methods: Between 1991 and early 2001, 237 SLN positive patients were identified among 1442 patients in a prospective SLN database. Several known stage III prognostic factors, including the above-mentioned AJCC criteria, as well as surrogates of microscopic tumor burden (largest SLN metastatic focus (mm), SLN tumor location [subcapsular only versus other]) were analyzed with respect to disease-specific survival (DSS). The Cox proportional hazards regression model was used to identify independent prognostic factors. Results: Of these 237 SLN-positive patients, nearly 2/3 had only one positive node, and 45% percent had ulcerated primary tumor. The median largest SLN tumor focus was 1.7mm and 65% were subcapsular only. At a median follow-up of 4 years, the 3-year DSS was 79%. A worsening DSS was associated with increasing tumor burden (either criterion). By multivariate analysis, # positive LNs, primary tumor ulceration, and microscopic tumor burden were independent predictors of DSS. A prognostic model was developed (Figure 1). Conclusions: While SLN-positive patients have a better survival compared to macroscopic LN-positive patients, the prognosis among SLN-positive patients is quite heterogeneous, and dependent on the extent of microscopic tumor burden, primary tumor ulceration, and # of positive nodes. These findings have significant implications for: (1) defining revisions to the AJCC/UICC staging system and (2) stratification criteria for future adjuvant therapy trials.

NOTES:

CL1-3 LYMPHATIC INVASION IDENTIFIED BY D2-40 AND YOUNGER AGE ARE PREDICTORS OF SENTINEL NODE INVOLVEMENT IN MALIGNANT MELANOMA.

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Background: The identification of lymphovascular invasion on H&E sections is difficult. Our previous study showed an increase in the frequency of identification of lymphatic invasion (LI) using Mab D2-40 in primary melanomas by 16%. The present study assesses whether lymphatic invasion identified by D2-40 is an independent predictor of sentinal lymph node (SLN) positivity. Material and Methods: 96 cases of cutaneous melanoma that had sufficient pathological material and had SLN biopsies were included in the study. Immunohistochemical staining with Mab D2-40 was performed on a single block of the primary melanoma. Circumferential staining of endothelial cells with D2-40 around the tumor cells was interpreted as positive for lymphatic invasion. The clinicopathological features, age, sex, vertical height, Clark's level, ulceration, lymphovascular invasion by H&E and LI by D2-40 were assessed using multivariable logistic regression. Results: 23/96 (23%) cases had positive sentinel lymph node biopsies. 32 (33%) showed lymphatic invasion identified by D2-40. LI by D2-40 was significantly related to SLN (p=0.008). Those cases that were LI positive by D2-40 were more likely to be SLN positive than those that were LI negative by D2-40 (Odds ratio [OR] 6.4, 95% C.I. = 1.6 - 25.3). Age was significantly associated with SLN (p=0.035 with older patients more likely to be SLN negative than younger patients (OR=0.95, 95% C.I. = 0.90 - 0.996). Clark's level, vertical height and ulceration were not significantly associated with a positive SLN. Conclusion: These results show that LI identified by D2-40 in primary melanomas, as well as younger age are independent predictors of a positive SLN biopsy. This information could be considered when recommending SLN biopsy. Follow-up studies relating LI by D2-40 to clinical outcome will be of interest.

CL1-4 PRIMARY MELANOMA MAY NOT ALWAYS METASTASIZE TO THE MOST RADIOACTIVE SENTINEL LYMPH NODES IN THE REGIONAL NODAL BASIN

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Objective: Intraoperative lymphatic mapping for sentinel lymph nodes (SLNs) using hand-held gamma probe usually identifies more than one SLN. Our goal in this study is to correlate the amount of radioactivity in each SLN in which occult metastasis is found. Materials & Methods: About 1054 patients underwent preoperative lymphoscintigraphy and selective sentinel lymphadenectomy at UCSF Melanoma Center from 4/7/94 to 2/25/03. When the SLNs were collected at the time of surgery, the ex vivo radioactive count of each SLN was recorded and submitted to pathology. A SLN was defined by its ex vivo count being 3 times higher than the background. All patients with one or more positive SLNs (174 patients, 16.5% of the total) for micrometastasis by H&E were included in this analysis. Each positive SLN was correlated with the radioactive count being calculated as a % of the hottest SLN. Results: The average number of SLNs being harvested per nodal basin was 2.08. Based on 181 positive SLNs from 174 patients, the distribution of radioactive of SLNs with micrometastasis is shown in the table below: %of Hottest Node # of lymph Nodes # of Positive Nodes % of Positive Nodes 0 86 7 8 10 55 13 24 20 48 13 27 30 34 16 47 40 23 5 22 50 26 10 38 60 17 4 24 70 14 7 50 80 13 9 69 90 16 10 63 100 182 143 79 Total 514 237 Conclusions: Although the hottest SLNs showed a significant number of positive SLNs, the distribution of radioactivity for positive SLNs ranged from 0 to 100% with no definitive break points. The hottest SLNs do not exclusively harbor occult metastasis. In order to minimize the false negative rate, it is important to harvest all SLNs in the regional nodal basin.

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CL1-5 MELANOMA METASTASES IN LYMPH NODES IDENTIFIED BY PROTON MAGNETIC RESONANCE SPECTROSCOPY OF FINE-NEEDLE BIOPSIES

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Introduction: Sentinel node (SN) evaluation offers advantages over SN removal. Proton Magnetic Resonance Spectroscopy (MRS) of lymph node needle aspirates accurately identifies melanoma micrometastases based on the presence of diagnostic metabolites. Validation of this technique would reduce the morbidity and costs of SN evaluation. Methods: Fine needle aspiration biopsies (FNAB) from 70 malignant and 42 benign nodes were obtained from patients undergoing node resection for metastatic melanoma. Proton MRS (8.5 T) was carried out using standard protocols (4). Four 5(m sections from each node block (3mm thick) were stained with H&E (2 sections) and for S100 protein and HMB45. MR spectra and histopathology were correlated using a statistical classification strategy (SCS) (3). Results: Figure 1 shows proton MRS of FNAB from benign and metastatic nodes. Resonances are those consistent with lipid (Lip), amino acids, lactate, creatine (Cre), phosphocreatine, choline (Chol) metabolites and inositol. An mathematical classifier was generated for benign and metastatic nodes using a Statstical Classification Strategy (SCS)-based approach. In four training sets, spectra from 47 metastatic and 28 benign nodes were subjected to SCS. Using the five most discriminatory spectral regions, metastases were predicted with a sensitivity of 97.3%, a specificity of 90.2% and an accuracy of 94.7%. In validation sets (duplicate samples not used in the training sets), including 23 metastatic and 14 benign nodes, the presence of metastases was predicted with a sensitivity of 93.5%, a specificity of 87.5% and an accuracy of 91.2%. The crispness of the data (% samples with a class probability >75%) was ~88% for training and validation sets. These data indicate that, following clinical validation of this technique, SN staging of melanoma may be achieved without surgical biopsy and histopathology. Conclusions: Proton MRS of FNAB of lymph nodes provides accurate diagnosis of metastatic disease in melanoma patients.

CL1-6 LYVE-1 IMMUNOSTAINING AS A SUBSTITUTE FOR SENTINEL NODE STATUS

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BACKGROUND: Although sentinel node status is the most important prognostic indicator in patients with cutaneous melanoma, there is some morbidity associated with the procedure. A lymphatic specific marker LYVE-1 offers the possibility of studying lymphangiogenesis and lymphatic spread within the primary tumour. PATIENTS AND METHODS: Double immunostaining for LYVE-1 and S100 was undertaken in cutaneous biopsies of malignant melanoma from eighteen sentinel node positive patients without lymphatic or vascular involvement on routine histology. These were compared with eighteen sentinel node negative patients matched for tumour thickness and ulceration. RESULTS: Vessels showing a fibrillary morphology were found within the tumour mass suggestive of active lymphangiogenesis. Tumour cells within lymphatics were detected in one of eighteen sentinel node negative cases compared to five of eighteen sentinel node positive patients. Of the latter, three showed clumps of S100 positive tumour cells and two showed single S100 positive cells within the lymphatics. Of note, the lymphatics containing the tumour cells were all located outside the tumour mass in well-formed vessels suggesting invasion of melanoma cells into preformed lymphatic vessels. There was no significant difference in lymphatic counts between sentinel node positive and sentinel node negative patients. Although peritumoural lymphatic counts were increased in ulcerated melanomas compared to non-ulcerated melanomas ($p = 0.029$), lymphatic counts did not vary with Breslow thickness of the tumour. CONCLUSION: LYVE-1 staining is a reliable means of demonstrating the distribution and density of lymphatic vessels in routinely processed tissue, but cannot be used as a substitute for sentinel node biopsy. Immunostaining with LYVE-1 is useful for detecting the presence of melanoma cells within lymphatic vessels, but fails to detect metastatic spread in more than two thirds of patients with positive sentinel nodes.

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CL1-7 METALLOTHIONEIN: OVEREXPRESSION INDICATES POOR PROGNOSIS - LONG-TERM FOLLOW UP OF THE INNSBRUCK MELANOMA COHORT

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Objective: Metallothioneins (MT) are ubiquitous small intracellular proteins with high affinity for heavy metal ions. In the past it could be shown, that MT-overexpression in a variety of cancers is associated with resistance to anticancer drugs or irradiation and is combined with a poor prognosis. The aim of the study was to examine the role of MT overexpression in melanoma patients as a prognostic factor for progression and survival. Material & Methods: 828 out of 1148 patients with primary cutaneous melanoma were investigated in a prospective study (1993 - 1998) by using a monoclonal antibody (E9) against MT on routinely fixed and paraffin-embedded tissues. Patients were followed up for progression or death due to melanoma (median observation time 60 month). Statistical analysis (e.g. Kaplan-Meier curves) of the MT data for progress-free interval and overall-survival were compared univariately and multivariately with other prognostic factors in Cox regression analysis. Results: The immunohistochemical overexpression of MT in tumour cells of patients with primary melanoma (194/828; 23,4%) was associated with a higher risk for progression of the disease (63/86; 73,3%) and reduced survival (52/67; 77,6%), than MT-negative lesions (both $p < 0,001$). Similarly, Kaplan-Meier tumour free survival and overall survival curves gave highly significant advantages for the MT-negative tumour group. Univariate analysis (compared with Breslow s tumour thickness: relative risk 4,5; CI 95% 2,7-7,4; $p < 0,001$ for progression and relative risk 4,4; CI 95% 2,4-8,0; $p < 0,001$ for survival), as well as multivariate analysis with other prognostic markers turned out MT-overexpression as a highly significant and independent factor for the prognosis in primary melanoma. Conclusion: MT-overexpression in primary melanoma is associated with an increased risk for progression of disease. This marker is independent from Breslow tumour thickness and helps to better outline those thin melanomas which are at increased risk for progression.

CL1-8 CLINICAL CORRELATES OF BRAF/NRAS MUTATION IN MELANOMA

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OBJECTIVE: We sought to determine clinical correlates for presence of BRAF and NRAS somatic mutations in a population-based series of melanomas. MATERIALS & METHODS: 110 first incident primary invasive cutaneous melanomas from a population-based series from designated counties of North Carolina in the year 2000 were analyzed for NRAS and BRAF somatic mutations. The cases had a mean age of 55 years at diagnosis and mean Breslow depth of 1.3 mm. The melanomas were screened for mutations in and around NRAS codons 12/13 and 61 and within BRAF exons 11 and 15, using single strand conformational polymorphism (SSCP) analysis with direct manual sequencing of SSCP positive PCR products. P-values were derived from the Fisher's Exact Test. Wildtype melanomas are defined as negative for both BRAF and NRAS mutation. RESULTS: BRAF and NRAS mutations were found in 44 (40%) and 22 (20%), respectively, of first incident invasive melanomas and were exclusive of each other. The chance of this distribution occurring randomly is $P<0.0001$. NRAS and BRAF positive melanomas differed from each other by mean age at diagnosis and presence of severe solar elastosis. Melanomas with NRAS mutation were more likely than those with BRAF mutation to be diagnosed at an older age ($P=0.0001$, t-test) and have severe solar elastosis histologically ($P=0.01$). Compared to wildtype melanomas, melanomas with either NRAS or BRAF mutation were more likely to be located on the trunk ($P=0.02$), of superficial spreading or nodular subtype ($P=0.0003$), deeper Clark level ($P=0.03$), and in vertical growth phase ($P<0.0001$). CONCLUSION: Melanomas differ in their clinical correlations based on BRAF/NRAS mutational phenotype. In addition, the data suggest that these mutations may be associated with progression or more aggressive subtypes.

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CL2-1 COMPARATIVE PERFORMANCE OF FOUR DERMOSCOPIC ALGORITHMS FOR THE DIAGNOSIS OF MELANOCYTIC LESIONS IN THE HANDS OF NON-EXPERTS

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OBJECTIVE: This study assessed four dermoscopy methods in an inexpert setting. Clinicians with significant dermoscopic experience tend to look at a dermoscopic image and immediately process all the information leading to a diagnosis without using any specific algorithm. Dermoscopic algorithms are more helpful in reaching a diagnosis for those with less experience in dermoscopy. This study aims to determine which dermoscopic diagnostic algorithm shows the highest sensitivity, specificity and diagnostic accuracy for the diagnosis of melanoma in a less expert setting. MATERIAL AND METHODS: Sixty one Australian medical practitioners, mainly primary care physicians, were given education in four dermoscopy algorithms. Participants then assessed macroscopic and dermoscopic images of forty melanocytic skin lesions. Each of the dermoscopic images was assessed with pattern analysis, the seven-point checklist, the ABCD rule and Menzies' method. RESULTS: The Menzies' method showed the highest sensitivity of 84.6% for the diagnosis of melanoma. This was followed by the seven-point checklist (sensitivity 81.4%), the ABCD rule (sensitivity 77.5%), pattern analysis (sensitivity 68.4%) and assessment of a macroscopic image (sensitivity 60.9%). Pattern analysis and assessment of the macroscopic image showed the highest specificity, 85.3% and 85.4% respectively. The ABCD rule showed a specificity of 80.4%, Menzies' method 77.7% and the Seven-point checklist 73%. The Menzies' method had a diagnostic accuracy of 81.1%, the ABCD rule 79.0%, the seven-point checklist 77.2%, pattern analysis 76.8% and clinical assessment 73.2%. CONCLUSION: All algorithms performed well in the hands of relatively inexpert practitioners who had undertaken self-guided training provided on compact disk. The Menzies' method showed slightly higher diagnostic accuracy and sensitivity for melanoma diagnosis.

CL2-2 DERMOSCOPY FALSE NEGATIVE MELANOMAS

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Objective: To describe the clinical and dermoscopic characteristics of false negative melanomas (FNM) by dermoscopy. Design: A set of FNM were retrospectively evaluated by 4 dermoscopists, classified by consensus in previously established categories and correlated with clinical characteristics of the lesions and the patients. Setting: All cases were obtained from the registers of 3 public Hospitals in Barcelona (Spain), Naples (Italy) and Graz (Austria). Patients: 93 tumors with a main preoperative diagnosis different from melanoma. Histopathologic report had been performed by at least 2 different pathologist with disagreement in the diagnosis of malignancy in 11 lesions. Main outcome measures: Age, sex, personal history of dysplastic nevi syndrome or melanoma from the patients; location, history of change from the lesion and mean reason for excision; dermoscopic description of the lesions according to pattern analysis in a two step procedure for melanocytic and non melanocytic lesion. Results: 23 (24.7%) in situ melanomas and 70 (75.3%) invasive melanomas, (Breslow thickness: mean of 0.936, median of 0.6, range 0.3-4.0 mm) were evaluated and classified. Concerning to the main groups of FNMMs, 16 cases lacked specific melanocytic nor non-melanocytic criteria (17.3%) (10 amelanotic (10.8%) and 6 hypomelanotic (6.5%)) and 77 (82.8%) presented specific dermoscopic criteria simulating non-MM tumours. In this second group, 11 FNMMs simulated non-melanocytic tumors (4 lesions simulated angiomas, 3 basal cell carcinomas, 2 seborrheic keratosis and 2 solar lentigos). The remaining 66 FNMMs, resembled benign melanocytic tumours, predominantly Clark nevus (51.8%), blue or combined nevus (4.4%), congenital o dermal nevus (3.3%) and Spitz nevus (11.8%). The main reason for removal Clark nevus like melanoma (52%) was the objective evidence of changes by dermoscopic digital follow-up. Conclusions: Based on the clinical and dermoscopic characteristics of our FNM an algorithm for minimizing misdiagnosed melanomas was elaborated.

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CL2-3 SAVING LIVES THROUGH THE EARLY DETECTION OF SMALL MELANOMAS (MM): THE ABCDS REVISTED

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The ABCD mnemonic was devised in 1985 by our group as an aid the the recognition of potentially curable melanomas by physicians and their patients. The incidence of MM continues to rise, thus early diagnosis remains an on-going public health concern. Most (~90%) MMs > 6mm fulfill the ABCD criteria. However, smaller evolving MMs may lack one or many of the ABCD features. In that context, we recently examined the concept of evolutionary (E) change in pigmented lesions in an attempt to identify small (<6mm) MMs during their evolution from inception (microscopic) through clinically visable growth/change. The earliest clinically evident MMs (1-2 mm) are generally not clinically distinguishable from early benigh melanocytic lesions. Evolving MMs 3-4 mm in diameter oftentimes exhibit subtle changes in color (tans-browns and black) before they begin to enlarge in diameter. In 17/24 small (2.3-5.7 mm) MMs, color change preceded all other evolutionary change. This was followed by change in diameter, symmetry and border respectively. MMs 4-5 mm in diameter oftentimes will exhibit more of the ABC features with change in diameter (D) representing the D component. In the aforementioned series, 22/24 of the small MMs were in situ and 2/24 were 'invasive' (up to 0.58mm). All of the invasive lesions were >4 mm in diameter. In sum, ABCD criteria for the assessment of pigmented skin lesions is a useful screening tool to aid in the diagnosis of MM. Scientific evidence of reasonable sensitivity and specificity using the ABCDs exists. Clearly CHANGE (evolution=E) in the ABCDs is a practical additional criterior in helping to distinguish MM from benign melanocytic proliferations. Remember the ABCDEs!

CL2-4 MELANOMA DIAGNOSIS BY FLUORESCENCE IN SITU HYBRIDIZATION

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OBJECTIVE: To develop a fluorescence in situ hybridization (FISH) assay that can assist in the differential diagnosis between benign melanocytic nevi and melanoma. MATERIALS & METHODS: CGH data from archival tissue of 190 melanoma and nevi specimens compiled at the Univ. Calif., San Francisco were analyzed to identify genetic loci differentially affected by copy number changes in nevi and melanoma. Specifically, we calculated sensitivities and specificities for distinguishing nevi and melanoma at 570 individual genetic loci, and combinations of these loci. FISH probes were prepared for loci that discriminated between the two groups. These probes were assembled into 3- and 4-color probe sets that were hybridized to tissue microarrays containing 34 nevi and 111 melanoma specimens. We counted hybridization signals in at least 20 nuclei per specimen and performed discriminate and combinatorial analyses. RESULTS: Analysis of the CGH database identified the following loci as most frequently aberrant in melanoma but not in nevi: 1q23, 6p24, 7q34, 8p22, 8q24, 9p21, 10q23, 17q25, 20q13, and centromeres 6, 7, 8, 9, and 10. FISH data from tissue microarray hybridizations using probes to each of these loci indicated the best performing individual loci were, in order, 20q13, centromere 8, 9p21, 17q25, 1q23, and 6p24. The best combinations of two probes were 9p21 with 20q13 and 9p21 with centromere 8, both of which provided >95% sensitivity and specificity. The 3-probe set of 9p21, 20q13, and 7q34 provided further improvement. CONCLUSION: FISH with panels of a limited number of probes targeting regions commonly aberrant in melanoma can achieve high sensitivity and specificity to discriminate between nevi and melanoma. These panels are now being applied to a larger cohort of clinical specimens to further verify the effectiveness of this assay.

CL2-5 A NARROW EXCISION OF MELANOMAS OF THE BACK LESS THAN 2 MM IN THICKNESS INCREASES REGIONAL RECURRENCES AND MORTALITY

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Objective: The WHO study indicated that in melanomas up to 2 mm in thickness a 1 cm excision margin is enough (Veronesi 1991). We participated in this study and were interested to check the long-term result to see if any prognostic variable, especially the trunk, changed over time. We compared our findings with the Cancer Registry. Methods: The 101 randomised patients had a mean follow up of 112 months; Patients in western Sweden had been treated with a 1 cm excision margin for melanomas up to 2 mm in thickness. 812 patients with melanoma on the trunk were analysed, 530 on the back (0.71 Breslow) and 282 on the front (0.65 Breslow) with a mean follow up of 50 months. Results: Looking at different subgroups in the randomised group of 101 patients we found an increased risk of nodal recurrences in melanomas of the back when a narrow 1 cm excision (20 patients, 0.97 Breslow) and not a wide 3 cm excision (17 patients, 0.84 Breslow) had been performed (p=0.048). In the Cancer Registry we also found a high risk of developing nodal metastases for melanomas of the back (22 of 530 patients) but not for the front of the trunk (2 of the 282 patients) p=0.0058. Regression analysis shows that melanomas on the back had a 3.8 (1.01 - 14.34) increased relative risk to die in melanoma. Discussion: Melanomas up to 2 mm in thickness on the back of the trunk do worse then those on the front when a 1 cm excision margin had been performed. This risk was eliminated with a wide 3 cm excision. These results should be checked with long-term analyses of other materials and if verified result in a recommendation of a wide excision margin in thin melanomas of the back.

CL2-6 EVIDENCE-BASED FOLLOW-UP SCHEDULES FOR PATIENTS WITH MELANOMA

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Objective: Current follow-up guidelines for patients with cutaneous melanoma are based largely largely on historical precedent rather than evidence. This study was performed to calculate recurrence rates, to establish factors of prognostic importance for recurrence development (RD) and to propose rationalised follow-schedule using this information. Methods: Information was extracted from a computerised database for 4726 American Joint Committee on Cancer (AJCC) Stage I and II patients treated between 1960 and 2002, whose first definite treatment had taken place in our centre. The distribution of RD by time was calculated and factors predicting RD and survival were analyzed using the Cox proportional hazards regression model. On the basis of the results, a follow-up schedule was developed. Results: Overall recurrence rates were 5.2% (95/1822), 18.4% (264/1436), 28.9% (215/750), 41.0% (213/524) and 45.2% (86/194) for AJCC Stages Ia-IIc respectively. Annual risk of RD was less than 5% in all Stage I patients, but varied from 6.4-18.4% for Stage II patients in the first two years after diagnosis (Figure 1). Tumor thickness, ulceration and mitotic rate were the most important predictors for RD (hazard ratios 1.4, 0.5 and 1.4 respectively, p-values all <0.0001). Conclusions: Based on the observed rates of RD we recommend annual follow-up visits for Stage I patients and 4-6 monthly visits for Stage II patients in the first three years after diagnosis, with annual visits thereafter.

CL2-7 THE INTRAOPERATIVE IDENTIFICATION OF THE SENTINEL LYMPH NODE IN MELANOMA PATIENTS USING 20% RADIOACTIVE THRESHOLD.

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Objective: Sentinel lymph node (SLN) biopsy has become a standard method of staging patients with cutaneous melanoma. Although SLN identification rates have improved with the addition of radioactive colloid to the blue dye technique, the identification of the 'true' SLN requires further definition. The objective of this study was to determine wether harvesting of nodes 20% or more of the ex-vivo counts of the 'hottest' node minimizes the false negative rate for recurrent nodal metastases in basins that are found to have negative SLNs after long term follow-up. Methods: All patients with primary stage I-II cutaneous melanoma who referred to the Nuclear Medicine Division of Pisa University Hospital, underwent radioguided sentinel lymphadenectomy. The criteria for g-probe identification of a SLN were based on detecting a focal zone of radioactivity with a count ratio > 10. Any additional lymph node whose counting rate exceeded 20% of the hottest node was considered an additional SLN. SLNs were assessed for tyrosinase and melanoma antigens recognized by T-cells (MART-1) mRNA expression using RT-PCR, in parallel with pathology and immunohistochemistry (PATH). Results : A total of 197 SLNs, belonging to 124 patients, were excised and examined with both PATH and RT-PCR techniques. Eighty-five patients (68.5 %) had SLNs that were negative by both PATH and RT-PCR (PATH-/PCR-). Thirty-nine of the 124 patients (31.5%) had positive SLNs by at least one of the two techniques (PATH or RT-PCR). Out of the 85 PATH-/PCR- patients, only 1 (1.2%, false negative rate) recurred locally (after 9 months) and 7 (8.2%) presented with distant metastases after a median follow-up of 30 months (range 10-53). Discussion: We considered 1.2% as an acceptable false negative rate and propose that all nodes with 20% or more of the ex vivo count of the hottest SLN should be harvested for optimal nodal staging.

NOTES:

CL2-8 RADICAL DISSECTIONE OF GROIN BASIN AFTER POSITIVE SNB: OVERTREATMENT OR STANDARD SURGERY?

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A retrospective review of patients observed between 1999 and 2004, at National Cancer Institute of Milan, was performed to determine the incidence of non-sentinel node metastases at completion groin dissection. This analysis includes patients with a diagnosis of positive sentinel node of the groin (clinically negative), who underwent completion groin dissection. The number of positive sentinel nodes (the SLNs were step-sectioned and stained with haematoxylin and eosin and immunohystochemical with S100 and HMB45) was considered, as well as the size of the metastasis (micro v/s macro). After radical dissection, the number and the localization (superficial or deep groin nodes) of the NSN nodes were analysed. 1191 patients with primary melanoma thicker than 1 mm, or Clark level IV-V, underwent sentinel lymphadenectomy. 440 patients had groin sentinel lymphadenectomy. 88 patients presented SN metastases and underwent completion superficial and deep groin dissection. The analysis of the specimens from completion nodal dissection showed additional metastatic nodes in 22/88 (25%) patients. Out of this 19,3% had 1 additional metastatic node and 5,7% 2 or more. In 17% the additional nodes showed a macrometastasis (> 1 mm deposit in the node) and 8% a micrometastasis (< 1mm deposit). Conscious that the impact of SL on survival of melanoma patients has yet to be defined, in order to obtain a clear nodal basin and regional control, we think that an inguinal-iliac radical node dissection is an appropriate procedure in presence of a SNB positive at groin level. In our series the incidence of further nodal metastases at CLND resulted 25%. In details 10,2 % showed further metastases at inguinal level while 9,1% at inguinal plus iliac basins level and 5,7% at iliac level. Thus confirming the role of SNB for an accurate microstaging and not as a therapeutic tool.

CL2-9 EVALUATION OF THE RATIO OF INVASIVE TO IN SITU LESIONS IN MALIGNANT MELANOMA

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Objective: To determine the ratio of invasive:in situ lesions among cases of malignant melanoma (MM) diagnosed at independent, nonhospital-based dermatopathology laboratories and to compare the distribution of lesions found with that reported through the National Cancer Institute (NCI) Surveillance and Epidemiology End Results (SEER) cancer registry. Accurate information on MM incidence is needed when evaluating the role of potential risk factors. Data from SEER indicate a preponderance of invasive vs in situ lesions among patients with newly diagnosed MM (61% vs 39%) (Jemal et al. CA Cancer J Clin 2002:52 ;23-47). Materials and Methods: Five dermatopathology laboratories participated in the study. Data for MM lesions diagnosed in 2000 and 2001 were collected retrospectively from each laboratory. Duplicate lesions (definitive excisions after diagnostic biopsy) were eliminated. Information on lesion thickness (invasive or in situ) was obtained and data on patient gender and age were collected when available. The ratio of invasive:in situ melanoma was calculated and compared with the SEER ratio published in 2002 using a two-sample test for binomial proportions. Results: A total of 9,143 MM cases were reviewed (54% specimens from males and 46% from females). This gender ratio was consistent with NCI data. There were 3,954 (43%) invasive and 5,189 (57%) in situ lesions. The ratio of invasive:in situ lesions diagnosed in the dermatopathology laboratories was significantly different than the SEER published estimate (z-score of 32.836, $P < 0.001$). Conclusions: The proportion of in situ vs invasive MM in the population was higher in this study compared with NCI estimates. Due to its method of hospital-based data collection, SEER may significantly underestimate the actual number of MM in situ in the population and the total number of MM cases. This discrepancy may grow over time as MM is increasingly diagnosed and treated in outpatient settings.

NOTES:

CL3-1 ROUTINE USE OF FDG-PET SCANS IN MELANOMA PATIENTS WITH POSITIVE SENTINEL NODE BIOPSY

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OBJECTIVE Melanoma patients with regional lymph node metastases by sentinel node biopsy (SNB) have a poor prognosis with many recurrences and a high mortality rate in spite of no detectable dissemination by conventional screening methods at the time of SNB. We wanted to investigate the utility of FDG-PET scanning for detecting subclinical metastases in stage III melanoma cases with positive sentinel node status. METHODS Thirty-tree patients with primary cutaneous malignant melanoma and sentinel node metastases were submitted to 18F-FDG-PET whole body scanning within 100 days after SNB and wide local excision. Before PET scanning all cases were screened conventionally with clinical examination, chest X-ray and blood liver tests and were found without evidence of dissemination. Positive PET findings were further evaluated by CT, MRI or ultrasonography and a biopsy was performed if possible. RESULTS Nine patients (27%) had positive PET scans and on verification, four cases (12%) were found to have melanoma dissemination not found by conventional screening and were thus upgraded to stage IV cases. Additionally, two cases (6%) with positive PET scans might also be disseminated but they refused further investigations. In one case (3%) the PET scanning was false negative. DISCUSSION Routine PET scanning of stage III melanoma cases after positive SNB revealed a number of cases to have further melanoma dissemination not found by conventional screening. Relevant therapy directed against stage IV disease can be instituted earlier on the basis of whole-body PET scanning after positive SNB.

CL3-2 SUPERFICIAL ULTRASONOGRAPHY USING SIMPLE DIAGNOSTIC CRITERIA IS MORE SENSITIVE AND SPECIFIC THAN PALPATION FOR THE DETECTION OF REGIONAL LYMPH-NODE MELANOMA METASTASES

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Objectives: The number and palpability of involved nodes are important prognostic factors of melanoma. Lymph-node sonography, an inexpensive procedure, might better detect invasion than palpation, but numerous criteria of invasion have been defined. Our aims were: 1/ to compare ultrasonography versus palpation to detect nodal invasion during initial staging and follow-up of melanoma patients; 2/ to assess, we believe for the first time, which ultrasound criteria should be used to define invasion in this setting. Methods: One-hundred-sixty new consecutive AJCC stage I-III melanoma patients entered a prospective single-center study performed in the Dermatology and radiology departments of a university hospital. Three-hundred-ninety-one paired palpation and ultrasonography (6-12 MHz) examinations were performed independently by experienced operators. Main outcome measures were as follows: 1/ Firm enlarged nodes found on palpation were considered metastatic; 2/ On ultrasonographic examination, circular or oval hypoechoic lymph nodes lacking hyperechoic hila were considered metastatic (stringent criteria). Nodes with 2 or fewer of these patterns and other published signs of metastasis (ie, intranodal nodular hypoechoic focus and irregularity of the node margin) were considered suspicious; 3/ Nodal invasion was confirmed by pathology. Results: Over the 6-year study period 33 patients developed nodal metastasis. For palpation and ultrasonography using the stringent criteria, respectively, sensitivity was 41.5% (95% confidence interval [95% CI], 29.6-53.5) and 76.9% (95% CI, 66.7%-87.2%) (P<.001) and specificity was 95.7% (95% CI, 93.5%-97.9%) and 98.4% (95% CI, 97.1%-99.8%) (P<.05). Including ultrasonographically suspicious lymph nodes significantly lowered specificity (86.2% [95% CI, 82.5-89.9]) (P<.05) without improving sensitivity. Previous lymphadenectomy had little impact on ultrasonographic findings. CONCLUSION: Ultrasonography using stringent criteria of nodal metastasis, which are easy to identify and reliable, is superior to palpation for early detection of regional lymph node metastases of melanoma.

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CL3-3 TARGETING MELANOMA VASCULARIZATION : A NEW THERAPEUTIC CONCEPT.

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Clinical oncologists have not been paying enough attention to features of tumour vascularization. Indeed, poor tumour penetration represents a major impediment to the efficiency of chemotherapy. It was shown that melanoma metastases have high interstitial fluid pressure and reduced permeability. In addition, tumour progression is strongly dependent upon angiogenesis. We have have shown that recombinant Tumour Necrosis alpha (TNF),first increases molecule penetration in the tumour and, second, destroys tumour associated microvessels, both in a selective manner. In the clinic, melanoma in-transit metastases were completely destroyed (complete remission) in about 80% of patients treated by isolated limb perfusion with TNF, Interferon gamma (IFNg) and melphalan. Impressive results were obtained in bulky highly vascularized melanoma metastases. Studies in our laboratory indicated that TNF and IFNg deactivate the integrin alphaVbeta3 an adhesion receptor that plays a key role in angiogenesis- on intratumoural angiogenic endothelial cells,resulting in apoptosis and vessels destruction, whilst resting vessels in normal tissues, which do not express this integrin,are spared. It was further demonstrated that cyclo-oxygenase-2 (cox-2) is required for alphaVbeta3 dependent angiogenesis and that cox-2 inhibitors exert a strong anti-angiogeneic effect. A patient suffering from recurrent extensive in-transit melanoma metastases on the lower limb, treated by cox-2 inhibitor, developed a nearly complete remission for two years without any other therapeutic intervention. Taken together, these observations, and the works by others, strongly support the concept that interfering with alphaVbeta3 integrin activation in melanoma metastases can: 1) increase tumour vessels permeability (low dose TNF), 2) disrupt angiogenic vessels (high dose TNF) and, 3) exert an anti-angiogenic effect on the long term (cox-2 inhibitors). Clinical trials designed to explore this concept are underway.

CL3-4 DETECTION OF CIRCULATING MELANOMA CELLS PREDICT TREATMENT RESPONSE TO NEOADJUVANT BIOCHEMOTHERAPY IN STAGE III MELANOMA PATIENTS

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Background: Detection of circulating tumor cells (CTCs) may be useful in assessing recurrent melanoma disease and response to treatment. We previously developed a quantitative RT-PCR (qRT) assay using four melanoma-associated markers (MART-1, GalNAc-T, PAX-3, and MAGE-A3) that upstaged immunohistochemistry and H&E negative SLN that predicted disease outcome. We applied this multimarker qRT assay to detect CTCs in blood of melanoma patients as a predictive surrogate of response to neoadjuvant biochemotherapy (BC). Patients and Methods: Four mRNA markers were assessed using qRT in 231 blood specimens collected at four intervals from 63 patients enrolled in a multicenter phase II clinical trial of BC before and after surgical treatment of high risk AJCC stage III melanoma patients. The BC regimen was comprised of cisplatin, dacarbazine, vinblastine, IL2, ±IFN, and GCSF, whereby patients received two cycles of BC before and after therapeutic lymphadenectomy. Results: Multimarker detection of CTCs in blood at post-BC significantly decreased compared to pre-BC in all patients ($P < 0.0001$). CTCs detection was correlated with BC response. In patients without relapse ($n = 19$), marker detection significantly decreased after each phase of BC (pre-surgery BC, $P = 0.036$; post-surgery BC, $P = 0.002$) and after overall treatment ($P < 0.0001$). Patients with marker detection after overall treatment showed significantly lower relapse-free survival (RFS) ($P < 0.0001$) and overall survival (OS) ($P < 0.0001$). Patients with CTCs detection after treatment demonstrated poorer survival (RFS, $P < 0.0001$, OS, $P < 0.0001$). The number of markers detected after treatment was a independent prognostic factor for OS (risk ratio = 12.6; $P = 0.0003$). Conclusion: Multimarker qRT assay for CTCs detection has clinical utility, whereby, serial monitoring of CTCs can indicate systemic subclinical disease and predict response to neoadjuvant BC.

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CL3-5 DURATION OF REMISSION AND SURVIVAL FOLLOWING ISOLATED LIMB PERFUSION: LONG TERM FOLLOW UP.

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Objective: To review the long term duration of complete limb tumour remission (CR) and survival following therapeutic isolated limb perfusion (ILP). Method: A retrospective case series of 124 consecutive ILPs performed in 111 patients between May 1984 and July 1997 using Alkeran, Cisplatin or Actinomycin in combination or as single agents. Results: Of the 120 assessable ILPs, 44.2% of patients had MD Anderson Stage IIIA melanoma, 32.5% had Stage IIIAB, while 11.7% and 10% had Stage II and Stage IVB disease respectively. CR was initially attained in 83 (69.2%) and partial remission in 19 (15.8%) of the ILPs. CR was maintained in 17 (20.5%) of the 83 cases without any further treatment (median follow-up 199 months, range 8-226). CR was maintained in a further 12 (14.5%) cases who had systemic treatment following the ILP. Disease recurred within the perfused limb in the remaining 54 cases (median time to recurrence 11 months, range 3-184 months). In 18 of these 54 cases, however, the limb was again disease free at last follow-up after further locoregional treatment. A long term disease-free state in the limb was achieved, with or without further treatment, in 47 (56.6%) of the 83 cases in which an initial complete remission occurred (median follow-up 65 months, range 8-244). There was no significant difference in long term remission between Stage IIIA and IIIAB patients. Ten-year overall survival for the case series was 14.2%, while survival for those who initially attained CR was 21.7% and 34.8% for those who continued to be in locoregional remission at last follow-up. Conclusion: ILP, with or without further locoregional treatment, achieves long term control of recurrent and metastatic limb disease in over half of cases who show an initial CR. An initial or maintained complete response to ILP is a positive prognostic indicator of survival.

CL3-6 STEREOTACTIC RADIOSURGERY (SRS) AS THERAPY FOR MELANOMA BRAIN METASTASES: IMPACT OF ADDED SURGICAL RESECTION AND WHOLE BRAIN RADIOTHERAPY

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Objective: Melanoma brain metastases have a poor outcome, with an approximately 3.6-4.1 month median survival when treated with whole brain radiotherapy. Several more recent studies have suggested a beneficial effect of stereotactic radiosurgery (SRS). We have evaluated our institutional experience with stereotactic radiosurgery in 46 sequential melanoma brain metastasis patients. Methods: Forty-six melanoma patients with brain metastases seen at this institution between 1999 and 2004 were included in the analysis. Twenty-four patients had a single metastasis and 22 had multiple metastases. Patients were treated with SRS alone (if (5 brain metastases) with or without the addition of whole brain radiation or surgical resection (in selected patients). SRS using multiple non-coplanar arcs was performed using a head frame and localization box fixed to the patient's skull using a Novalis linear accelerator. All patients had KPS>70. Survival was calculated from the time of diagnosis of brain metastases using the Kaplan-Meier product-limit method. Statistical significance was calculated using the log-rank test. Various factors influencing survival were evaluated including surgical resection, use of whole brain radiotherapy (WBRT), sex, and number of SRS treatments, using Cox multivariate models in this retrospective analysis. Results: The median survival of melanoma patients with brain metastases treated with SRS was 13.4 months (CI 10.8-16.0 months), which appears superior to 3-4 month survival obtained with conventional whole brain radiotherapy (WBRT). Neither the addition of surgery or WBRT to SRS provided an increase in survival in our patients. Conclusions: Systematic screening for brain metastases in patients with stage IV melanoma and aggressive use of stereotactic radiosurgery appears to result in improved survival for patients with melanoma-derived cerebral metastases. The addition of surgical resection or whole brain radiation did not significantly improve survival. Most of our patients were able to successfully tolerate subsequent systemic chemotherapy or immunotherapy following SRS.

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CL3-7 LOW EFFICACY OF SHORT COURSE 5% IMIQUIMOD CREAM IN LENTIGO MALIGNA AS ASSESSED BY COMPLETE SURGICAL EXCISION

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OBJECTIVE To determine whether a significant inflammatory response sustained for at least 7 days would be sufficient to histologically clear lentigo maligna (LM) treated with 5% imiquimod cream. Complete surgical excision was used as the study endpoint rather than random partial biopsies. MATERIALS & METHODS Nine patients who had biopsy proven LM confirmed by two experienced dermatopathologists were recruited into this study. The border of the LM was tattooed and patients were required to apply 5% imiquimod cream daily for 5 days per week until there was a significant inflammatory response for at least 7 days, or if there was no inflammatory response, for a maximum of 12 weeks. The end point of the study was full surgical excision of the lesion plus a 5mm margin of macroscopically normal skin as assessed by Wood's light examination. Excision specimens were examined by the same two dermatopathologists. RESULTS Nine patients completed the trial. The mean duration of treatment was 7.5 weeks, with the range being between 2 and 12 weeks. Five patients completed treatment with imiquimod before 12 weeks. Only 2 of the 9 patients achieved histological clearance of their LM and one of these required a full 12 weeks of treatment. Six of the 9 showed inflammation and of these, 2 had clinical clearance but only 1 of these had histological clearance. One of the 3 patients who had no inflammation managed to achieve histological clearance. CONCLUSION This study suggests that short term inflammation is not adequate to clear LM. Significant discordance may be seen between the occurrence of an inflammatory response, apparent clinical response and histologic clearance. A cautious approach to the use of this treatment is advocated. Complete surgical excision is recommended as the endpoint for future studies on the efficacy of imiquimod.

CL3-8 SURGICAL MANAGEMENT OF TUMOR-POSITIVE SENTINEL LYMPH NODES IN THE GROIN: ROLE OF COMPLETION DISSECTION

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Objective: Sentinel lymphadenectomy (LM/SL) has become the standard surgical approach for staging the regional lymph nodes (LN) in early-stage melanoma. LM/SL has great advantage for the groin where the morbidity of superficial inguinal (SGD) or deep iliac dissection (ILND) can be high. Yet the therapeutic value of SGD or ILND is unknown for patients with tumor-positive sentinel lymph nodes (SN). Methods: Analysis of 308 patients undergoing LM/SL over 16 yr. LM/SL performed with uniform method: lymphoscintigraphy, blue dye and gamma probe directed SN dissection. All SN examined by H&E and IHC to S-100 and HMB-45. Patients with tumor-positive SN underwent complete groin dissection. Median follow-up 63 months. Results: Of 308 patients, 185(60%) were women. Median age 50yrs (range 15-89). Most (87%) of the primaries arose on the lower extremities. 58% were Clark IV/V & mean thickness 1.89+1.59mm. 66(21%) of patients were found to have tumor-positive SN. 7(2.9%) patients recurred in the dissected basin after tumor-negative LM/SL. After LM/SL+SGD+ILND, 44(67%) were found to have only 1 tumor-positive LN. 56(85%) patients underwent SGD for tumor-positive SN from lower extremity primaries. 31 had sampling of Cloquet's node to determine need for ILND. In 4(12%) cases Cloquet's node was tumor-positive, 2(50%) had tumor-positive ILND. 10(15%) underwent SGD for tumor-positive SN from trunk primaries. None had tumor-positive ILND. 1 patient recurred in the iliac basin after tumor-negative LM/SL. Survival was significant better (p=0.0003) for SN- than SN+. Multivariate analyses with a Cox regression model identified lymph node tumor burden (p=0.045), primary thickness (p=0.033) and ulceration (p=0.003) as predictive of survival. Conclusions: Our results demonstrate the prognostic significance of LM/SL for early-stage melanoma draining to the groin basin. While the majority of patients have lymph node metastases confined to the SGD, sampling of Cloquet's node may be essential for determining the role of ILND.

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SS1-1 INTERFERON-ALPHA AS ADJUVANT THERAPY FOR MELANOMA: A META-ANALYSIS OF THE RANDOMISED TRIALS

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Objective: Several randomised trials have evaluated the role of adjuvant interferon-((IFN) in high-risk melanoma, some suggesting benefit and others not. To assess the totality of the current evidence, a meta-analysis has been performed of all available trial results.
Materials & Methods: Standard meta-analysis methods were used. Published data were mostly used, but with individual patient data (IPD) available for three trials. The endpoints evaluated were recurrence-free survival (RFS) and overall survival (OS). The only subgroup analysis performed was by dose of IFN – high (20MU/m2), intermediate (5 or 10MU), low (3MU) and very low (1MU). Results: Data were available from 11 trials of IFN versus control (i.e. no IFN) that recruited over 5200 patients, with over 2800 and 2100 events in the RFS and OS analyses respectively. For RFS, there was clear benefit for IFN: odds ratio (OR) = 0.85, 95% confidence interval (CI) = 0.79-0.92, p=0.00002. There was no clear advantage for OS (OR=0.92, CI=0.84-1.01, p=0.07) and the upper CI is compatible with no survival benefit. There was no evidence of heterogeneity between trials, though there was weak evidence of a dose response relationship for RFS (test for trend: p=0.05), but not for OS (p=0.9). For RFS, the benefit of IFN increased with increasing IFN dose: high dose: OR=0.77 (CI=0.66-0.90), intermediate dose: OR=0.83 (CI=0.69-0.99), low dose: OR=0.85 (CI=0.77-0.95) and very low dose: OR=1.04 (CI=0.84-1.29). Conclusion: This meta-analysis demonstrates some evidence for dose-response in terms of RFS but not OS, and hence uncertainty remains regarding the benefits of IFN in melanoma. An IPD meta-analysis is now under way, that will clarify issues of dose-response and investigate any differences in treatment effect by type of patient and over time.

SS1-2 LONG TERM SURVIVAL BENEFIT AFTER ADJUVANT TREATMENT OF HIGH RISK CUTANEOUS MELANOMA WITH DACARBAZINE AND LOW DOSE NATURAL INTERFERON ALPHA: A CONTROLLED, RANDOMISED, MULTICENTRE TRIAL

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Two hundred and fifty-three patients with cutaneous melanoma stages Iia to IIIb of disease were randomised after complete tumour resection to receive either two monthly applications of dacarbazine (DTIC 859 mg/m2) followed by a 6-month treatment with highly purified natural interferon-alpha (nIFN-a, 3 MIU, thrice weekly, arm A) or no adjuvant treatment (arm B). The primary ITT target group was patients with high-risk (HR) melanoma (stage IIb, IIIa and IIIb), comprising 80 A and 76 B patients who were all, except one patient, followed up for 7 years or more. Treatment with DTIC and nIFN-a was well tolerated. The estimated 5-year relapse-free survival (RFS) rates in the HR group were 42% and 17% in arm A and B, respectively (log rank test, p=0.0018). The actual 7-year OS rate, calculated using a worst case scenario, was significantly higher (51% vs 30%, p=0.0077), and the estimated 9-year survival was more than twice as high, in the treated as in the non-treated patients (51% vs 24%). The benefit of adjuvant treatment was particularly evident on late mortality. Supportive evidence for the efficacy of the regimen used were the findings of highly significant differences between the two arms of the study in terms of distant-metastases-free survival (DMFS). There were no statistical differences regarding RFS, OS or DMFS in stage IIa, low-risk, patients. These results imply that low dose nIFN-a therapy following DTIC is an effective, durable and tolerable adjuvant treatment for patients with cutaneous melanoma, and suggest that the treatment is more effective in high-risk than in low-risk patients.

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SS1-3 EVALUATION OF HIGH DOSE DNA/MVA HETEROLOGOUS PRIMEBOOST IMMUNOTHERAPY IN STAGE III/IV METASTATIC MELANOMA PATIENTS.

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Objective: This study evaluates safety, immunogenicity and tumour response of increasing doses of plasmid DNA (DNA.Mel3) and Modified Vaccinia virus Ankara (MVA) vector (MVA.Mel3) expressing 7 human CTL epitopes from 5 melanoma antigens (tyrosinase, melan-A, MAGE-1, MAGE-3, NY-ESO-1). Materials and methods: 38 HLA-A2+ patients with measurable stage III/IV melanoma were enrolled and sequentially allocated to 7 treatment groups, with >5 patients per group. All patients received one high dose (4mg) or two low doses (2x2mg) of DNA.Mel3 (i.m.) then two doses of MVA.Mel3 (i.d.) at 5x107pfu, 2x108pfu, 5x108pfu or 1x109pfu. Immunisations were 3 weekly. Safety was monitored throughout and reviewed before MVA dose escalation. Epitope-specific CD8+ T cell response was monitored using ex vivo tetramer staining and IFN-g ELISPOT assays. Tumour response was evaluated by RECIST. Results: In groups 1-3, 8/15 immunologically evaluable patients to date showed a significant increase of melan-A-specific CD8+ T cells. One low dose (5x107pfu) recipient with a partial tumour response, confirmed at week 32, had ELISPOT responses against both melan-A and tyrosinase leader epitopes which increased on continued MVA boosting at week 16 and 24. No immunological response difference was observed between one high dose (4mg) vs two low dose (2x2mg) DNA primes followed by low dose (5x107pfu) MVA boost. Transient grade 1-2 injection site reactions were observed predominantly following MVA (up to 5 x108pfu). Following high dose MVA (1x109pfu) transient grade 3 injection site reactions (pain, erythema, swelling and ulceration) were observed in 3/10 patients immunised to date. Immunology and tumour response data from all patients will be presented. Conclusions: Low dose heterologous PrimeBoost immunotherapy was safe and stimulated immune responses in >50% of patients treated. Continued MVA boosting appears beneficial in some patients. High doses of MVA (up to 1x109 pfu) administered here for the first time has the potential to be more immunogenic.

SS1-4 MEDI-522, A HUMANIZED MONOCLONAL ANTIBODY DIRECTED AGAINST THE HUMAN ALPHA V BETA 3 (AVB3) INTEGRIN, +/- DACARBAZINE (DTIC) IN PATIENTS WITH METASTATIC MELANOMA (MM) APPEARS TO PROLONG SURVIVAL IN A RANDOMIZED, MULTICENTER PHASE II STUDY

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Background: MEDI-522 targets integrin avb3, QUOTE "" ADDIN REFMAN ÿ\11\05'\19\01\00\00\00\00\01\00\00\0FK:\5CRefman\5CDRUGS\03\00\042192#Wu, Beuerlein, et al. 1998 2192 /id\00#\00 QUOTE "" ADDIN REFMAN ÿ\11\05'\19\01\00\00\00\00\01\00\00\0FK:\5CRefman\5CDRUGS\03\00\042209#Brooks, Clark, et al. 1994 2209 /id\00#\00 which plays a critical role in angiogenesis, bone resorption, tumor growth and metastasis and is highly expressed in MM. Methods: To select the better arm for a Phase III study, this non-comparative study explored the antitumor activity and safety of MEDI-522 (8 mg/kg/week) with or without DTIC (1000 mg/m2 once every 3 weeks) in patients (pts) with Stage IV MM. Eligibility- ECOG 0/1, no prior therapy for MM. Endpoints included response rate (RR), progression-free survival (PFS), and overall survival (OS). Stratification was by AJCC staging and institutional region. DTIC results from Phase III Genasense trial was used for descriptive comparison. Results: 112 patients (57 to MEDI-522 [Arm 1] and 55 to MEDI-522 + DTIC [Arm 2]) were randomized. Median age was 59 years, M/F (68%/32%), ECOG 0/1 (79%/21%), and Stage IV M1a/M1b/M1c (10%/30%/60%). Most adverse events (AEs) were Grade 1/2. Grade (3 AEs occurred in 21 (37%) pts in Arm 1 and 26 (48%) pts in Arm 2. MEDI-522-related deaths (myocardial infarction and pulmonary embolism) occurred in 2 pts, 1 pt per arm. Most common Grade 3/4 AEs in Arm 2 were hematologic (17% neutropenia, 9% thrombocytopenia, and 6% each for anemia/leukopenia). 1 (2%) pt in Arm 1 had Grade 3 thrombocytopenia. Arm 1: Median OS was 12.7 months; median PFS was 57days, and ORR was 0%. Arm 2: Median OS was 9.4 months; median PFS was 78 days, and ORR was 13%.DTIC historical control: Median OS was 7.9 mos; median PFS was 49 days, and ORR was 7%. Conclusions: MEDI-522 with or without DTIC appears well tolerated. The preliminary OS results in both arms compared to DTIC historical control suggest potential clinical activity of MEDI-522 (DTIC in MM. Based on OS, MEDI-522 appears to be the better arm to test in Phase III.

SS1-5 BENEFICIAL IMPACT OF ADDITION OF INTERLEUKIN-2 (IL-2) TO SYSTEMIC THERAPY ON THE LONG-TERM SURVIVAL OF PATIENTS WITH METASTATIC MELANOMA (MM).

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Background: The impact of therapy on long-term survival of patients with metastatic MM remains uncertain. This retrospective study compares the effect of combinations including cisplatin (C), Vinblastine (V), DTIC (D), Taxol (T), Tamoxifen (Tam), interferon (IFN) and IL-2 on long-term Survival. Methods: 616 patients with primary MM of Skin (498), unknown primary (81) mucosal (21) and choroid (16) were treated on 8 frontline clinical trials completed between 1987 and 2001. Results: The characteristics of the patients who received CVD-IFN-IL-2 biochemotherapy (BCC) and those treated with chemo/chemoimmunotherapy combinations without IL-2 (CIC) were compared. The 2 groups were similar in terms of median age, gender, sites of primary, serum LDH, and number of Stage M1C cases. Patient characteristics favored ($p < 0.001$) the 264 patients who received BCC in terms of younger age, no bone metastasis and normal serum albumin level, and favored the 352 patients with CIC in terms of patients with no brain metastasis and Zubrod Performance Status (PS) = 0. There was no statistically significant difference in survival between the patients treated with CVD, CTD or CVD-IFN±TAM and similarly between the different BCC regimens. The overall 5 and 10-year survival of BCC and CIC were 14.5 % and 7.95 % ($p= 0.020$) and 11.94 % and 4.47 % ($p= 0.002$) respectively. The chance to have complete response and 10-year survival of CRs was increased by 2.7 and 3.5 fold respectively when IL-2 was included in the treatment. Multivariate analysis identified treatment with BCC ($p = 0.001$), normal serum albumin level ($p < 0.001$), normal LDH level ($p <0.001$), PS = 0 ($p = 0.03$), and one visceral metastasis ($p = 0.02$) as the favorable prognostic factors for long-term survival. Conclusions: Addition of IL-2 to combination chemotherapy significantly improved the impact of therapy response rate and long-term survival.

SS1-6 VACCINATION WITH AUTOLOGOUS AMPLIFIED NAKED TUMOR-MRNA IN PATIENTS WITH METASTATIC MELANOMA - ANALYSIS OF SAFETY AND IMMUNE RESPONSES IN A PHASE I/II CLINICAL TRIAL

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OBJECTIVE: Direct injection of globin-UTR (Untranslated Regions) stabilized mRNA triggers an immune response against the antigen encoded by the mRNA in mice (Hoerr et al., EJI, 2000). A phase I/II trial was performed as a pilot study in order to evaluate safety and immune responses of vaccination with an individualized composition of naked autologous tumor mRNA applied by repetitive intradermal injections and GM-CSF as an adjuvant in patients with metastatic melanoma. MATERIALS & METHODS: 15 melanoma patients (9 stage III, 6 stage IV) were enrolled in this protocol and received between 4 and 16 monthly injections of mRNA. Total mRNA was extracted from solid tumor tissue of the individual patient followed by construction of a cDNA-libary and in vitro transcription. 24h after intradermal injections of 200µg mRNA, 1,4 Mio.IE GM-CSF were applied subcutaneously. Cellular immune response was analyzed by FACS after in vitro stimulation of CD8+ and CD4+ T-cells with vaccine-transfected autologous PBMCs followed by IFN-Gamma staining. Humoral immune response was measured by FACS after incubation of patient sera with cultured SK-Mel-28 cells and staining with PE-labeled Anti-human IgG antibody. RESULTS: No severe toxicity (WHO III/IV) was observed. Side effects comprised an inflammatory DTH-like local site reaction in 12/15 patients. Flu-like symptoms were reported by 8/15 patients. An increase of vaccine-specific T-cells was found to be present in 6/13 evaluable patients, an increase of melanoma-specific antibodies was detected in 5/13 patients. CONCLUSION: Intradermal vaccination with Tumor-mRNA in combination with GM-CSF is safe and can induce tumor-specific cellular and humoral immune responses in humans. Advantages compared to vaccinations based on dendritic cells loaded with peptides are the lack of HLA-Haplotype restriction, less workload and the possibility to reach both arms (CD4 and CD8) of acquired cellular immunity.

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SS1- 7 CLINICAL EXPERIENCE TARGETING THE CANCER TESTIS ANTIGEN NY-ESO-1 IN MALIGNANT MELANOMA

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NY-ESO-1 is a Cancer Testis antigen expressed in over 45% of melanomas. If patients with relapsing melanoma are studied longitudinally, it can be found in approximately 75% of patients. It is highly immunogenic and frequently induces spontaneous immunity in patients with advanced disease. There are some indications that this can modify the natural history of melanoma. In a program that combined NY-ESO-1 protein with ISCOMATRIX® adjuvant (IMX), a vaccine was created which was studied in vitro, pre-clinically and in early phase clinical trials in patients with high risk resected melanoma as well as in pts with evaluable metastatic disease. PROGRAM OBJECTIVE: To determine whether immune targeting of NY-ESO-1 protein can modify the natural history of malignant melanoma. MATERIALS & METHODS: In a phase I study, 46 patients (pts) received NY-ESO-1/IMX vaccine. Endpoints were safety and immunogenicity assayed by Delayed Type Hypersensitivity (DTH) and ELISA. T-cell responses were assessed by A2 tetramer and against panels of overlapping peptides (OLPs). In an ongoing phase II study, patients with advanced disease are being evaluated clinically and immunologically. RESULTS: High risk patients. Antibody and DTH responses were significantly greater in pts who received NY-ESO-1 with adjuvant than in those who received protein alone. T cell responses involving CD8 and/or CD4 lymphocytes against multiple NY-ESO-1 epitopes were demonstrated in every vaccinated patient evaluated with OLPs. In an unplanned analysis, disease free survival in melanoma vaccine recipients with resected disease appeared longer than in controls. In those with advanced disease, preliminary data show a similar safety and immune response profile CONCLUSION: A prospective evaluation is underway to determine whether this vaccine can modify the survival of pts with NY-ESO-1 +ve tumours. Ongoing studies will further map the epitopes of NY-ESO-1, evaluate impact on clinical outcomes and investigate methods for further optimizing vaccination.

SS1- 8 HIGH RESOLUTION IN VIVO MULTIPHOTON TOMOGRAPHY MELANOMA

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Keywords: optical tomography, multiphoton, NIR, femtosecond laser, skin, melanoma Background Multiphoton optical tomography provides non-invasive optical biopsies of skin with high intracellular resolution and high NIR (near infrared) light penetration depth. 3D and 4D (FLIM, fluorescence lifetime measurements) images can be obtained by two-photon excitation of endogenous fluorophores with a femtosecond NIR laser. Objectives Multiphoton tomography of patients with melanoma. Methods Optical tomography studies of human skin were performed with subcellular resolution using the novel imaging system DermaInspect (JenLab GmbH, Jena, Germany). Patients with abnormal pigmented tissues were imaged in vivo. Afterwards, biopsies were taken and analyzed. A compact, solid-state, turnkey, mode-locked 80-MHz titanium sapphire laser MaiTai (Spectra Physics, Mountain View, USA) with a range of 750 850 nm was coupled into a scanning module with fast x,y galvoscanner, piezodriven 40x focusing optics with high NA of 1.3 (oil) and 200 µm working distance; a PMT (photomultiplier) with short rise time, a module with a 0.17 µm thick glass window for in vivo measurements and a single photon counting unit for FLIM measurements. Results The 3D and 4D distribution of the endogenous fluorophores of the epidermal and dermal tissue, like keratin, NAD(P)H, flavins, melanin, collagen and elastin was visualized in situ and in vivo. Pigmented cell clusters based on non-linear luminescence could be clearly distinguished from non-pigmented cells. Dendritic cells and morphological modifications were detected with 1 µm spatial resolution. Conclusion The high resolution system DermaInspect offers the unique chance of high resolution in vivo non-invasive screening of tissues and tissue structures up to a molecular level and might become a high resolution diagnostic tool for dermatological diagnostics, particular for early detection of melanoma.

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6th World Congress on Melanoma

POSTER PRESENTATIONS

P-001 MC1R VARIANTS AS RISK MODIFIERS IN CDKN2A MUTATION CARRIERS OF SOUTHERN SWEDISH MELANOMA FAMILIES

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The melanocortin-1-receptor, encoded by the MC1R gene on 16q24.3, stimulates eumelanin production in response to ±MSH and UV-radiation, being a major determinant of hair colour and skin type. The gene is highly polymorphic in Caucasian and Asian populations, but not in native African populations. Some MC1R variants are associated with Red Hair Colour phenotype (RHC), and an increased risk of malignant melanoma and other skin tumors. OBJECTIVE: The present ongoing study addresses the role of MC1R variants in Swedish cutaneous malignant melanoma families with germline CDKN2A mutation. MATERIALS & METHODS: Eleven families (31 patients) were screened for MC1R variants by direct sequencing of the complete single exon. All patients were carriers of the CDKN2A 113insArg founder mutation, and half of the patients (15/31) had developed melanoma. RESULTS: Nine different MC1R variants were found in 26/31 patients. Two of the three known RHC variants, R151C and R160W, were each identified in 10 patients (4 families and 7 families respectively). One patient, that developed multiple melanomas at age 18 and 26 years, was homozygous for R151C. Another patient with multiple melanomas at 23 and 29 was found to carry an R151C allele and an 86insA allele, that give rise to an N-terminal truncation. Along with these observations, a patient with melanomas at the age of 37 and 61 had a wild-type MC1R sequence, and another CDKN2A mutation carrier, unaffected by age 47, was found to carry two RHC alleles (R151C and R160W). Other identified variants are: V60L affecting 5 individuals in one family, D84E, V92M, T95M, R163Q and T314T (g. A942G). CONCLUSION The study reveals a high frequency of MC1R variants in affected CDKN2A mutation carriers, suggesting a modifying effect on age of onset and risk of melanoma. However, the significance of these findings remains to be evaluated in a healthy population.

P-002 A NOVEL MELANOMA-PREDISPOSING MUTATION, CDKN2A E27X, WITH A FOUNDER EFFECT IN NORTHERN ITALY

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Melanoma families from the northwestern Italian region of Liguria display a prevalent founder mutation, G101W, in the CDKN2A melanoma susceptibility gene. This study aimed to verify whether other CDKN2A germline mutations are present in familial melanoma patients from surrounding, non-urban areas. Melanoma cases from areas bordering Liguria, particularly southern Piemonte, are increasingly referred to the National Cancer Institute in Genova and therefore seen at our Genetics Service if familiality is ascertained. Between 2002 and 2004 7 such familial patients underwent routine screening of the CDKN2A gene by direct sequencing. Mutation positive cases and relatives were haplotyped for microsatellite markers at the 9p21 locus. A novel CDKN2A germline mutation that determines a stop codon in exon 1, E27X, was identified in 3 kindreds from neighboring towns in the south of Piemonte (bordering Liguria). Presence of the same mutation was confirmed by sequencing in 2 non-familial cases whose families originally came from those same towns. Haplotype analysis revealed a conserved haplotype at 9p21 for 6 surrounding markers in 2 families, while the third family showed discrepant allels at marker D9S942, which may have arisen through separate slippage or conversion events. Haplotype analysis of another E27X positive family living in Florence revealed the haplotype conservation at three sorrounding markers. The novel E27X mutation is the most frequent CDKN2A germline mutation after G101W in melanoma patients from northern Italy. Our results suggest that it is a founder mutation, and highlight the likelihood that founder mutations may underlie melanoma susceptibility in other poorly admixed Italian populations.

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P-003 AN EXONIC TILING CGH MICROARRAY TO INTERROGATE 1P22: HIGH-RESOLUTION ANALYSIS OF FAMILIAL MELANOMA

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Mutations in known melanoma susceptibility genes, CDKN2A and CDK4, account for only ~20% of families with multiple cases of cutaneous malignant melanoma (CMM). In collaboration with the International Melanoma Genetics Consortium, we previously performed a genome-wide linkage scan of 82 CMM kindreds with no involvement of CDKN2A or CDK4, and have identified a novel CMM susceptibility locus of approximately 15 Mb on chromosome band 1p22. Additionally, we demonstrated that LOH occurs within this critical region in at least 29% of melanoma cell lines, 38% of sporadic melanomas, and 80% of tumors derived from 1p22-linked family members. The development of oligonucleotide CGH microarrays using 60-mers designed to have the sensitivity to detect single-copy number changes with a single probe has greatly improved our ability to perform high-resolution analyses of candidate tumor-suppressor loci such as 1p22. Here we present the application of these arrays to a panel of malignant melanoma cell lines, sporadic melanomas, and germline DNA from 1p22-linked melanoma patients for the purpose of identifying small DNA deletions that may better highlight the position of the 1p22 melanoma susceptibility gene. Samples were whole-genome amplified, labeled via random-priming, and co-hybridized with labeled normal female DNA to 44,000 element human genome-wide microarrays designed specifically for CGH. In addition, these samples were hybridized to custom 1p22 region-specific arrays containing probe features designed to cover gene exons, along with additional features designed to cover intronic and intergenic sequence at a density of one probe per kb. Using these arrays, we have demonstrated the ability to reliably detect single copy differences, identified alterations of genes previously implicated in melanoma (CDKN2A, BRAF), and found and confirmed other novel small genomic deletions and amplifications. The data from 1p22 probe features are currently guiding mutation detection efforts in order to locate the melanoma gene at 1p22.

P-004 FREQUENCY OF COX-2 AND C-KIT IN MAL0GNANT MELANOMA

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Malignant melanoma is a chemoresistant tumor and has poor prognosis even in locally advanced stage. Determination of prognostic and predictive factors are of great importance. Frequency and predictive importance of c-kit and Cox-2 were retrospectively investigated in 50 patients with malignant melanoma. Of the patients, 34 presented with skin, 5 with mucosal, 10 with lymph node and 1 presented with bone marrow involvement. Cox-2 positive tumor rate (94%) was higher than c-kit (62%) positive tumor rate. Of the tumors, 25 (50%) were strongly positive for Cox-2 (3 ++). This rate was 16/50 (32 %) for c-kit positivity. Both c-kit and cox-2 positivity of 3 ++ was seen in only one of metastatic lymph nodes (10%), which was lower than skin (50%) and mucosal (40%) lesions. In our patient population 40 % of the patients had both c-kit and Cox-2 strongly positive tumors. In 4 / 6 (66%) of our patients c-kit was negative in lymph nodes, as it was positive in skin lesions. Of the skin melanomas 19/ 34(56%) had 3++ positive staining with Cox-2. The bone marrow biopsy specimen was strongly positive for cox-2 and c-kit mutation. We couldnt get any other biopsied distant metastatic lesion. Patients with c- kit negative skin tumors had more advanced tumor and shorter disease free survival (DFS) (Mean DFS ± SD= 19 ± 6 months) than c-kit positive cases (Mean DFS = 35 ± 8 months) . As conclusion of this study, it was decided that tumor looses c-kit gene mutation as it progresses. To obtain more reliable results, it is necessary to increase the number of patients and evalute the data with longer follow-up,.

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P-005 QUANTITATIVE TRAIT LOCI UNDERLYING HEREDITARY CUTANEOUS MELANOMA IN THE MELIM SWINE MODEL

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OBJECTIVE : Human cutaneous melanoma is of poor clinical prognosis and therapy, and has been yearly increasing over the past several decades. To search for melanoma susceptibility loci, we started the genetic analyses of the MeLiM (melanoblastoma-bearing Libechov minipig) swine model, and comparatively localized the homologous genomic segments in human. MATERIALS & METHODS : We produced the first filial (F1) animals (affected MeLiM × healthy Duroc) and backcross families (affected F1 × healthy Duroc), and performed a genome-wide scan using 347 backcross animals after clinical and histological phenotyping. Up to now, interval mapping and single marker association test have been done with 200 animals, and data from 347 backcross animals will be processed and presented. RESULTS : Quantitative trait loci (QTLs) on Sus Scrofa chromosomes (SSC) 1, 7 and 10, were found to be associated with melanoma development, as well as some regions on SSC 2, 6, 8 and 14 after linkage and association tests. Massive radiation hybrid (RH) mapping comparatively localized orthologous genes from Homo Sapiens chromosome (HSA) 9p21 on SSC1 chromosomal regions containing some putative QTL(s) in pigs. Our previous work and the characteristic composition pattern of SSC 1 putative QTL peaks compared to HSA9p21 region suggested that novel putative gene(s) underlying melanoma susceptibility might be in these regions, and needs further investigation. Furthermore, after sequencing the entire MC1R (melanocortin-1 receptor) coding region, and single-stranded conformational polymorphism identification, black pigs with allele2 were found to be significantly predisposed to melanoma, after the calibration for coat color and gender effects. CONCLUSION : QTLs underlying melanoma susceptibility in the MeLiM swine have been found, and homologously localized on human genomic counterparts. These results may be useful for human melanoma study.

P-006 ASSOCIATION OF THE 609 C/T NAD(P)H: QUINONE OXIDOREDUCTASE (NQO1) POLYMORPHISM WITH DEVELOPMENT OF CUTANEOUS MALIGNANT MELANOMA

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Cutaneous Malignant Melanoma (CMM) is a life threatening disease whose incidence and mortality rates have risen rapidly in the White Caucasian population in recent decades. The aim of the current study was to investigate the association between polymorphisms in genes involved in DNA-repair and detoxification of reactive metabolites and the development of CMM. The patient cohort consisted of 69 individuals while the control population consisted of 100 individuals. We found a statistically significant association between the presence of the wild type NQO1 C allele and development of CMM [P = 0.04; odds ratio = 2.35]. The NQO1 CC genotype was more strongly associated with CMM development [P = 0.016; odds ratio = 2.92]. The NQO1 gene codes for a protein that has been widely considered to be protective through its ability to detoxify quinones. However recent studies have also linked it to an important source of reactive oxygen and to NF-?B-dependent proliferation of cultured melanoma cells. In conclusion these results link molecular epidemiology and experimental evidence for the role of the NQO1 gene product in development of CMM.

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P-007 MALIGNANT MELANOMA IN ISFAHAN, IRAN

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Background: Skin cancers are common in the east countries like the west countries but some factors including genetic and local carcinogens affect the demographic characteristic skin cancers. The goal of this investigation was to determine the prevalence of melanoma in Isfahan as determined by age, sex and location from 1998 to 2002. Methods: The data were obtained from 2383 cases of skin cancers including melanoma and non-melanoma in a period 4 years by information in cancer registry part of health vice department Isfahan university of medical sciences as a sample from Iran. The data analyzed included age, sex, anatomical distribution, clinical features of primary lesions and pathology reports. Results: Results revealed in our study of the skin cancer registry for the period 1998-2002 that 159 cases had melanoma and 2224 cases had non-melanoma that 1493 cases were males and 790 cases were females. The male-to-female ratio was 1.8 to 1, the commonest site of melanoma in both sexes was the extremity and with respect to Clark's subtype, acral lentiginous melanoma was commonest, accounting for about half of all melanomas. The mean age incidence of melanoma was in seven decades. Discussion: In Iran melanoma was more common in lower extremity like as Japan and the prevalence of melanoma in Iran is less than the west countries.

P-008 MAPPING OF A NOVEL OCULAR AND CUTANEOUS MALIGNANT MELANOMA SUSCEPTIBILITY LOCUS TO CHROMOSOME 9Q21.32

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The incidence of cutaneous malignant melanoma (CMM) is increasing worldwide, likely due to increased sun exposure in interaction with genetic factors. An estimated 10% of all CMM cases are inherited. Whereas mutations in the CDKN2A and CDK4 genes account for 20-25% of families with multiple cases of CMM, the genetics of remaining familial CMM is largely unknown and probably heterogeneous. Ocular malignant melanoma (OMM) is rare, but may be associated with CMM in familial settings. Objective: To localize additional genes responsible for inherited malignant melanoma. Material & Methods: Two extensive pedigrees from western Denmark with multiple OMM (ten) and CMM (three) cases, as well as other malignancies, in which CDKN2A, CDK4, BRCA1 and BRCA2 had been excluded was used for genome-wide scan for linkage using the ABI Prism Linkage Mapping Set with an average spacing of 10cM. Furthermore, we performed quantative PCR for characterization of candidate genes. Results: Evidence for linkage was found for several markers localized to chromosome 9p13-q22 with a peak two-point parametric lod score of 2.2 obtained at D9S167 on 9q21. The families segregated two different haplotypes and recombinations narrowed the linked region to ~11cM. Loss of the non-segregating allele was seen in several OMM tumors. Subsequently, targeted analysis of a third west-Danish family with cases of OMM and CMM confirmed 9q21 linkage, providing a combined four-point parametric lod score of 3.02. Moreover, two of the families shared a common haplotype comprising three consecutive and highly polymorphic markers, limiting the region to less than 5cM and 3Mbp at 9q21.32. Public databases disclose four predicted and three known genes in the region, including biologically relevant genes. Conclusion: This study provides suggestive evidence of a novel melanoma susceptibility locus on chromosome 9q21.

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P-009 GENOMIC PROFILING OF MALIGNANT MELANOMA CELL LINES USING HIGH-RESOLUTION BACARRAY COMPARATIVE GENOMIC HYBRIDIZATION

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The incidence of cutaneous malignant melanoma has increased dramatically in recent years, probably due to increased sun exposure. The etiology of melanoma is complex involving both environmental and genetic factors. OBJECTIVE: To characterize chromosomal alterations and potential oncogenes and tumor suppressor genes involved in tumor development. MATERIALS & METHODS: 40 different cell lines derived from primary and metastatic cutaneous or ocular malignant melanoma were analyzed for DNA copy number alterations using microarray-based comparative genomic hybridization (arrayCGH). Arrays were produced from a recently assembled unique set of 32,433 genomic BAC clones (BACPAC Resource Center at CHORI, Oakland CA) that span the human genome with a contiguous and tiling coverage of >99% at an average resolution of <50kb. RESULTS: Using this 33K aCGH platform we found several distinct copy number alterations, including minute amplifications and homozygous deletions, but also larger changes including losses or gains of whole chromosomes. Frequent losses were found on chromosomes 1p, 4, 6q, 9p, 10 and 11q and frequent gains were found on chromosomes 1q, 6p, 7, 17q and 20. High level amplification peaks and complex amplification patterns were found on 1p11, 5p and 19q12, occasionally including a single or a few genes per amplicon. We found several homozygous deletions pinpointing known or novel potential tumor suppressor genes of importance in melanoma development. Homozygous deletions on chromosome 9p21 varied in size from ~160kbp to ~4Mbp, always including CDKN2A and CDKN2B. Other affected chromosomal regions, such as 11q and 4q, harbor biologically relevant cell cycle-associated and candidate melanoma tumor suppressor genes. CONCLUSION: Genomic profiling may reveal patterns of DNA alterations useful for melanoma classification. We also aim to compare arrayCGH with global gene expression analysis and mutation screening of known melanoma oncogenes. Furthermore, these findings in melanoma cell lines will eventually be tested clinical specimens.

P-010 COMBINATION OF RNAI AND PROTEOME TECHNOLOGY FOR THE ESTABLISHMENT OF NEW TREATMENT MODALITIES FOR METASTATIC MALIGNANT MELANOMA

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Malignant melanoma is an aggressive tumor of high metastatic potential. In late-stage metastatic disease few established therapies exist with response rates of only 10-15%. In recent years so-called RNA interference (RNAi) technology has been established by which particular genes may be down-modulated in a large variety of cell types. Pre-clinical models were indeed promising fostering hopes for a clinical application in the near future. However, much has to be done to make this innovative approach an efficient and safe treatment modality. In the present report melanoma cells were transfected in vitro with RNAi molecules against tumor progression genes such as osteopontin. The latter were identified in own large-scale gene expression studies of primary melanomas and melanoma metastases after laser-microdissection of tumor cells. Since cellular proliferation assays and apoptosis assays may be not accurate enough to test efficacy and putative side-effects of gene interference executed by RNAi molecules proteome analysis was performed. By this means important protein networks and also interesting downstream targets of melanoma progression genes were identified. Among these were cell cycle molecules and molecules involved in DNA repair. Taken together, the present approach may open new perspectives for the generation of specific or maybe even tailor-made therapies for malignant melanoma. Experiments are currently underway using adenoviral gene transfer to target larger series of genes and thereby change the aggressive phenotype of melanoma cells.

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P-011 BRAF V599E MUTATION IN SPITZ AND REED NEVI, IN RADIAL, VERTICAL GROW PHASE AND METASTATIC MELANOMA

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Background Spitz/Reed nevi which occur typically in childhood and adolescence showing histopatologic characteristic features resembling melanoma, are usually considered a benign lesion. In this connection it is clear that molecular pathology may be a useful aid in establishing a correct diagnosis. Since recently, B-RAF mutation in V599E was demonstrated to occur in a high percentage of melanoma, herein we have analysed in Spitz/Reed nevi, in RGP, in VGP and lymph nodal metastasis the possible presence of V599E BRAF mutation. Methods DNA was extracted by biopsy and BRAF exon 15 was PCR amplified using forward and reverse primer sequences. The purified PCR products (4ng) were directly sequenced on both strands using a 3730 XL DNA sequencer (Applied Biosystem). The latter is the last generation of Applied Biosystem sequentiator and it is sufficient 2-4ng to obtain a very good sequence. A375 melanoma cells were used as positive control, since they are homozygous for BRAF V599E mutation, and serial dilution with Jurkat DNA without such a mutation was carried out to estimate of the percentage of mutant DNA detectable. Results All the bioptic samples of Spitz/Reed nevi and VGP/metastatic melanoma showed such a mutation, while in RGP V599E mutation seems to be extremely rare. Conclusion Our findings show that, at least regarding to V599E BRAF mutation, Spitz/Reed nevi like as other benign melanocytic lesions, present the same molecular characteristics of VGP/metastatic melanoma.

P-012 IMPACT OF BOVINE SERUM ALBUMIN ON THE GENE EXPRESSION IN BLACK, GREY, AND WHITE HUMAN MELANOMA SPECIMENS

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Background and Hypothesis: It has been noted that melanin inhibits RNA expression in melanoma samples and the addition of bovine serum albumin (BSA) overcomes such inhibition. As real time quantitative Polymerase Chain Reaction (rtq-PCR) has been successfully developed showing superiority over the regular PCR, we studied the effect of BSA on melanin inhibition in rtq-PCR regarding black, grey and white melanoma samples. We hypothesize that the most significant impact of BSA will be on the black samples. Materials and Methods: Cycle threshold (Ct) difference was used to determine the extent of melanin interference with rtq-PCR in three different pigmented samples. We screened for the expression of eight melanoma-associated and other genes in 22 samples (11 black, 5 grey, and 6 white): gp-100, Mart-1, Mage-A3, Tyrosinase, Pax-A3, Survivin, IL-10, and CCR-7 plus house keeping gene Gus. Results: The following table depicts the percentages of black, grey and white samples having Ct values consistent with gene expression: Of the eight genes, we found that of 11 black samples, BSA reversed melanin inhibition of gene expression significantly in 73% gp-100, 64% Mart-1, 55% Pax-3, 36% Gus, 28% CCR-7, and 18% Tyrosinase samples. The grey and white melanoma samples were not significantly affected by the addition of BSA since Ct cycles remained relatively constant, except for white CCR-7, Survivin and Mart-1 samples. The expression of the eight genes in different melanoma samples with respect to Gus varied among the samples. Conclusions: We conclude that melanin in a significant number of black samples inhibits gene expression and may give false-negative results in rtq-PCR. BSA can restore the gene activity in the majority of black samples, thus, enhancing gene expression in black samples as detected by rtq-PCR. Further, gene expression in different melanoma samples is heterogeneous.

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P-013 MOLECULAR MARKERS FOR CLASSIFICATION AND STAGING OF MELANOMA

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Gene expression and mutation analyses can be used to identify differences in tumor biology and to score markers for diagnostics. Using real-time quantitative RT-PCR, we interrogated 36 samples (30 melanomas, 4 benign nevi and 2 reactive lymph nodes) for the expression of 20 melanoma related genes that function in cell growth and differentiation (EGFR, WNT5A, BRAF, FOS, JUN, MATP, TPM1), cell proliferation (KI67, TOP2A, BUB1, BIRC5, STK6), melanoma progression (CD63, MAGEA3, GALGT), and melanin synthesis (TYR, MLANA, SILV, PAX3, MITF). In addition, samples were tested for mutations in BRAF (exons 11 and 15) and NRAS (exons 2 and 3). Hierarchical clustering analysis of the expression data distinguishes between the melanoma and non-melanoma samples and further stratifies melanomas into 2 groups differentiated by high expression of genes involved in b-catenin activation (EGFR and WNT5A) and the MAPK/ERK pathway (BRAF, FOS, JUN). Eighteen (64%) out of 28 patients had mutations in either exon 15 of BRAF (V599 substitution) or codon 61 of NRAS. Mutations were found in both expression subtypes with similar frequency. We found that the best discriminators for molecularly distinguishing between melanoma, benign nevi, and lymph nodes were MLANA, CD63, and BUB1. These markers could have utility in diagnostics for the detection of melanoma micrometastasis in sentinel lymph nodes.

P-014 A STATISTICAL VALIDATION STRATEGY FOR THE PREDICTION OF THE OCCURRENCE OF DISTANT METASTASIS IN CUTANEOUS MELANOMA USING MICROARRAYS

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Recently, data from seven published studies that have attempted to predict prognosis of cancer patients on the basis of DNA microarrays have been reanalyzed. (Michiels et al.; Lancet 2005; 365:488-92). It was shown that that the list of genes identified as predictors of prognosis was highly unstable and the use of validation by repeated random sampling was advocated to assess prognostic value of microarray data in cancer. Our objective was to apply this random validation strategy to the largest gene expression study of primary cutaneous melanomas (CM), using high-density oligonucleotide genome-wide Agilent microarrays. The standard strategy is to identify a molecular signature (ie, the subset of genes most differentially expressed in patients with different outcomes) in a training set of patients and to estimate the proportion of misclassifcations with this signature on an independent validation set of patients. We expanded this strategy (based on unique training and validation sets) by using multiple random sets, to study the stability of the molecular signature and the proportion of misclassifications. We focused on the binary classification problem between patients who developed a distant metastasis within 4 years and patients who did not. We divided the size N dataset using a resampling-without-replacement approach into 250 training sets (size n) and 250 associated validation sets (size: Nn) for different values of n. We identifed a molecular signature for each training set (defined as the first k genes most associated with prognosis) and estimated the proportion of misclassifications for each associated validation set. In conclusion, the list of genes identifed as predictors of prognosis in CM is highly unstable; molecular signatures strongly depended on the selection of patients in the training sets. The proportion of misclassified cases decreased as the number of patients in the training set increased, with a minimum proportion of 30%.

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P-015 CORRELATION BETWEEN CLINICAL OUTCOME AND GENE EXPRESSION PROFILE IN CUTANEOUS MELANOMAS

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The cutaneous melanoma originates from the malignant transformation of the melanocytes which are located in the basal layer of the epidermis. UV radiation is one of the main etiologic factors for melanoma development but, beyond UV radiation, the combination of genetic alterations play an important role in the biology of melanomas. In order to contribute to the understanding of variability in clinical behavior, we are determining the gene expression profile of tumors from patients with primary melanomas and metastatic melanomas. Equally important is the understanding of the mechanisms that distinguish melanomas from the benign compound nevi. Hence, we are also comparing gene expression profile of malignant and non-malignant lesions. These data are obtained by cDNA microarrays and will be compared with the clinical datas of the same patients. The clinical databank provides data about predisposing factors, clinical and histopathological characteristics of the tumor and close follow up of each patient. Using mathematical and statistical tools we shall determine the association between a given expression profile and clinical outcome as well as the correlation between malignant and non malignant lesions.

P-016 A MICROSATELLITE POLYMORPHISM IN THE HEME OXYGENASE 1 GENE PROMOTER IS ASSOCIATED WITH TUMOR STAGE AND RISK FOR MELANOMA

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Objective. Recently, Heme oxygenase-1 (HO-1) has been demonstrated to play a role in the regulation of signaling systems which are involved in the control of cell cycle progression and apoptosis. A (GT)n dinucleotide repeat polymorphism in the HO-1 promoter was suggested to modulate HO-1 gene expression, whereas short repeats were associated with HO-1 upregulation. Malignant melanoma (MM) is the most serious cutaneous malignancy with its tendency to early metastasis and resistance to apoptosis. Therefore,we sought to determine the influence of this polymorphism on the progression of MM. Patients and Methods. We determined the HO-1 promoter genotype in 152 patients with MM and 398 healthy controls. The homozygous short (<25) repeat genotype and long (e25) repeat allele carriers were compared in regard to Breslow thickness (BT) and disease free survival. Results. Allele class S/S was found more frequently in the MM group compared to the healthy control population. Within the MM group (n=152), allele class S/S was present in 21%(32/152) instead of 12% (46/398) in the healthy control population(p=0.005). Our data also showed, that the S/S genotype was significantly associated with primary tumors with deeper BT compared to L allele carriers (Mean BT:4.0mm ±2.9mm versus 3.1mm ±1.7mm, p=0.03), however, no significant differences were observed in disease free survival (67 months (±49) in L-allele carriers versus 56 (±42) in S/S individuals, p=0.3). Conclusion. Homozygous carriers of short repeats in the HO-1 gene promoter were significantly associated with a significantly increased risk for MM and deeper BT.However,the HO-1 genotype did not significantly influence disease free survival of patients. These data suggest that HO-1 might render a higher risk for MM in S/S genotype individuals and could represent an important candidate gene in the pathogenesis and growth of malignant melanoma.

P-017 INTEGRATING SINGLE-COPY SINGLE-EXON ARRAY-CGH (ACGH) AND EXPRESSION PROFILING TO MAP FUNCTIONAL CONSEQUENCES OF INDIVIDUAL GENE COPY NUMBER CHANGE IN MALIGNANT MELANOMA

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Changes in DNA copy number and gene expression in tumors lead to altered cellular behavior and tumor progression. Recognitions of such genetic alterations in a variety of cancers has lead to improved disease classification, disease course prediction and identification of novel therapeutic targets. However, despite significant effort, a comprehensive picture of integrated expression and single-copy change in malignant melanoma has not yet appeared. Here we present an integrative approach for comprehensive analysis of malignant melanoma using genomic and transcriptional level profiling which makes use of a novel aCGH platform coupled with expression profiling analysis. The CGH microarray used in this study utilizes 44,000 60-mer oligonucleotides specifically designed to have sensitivity to detect single-copy number changes at single exon resolution. This approach allows identification of previously undetectable small intra-genic deletions, gene duplications and unbalanced translocation leading to identification of novel recurrent abnormalities. A total of 29 flash frozen clinical melanoma samples were collected and DNA and RNA were isolated from the same frozen blocks. DNA was whole-genome amplified, labeled via random-priming and co-hybridized with labeled normal DNA for aCGH. Information will be presented regarding the presence of small homozygous deletions detected in loci previously suspected to harbor tumor suppressor genes, as well as in novel loci not previously recognized. We are currently evaluating the frequency at which these genetic changes occur in a larger cohort of sporadic melanomas. In addition, expression-profiling analysis was performed using the RNA extracted from the same set of frozen blocks. Expression profiling data was integrated against the aCGH and patterns of expression changes for the most common gene copy number changes were mapped. This approach identified dynamics of gene expression as functional consequences of individual gene copy number changes.

P-018 ASSOCIATION OF ENDOTHELIN RECEPTOR B NON-SYNONYMOUS VARIANTS WITH MELANOMA RISK

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The endothelin system plays a crucial role in melanocyte differentiation and migration. In this study, we hypothesized that germline mutations of endothelin receptor B (EDNRB), a gene involved in Hirschsprung disease (HSCR), could also predispose to melanoma (MM). The coding region of EDNRB was entirely sequenced in 137 MM patients and 130 ethnically matched Caucasian controls. The association between EDNRB and MM was assessed by comparing number of patients and controls carrying non-synonymous EDNRB variants using Chi2 and two-sided p tests. EDNRB nucleotide diversity in patients and controls groups was also assessed. Multiple logistic regression analysis was performed taking into account all clinical and genetic melanoma-risk factors. Six EDNRB mutations were found in 15 patients (11%), but only 2 in 4 controls (3%, p-value, two-sided=0.012, OR: 3.87 [1.25-12]). Interestingly, one patient with an explosive melanoma of the scalp was harboring two EDNRB mutations. Overall, 14/15 MM patients carried EDNRB mutations reported in HSCR disease, some of which were previously shown to be loss-of function. In addition, the pattern of EDNRB nucleotide diversity showed an excess of non-synonymous substitutions in patients. Finally, in multiple logistic regression analysis, the effect of EDNRB mutations on MM risk remained significant (p = 0.030). Our results strongly support that EDNRB can be involved in predisposition for two different multigenic disorders, Hirschsprung disease and melanoma.

P-019 A CASE/CONTROL STUDY OF THE FREQUENCY OF P53 CODON 72 POLYMORPHISM IN PATIENTS WITH CUTANEOUS MELANOMA

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Background. The substitution of an arginine to a proline in codon 72 of p53 gene results in two structurally and functionally different proteins. Because p53 plays an important role in carcinogenesis, this polymorphism has been studied in various human malignancies, including cutaneous cancers, with inconclusive results. Materials & Methods. In this hospital-based case-control study we used allele-specific polymerase chain reaction to investigate the frequency of p53 codon 72 polymorphism in blood specimens from 90 Greek patients with malignant melanoma and 108 controls. Results. Study populations did not deviate from the Hardy-Weinberg equilibrium. The genotype distributions between cases and controls were calculated, and univariate and multivariate analyses were performed, in order to evaluate whether a codon 72 variant is associated with increased risk of cutaneous melanoma. The genotype frequencies were not statistically different between cases and controls (frequencies of Arg/Arg, Arg/Pro and Pro/Pro were 52.2%, 37.8% and 10% respectively in the cases and 46.3%, 49.1% and 4.6% in the controls, p=0.15). Further analysis of the clinical data, however, revealed a statistically significant association between Pro/Pro genotype and malignant melanoma patients with dark eyes versus controls (p=0.015). Conclusions. In conclusion, an association between p53 codon 72 polymorphism and malignant melanoma was not established in this study.

P-020 GENE EXPRESSION SIGNATURE OF IFNA2 SENSITIVITY IN HUMAN MELANOMA CELLS

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Objective IFNa2 therapy is part of the management of malignant melanoma with unpredictable individual success rate, therefore the identification of IFN-sensitivity gene signature would have potential clinical significance. Method We have used human melanoma cell lines characterized by high or low in vitro sensitivity for IFN-a2. Gene expression profiles have been determined by microarray analysis using a 3.2K custom made human cDNA chip. Expression differences were considered significant in case of >2-fold increase or >50% decrease. Results The high and low IFN-sensitive human melanoma cells differed significantly in their altered gene expression profiles involving 19 known genes. On the other hand, out of the 3200 genes tested, another 24 showed differential expression in the two cell lines following in vitro IFN-a2 exposure. The majority of the genes (19/24) were found to be down-regulated and only a few were upregulated in the two human melanoma cell lines following cytokine exposure. 9/24 genes responded similarly to IFN-a2 in the two cell lines suggesting irrelevant gene alterations. Upon IFNa2 treatment 7/24 genes were changed in the sensitive cell line exclusively (5 down- and 2 upregulated) while 8/24 genes were different in the resistant cell line only (4 up- and 4 downregulated) defining the sensitive and resistant signature. Conclusion Comparison of the gene expression profiles of IFNa2 sensitive and resistant human melanoma cell lines defined a cytokine signature of 19 genes out of which 12 can be considered specific for the biological response. Further analysis of the 12-gene set in vivo may help to define a clinically usefull cytokine sensitivity signature. This work was supported by the Ministry of Economy (GVOP 3.1.1.-2004-05-0090/3.0).

P-021 MOLECULAR CHARACTERIZATION OF THE INFLAMMATORY GENE PROFILES IN SENTINEL NODES IN MELANOMA

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Introduction: Sentinel node dissection is a minimally invasive procedure for staging melanoma. This procedure has proven extremely accurate for diagnosing metastases, and enables us to investigate the inflammatory interactions of the primary melanoma and sentinel nodes (SN). We hypothesized tumor-positive SN (SN+) would posses an immunosuppressive cytokine profile not seen in tumor-negative SN (SN-). Methods: We evaluated 23 fresh SN (13 AJCC stage-I/II and 10 stage-III with micromet<2mm) using a 96-gene cDNA-microarray. Clinical and pathologic features of patients are not significantly different except SN status. RNA were extracted and converted into biotin-16-dUTP labeled cDNA, then amplified with PCR. Gene expression was normalized to 2-actin and compared between SN+ and SN-. Over- or under expression were defined as a 2-fold increase or decrease. Statistical analysis used Students t-test, Receiver-Operator Characteristics (ROC) and discriminant analysis. Results: Of 96 inflammatory cytokine-associated genes evaluated, 73 genes were similarly expressed in SN+ vs. SN-. 19 genes were up-regulated in SN+. IL-13, leptin, lymphotoxin beta receptor (LTbR), and macrophage inflammatory protein (MIP)-1b expression level were statistically higher in SN+ vs. SN-(24.4-fold, $p<0.04$, 3.5-fold, $p<0.01$, 2.3-fold, $p<0.05$, and 2.1-fold, $p<0.01$). IL-11Ra expression level was statistically lower in SN+ than SN-(0.1-fold, $p<0.03$). ROC curve show the area under the curve for IL-13, leptin, LTbR, MIP1b and IL-11Ra are 0.79, 0.83, 0.75, 0.81, and 0.77. When combined, the 5 genes ROC was 0.957 suggesting the high level of concordance of gene-expression with SN staging. For SN+ patients, these genes were less evident in the primary tumor. Conclusions: SN+ have a cytokine gene profile suggestive of local immunosupression with increased expression of IL-13, leptin and MIP1b, along with diminished expression of IL-11Ra (macrophage maturation/activation gene) and LTbR, a gene associated with inflammatory cell apoptosis. This cytokine profile in SN+ may be important for melanoma invasion and propagation though the SN.

P-022 XENOVACCINATION OF PATIENTS WITH SKIN MELANOMA: PHASE I/II CLINICAL TRIAL

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It is reported that xenovaccination of BDF1 and C57B16 mice with reinoculated human melanoma (SK.MEL-28) cells or with primary cell cultures of human melanoma encapsulated into polyacrylamide gel (PAAG) results in a biologically significant (>70%) inhibition of growth of the syngeneic melanoma B-16.This effect is accompanied by the appearance of activated cytotoxic splenocytes in the early post-vaccination period (up to 12 weeks). Materials and Methods: The main goal of this study was to assess the efficiency and toxicity of PAAG xenovaccination in patients with high risk skin melanoma. The study included 20 patients with histologically verified skin melanoma grades IV-V invasion (according to Clarke) who underwent radical or cytoreductive operations after signing an informed consent. The tested material included PAAG and the PCMM B-16 vaccine (cell cultures of murine melanoma B-16). PAAG was injected subcutaneously into the outer surface of a shoulder. Three to six weeks thereafter, the PCMM B-16 vaccine was injected under ultrasound control into a subcutaneous capsule formed by PAAG. The resulting depot retained its shape and size for a long period of time and was visualized as a shapeless hypoechogenic structure. Results:No systemic toxic or allergic responses were noticed. Local responses: DTH response - 65%, slight itching -10%, peeling - 5%, tenderness -15%, slight burning -10%. Stimulation of immune response was noticed on the 14th and 30th post-vaccination days and was manifested in increased populations of CD3+, CD4+ and CD8+ T-lymphocytes (P=.02, P=.03 and P=.05, respectively).The activation peak of CD4+ cells was observed on day 14(P=.02), CTL CD8+ cells - on day 30(P=.05). This effect and the DTH response testify to the indisputably high immunogenicity of the tested vaccine. Conclusion:Xenovaccination is generally well tolerated, does not elicit pronounced local and general toxic responses, is associated with DTH and stimulates the formation of CTL.

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P-023 THE SCREENING OF MELANOMA IN A MEDITERRANEAN ISLAND : NEW TECHNOLOGIES, PRELIMINARY RESULTS AND OBJECTIVE DATA

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The Government of Malta promoted for the first time worldwide the computerized screening of the whole population of the Gozo Island, 28,000 abitants. The homogeneity of the population renders this study particularly rich of informations. The Computerized System, DB-Mips, equipped with exaustive patient's forms databases help the clinicians to store the patients informations, to provide high resolution total body pictures and to process in real time all of the observed lesions providing expert-level aid to the early diagnosis of melanoma. Innovative mathematical algorithms are actually employed in order to obtain objective variables to be linked with the patient's personal history and compared to other populations. The instant computerized diagnostic aid is given on live images at a frame rate of 15 frames/second, providing full fast support with an accuracy higher than 90% [1-2] : patterns analysis, skin measurements and other 49 objective variables are linked with the patients data. The digital images of the patients lesions are grabbed both through epiluminescence technique, macro photography and global view in order to provide the most complete visual description : the most suspicious lesions are transmitted through telemedicine to 4 reference centers in Italy, Germany and Switzerland. The objective analysis of the pigmented skin lesions belonging to the Gozo abitants will be compared with other 27,000 lesions grabbed and processed in other countries with the same equipment in order to evaluate morphological and chromatic differences among different people. [1] Burroni M, Corona R, Dell'Eva G, Bono R, Perotti R, Nobile F, Andreassi L, Rubegni P. Melanoma computer-aided diagnosis: reliability and feasibility study. Clin Cancer Res. 2004 Mar 15;10(6):1881-6. [2] Rubegni P, Cevenini G, Burroni M, Perotti R, Dell'Eva G, Sbano P, Miracco C, Luzi P, Tosi P, Barbini P, Andreassi L Automated diagnosis of pigmented skin lesions. Int J Cancer. 2002 Oct 20;101(6):576-80.

P-024 DEVELOPMENT OF A HUMAN IN VIVO METHOD TO STUDY THE EFFECT OF SUNSCREENS AND ULTRAVIOLET RADIATION IN MELANOCYTIC NEVI

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BACKGROUND: UV radiation (UVR) is generally accepted to play an important role in the development of acquired melanocytic skin lesions. Sunscreens have shown a great impact in the prevention of UVR damage in keratinocytes. However their role in protecting melanocytes against UVR has not been well established. OBJECTIVES: To design and validate an in vivo human model to study the influence of UVR and sunscreens protection in nevi. METHODS: Based on previous studies about UVR effect in melanocytic nevi, a model describing clinical, dermoscopy, histopathological, immunohistochemical, and molecular changes has been elaborated. RESULTS: First step consists of an in-vivo methodology. UVB minimal erythematous dose (MED) is calculate for each volunteer patient, and two MED-UVB is irradiated to a half of selected melanocytic nevi, whereas the other half of lesions are randomized between remaining with no irradiation, or applying topical sunscreen before the same irradiation. Clinical and dermoscopic features are compared in two halves of each nevus. Second step of the study is performed ex-vivo, after excision of all irradiated nevi 7 days afterward. Histopathological features and immunohistochemical demonstration of melanocytic activation markers (Melan-A, HMB45) and cell cycle-regulators expression (p53, p16, CyD1, Bcl2, MMP2, TIMP2, survivin, Ki67) in each part of irradiated and non-irradiated nevi are studied. In addition, mutations in genes implicated in nevogenicity and melanoma development (BRAF, CDKN2A) are studied in tissue specimens from nevi. CONCLUSION: We have developed a human model to study the UVR influence, and efficacy of sunscreens, in melanocytic lesions. In vivo and ex vivo differences between irradiated nevus versus non-irradiated and versus irradiated plus sunscreen, should provide a useful and secure method to study UVR influence and sunscreen protect role, in benign melanocytic lesions.

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P-025 TOPICAL PHOTOPROTECTION IN THE PREVENTION OF CUTANEOUS MELANOMA: CONSUMER' NEEDS, THE SPECIALIST'S DUTY.

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Objective. When spring comes, the issue of photoprotection is covered in depth by newspapers and, in general, by mass media. Despite the fact that a lot is said about sun photoprotection and tropical protection for the prevention of cutaneous melanoma, nowadays there is still some confusion about the real utility of those substances and the most effective way of using them. How have consumer' needs changed in the third millennium? Our survey aims at investigating consumer' most urgent needs concerning sun filters. Materials and methods. Our study was carried out on 650 subjects randomly chosen among the customers of various chemists' and beauty shops in the province of Naples. They wee asked to answer a detailed questionnaire concerning not only their most common habits of photoexposure, but, most of all, about which factors affect their choice of sun filters, about what they expect from a good sun filter, as well as about the importance they give to its cosmetics properties and to whom they apply for solution of such problems. Results. Our survey has revealed that 82.4% of the population concerned use sun product and that 69% of them are women. Most males (67%) do not use any protection. They do not use solar filters because 37 percent of them believe they are useless. Too many, among the responders self administer the filters (62 %) while only a few of them know what SPF means. Most of them think the terms high protection and minimum protection are too complicated. Conclusion. Italians love the sun though they consider it as responsible for cutaneous melanoma and other damage. They would like a clearer and simpler terminology from producing firms and more answers to their questions from the specialist. *supported by Johnson-Johnson.

P-026 ADVERSE SIDE EFFECTS FROM HERBAL REMEDIES IN DERMATOLOGY

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Background and objectives: Herbal treatments are becoming increasingly popular, and are often used for dermato-cosmetical conditions. Thus dermatologists should know about their potential to cause adverse events. This review is aimed at addressing this area of medicine as an important reminder to dermatologists. Some agents, particularly ; cedre, calendula,and rosemary ,have been shown repeatedly to be used instead of antifungals ,antibiotics and corticosteroids. Virtually all herbal remedies can cause allergic reactions and several can be responsible for photosensitization especially furocoumarine containing groups which cause photoinduces lesions and sun -related cancers and change the benign lesions to a melanoma for example . Some herbal medicines, contain arsenic or mercury that can produce typical skin lesions and malignancies as well. Other popular remedies that can cause dermatological side-effects include St John's Wort, sesame oil, aloe vera, eucalyptus, camphor, henna and clover leafs. Finally, there are some herbal treatments used specifically for dermatological conditions, e.g. calendula officinalis for atopic eczema, or sage for acne and alopecia which have the potential to cause allergic or even systemic adverse effects. Conclusion: It is concluded that adverse effects of herbal medicines are an important albeit neglected subject in dermatology which deserves further systematic investigation. We can add to the list of raedy to use OTC in dermatology the vast species of herbs and plants which are not at all safe for practice. Keywords: Herbal agents,side effects,dermatological diseases.

P-027 SPECTROMETRY AND CUTANEOUS MALIGNANT MELANOMA: AN EXPERIMENTAL STUDY

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Objective: As early diagnosis is the gold standard in the treatment of melanoma, the Unit of Plastic and Reconstructive Surgery at The National Institute for Cancer Research in Genoa, Italy is carrying on a new project based on spectrometry in collaboration with DV S.r.l. (Padua, Italy). The purpose is to develop, test and validate a prototype system (Spectramed) for the analysis of skin lesions, providing acquisition and visualization of the affliction in visible and near-infrared light bands. The non-invasive system is based on fast spectral imaging to extract high level features for melanoma detecting and diagnosis. Methods: The system is based on different modules: 1. spectral acquisition module (hardware core), obtained by integrating the spectrograph with the matrix optical sensor (CCD), so as to form a scanning head; 2. diagnosis support module used in the diagnostic phase (images visualization, possibility to analyse and extract colorimetric features, data collection and retrieval for the patients follow-up); 3. telemedicine module to share information using a telemedicine support media, to implement remote diagnosis. The spectrographic system is based on the PGP (Prism-Grating-Prism) technology, which analyses the light spectrum (from infrared to ultraviolet) independently of light polarization. The spectrometer, obtained by the combination of the spectrograph with the matrix optical sensor, allows the colour measurement along a line on a large number of points, with high spatial and spectral resolution. Results: By now we have found 4 melanomas out of 121 lesions examined. The preliminary results of the analysis and comparison between spectrographic and hystological features will be soon available. Discussion: Clinical impact is validated by a program coordinating the activities of the medical units involved. After gathering an informed consent, lesions at risk are thus excised and tested with Spectramed. At the moment, the data collection is allowing the creation of a database for diagnostic correlations between test data and hystological features.

P-028 IMPROVING PROTECTION IN SUNSCREEN USERS A RANDOMIZED STUDY SHOWING THE MAJOR EFFECT OF LABELING AND COST

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Background: Although sunscreens (SCs) are not ideal protection against sun-induced skin cancers, they are widely used. It is thus crucial to correct the main factors which compromise protection by SCs: insufficient amount per application, and incorrect SCs choice. An hardly understandable labelling for the layman (Sun Protection Factor, SPF), and a high cost may account for misuse. Design: 3-arm prospective randomized survey in 364 individuals in 3 beach resorts to assess whether an explicit labelling and a low cost could influence favorably the use of SCs and the subsequent skin protection. Intervention:1/ Free SCs (FS) = 4 types of SCs with their usual sun protection factor B and A (SPF) label (60B-60A, 20B-20A, 12B-12A, 6B-3A) at free disposal; 2/ Same free SCs with new explicit labelling (FNL), summarizing their properties in terms of sunburn protection, likely protection against long-term effects of UV, and possibility to get a tan; and 3/ No intervention (NI). Results: As compared to FS, FNL significantly increased the quantity of SCs applied (+ 85%;much more in individuals who did not consider tanning as a major objective than in "tan-seekers"), reduced sunburns particularly in sun-sensitive individuals (25.6% vs 58.3%, p=0.005), and induced a shift in the level of SCs chosen. As compared to paying SCs (NI), free-SCs delivery was associated with a more systematic application of SCs in case of sun exposure, and a decreased sunburn occurrence, without increase of sun exposure. Conclusion: As compared to the SPF, an explicit labelling of SC bottles dramatically improves the use of SCs and the skin protection in those who search for safety, and gives a better perception of the risks in "tan-seekers". Pragmatic public health measures as to SCs labelling and cost could improve a lot the protection in SCs-users, without inducing a more risky behavior.

P-029 MELANOMAS THAT ESCAPE EARLY DETECTION - THE CHARACTERISTICS AND ASSOCIATIONS OF RAPIDLY GROWING MELANOMA

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Objective: Anecdotal experience suggests that fast growing melanomas escape early detection. Little information is available on characteristics and associations of such tumours. This study aimed to describe the spectrum of melanoma growth rates and to explore the clinical associations of fast growing melanomas. Methods: A cross-sectional study was conducted between April 2003 and September 2004, involving participant interviews, skin examinations and independent pathology reviews. Rate of growth (ROG) was calculated as the ratio of Breslow thickness to time delay between melanoma appearance and excision, based on previously reported methodology. Geometric mean (GM) of ROG was compared between subgroups of patients and expressed as the GM ratio (GMR). Three tertiary centres and two private practices in Australia participated. Results: A final cohort of 404 participants with invasive malignant melanoma was included. Median ROG (mm/month) was 0.12 for superficial spreading melanoma, 0.13 for lentigo maligna melanoma, 0.49 for nodular melanoma (NM), 0.13 for females, 0.28 for males, 0.17 for those <70 years of age and 0.52 for those >=70. Fast melanoma growth was found in thicker tumours (<1mm, GMR 1.0; 1-4mm, GMR 2.5; >4mm, GMR 6.3) and mitotically active tumours (<1/mm2, GMR 1.0; 1-4mm/mm2, GMR 1.6; 5-10/mm2, GMR 2.3; >10/mm2, GMR 3.0). Faster tumour growth was also found in males (GMR 1.9), the elderly (>70, GMR 3.4), individuals with fewer naevi (<50, GMR 1.9) and fewer freckles (GMR 2.2). NMs grew faster than other radial growth phase melanomas (GMR 2.1). All above associations were statistically significant (P<0.05) on multivariate analysis. Conclusion: This is the first study to describe the spectrum of melanoma growth rate. We found evidence to suggest that fast tumour growth is associated with poor prognostic indicators and occurs in males, the elderly and those with fewer naevi and freckles.

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P-030 WHAT FEATURES DO PATIENTS NOTICE THAT HELP TO DISTINGUISH BENIGN PIGMENTED LESIONS FROM EARLY MELANOMAS? THE ABCD(E) RULE VERSUS THE 7-POINT CHECKLIST

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Objective: The ABCD(E) rule and the 7-point checklist are well-established tools used by clinicians to aid the early detection of melanoma. However, little is known of their value to patients with respect to aiding self-detection. This study aimed to investigate features that patients notice when identifying early melanomas and to explore how well these features correspond to the ABCD(E) rule and the 7-point checklist. Methods: A retrospective, modified case-control study involving patient interviews with respect to their observations of their pigmented lesions. All interviews were conducted in the time period between lesion removal and the results of pathology being known to the patients and the interviewers. Private consulting rooms of a Melbourne dermatologist (JWK) and a Newcastle plastic surgeon (CH) participated in recruitment. Results: 67 patients with benign pigmented skin lesions and 54 patients with early melanomas that were less than 1mm in Breslow thickness, and possessed an identifiable radial growth phase. Using a logistic regression model, change in size or colour, as listed under the major criteria in the 7-point checklist, were most useful in differentiating early melanomas from benign pigmented lesions in the hands of patients (OR 4.18, 95% CI 1,74 -10.02, P<0.01; OR 3.12, 95% CI 1.27-7.66, P < 0.05, respectively). The ABCD(E) rule failed to discriminate melanoma from other benign pigmented skin lesions. Conclusion: In the patient's perception, change in size or colour were most important in distinguishing benign pigmented lesions from early melanomas. Such features enable patients to identify early melanomas and therefore should be emphasised in public education campaigns. Medical professionals should also remember to seek a history of change in assessing pigmented skin lesions.

P-032 FIVE YEARS OF EUROMELANOMA DAY IN BELGIUM

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Since 1999, a Melanoma Day has been organized once a year in Belgium during spring. During that day, 65 % of Belgian dermatologists were mobilised to do free skin examinations. A media campaign was launched a month before in order to announce the day, try to target the population at risk and provide general information about melanoma with a primary and secondary preventive goal. To define the population screened, a questionnaire was filled in on all the patients screened that day and another was sent a month later to the participating dermatologists Despite the dermatologists impression that a population at risk was seen only during the month following the Melanoma Day, analysis showed that the population screened on that day was at risk. In fact, repetitively, that day, the number of the melanoma screened showed an incidence largely above the estimated European melanoma incidence. The results concerning this population screened will be presented. One can debate the need for such a mobilisation. However, the Belgian Melanoma Day creates a national event readily supported by the media, which, following the advice of 80% of the participating dermatologists changes the attitude of the population regarding a more regular scanning (secondary prevention). The effect regarding primary prevention, melanoma and other skin cancers incidence, morbidity and mortality in Belgium still has to be determined.

P-033 IN-VITRO COMPARISON OF IRANIAN AND EUROPEAN SUNSCREENS IN UVA BAND (320-400 NM)

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Objectives This work compares the transmission of various Iranian sunscreens with European brands of the same SPF by the facilities provided at UMIST (UK) Atmospheric Physics laboratories. In addition, UVA star ratings (UVA/UVB) of Iranian samples were determined according to the Boots Star Standard and compared to the European samples. Materials and Methods Sunscreens were evenly applied on substrate Transpore 3M tape (25.4x40.0 mm) fixed in the centre of a 45 mm quartz plate. A 1000W UV Xenon Arc Lamp (Oriel 6271) mounted on the solar simulator (Oriel 66020) was used as the light source. A collimator was used between the simulator and samples to ensure uniform exposure. Transmissions were measured by an Optronic 742 spectroradiometer. Significant differences were determined by statistical methods. Results and conclusions The results show that the UVA transmission of many Iranian sunscreens is much higher (P<0.05) compared to European brands of the same SPF (Fig.1). The differences appear from 335 nm and get larger (P<0.05) for longer UVA wavelengths (Fig.2,3). Due to high solar UV level in Iran and increasing application of sunscreens, the results suggest that Iranian sunscreens need more routine physical and chemical in-vitro checks by the health authorities (Ministry of Health and Medical Education and Standard Organization). Keywords: In-vitro sunscreen test, Iranian sunscreens, UVA rating. References 1 B.L. Diffey, A method for broad spectrum classification of sunscreens, Int. J. Cosm. Sci., 16, 47-52(1994) 2- F. Ahmadi, Jorjani, M., Amirjavanbakht, A. SPF Comparison of various Iranian Sunscreens. Pazkouhesh (Iranain Journal of Medical Research), No.3, 217-221, (2002)

P-034 DIFFERENCES AND SIMILARITIES OF THE EUROMELANOMA SCREENING CAMPAIGN IN TWO EUROPEAN COUNTRIES OF NORTHERN (BELGIUM) AND SOUTHERN LATITUDE (GREECE)

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Background: The Euromelanoma screening campaign has been organized by dermatologists in several European countries, including Belgium and Greece. The screening event occurs annually with an aim to detect melanoma at an early curable stage and increase public awareness about skin cancer through an intense mass media campaign. Methods: We performed a comparative analysis of the demographic, epidemiologic and clinical data derived from the questionnaires completed during the screening event in the two countries. Results: A total of 22,951 Belgians and 9,723 Greeks were screened on the Euromelanoma screening campaign in the years 2000-2004. Interestingly, the gender, the mean age and the skin phototype of the screened population were similar in both countries. A history of sunburns during childhood was reported equally high in both screened populations (approximately 50% of screenees). In contrast, the use of sunbeds was reported by 30 % of Belgian compared to only 3% of Greek screenees. A family or personal history of melanoma was reported twice as highly in the Belgian population. Dysplastic naevi were observed in 20% of Belgian and in 30 % of Greek screenees. The average detection rate of melanoma in the years 2003-2004 was 0.3% in both countries, with a higher proportion of thin melanomas (thickness <1 mm) diagnosed in Belgium (70%) than in Greece (50%). Conclusion: Despite latitudinal differences between Belgium and Greece, the 5-year results of the Euromelanoma screening campaign showed interesting epidemiologic and clinical similarities. The high incidence of personal or family risk factors among screenees suggested that, in both countries, the campaign targeted a population at risk for melanoma. Although its impact on the early diagnosis of melanoma is unclear, the Euromelanoma screening campaign has created an event readily supported by the media, which has changed the attitude of the population regarding a more regular screening.

P-035 TARGETING MIDDLE-AGED AND OLDER MEN TO IMPROVE MELANOMA AWARENESS AND EARLY DETECTION

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Objective: Melanoma incidence and mortality have increased dramatically in the United States, particularly among middle-aged and older men. Spouses/partners of male cases have been shown to play a vital role in melanoma discovery. We sought to explore behavioral and medical care antecedents and the role of the spouse/partner in men with thin (≤2mm) and thick (>2mm) primary melanoma. Materials and Methods: 200 men 40+ and their spouse/partner are being surveyed in the melanoma centers at Stanford, Michigan, and Pittsburgh within 3 months of diagnosis. Behavioral factors including risk factor/warning sign awareness, skin self-examination practices, and medical care antecedents are being assessed, along with the role of the spouse/partner in the subject's health maintenance. To develop a toolkit to boost men's awareness of early detection, we have extensively reviewed the literature on men's access to health information and patient empowerment. Results: We have paired data (cases and spouse/partner) for 78% of subjects; 33% of cases were diagnosed with lesions >2mm. More than 60% of cases (prior to diagnosis) were not confident that they knew the difference between melanoma and ordinary growths or knew what kind of moles to look for if they examined their skin. Most (62%) never had a nurse or physician-led discussion about melanoma. Less than a quarter paid careful attention to information about skin cancer detection. While cases and spouse/partners agreed on many questions, spouse/partners were more likely than their husbands to have received instruction on how to look at the skin for signs of melanoma. Conclusions: Men over 40 should be targeted for improved melanoma awareness. Educational health messages should be directed to the spouses/partners to enhance discovery of early melanoma. Moreover, outreach strategies for melanoma detection will be boosted by an understanding of corporate and other health marketing to men of this age group.

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P-036 LINKING REAL-TIME SOLAR ULTRAVIOLET RADIATION EXPOSURE WITH THE SOCIAL AND PHYSICAL ENVIRONMENT, ACTIVITIES, KNOWLEDGE AND ATTITUDES OF NEW ZEALAND SCHOOL CHILDREN

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Although New Zealand experiences high levels of summer-time erythemal solar ultraviolet (UV) radiation, and excess exposure early in life is a risk factor for melanoma, to date, no measures of exposure and risk factors among a large, random sample of school children have been available. To meet this lack, schools from five geographically different regions from throughout New Zealand were randomly selected and 28 schools and around 500 students in two age groups (modal ages 8 and 12 years) consented to participate. The study aim was to create a comprehensive research database with the goal of helping to inform health promotion interventions targeted at preventing harmful UV radiation exposure in early life. Electronic UV radiation dosimeters (cross-calibrated against meteorological grade instruments at NIWA, Lauder), which calculated erythemally-weighted UV radiation at the lapel site, were used to measure actual individual solar UV radiation exposure at 8 second intervals over a one week period during the summer of 2004-5. Concurrent activity diaries were self-recorded, and social and physical environmental factors assessed, including the influence of school and community factors and local weather conditions. Students: sun exposure attitudes, behaviour and knowledge were ascertained using age-appropriate questionnaires. A skin phototype assessment was conducted using the Fitzpatrick classification and a Munsell colour chart. The response rate among eligible schools was 96%, with two randomly selected replacements invited to participate before fieldwork began. The overall student participation rate was 76%. Preliminary results will be presented from this study which is unique in being able to link a comprehensive range of risk and protective factors in an integrated research database.

P-037 TOTAL NUMBER OF POSITIVE NODES AND PRIMARY MELANOMA MITOTIC RATE AS MAJOR PROGNOSTIC FACTORS IN POSITIVE SENTINEL NODE PATIENTS.

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INTRODUCTION: Factors influencing the presence of positive non-sentinel nodes (+ non-SNs) and their impact on survival in patients with +SNs have not previously been examined in detail. METHODS: This retrospective Sydney Melanoma Unit study involved 244 clinical Stage I and II patients with primary tumor thickness >1.0mm or <1.0mm and Clark level IV. Between 1992 - 2004 these patients had a SN biopsy, were found to have at least one +SN and underwent an immediate completion lymph node dissection. RESULTS: At least one +non-SN was found in 45 (18%) patients. No primary tumor characteristic examined (thickness, mitotic rate or ulceration) nor the number of +SNs was predictive of +non-SNs. Actuarial 5-year survival in 159 patients with one +SN was 64%, in 75 patients with a total (both SN and non-SN) of 2-3 +nodes it was 52% and in 10 patients with a total of >3 +nodes it was 26%. Multivariate analyses utilizing 5 separate Cox models reinforced these findings and indicated that the total number of +nodes (SN and non-SN) was the most important independent predictor of survival. Primary melanoma mitotic rate surpassed tumor thickness in its prognostic strength in each model. Four distinct, significantly separate risk groups were identified by the exponential survival tree method. These were in descending order of survival: 1. +SN only, primary tumor thickness <2mm, 2. +SN only, primary tumor thickness >2mm, <10 mitoses/mm,2 3. +SN only, primary tumor thickness >2mm, >10 mitoses/mm2 or a total of 2-3 +SN and +non-SN nodes and 4. a total of >3 +SN and +non-SNs. CONCLUSION: If the independent significance of mitotic rate in +SN patients is confirmed by other large series, this may have important implications for stratification in future trials and further revisions of the AJCC Staging System.

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P-038 PATTERN OF MELANOMA RELAPSE FOLLOWING SENTINEL NODE BIOPSY - COMPLETION LYMPHADENECTOMY DOES NOT INCREASE THE RISK OF IN-TRANSIT DISEASE

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Purpose The aim of this study was to compare the clinical pattern of first disease recurrence between sentinel lymph node biopsy (SLNB) negative and SLNB positive melanoma patients within our own and other cohorts relative to non-selected melanoma patient cohorts. Patients and Methods Data were extracted from the hospital records of 226 patients who underwent SLNB in our unit to December 2002. A systematic review of published studies employing a combined technique identified 9 papers providing data on sentinel nodal status and clinical pattern of first relapse. Results Overall there were 736 relapses amongst 4713 subjects who had undergone SLNB (966 SLNB positive), a relapse rate of 15.6%. Of these relapses; 98 (2.1%) were nodal, 232 (4.9%) were either in transit or local recurrence, and 406 (8.6%) were distant. In a large reported cohort not subjected to selective lymphadenectomy the equivalent figures are 7.8% nodal, 3.4% in transit and 4.4% distant relapses. The proportion of relapses due to in transit disease was virtually identical in SLNB negative (30.2%) and SLNB positive (32.6%) cohorts. Conclusion Selective lymphadenectomy reduces the absolute rate of nodal relapse mainly at the expense of an increased rate of distant metastases. The modest increase for in-transit disease is probably explained by patient selection. Completion lymphadenectomy per se does not appear to increase the risk of in transit disease

P-039 EVALUATION OF PACLITAXEL AND DACARBAZINE COMBINATION IN METASTATIC MELANOMA

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Background: Metastatic melanoma has a poor prognosis, with less than 11 months median survival rate from the diagnosis. Response rates for both dacarbazine and paclitaxel as single therapy are around 20%. High levels of S100 protein were found in the serum of patients with metastatic melanoma. Objective: The purpose of this study was to determine the safety and efficacy of Paclitaxel + Dacarbazine combination in metastatic malignant melanoma. Secondary we want to evaluate the correlation between serum level of S100 protein and therapeutic response. Materials and methods: 63 patients were included between January 2001 and December 2002. The median age was 54 years. Patients could have prior adjuvant immunotherapy. Performance status was ECOG 0 - 2. Metastatic sites were: liver - 29 patients; lung - 19 patients; soft tissue - 11 patients; lymph nodes - 9 patients; bone - 6 patients. Treatment schedule: Paclitaxel 175 mg/sqm on day 1 and Dacarbazine 400 mg/sqm/day on days 1, 2, repetead at 3 weeks for six cycles. Serum S100 was determined at start, every two chemotherapy cycles, and in follow-up visits (every three month). Results: All patients were evaluable for toxicity and 61 patients were evaluable for efficacy. Main grade 3-4 toxicity was: hematology 12 patients, no neutropenic fever, neuropathy 8 patients, emesis 9 patients. Overall response rate was 23.8% (15 patients partial response); 23 patients had stable disease and 25 patients had progressive disease. 59 patients had elevated S100 levels. The highest were among patients with liver metastasis. The responders registered decrease of more than 50% in S100 level. Conclusion: our results suggest that the combination is not significant superior to single agent therapy. The regimen was well tolerated, with no treatment related serious adverse events. Serum levels of S100 were correlated with the treatment efficacy and could be used for easy follow-up.

P-040 VOLUME DOUBLING TIME AS A NOVEL PROGNOSTIC FACTOR IN CUTANEOUS MALIGNANT MELANOMA

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OBJECTIVE: To estimate the kinetic parameters of skin melanoma growth and their prognostic significance. METHODS: Tumor growth rate (TGR) and volume doubling time (VDT) have been calculated using digital dermoscopic photographs in 127 malignant melanomas of the skin. We got standard scaled images twice in a range from 3 to 141 days (mean 11.4) and processed them by graphic gauge editor. TGR and VDT were estimated respecting visual type of melanoma according to expansion of tumor image area: TGR = k*(lnS2-lnS1)/t and VTD = ln2/TGR, where S1 and S2 - tumor image areas on first and second photograph, t - time between photographs, k = 1 for superficial and k = 1.5 for nodular melanomas. Obtained kinetic parameters have been tested in univariate and multivariate Cox regression models. RESULTS: Superficial melanomas had a median VDT of 127 days, nodular melanomas - 35 days. Median growth rates of superficial and nodular components in two-component melanomas did not differ from the same in superficial and nodular tumors. Kinetic parameters of nodular component depended on prevalent cellular type and tumor ulceration. Sex, age, location, size, degree of lymphoid infiltration, pigmentation and mitotic activity showed no influence on the growth rate. TGR of melanomas of all types had a positive correlation with tumor thickness. In univariate and multivariate analysis, VDT was found to be an independent prognostic factor of disease-free and overall survival ($P<0.05$). DISCUSSION: A simple technique of precise estimation of skin melanoma kinetics is proposed. Volume doubling time seems to be a novel prognostic factor in cutaneous malignant melanoma. The role of kinetic parameters among well-known predictors should be further specified.

P-041 IN VIVO CONFOCAL MICROSCOPY AS A GUIDE FOR MARGIN MAPPING OF MALIGNANT MELANOMA.

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Objectives: In vivo confocal laser scanning microscopy (CLSM) allows for non-invasive high resolution imaging of cellular and architectural details of human skin. This imaging modality is particularly useful for melanocytic lesions, where melanin and melanosomes provide image contrast, permitting the evaluation of melanocyte distribution and morphology. These characteristics can be helpful in margin assessment of lentigo maligna melanomas and amelanotic melanomas, where clinical assessment may be equivocal and multiple biopsies may be required for precise margin delimitation prior to surgery. Methods: We present a case series of challenging melanomas where confocal microscopy was compared to routine histology for margin mapping. Results: We found overall good correlation between margins assessed by in vivo confocal microscopy and pathology. CLSM detected margins accurately in comparison to histology in the majority of cases, but factors such as severe sun damage and use of topical medications appear to influence cellular distribution, making margin definition less accurate. The presence of pagetoid melanocytes greatly facilitates the recognition of melanoma on CLSM. A limitation of this technique with the technology utilized in this study was the need to reapply the tissue stabilizing ring at multiple sites for large lesions. Conclusion: Newer generation CLSM instruments may significantly improve presurgical margin assessment for large indistinct and amelanotic melanomas.

P-042 ARTESUNATE IN THE TREATMENT OF METASTATIC UVEAL MELANOMA - FIRST EXPERIENCES

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Artesunate (ART) is a derivative of artemisinin, the active principle of the Chinese herb Artemisia annua L.. Artesunate is approved for the treatment of multi-drug resistant malaria and has an excellent safety profile. Recently it has been shown that Artesunate, apart from its anti-malarial activity, has cytotoxic effects on a number of human cancer cell lines, including leukemia, colon cancer and melanoma. Here we report on the first long-term treatment of two cancer patients with ART in combination with standard chemotherapy. These patients with metastatic uveal melanoma were treated on a compassionate-use basis, after standard chemotherapy alone was not effective to stop tumor growth. The therapy-regimen was well tolerated with no additional side effects other than that caused by standard chemotherapy alone. One patient experienced a temporary response after addition of ART to Fotemustine while under therapy with Fotemustine alone the disease was progressing. The second patient first experienced a stabilization of the disease after addition of ART to Dacarbazine followed by objective regressions of splenic and lung metastases. This patient is still alive 47 months after first diagnosis of stage IV uveal melanoma, a situation with a median survival of 2-5 months. Despite the small number of treated patients, ART might be, in combination with standard chemotherapy, a promising adjuvant drug for the treatment of melanoma and possibly other tumors. Its well tolerability and lack of serious side effects will facilitate prospective randomized trials in the near future.

P-043 TREATMENT MODALITIES FOR LENTIGO MALIGNA: A SURVEY OF THE AMERICAN COLLEGE OF MOHS MICROGRAPHIC SURGERY AND CUTANEOUS ONCOLOGY (ACMMSCO)

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There are numerous methods available to dermatologists for the treatment of lentigo maligna (LM). We surveyed all members of the American College of Mohs Micrographic Surgery and Cutaneous Oncology (ACMMSCO) on what modalities they are currently utilize. A one-page survey was sent to all members of the ACMMSCO. Recipients were given a list of treatment modalities that included: techniques with permanent sections, techniques without permanent sections, and non-surgical techniques. We asked them to clarify the use of any special staining, and/or adjuvant therapies. A total of 650 surveys were mailed. The response rate is presently 50% (325/650). Analysis shows that 53% of respondants use Mohs surgery, 30% use wide local excision, and 17% use a staged square/polygonal excision. Sixty-four percent use one surgical modality for treating LM, while the remainder use a variety of techniques depending on the clinical scenario. When asked about the processing of tissue, 85% utilize permanent sections (or a combination) and 26% rely on frozen sections. The most commonly used surgical adjuvant was imiquimod (30%). Immunohistochemical stains are used by 18%. Members of the ACMMSCO appear to use some form of Mohs surgery as their primary treatment modality for lentigo maligna. However, wide local excision is still a commonly used approach. The vast majority of respondants rely on permanent sections to verify their margins.

P-044 IS REALLY IMPORTANT THE ANALYSIS OF CUTANEOUS MELANOMA BY AN EXPERIENCED DERMATOPATHOLOGIST?

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BACKGROUND: Surgical pathology analysis of biopsies from cutaneos melanoma directly determines the diagnosis, and it's characteristics are useful for the establishment of the prognosis and for the treatment. If there where unreported characteristics, or worse, mistaken diagnosis, the treatment and the prognosis may be compromised. OBJECTIVE: The main objective of this paper was to analyze if there are differences between anatomopathological records of cutaneous melanoma from common pathologists and experienced dermatopathologists. METHODS: One hundred and five consecutive patients of cutaneous melanoma presented at the Skin Oncology Group of the Hospital do Câncer, A. C. Camargo, São Paulo, Brasil have had their anatomopathological records reviewed by the main dermatopathologist of our Institution, based on WHO criteria. RESULTS: From the 105 patients, 43 had lesions located on trunk, 51 on extremities and 11 head and neck. The Breslow thickness reported initially from common pathologists remained unchanged in 70 patients (66,6%) after the analysis of the dermatopathologist, but in 35 (33,3%) the depth changed, and in 32 of them (31,5%) the treatment was changed based on the new measured depth. Clark's level have been changed in the same way in 51 patients (48,6%). The growth phase was not mentioned initially in 88 patients, and among these patients 66 had vertical growth phase after the pathological study. Ulceration was not reported initially in 80 cases (76,19%) from other pathologists, and the revision showed ulceration on 13 of them. CONCLUSION: The surgical pathology analysis by an experienced dermatopathologists is obligatory on cutaneous melanoma. This paper showed that the surgical treatment was changed in 31,5% of the patients after the analysis of the initial biopsy by a dermatopathologist.

P-046 EVALUATION OF CLINICAL RESPONSE OF ACTIVE SPECIFIC IMMUNOTHERAPY USING AUTOLOGOUS TUMOR CELLS IN PATIENTS WITH STAGE IV CUTANEOUS MELANOMA.

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Objective. Evaluate response in patients stage IV cutaneous melanoma using attenuated autologous melanoma cells Materials and Methods. Thirty-nine patients with stageIV cutaneous melanoma were treated with 6 doses of autologous whole tumor cell vaccine associated with BCG and low doses of cyclophosphamide and GM-CSF. The total period of treatment was 13 weeks Results: Ten patients died during vaccination therefore before finishing the treatment, and 4 abandoned the protocol. Therby,25 patients were evaluated. Eight had clinical response (32%). None of the patients developed side effects other than skin ulcer at the site of the first 3 doses and nausea after cyclophosphamide injection. The median survival time of the responders was 23,26 months and of the non-responders was 6,74 months (p=0,0064, Kaplan-Meier, Log-rank test). The median survival time of the patients that did not respond but had a positive cutaneous reaction (DTH)was 6,74 months, and for the patients that had a negative DTH was 2, 01months (p=0,45). Conclusion: This treatment seems to be effective for patients that do not have rapidly progressive disease, and multiple visceral metastases. The treatment had low morbidity and no mortatlity.

P-047 METASTATIC MELANOMA OF UNKNOWN PRIMARY TUMOR. AN ANALYSIS OF 97 PATIENTS AT A SINGLE INSTITUTION

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Objective: A retrospective analysis of 97 patients with metastatic melanoma and unknown primary tumor was undertaken to analyse the clinical behaviour of this presentation Material an Methods: The data were obtained through the consultation of the patients chart. The statistics methods applied were: descriptive statistics, chi-square test and survival analysis by Kaplan-Meier Results: From 97 patients, 57 were male and 40 female, Sixty-six percent of the patients presented with lymph node metastasis, 50,5% presented with subcutaneous nodules, 16,5% with lung metastasis, 16,5 had visceral lesions, and 9,3%had brain metastasis.The prevalence of displastic nevi was 5,2%. The median follow-up was 25 months(1,35 - 403,85). The survival of the operated patients(69%)was 45,6% and the patients that were not operated on was 14% (p<0,01). The 5 year overall survival for the patients that were operated on was 49 months and the patients that were not operated on had an overall survival of 15 months. Conclusion: The patients presenting with metastatic melanoma of unknown primay that have the possibility to be operated on had a better prognosis than the patients that did not have the chance to have an operation.

P-048 MUCOSAL MELANOMAS OF THE UPPER AERO-DIGESTIVE TRACT: AN INDIAN EXPERIENCE.

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Though common in the fair skinned population,cutaneous melanomas are rare in India. Mucosal melanomas(MM) in the upper aerodigestive tract is still rarer. We did this study to see the various clinical, pathological parameters associated with MM in Indian patients. Methodology Retrospective review of the charts of Head and Neck cancer patients visiting our hospital from 1985 till date. We could locate 17 patients. Result: The age ranged from 28-70 years. There were 9 males and 8 females. Lower alveolus was the commonest site of involvement (8/17).Upper alveolus was involved in 3, nasal cavity in 3, palate in 2 patients and nasopharynx in 1. All patients had undergone wide excision of the lesion and 11 patients required neck dissection. 8 of these patients underwent adjuvant RT. The follow up data is available for 13 patients. 7 of these patients developed metastatic disease within 1 month to 2 years of surgery and died of disease. 2 patients have completed 2 years of follow up and are disease free. Remaining 4 patients have completed only 1 year follow up and are disease free. Conclusion: This is one of the largest series form India.Our findings are different from the pattern of MM reported in the literature.The rarity of the entity could be assessed by the fact that we could find only 17 cases in last 20 years. The disease is very fatal and 3 years survival is 0%. The site predilection is also quite different from other reports. Lower alveolus being the commonest involved site has never been reported in past. We did not find any sex predilection as reported in the past.

P-049 SENTINEL LYMPH NODE BIOPSY FOR MELANOMA: CENTRE HOSPITALIER UNIVERSITAIRE DE QUÉBEC EXPERIENCE WITH 533 PATIENTS

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OBJECTIVE : To evaluate the role of sentinel lymph node (SLN) biopsy as a prognostic factor in patients with melanoma. MATERIAL & METHODS : We evaluated 533 patients with melanoma (Breslow > 1,00mm or Clark IV) at our Melanoma Clinic for SLN biopsy between 1996 and 2004. Cox regression analysis was performed to evaluate the hazard ratio to predict the time to recurrence. RESULTS: The patients mean age was 54 years old (6 to 95 y.o.), 78% had negative SLN and 22% had one or more positive SLN. We found sentinel nodes in 2 or more sites in 19% of the patients. The median Breslow thickness was 2,23mm. Patients with negative SLN had a mean Breslow thickness of 1,91mm as compared to 3,37mm for those with positive SLN. Disease free survival at 3 years demonstrated 89% of patients were free of disease if the SLN was negative as compared to 60% for patients with positive SLN ($p < 0,05$). Hazard ratio to predict the time to recurrence in these patients was 4,6 times greater in positive SLN patients. Stratification according to the burden of disease in the SLN measured in millimetres demonstrated a strong correlation: 2,7 times more chance to recur than negative patients for nodal disease < 2mm and 12 times more chance if nodal disease > 2mm. CONCLUSION: We observed a strong correlation between SLN status and Breslow thickness of the melanoma. The SLN status and the amount of melanoma disease in the SLN are strong prognostic factors.

P-050 SENTINEL LYMPH NODE BIOPSY FOR MELANOMA: THE INFLUENCE OF EXCISIONAL BIOPSY ON LYMPHOSCINGRAPHY

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OBJECTIVE: To evaluate the influence of excisionnal biopsy of melanoma on lymphatic drainage. MATERIAL & METHODS: We studied 81 patients at our melanoma clinic with lesions strongly suspicious for invasive melanoma. In those patients, a lymphoscintigraphy was performed prior to the excisional biopsy to identify lymphatic drainage bassins and sentinel lymph node(s). Biopsies were performed following the expected lines of drainage with a 2 mm margin. A few weeks later, a second lymphoscintigraphy was done to compare. RESULTS: The drainage was significantly modified after excisionnal biopsy in 11 patients. In 8 of them, a bassin was missing and in 3 of them, an additionnal bassin was found. In all modified cases, lesions were located on the trunk or proximal limbs. CONCLUSION: Our data suggest that lymphoscintigraphy should be performed prior to excisionnal biopsy of suspicious invasive melanoma of the trunk or proximal limbs. This could avoid missing some bassins of drainage or sampling wrong ones.

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P-051 METASTATIC MELANOMA TO LYMPH NODES IN PATIENTS WITH UNKNOWN PRIMARY SITES: STAGE III OR STAGE IV?

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Objectives: The natural history of patients with metastatic melanoma in lymph nodes (LN) in the absence of a known primary site (MUP) is poorly defined. Therefore, it is unknown whether MUP represents stage III or stage IV (M1a, distant nodal disease). Prognostic factors and survival for patients with MUP were compared to patients with known concurrent primary melanoma with matched nodal (N) status (controls) according to the 2002 AJCC staging system. Materials & Methods: A retrospective analysis was performed on consecutive melanoma patients treated with surgical resection of melanoma metastatic to regional LN. Of these, 71 patients with MUP and 465 matched controls were identified. Associations between clinicopathologic factors and overall survival (OS) were estimated using the Cox proportional hazards model. Results: The most common presentation for patients with MUP was a palpable mass in the axillary basin (76%), in a male (72%), with a median age of 51 years. Following completion LN dissection, MUP patients were classified as having N1b (47%), N2b (14%), or N3 (39%) disease. Regional nodal control was accomplished in 82% of MUP patients. With a median follow-up of 7.6 years, 5- and 10-year OS was 55% and 42%, respectively, for patients with MUP compared to 42% and 32%, respectively, for controls (p = 0.04). In multivariate analyses, age 3 50 years, male gender, and N2b or N3 status were identified as adverse prognostic factors for OS. MUP patients had improved OS compared to controls (hazard ratio = 0.61, 95% CI 0.43-0.87, p = 0.006). Conclusion: The relatively favorable long-term survival of MUP patients suggests that patients with MUP have a natural history similar to that of stage III rather than stage IV patients. Therefore, patients should be treated with aggressive surgical approaches for curative intent and be considered for stage III adjuvant therapy protocols.

P-052 EXTRAESCLERAL EXTENSION AS THE PRESENTING FEATURE OF THREE UVEAL MELANOMAS

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Uveal melanoma is the most common primary malignant intraocular tumor in adults, representing 70% of all malignant ocular tumors. Visual loss is the primary presenting feature in the overwhelming majority of the cases. However, in patients who are asymptomatic and do not seek ophthalmic evaluation or in those who refuse recommended treatment and allow tumor growth to continue, advanced progressive disease may occur. The reported incidence of extraescleral extension of uveal melanomas ranges from 10 to 15% and it usually denotes a bad prognosis. Mortality with extraescleral extension is 2-3 times greater than that of cases in which the tumor is totally confined within the eye at the time of enucleation. We report 3 cases of choroidal melanoma presenting with extraocular extension as their primary feature. The appearance of the first case mimicked that of a conjunctival melanoma, leading to the enucleation due to the scleral and uveal compromise found at the operative procedure. The second case presented at diagnosis with a round-shaped pigmented and ulcerated tumor in the right eye, protruding over the lower eyelid. Despite the unusual clinical presentation, a diagnosis of melanoma was reached by biopsy of this extraescleral lesion. The third case denotes the natural history of an uveal melanoma, having a two year period of growing without consulting (despite the visual loss it implied), and coming for the first time to the physician with the pigmented lesion affecting the extraescleral structures. This three cases are analyzed and their evolution is reported.

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P-053 OVERVIEW OF ADJUVANT THERAPY OF MELANOMA WITH INTERFERON ALFA 2B IN A REGION OF NORTHERN BOHEMIA.

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Patients with intermediate-thickness melanoma (2- 4 mm thick) have an increased risk of metastases and therefore may benefit from adjuvant therapy after excision of the primary tumor.Analysis of multicenter study conducted by the WHO Melanoma Program showed a 2-year disease- free survival of 46% in patients receiving long-term IFN ± 2a compared with 27% in those who had no further therapy after surgery. These results confirms Kokoschka using IFN ±2b.We analysed the treatment benefit of 32 patients (AJCC classification-stage I pT2,N0,M0, stage II pT3,N0,M0, stage III pT4,N0,M0) treated with interferon ± 2b. Age 50.29± 12.4. The patients were treated to the regimen 10 MIU IFN± 2b daily 5 days a week/up to 4 weeks, then 5MIU INF± 2b three times a week for 48 weeks. After median follow-up of 24 months, had complete remission 71% patients, Patients from this group are still alive (35±20.6 months). 29% patients had died from melanoma (16% patients had died before the end of study, after 10.4±6.5 months from the beginning of study and 13% patients had died after the end of study, after7±3.74 months). Interferon ± 2b has the potencial in preventing relapses and prolonging survival in most of our patients rendered surgically disease free, but at high risk of recurrences. Adjuvant immunotherapy with IFN ± 2b has shown great promise in recent trials.

P-054 ADVERSE EVENTS OF ADJUVANT INTERFERON-± TREATMENT IN HIGH RISK CUTANEOUS MELANOMA - COMPARISON BETWEEN PEGYLATED INTERFERON-±2B AND CLASSIC INTERFERON-±2B

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Adverse events of adjuvant interferon-á treatment in the induction phase over 12 weeks were analyzed. Adjuvant treatments were performed in an European multicenter prospective randomized phase III trial evaluating the efficacy of interferon-á2b (IFNá) for 18 months (3x3MIU sc/week) vs. pegylated interferon-á2b (PEGIFNá) for 36 months (100µg sc/week). Eligible for this trial were patients after the local excision of an intermediate risk cutaneous melanoma (tumor thickness >1,5mm and absence of regional nodal macrometastases). A total of 108 patients were evaluable. 55 individuals were treated with PEGIFNá and 53 with IFNá. Fatigue (40.1%) and myalgia (32.7%) were the major clinical adverse events (CTC1-3), whereas fatigue was more frequent and more intensive in the PEGIFNá treated patients (77 vs. 53 events; p=0.018). Also, depressive mood, occurring in 7.1% of all cases, appeared more frequent in the PEGIFNá arm (31 vs. 15 events; p=0.018), as well as an erythema at injection side (23 vs. 5 events; p=0.0007). There were no significant differences between PEGIFNá and IFNá treatment concerning fever, weight loss, myalgia, nausea, constipation, hair loss, pain, cardiovascular function, pulmonal and nervous status. Regarding laboratory events (CTC 1-3) leukocytophenia was the most frequent event occurring in 36.0% of all cases, whereas this was observed clearly more frequently in the PEGIFNá arm (76 vs. 35 events; p=0.00004). Also, with respect to granulocytopenia which was present in 17.7% of all treated patients, PEGIFNá was more toxic (38 vs. 15 events; p=0.0007) as well as in respect to SGOT (20 vs. 8 events; p=0.02) and SGPT (33 vs. 14 events; p=0.003). There were no significant differences in terms of anemia, thrombopenia, bilirubinaemia, BUN and proteinaemie. Both PEGIFNá and IFNá are well tolerated; there were no grade 4 toxicities observed. However, PEGIFNá caused more frequent and more intensive fatigue, depressive mood, erythema at injection side, leuko- and granulocytophenia as well as an increase of liver enzymes.

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P-055 A CRITIQUE OF OBSERVATIONAL STUDIES OF THE THERAPEUTIC BENEFIT OF SENTINEL LYMPH NODE BIOPSY

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Two groups have reported results of observational studies of survival in relation to sentinel lymph node biopsy (SNB) (1,2). Both groups compared the survival of patients with positive SNB and immediate lymphadenectomy with the survival of patients who had wide excision alone and who had delayed lymphadenectomy when they developed palpable lymph nodes. In both analyses, survival was superior among those patients who underwent lymphadenectomy following a positive SNB and both groups of authors concluded that this sequence had therapeutic benefit. Are their results valid? Consider a hypothetical randomised controlled trial (RCT) evaluating the potential efficacy of SNB. The appropriate statistical analysis of such a trial is on the basis of intention-to-treat. All patients randomised would be included regardless of what happens to them after randomisation. Thus, from the SNB arm, we would include those patients who have a negative SNB as well as those with a positive SNB, and from the control group (wide excision alone) we would include those who later develop palpable nodes as well as those who do not. The analyses of Morton et al (1) and Kretschmer et al (2) left out those patients with a negative SNB and those patients who had wide excision alone and who did not develop palpable nodes. Their analyses do not test the hypothesis that SNB offers therapeutic benefit. Rather, they test the hypothesis that patients with micro-metastases in the regional lymph nodes at diagnosis have better survival than patients who may or may not have micro-metastases at diagnosis and who later develop macro-metastases. Therefore, because their analyses were based on subgroups only, they provide no evidence on whether SNB offers therapeutic benefit. References: (1) Morton et al. Ann Surg 2003; 238: 538-49 (2) Kretschmer et al. Eur J Cancer 2004; 40: 212-8

P-056 MANAGEMENT OF LOCO - REGIONAL MELANOMA METASTASES IN THE UK

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Multiple dermal and subcutaneous loco-regional metastases occur in about 5% of melanoma patients and may be a difficult management problem. Effective treatment options include surgery, CO2 laser and regional chemotherapy with isolated limb perfusion (ILP) or infusion (ILI). Treatments of uncertain efficacy include local radiotherapy and systemic chemotherapy. We have conducted a questionnaire based audit to clarify which treatment modalities are used by UK specialists. The questionnaire was distributed to members of the UK Melanoma Study Group and Chairs representing the 29 UK Cancer Networks. We asked: 1) number of new cases of primary cutaneous melanoma and 2) new cases of loco-regional limb metastases diagnosed annually; 3) and 4) first and second line treatment(s) used; 5) ILI / ILP eligibility; and 6) reasons for referral. Twenty three centres responded: 2325 new primary melanomas are seen annually; 115 develop loco-regional disease, and 68 would be considered for ILI /ILP. First treatment choices for loco-regional disease are: a) surgery (20); b) CO2 laser (6); c) ILI /ILP (5); d) radiotherapy (3); e) systemic chemotherapy (1). Second treatment choices are: a) 4; b) 5; c) 13; d) 5; e) 7 respectively. Commonest reasons for referral for ILI / ILP included lesions that were too numerous, bulky, and rapidly progressive for surgery / CO2 laser. Less common reasons were: pain, deep lesions or threat to limb viability. Thirteen centres would refer for all these reasons. Current UK practice in managing loco-regional disease in melanoma varies widely. Surgery remains the preferred first treatment option, followed by regional chemotherapy. Overall published response rates over 80% are obtainable with both methods; ILI / ILP would normally be considered after surgery. Surprisingly, local radiotherapy and systemic chemotherapy are used despite little / no evidence of efficacy. Greater awareness of regional chemotherapy with ILI / ILP is required.

P-057 PERFORATOR FLAP RECONSTRUCTION IN PRIMARY CUTANEOUS MALIGNANT MELANOMA WIDE LOCAL EXCISION AND SENTINEL NODE BIOSPSY

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Perforator flaps have evolved to allow reconstruction in a range of locations, minimising contour deformity, colour mismatch and loss of function. This presentation describes their application in malignant melanoma management. In accordance with St Thomas' Melanoma Group Protocol a consecutive series of 332 patients with biopsy proven melanoma, Breslow thickness 1 mm or greater, underwent simultaneous sentinel lymph node biopsy and 2 cm wide local excision. The resulting defects, ranging in anatomical site, measured 4 - 8cm (mean 6) in diameter. Where they could not be closed directly, more complex reconstructive methods were used depending on specific local requirements including sensation, tissue durability and aesthetic. Where flaps were based on perforators, these were identified either from their known anatomical location, through Doppler ultrasound mapping or exploratory incisions and the appropriate flap design chosen to incorporate them. Where the primary site was close to the sentinel node the flap orientation permitted access to the node without an additional incision. Perforator flaps were used in 143 cases (43% of the series), 1.5% of these showing total and 3% partial flap necrosis and minor complications in a further 5%. This series illustrates that perforator flaps are reliable and effective in achieving satisfactory functional and aesthetic reconstruction in melanoma wider excision with sentinel node biopsy.

P-058 MODERN CONCEPTS IN MELANOMA RECONSTRUCTION

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Reconstructive plastic surgery is a rapidly evolving field, with developments in surgical technique availing of increasing knowledge of vascular anatomy, tissue engineering and aesthetic design concepts. Illustrative examples from a series of over 400 melanoma cases demonstrate the role of local, distant and microvascular flaps, composite tissue transfer, Integra and Vacuum Assisted Wound Closure in repairing wide local excision defects in conjunction with sentinel node biopsy. Optimal aesthetic and functional outcomes can be achieved with appropriate application of one or more of these reconstructive modalities

P-059 PROTECTION OF FEMORAL VESSELS WITH INTEGRA IN RADICAL DISSECTION OF THE GROIN

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Prophylactic sartorius muscle transposition cover of the femoral vessels in groin radical dissection may lead to complications. This presentation describes the alternative, novel use of Integra in protecting the femoral vessels. In 12 consecutive radical groin dissections for metastatic melanoma the silicone layer was removed from the sheet of Integra, which was then applied to the skeletalised femoral vessels and secured with 4/0 Vicryl Rapide. Two large bore closed suction drains were placed in the wounds, which were closed directly with 3/0 Monocryl subcutaneously. Of the 12 groin dissections, one developed a large haematoma at 24 hours requiring evacuation but went on to heal primarily despite prolonged blood and serous drainage. One groin developed an infected seroma requiring surgical drainage and packing, revealing well vascularised cover of the femoral vessels. A similar picture was seen in the two groins which broke down due to skin flap marginal necrosis. One groin developed a persistent seroma but no wound breakdown. In this series of 12 radical groin dissections Integra was shown to be effective in protecting skeletalised femoral vessels in the event of wound breakdown.

P-060 PROGNOSIS OF STAGE III PATIENTS WITH THICK (>4.0 MM) MELANOMA OF LOWER EXTREMITY IS DEPENDING ON THE TIME OF LYMPH NODES INVOLVEMENT.

K. Herman (Cancer Centre, Poland)*

The prognosis of stage III melanoma patients is heterogenic, therefore the new TNM system was verify in the prospective material. Between 249 melanoma patients who had selective ilio-inguinal lymphadenectomy 185 patients with thick (> 4.00mm) melanoma were analyzed. The average depth of invasion was 5.85mm, tumor was ulcerated in 67 of all cases (36.2%) and Clark V was assessed in 82 patients (44.3%). The median interval between primary excision and the time of lymphadenectomy was 11.1 months. In 150 patients recurrent disease were reported, including skin (15.7%), lymph nodes (13.5%) and distant metastases (28.7%) as the first failure. Skip metastasis (positive iliac and negative inguinal) were found in 26 patients (14 %). The median survival was 54.2 months. In unilaterally analysis the number of involved nodes influenced overall 5-year survival rate (49.8% - pN1, 29.6% - pN2, 20.3% - pN3; p=0.00279). Overall 5-year survival rate was 54.5% for subgroup of patients when lymphadenectomy was performed more than 12 months after primary excision, 10.4% for subgroup of patients when lymphadenectomy was performed simultaneously with primary excision and 27.2% for others (lymphadenectomy 1-12 months after primary surgery); p=0.00000. The presence of ulceration was also confirmed to be significant parameter (5-year survival without ulceration was 34.8% compared to 19.3% with ulceration; p=0.01551). In multivariate analysis the time between first surgery and lymphadenectomy and the number of involved nodes were significant. Relative risk of death was 5.2 times higher for subgroups which had simultaneously lymphadenectomy (compared to dissection performed more then 1 year after primary excision), and circa 2.7 times higher for more advanced N subgroups (pN3v pN1). The long time to develope lymph node metastases and to node dissection is favorable prognostic factor independent of other parameters. The value of early lymphadenectomy (including sentinel procedure) in this group should be reanalyzed very carefully.

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P-061 SENTINEL NODE BIOPSY IN SKIN MELANOMA

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OBJECTIVE: To present our experience in sentinel lymph node biopsy (SLNB) in cutaneous melanoma in Helsinki University Hospital since October 2000. MATERIALS AND METHODS: Patients with primary melanoma of Breslow thickness 1-4 mm and those <1 mm but Clark 4 were the original study group (intermediate risk group, n=323). Later also thick melanomas (n=40) and some cases with thin melanomas (n=12) were included. Altogether, 375 patients went to lymphoscintigraphy (LSG). LSG using Tc99m with Albures or Nanocoll was performed the previous day and at operation blue dye and gamma-probe were used. The more active half of SLN was fixed in formalin and serial sections of 1 mm thickness were made for haematoxylin-eosin staining and immunohistochemistry (IHC) with tyrosinase and MART-1. In metastatic cases reoperation: lymph node dissection or re-excision outside lymph node basin, was performed. RESULTS: SLNB was successful in 97 % of cases. The 9 cases with non-visible SLN included 2 with clinical LN metastases (one of thick melanoma, fatal) within 6 months suggesting tumor occluding lymph flow, and 5 of head and neck melanoma, one breast and one upper arm melanoma, all disease free. In 2 additional cases SLN could not be found at operation, one later developed LN and pulmonary metastases. In the intermediate risk group metastases were found in 78 cases (25%) of 312 with SLNs examined, in 32 cases of 78 not without IHC (41%). The median follow-up time is 16.4 months (1-50). In SN + cases, 15 recurrences (19%) and 8 deaths due to melanoma (10%) have occurred. In the 234 SN - cases 16 recurrences (7%) and 6 melanoma deaths (2,5%) have been recorded. CONCLUSIONS: SLNB is a valid method for staging patients, also in our hands. The number of recurrences at this early stage of follow up is within predictable limits.

P-062 INFLUENCE OF PARTIAL REGRESSION AND LYMPHOCYTIC TUMOR INFILTRATION OF PRIMARY TUMOR ON MELANOMA SENTINEL LYMPH NODES METASTASIS

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OBJECTIVE: Sentinel lymph node (SLN) affection is thought to have a significant prognostic impact in melanoma patients. The sentinel node biopsy (SLNB) is a well established low-morbidity procedure and is widely used. Whether partial regression or tumor infiltration of a primary melanoma has an adverse impact on prognosis is controversial. MATERIALS & METHODS: Consecutive patients with SLNB for melanoma treated at the Charité Skin Cancer Center were analyzed between October 1996 and January 2002 with special emphasis put on regression of the primary tumor. RESULTS: A total of 808 patients were analyzed, including 406 patients with partial tumor regression and 402 patients without regression of the melanoma. Median tumor thickness in patients with partial regression was 0,95 mm (range 0,19-30,6), and in patients without regression 1,85 mm (range 0,25-53,0). 141 patients (17,5%) patients showed involvement of the SLN. In 10,8 % of patients (n=44) with partial regression of the primary tumor the SLN was positive as compared to 22,6 % in patients (n=92) without regression. Regarding thin melanomas (<1mm) in patients with regression a SLN involvement was observed in 7,1 %, whereas in patients without regression the SLN was affected in only 2,5 %. Interestingly, in primary melanomas with abundant lymphocytic infiltration only 10,7 % of the patients showed SLN involvement, whereas in patients with absent or moderate infiltration 23,3 % SLN metastasis were observed. CONCLUSION: Thin melanomas deserve special attention since SLNs are more often affected when regression is present. Dense lymphocytic infiltration of the primary tumor is correlated with a less frequent SLN affection.

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P-063 IN VITRO MODULATION OF NK CELL ACTIVITY AND EXPRESSION OF ACTIVATING AND INHIBITORY RECEPTORS WITH INTERFERON-ALPHA, RETINOIC ACID AND IL-2 IN METASTATIC MELANOMA PATIENTS

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As NK cells are the major effector subpopulation of the innate antitumor immune response, in this study, we evaluated the cytotoxic activity of this subpopulation and the expression of NK cell activating (CD161, NKG2D) and inhibitory (CD158a, CD158b) receptors prior and following predictive in vitro treatments. The study was performed on freshly isolated and 18 h in vitro treated peripheral blood lymphocytes (PBL, 2.5x106/ml) with interferon-± (IFN, 250U/ml), 13-cis retinoic acid (RA, 10-6M), their combination and rh IL-2 (200U/ml), obtained from 40 metastatic MM patients, prior to therapy and 20 healthy controls. Analysis of NK cell cytotoxic activity and the expression of CD161 and NKG2D activating receptors of freshly isolated PBL of MM patients showed significant decrease compared to healthy controls. IFN, IFN and RA and IL-2, unlike RA alone, gave a significant increase in NK cell activity of MM patients. Contrary to the lack of any significant effect in healthy controls, in MM patients, IFN, alone, also induced a significant increase in CD161 activating receptor expression. The performed treatments gave no change in the expression of CD158b, while RA, alone, induced significant decrease in the expression of the inhibitory CD158a antigen. Further investigation showed that IRF-1, as a transcription factor, is involved in the obtained effect of IFN and IL-2. As NK cell activity is regulated by the balance of activating and inhibitory signals, in this study it is shown that IFN-induced increase in NK cell cytotoxic activity of MM patients is the consequence of the up-regulation of the activating signals, mediated through increased expression of CD161, rather than of the decrease in inhibitory receptors. This predictive in vitro investigation gives an insight into the mechanism involved in the effect of the applied immunomodulating agents and can indicate novel immunological parameters for monitoring in MM patients during immunotherapy.

P-064 ADJUVANT IFN-A THERAPY OF HIGH RISK CUTANEOUS MELANOMA

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There are more than 100 new melanoma patients registered at the Dermato-oncology Dept. of the GTH of Prague. About 40% of them have been classified as moderate / high risk tumors. For those patients we do consider adjuvant IFN-a therapy as the reasonable treatment approach with regard to prolongation of PFS and hopefully OS too. Currently there are 478 patients being indicated for adjuvant IFN-a therapy in a single arm, open label prospective study.. Patient indicated for adjuvant IFN-a therapy should be classified at least AJCC Stage IIB cutaneous melanoma. In case of OS evaluation we do consider treatment of the first relapse of the disease. In general, since the year 1996 we have been using recombinant IFN-a2b in the following treatment schedule: Induction: 10 MU flat dose, daily for 4 weeks, followed by maintenance: 10 MU TIW, for at least next 48 weeks. Patients having no SAE and/or progression of the disease can continue on the maintenance therapy for more than another 48 weeks. At the end of the year 2004 there were 9 patient treated for more than 4 years, 52 patients for at least 36 months, 87 patients for 24 months, 214 patients for 12 months. There are only 36 patients who did not finished the basic treatment protocol for one year, majority of them due to the intolerable AE. Primary end point of the study has been 3 and 5 year PFS. Secondary end points have been OS, survival time from first relapse till the last follow-up/death and distribution of metastases within the first and/or repeated relapse. Full, up-dated clinical data, reflecting primary and secondary end points will be presented at the conference.

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P-065 THE SENTINEL LYMPH NODE EXAMINATION IN MELANOMA PATIENTS: IMPACT OF SLN POSITIVITY ON OVERALL SURVIVAL OF MELANOMA PATIENTS.

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Since the year 2001 there were 219 patients indicated for SLN examination, and all but one have been successful in SLN identification, leaving the sensitivity of SLN detection as 99,5%. For the final evaluation data from 177 patients were available. Primary tumor location was on trunk in 51% , on lower extremity in 28%, on the upper extremity in 19% of cases. Median value of the tumor thickness measured according to Breslow was 1,9 mm, while the mean value was higher, 2,3 mm (SD 2,2 mm). In the sample of 177 examination, there were 316 SLN identified, i.e. average 1,8 nodes per single examination (range 1 - 6, median 2 SNL). Positivity of SLN was proved in 47 cases out of 177 examined, i.e. 26,6%. Positivity of SLN related to the value of the tumor thickness was as follows: Patients with Breslow < 1.1 positivity 2/39 (5%) Patients with Breslow 1.1 - 2.0 positivity 17/70 (24%) Patients with Breslow 2.1 - 4.0 positivity 16/46 (34%) Patients with Breslow > 4.0 positivity 12/20 (60%) Forty two out of 47 patients with positive SLN, were indicated for radical regional lymphadenectomy. Metastases in any resected lymphnodes have been found in 1 case only (2.5%). Median of a follow-up has been 18 months from the time of SLN biopsy. Among patients with positivity of SLN there were 19% of relapses (9/47), while in group of SLN negative patients it was 5% of relapses only (6/130). Despite the relatively low median value of follow-up (18 months), there is statistically significant difference in 3 year overall survival in favor of SLN negative versus positive patients.

P-066 DOES REGIONAL LYMPH NODE CLEARANCE MEANS SOMETHING , STUDY OF THE VARIATION BETWEEN SURGEONS

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The aim of this study was to analyze outcome in melanoma patients undergoing axillary or inguinal lymphadenectomy. Material and methods: 350 positive sentinel node or nodal recurrence patients were enrolled in a retrospective study. In each case the authors collected primary site and type of the tumour, and clinical stage indicating lymph node clearance, surgical technique including skin incision and landmarks of tissue removal, and number of lymph nodes removed. Outcome was analyzed including single or multiple sites and visceral or non visceral recurrences. At last duration of remission and survival were noticed. Results: In axillary dissections operative techniques were quite standardized with little variability. Inguinal lymphadenectomies were a matter of huge interindividual variations due to non standardized surgical procedures. Kruskal-Wallis tests correlated duration of remission and mean survival to extensivity of lymphadenectomy. Conclusions: Lymphadenectomies provide huge variability due to surgeon's identity and own technique in inguinal dissections although the extensivity of lymphadenectomy is an important prognostic factor of remission and survival. In conclusion surgical techniques must be revisited and valuate to be taught homogenously in schools of surgery to provide standardized optimal patients management.

P-067 EVALUATION OF A METHOD TO REDUCE THE NUMBER OF SENTINEL NODE REMOVED IN MELANOMA PATIENTS: PROSPECTIVE STUDY

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Evaluation of a method to reduce the number of sentinel node removed in melanoma patients: prospective study two major problems were noticed in the sentinel's nodes procedure: seven blue dye anaphylactic shocks and a variation in the number of nodes removed (1 to 9 SLN in the literrature). The aim of our study is to decrease the number of nodes removed without lack of efficiency. We included, in a prospective study, patients with melanoma > 1mm Breslow, stage I. Preoperative lymphoscintigraphy and gamma-probe detection were used. The hottest node was dissected, and others if their radioactivity is > 70% of the hottest. We compared our efficiency to two major published surveys. 50 patients, mean breslow 2.96 [0.3-20], mean age 62.8. SLN was identifiable in 100% of procedures. In 74% of cases, we removed one SLN, in 22% two, 4% three. 22% of micrometastasis. 2.5% of patient with negative SLN have recurrence, versus 18% with a positive SLN. No statistical difference in term of staging efficiency with the comparative survey, but a significant reduction of the number of extracted nodes was found. A high selectivity for SLN's dissection is effective at detecting micro-metastatic disease and helpful in reducing costs. These findings seem to us a come back to the original concept of Morton.

P-068 ISOLATED LIMB INFUSION -THE UK EXPERIENCE

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Isolated limb infusion (ILI) is a simplified, less invasive method for delivering regional chemotherapy. Since 2002 15 patients (aged 61 to 87, 2 male, 13 female) have been treated at 3 UK centres: Birmingham (n=12), Leeds (n=2) and London (n=1). All had melanoma (stage 3C, n= 11 , stage 4 n=2) or Merkel cell tumour (stage 4, n=2) with progressive lower limb disease not amenable to surgery. In 10 patients disease was below the knee, and in 5 also involved distal thigh; 11 had superficial and 4 had deep metastases; tumour size was 2-40 mm; 1 had consecutive inguinal dissection. Chemotherapy dose was melphalan 5 mg/L and actinomycin 50 mcg/L (n=7) and melphalan 7.5 mg/L and actinomycin 75 mcg/L (n=8). Median limb volume was 5.5 L (range 2.7 - 10). Starting limb temperatures were 36.8°C (33.7-37.8) and 37.5°C (34.5-39.2) at completion; starting pH and pO2 were 7.42 (7.32-7.47) and 13.4 (29.0-11.6), and at completion were 7.07 (7.04-7.18) and 1.9 (1.4-3.7). Recirculation volume was 1.3 L (0.6-2.5) and flow rate was 63 L/min (20-87). There were 2 CRs, 10 PRs, 3 stable disease or not evaluable. Toxicity was grade 2 (n=8); grade 3 (n=6); grade 4 then 5 (n=1) due to inadequate temperature control, low flow rates, and extensive disease. Creatine kinase levels were > 1000 IU/L by day 6 in 4 patients. Hospital stay was 9 days (7-38). ILI is a safe, effective treatment, but requires careful attention to detail to minimise risk of severe local toxicity. It should be directly compared with ILP; the therapeutic index may be improved by increasing selectivity of uptake of melphalan, or by using less toxic agents.

P-069 IMIQUIMOD IN THE MANAGEMENT OF LENTIGO MALIGNA - A CASE REPORT AND REVIEW.

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A 68 year old lady was first diagnosed with lentigo maligna (LM) affecting the skin of the right upper cheek/lower eyelid at the age of 46. This was treated with cryotherapy at a different unit, but recurred into invasive lentigo maligna melanoma (LMM) three years later. Excision and grafting was performed. The LM recurred in and around the graft a total of four times over the ensuing 18 years. It was re-excised and re-grafted on each occasion. The LM recurred in 2003 and was confirmed histologically. She was reluctant to have further surgery. Radiotherapy was considered inappropriate as it could exacerbate the mild right ectropion. After obtaining informed consent, she was treated with 5% imiquimod 3 times a week for 8 weeks with a 5mm margin. There was no irritation or improvement. The dose was increased to daily for a further 6 weeks, which resulted in mild inflammation but no improvement. She was eventually treated with further surgery. Imiquimod is widely considered an attractive treatment option for LM. At the time of writing, the literature contains 10 publications involving 55 patients who have been treated with imiquimod for LM. Including our case, complete response has been reported in only 80% of patients (45/56). Treatment periods ranged from 5 to 28 weeks, and dosing schedules ranged from 3 times a week to daily. Follow up periods ranged from 3 to 18 months. Among the 20% (11/56) that did not respond, 2 cases developed LMM while on treatment. In another non responder, the lesion appeared to clear clinically but remained histologically, effectively converting a pigmented LM to an amelanotic LM. This implies imiquimod may inhibit melanogenesis without affecting disordered cellular growth in some cases; this is potentially harmful. Imiquimod should therefore still be considered an experimental treatment in LM.

P-070 THE PROGNOSTIC SIGNIFICANCE OF SENTINEL NODE STATUS IN PRIMARY MELANOMA OF THE HEAD AND NECK COMPARED WITH OTHER PRIMARY SITES.

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OBJECTIVE To compare the prognostic significance of sentinel node (SN) status in primary melanoma of the head and neck (H&N) with that of SN node status in the axilla and groin. PATIENTS AND METHODS SN biopsy is more challenging in the H&N because of unpredictable lymphatic drainage and more complex anatomy. Therefore it is hypothesized that the prognostic reliability of SN status maybe less than for other node fields. The Sydney Melanoma Unit (SMU) database was used to identify all patients undergoing SN biopsy for melanoma between 1992 and 2004. RESULTS 11784 primary melanomas were treated during the study period and 2045 were located in the H&N region. 2691 SN biopsies were performed including 358 in the H&N. Men had 257 SN biopsies for H&N melanomas, compared with 82 in women. Median thickness of the primary melanomas was 2.3mm and 32% were ulcerated. In 92% of patients all SNs identified by preoperative lymphoscintigraphy were localised and removed, 44(12.8%)of which were positive. There was no significant difference between the prognosis indicated by SN status for the three node fields, but a highly significant difference between SN +ve and SN ve patients at all sites. Median follow up was 32.1 months. Comprehensive multivariate analysis of the data will be presented. SN + VE 5YS% SN VE5YS% SIGNIFICANCE H&N 69 (n=41) 86 (n=268) P < 0.001 AXILLA 60 (n=91) 91 (n=577) P < 0.0001 GROIN 69 (n=73) 91 (n=386) P < 0.0001 Table 1 CONCLUSION Despite the technical difficulties of SN biopsy in the head and neck, the prognosis associated with SN status is similar to that for other SN locations.

P-071 SENTINEL NODE BIOPSY FOR MELANOMA. IS IT PREOPERATIVE LYMPHOSCINTIGRAPHY NECESSARY?

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Objective: Nodal metastases in patients with melanoma identify a reduction of survival by 50%. Sentinel node biopsy has been shown to stage the patient accurately. The frequency of interval nodes are 5%. We report the use of technetium rhenium colloid in transoperative setting. Methods: 95 patients with new AJCC stage Ib to IIc melanoma seen at Instituto Nacional de Cancerología in Mexico City between March 2001 to july 2003 were enrolled prospectively to undergo sentinel node biopsy. Intraoperative injection of 1mCi of technecium 99 rhenium colloid was done 15 minutes before the skin incision; five minutes later 1 ml of patent blue dye was injected too. A hand-held gamma probe was used intraoperatively as a guide to the first draining node. Blue-stain lymphatic channels aided in the dissection. We recorded the time between injection and localization of the sentinel node and the successfulness of the procedure. All sentinel nodes were evaluated by HE, HMB-45 and S-100 protein. Results: Sentinel node localization was successful in 90 of 95 patients, for an overall success rate of 94.7%. On average, 1.6 sentinel nodes per patient were identified at surgery (0-4). Of all, 28 patients (30.5%) had a positive sentinel node. The mean Breslow was 3.4 mm (2.9 mm in negative vs. 4.75 mm in positive sentinel node p=0.0002). Three patients experienced recurrence in a regional basin after negative pathological and inmunohistochemical evaluation, but in re- revision were positive. No preoperative lymphoscintigraphy was needed. No interval nodes were detected or nodal recurrence different from basin detected. Conclusion: The use of Sentinel node biopsy is a feasible technique with a high success rate (95.7 %), but it requires a multidisciplinary approach. This study validates the clinical usefulness of technetium rhenium colloid for lymphoscintigraphy in a transoperative setting.

P-072 CUTANEOUS DESMOPLASTIC MELANOMA.

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Desmoplastic melanoma (DM) is a rare histological variant of melanoma. It often presents a higher local recurrence rate than other forms of melanoma. We reviewed the cases of DM treated at National Cancer Institute of Milano, in order to evaluate if wider margins of surgical excision for primary tumor are related to lower rate of local recurrence and a longer overall survival. Twenty-one patients with DM underwent surgical excision between 1994 and 2004. Eleven male and ten female patients averaged 59 years of age (range 33-82).Thirteen tumors (62%) were located on the head and neck; the median thickness of lesions was 5,66 mm. In all cases treatment of primary tumor was surgical excision. In eight cases (38%) the surgical clearance margin was d 1 cm, in eleven cases (52%) was 1,5-2 cm, while two primary DM were incompletely excised. The average lenght of follow-up was 54 months. Ten patients (48%) developed one or more local recurrences: for eight patients of this group, surgical clearance margin of primary lesion was d 1 cm. Regional node metastases were two.Seven patients (33%) died from disseminated disease: five of them had local recurrences before the development of distant spread. One patient died with inoperable local recurrence, one died from other disease. Overall survival rate is 57%. Eleven patients are alive and free from tumor: in nine of them the surgical clearance margins of primary tumor was 1,5-2 cm, one is alive with inoperable local disease. Local recurrence may be related to inadequate initial surgical treatment (clearance margins d 1cm) and also distant metastases seem to be less frequent in those patients who underwent a wider surgical excision of primary tumor.Treatment should be aggressive local excision (2 cm margins where possible) with a close follow up, necessary to detect resectable recurrent lesions.

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P-073 SURGICAL TREATMENT IN HORIZONTAL GROWTH PHASE MELANOMA. RESULTS OF A PROSPECTIVE STUDY AT NATIONAL CANCER INSTITUTE, MILAN

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Assuming that horizontal growth phase (HPG) invasive cutaneous melanoma is, in terms of biological behaviour, more similar to an in situ than to an invasive lesion, between March 1997 and December 2002, 79 (HGP) invasive cutaneous melanoma, in 73 patients (27 men and 46 women,) were treated at the out-patient clinic under local anesthesia. The margins of resection were 5 mm and the subcutaneous fat was cleared to the deep fascia, which was respected. In most cases patients had previously undergone an excisional diagnostic biopsy. The size of the lesions ranged from 2 mm to 20 mm (median 7 mm). Lesions thickness ranged from 0.11 mm to 0.62 mm (median 0.30 mm). All patients were followed with physical examination every six months. No instrumental analysis was performed, because of the limited thickness of the primary. The median follow-up was 37 months (range, 24-92 months). No loco-regional or distant relapses were observed during this period. This study suggest that a very limited surgical excision (5 mm)could be considere a safe procedure. Moreover, this conservative surgical approach started in our Unit, produce advantages in terms of cosmetic outcome.

P-074 ADJUVANT INTERMITTENT HIGH-DOSE I.V. INTERFERON-ALFA-2B THERAPY IN STAGE IIC/III MALIGNANT MELANOMA: A PHASE II STUDY

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OBJECTIVE: Adjuvant high dose Interferon-alfa-2b prolongs overall survival in two out of three randomized, multi-center trials. This cytokine treatment is unfortunately associated with severe side effects. Since the i.v. phase of this Interferon-treatment may be the most relevant part of this therapy, we developed a schedule for administering high dose Interferon-alfa-2b by repeating the 4 week i.v. phase (5 x 20 Mio./m2 I.E. IFN-a-2b per week) three times, each i.v. cycle followed by three months without treatment. Toxicity and efficacy was analysed. METHODS: 46 patients with a large primary melanoma (n=4) or regional lymph node metastasis (n=42) were treated according to the new regimen. 45 patients are evaluable at present. RESULTS: Only 9 evaluated patients (20,0%) experienced grade III/IV toxicity. No treatment related death was monitored. Only in 10 (22,2%) patients a short-term interruption of the IFN treatment was necessary. 7 (15.5%) patients required a 50 % dose reduction. Interestingly, the IFN-effects as measured by the drop in leukocytes, by the rise in liver enzymes (GOT, GPT, LDH) gradually decreased from treatment period to period suggesting a long-term alteration of the IFN sensitivity in the treated patients. Within 36 months only 17/45 (37%) patients experienced a recurrence of the malignant melanoma and 13/45 (28,8%) died. DISCUSSION: The data demonstrates the feasibility of this treatment schedule. Toxicities observed in this study were less frequent than in the three ECOG studies. Our study population was less favourable regarding stage groups. Since only 37 % recurrences of the disease was reported within three years, the therapy might well be as effective or more effective than the Kirkwood regimen. A randomized multi-center study comparing the classic high-dose regiment with the new sequential i.v. regiment has been started within DeCOG.

P-075 CLEAR CELL SARCOMA AND SENTINEL LYMPH NODE BIOPSY. CASE REPORT AND REVIEW OF LITERATURE

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INTRODUCTION: The procedure of sentinel node already has been used previously in clear cell sarcoma. There are few studies reported. Due to the similar biological features with melanoma this procedure can be efective. OBJETIVE: Sentinel biopsy is an option to detect nodal subclinical metastases. MATERIAL AND METHODS: A 19 year old man presented with ulcerated lesion in the fifth finger of the left hand. The Biopsy report clear cell sarcoma. Immunohistochemistry positive for Vimentin and S-100. There was no evidence of regional disease. The sentinel lymph node biopsy was made using patent blue and Tc-99 rhenium. RESULTS. The sentinel node was positive to metastases. The axilar dissection was made. The final report confirmed 3 metastasic nodes. The patient received adjuvant chemotherapy. Key Words: Clear Cell sarcoma, sentinel lymph node biopsy, adjuvant chemotherapy.

P-076 SENTINEL NODE BIOPSY: STANDARD TREATMENT FOR MELANOMA? RESULTS AT THE NATIONAL CANCER INSTITUTE OF MILAN.

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SN status in melanoma patients accurately reflects the status of the entire regional node field and is a critically important prognostic indicator. However, randomized trias have yet to determine the therapeutic value of this procedure. Aim of this retrospective study is to identify if the sentinel node status or other primary melanoma risk factors may influence recurrence and overall survival in melanoma patients. Between 1999 and 2004, at National Cancer Institute of Milan, 1191 patients with a diagnosis of cutaneous melanoma thicker than 1 mm, or Clark level IV and V, underwent sentinel node biopsy. The parameters that were considered are the sentinel node status, Breslow thickness, ulceration, Clark level, the number of metastatic foci in the sentinel node, the diameter of major metastatic focus and the location of metastatic focus in the sentinel node. Chi-square and Cox proportional hazard ratio was used to identify factors associated with overall survival. 852 patients were considered for this analysis having an adequate follow up period and all the parameters for statistical evaluation. OS was statistically significant (P 10-5) when SNB is negative. Breslow's thickness, seems not to impact on OS. The results of our study seem to confirm the value of SN biopsy for staging of melanoma patients. It provides a more reliable estimate of prognosis, foundamental for accurate stratification of patients for entry into adjuvant therapy clinical trias. The impact of the procedure on patients outcome was not fully determined in this study; according to the characteristics of SN metastases, a direct correlation between diameter and survival, nor between site and survival was not identified.

P-077 DESMOPLASTIC MELANOMA- PATTERNS OF SPREAD

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Desmoplastic melanoma (DM) is an uncommon variant of melanoma characterised by spindle cells within a fibrous stroma, often accompanied by a lymphocytic infiltrate. Recent studies have reported a lower incidence of positive sentinel nodes in patients undergoing selective lymphadenectomy (SNB). This presentation describes the Sydney Melanoma Unit experience with SNB in patients with DM. In the twelve years between January 1993 and December 2004 some 230 patients with melanoma, who had desmoplasia present, underwent SNB. Median age was 65 years with two thirds of patients being male. Median thickness was 2.4 mm and in 20% ulceration was noted.These melanomas were further subclassified into the degree of desmoplasia present.Median follow up was 30 months at this time. The number of patients with sentinel node positivity was less than expected (6.5 % overall). However the number of patients with lung as the site of first systemic recurrence was more than expected (87%). These findings once again question the inherent biology of this uncommon varant and may suggest modification of their management plan.

P-078 LOCAL DISEASE MANAGEMENT OF MELANOMA METASTASES WITH ELECTROPORATION PLUS INTRALESIONAL BLEOMYCIN

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Objective: To date, there is no recommended standard therapy for advanced melanoma with skin and soft tissue metastases. Although a number of therapies such as surgery, limb perfusion, systemic chemo- or immunochemotherapy, cryotherapy, radiotherapy, intratumoral chemotherapy and intratumoral immunotherapy are currently in use for this situation, management of advanced melanoma with skin and soft tissue metastases can be a major challenge, particularly in cases of rapidly progressive disease, multiple recurrences, and extensive previous therapy. Intralesional therapy modalities generally lack the severe side effects of systemic therapies and, provided there is sufficient efficacy, can be desirable alternatives to surgery, systemic therapy or radiotherapy. The therapeutic value of several intralesionally administered cytostatic agents and cytokines, such as bleomycin, fotemustine, cisplatin, interferon-alfa, interferon-beta, GM-CSF and IL-2 has been investigated with varying outcome. However, none of these therapies has yet been shown to be a convincing alternative to conventional therapies. Materials and Methods: Electroporation therapy is a novel treatment, using pulsed electrical currents to enable the delivery of large molecules such as bleomycin into cells. The effectiveness and feasibility of this new treatment option have been reported for tumors of the bladder, pancreas, colon and rectum, prostrate, brain, head and neck and recently in various types of skin cancer and especially in malignant melanoma. Results: Treatments were administered on an outpatient basis under local anaesthesia; patients discomfort was brief and objective response rates appear to be greater than results achieved with other therapies, including intralesional bleomycin without electroporation. To substantiate using electroporation in combination with intralesionally administered bleomycin prospective multicenter clinical trials are currently being conducted throughout Europe. Conclusion: Electroporation is an effective, outpatient based, well-tolerated and non-invasive therapeutic option for patients presenting with cutaneous and soft tissue melanoma metastases and may potentially be useful for the treatment of visceral metastases, resulting from melanoma.

P-079 SURGERY AND RADIOTHERAPY FOR LENTIGO MALIGNA - WHAT IS THE EVIDENCE?

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Surgical excision is the standard of care for Lentigo Maligna (LM) as it permits histological confirmation, accurate staging and margin control. Approaches include conventional excision, staged excision and standard or modified Mohs' micrographic surgery (MMS). Comparing the efficacy of these interventions is restricted by the lack of randomised controlled data, small sample sizes and variable methodology, though the available evidence suggests that all three surgical approaches may achieve cure rates of 95% or more. This evidence is presented. However, surgery for LM may not be feasible in certain situations due to potential aesthetic and functional impairment, comorbidity and patient preference. Non-surgical interventions include radiotherapy, cryotherapy, laser, intralesional interferon, topical 5-FU, azaleic acid and most recently imiquimod. Radiotherapy is the most evaluated non-surgical modality and is an effective alternative to surgery as long term cure rates are comparable. Evidence for the efficacy of radiotherapy is also presented.

P-080 A PREDICTIVE MODEL FOR NON-SENTINEL NODE INVOLVEMENT IN PATIENTS WITH A POSITIVE SENTINEL NODE: IMPACT ON SURGICAL MANAGEMENT

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Objective: While selective lymphadenectomy using sentinel node biopsy (SNB) limits the morbidity of formal node dissections to those most likely to benefit, only 12-20% of patients with a positive SN have disease identified in the completion lymph node dissection (CLND) specimen. Therefore, some argue that a selective approach to CLND is warranted. We sought to establish a predictive model to better stratify patients according to risk of non-SN involvement. Methods: Between 1991 and 2001, 247 patients with at least 1 positive SN and who underwent a CLND were identified. Several primary tumor (thickness, ulceration, Clark level), host (site, age, gender), and SN (number SNs removed/involved, SN tumor burden) factors were analyzed according to risk of non-SN metastases; Cox proportional hazards regression model was used to identify independent prognostic factors. A predictive model was developed to estimate risk of non-SN metastasis. Results: The overall incidence of non-SN involvement was 18.2%: primary tumor thickness (< or > 2mm) and measures of SN tumor burden were independent predictors. Primary tumor factors were assigned "T" scores of 0 or 1 (<2mm and > 2mm, respectively) and SN tumor burden was assigned an "N" score of 0, 1, 2, or 3 according to largest SN tumor focus (mm): < 0.5, 0.5-2, >2<=10, and >10mm, respectively. Four groups were defined (Table 1). Conclusions: The model identifies a very low risk group (TN score=0), defined by primary tumor thickness and small SN burden, but represents only 12% of the SN positive population. While it appears rational to consider careful observation in this group, such an approach is best implemented as part of a multi-center prospective registry. For all other patients, CLND should be promoted as part of a multi-center, prospective experience to validate the model and/or to identify other predictive factors.

P-081 PAROTID LYMPH DRAINAGE FOR A MELANOMA LOCALIZED ON THE BACK.

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Sentinel lymph node technique is increasingly used for cutaneous melanoma staging. Preoperative lymphoscintigraphy allows to detect lymphatic drainage pathways, location and number of SLN. So, LN located in unexpected LN areas can be found. Case report: We are reporting the case of a patient who had an unclassified melanoma in September 1998 at the age of 56, with 9.7 mm Breslow thickness, Clark 4, mitotic index of 3/3. The tumor was located in the left scapular area. Brain MRI and thoraco-abdominal CT scan were normal. The lymphoscintigraphy for SLN (4 intradermal injections of 99mTC-labelled nanocolloïds) showed various lymphatic channels leading to three SLN : one in the left axilla, another in the left triangular intermuscular space and a third in the left parotid. Late images confirmed the absence of other alternative drainages. The wide excision as well as the removal of the SLN (Patent blue and gamma probe) were carried out the same day as the lymphoscintigraphy. Histological and immunohistological (S100B; HMB45) examination of the SLN were negative. No adjuvant treatment was proposed and after six years of follow-up the patient still alive without recurrence. Discussion: Lymphatic drainage of the trunck is known to be highly variable. Since the systematic use of lymphoscintigraphy to pick-up SLN, unexpected drainage areas have been frequently reported. However, no drainage to the parotid basin, that is usual for melanomas occurring in the face and scalp, have been reported up-to-now for a melanoma of the back. Without the aid of scintigraphy, if this patient would have developped a parotid metastase, she would have been considered as in stage IV in place of a stage III. Conclusions: Melanomas of the back may be drained onto the parotid area. Only an adequate preoperative lymphoscintigraphy may allow the visualization of such drainage.

P-082 INTERVAL LYMPH NODES MORE FREQUENT THAN EXPECTED !

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Material and Methods: Between November 1997 and January 2001, 150 patients having melanoma located in limbs (26 upper; 62 lower) and in trunck (24 ant.; 37 post.), entered the study. Technetium labelled nanocolloïds were intradermally administered in 4 injections. Serial images were taken after each administration. Late images were also collected 2 hours later. Patent blue and gamma probe were used for SN biopsy. A complete LND was performed if at least one of the sentinel nodes was invaded. If a interval LN was positive, a LND of the downstream area was done. Results: We have found interval SLN in 18% distributed as follows: posterior trunck: 41% (triangular intermuscular space, bottom, lateroaortic), anterior trunck : 25% (para mammary and internal mammary); upper limbs:15% (epitrochlear), lower limbs: 3% (popliteal). All these nodes were shown on lymphoscintigraphy. The internal mammary and latero-aortic SLN were not removed. The overall LN positivity was of 28%. Among the 5 patients with positive interval SLN (18% of removed LN), it was the only positive SN in three cases. One out of the latter 3, had a positive LN in the downstream area (detected after complete node dissection). Discussion: Our 18 % score of interval LN that is much higher than the one reported by others (3,1 to 7.2%), may be explained by the use of a particular lymphoscintigraphic method as well as the systematic prospective search of interval LN.The level of interval LN invasion was comparable to most published series and confirms that, if the staging was to be improved, the removal of these LN is mandatory. Conclusions : We found SLN outside the classical draining areas in 18% of the cases. They must be systematically searched for lymphoscintigraphic images so they could be removed and examined.

P-083 LYMPHOSCINTIGRAPHIC ASSESSMENT OF LYMPHATIC DRAINAGE OF THE EAR IN MELANOMA PATIENTS

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Purpose: The ear is known to have a variable pattern of lymphatic drainage. Lymphoscintigrams and sentinel node biopsy (SNB) results were studied to determine whether lymphatic drainage patterns could be predicted in patients with primary melanomas of the ear. Methods: The ear was subdivided into anatomical subunits and patients grouped accordingly. Sentinel node (SN) location was determined by lymphoscintigram alone or in conjunction with SNB. Lymphatic drainage patterns were assessed by relating each anatomical sub-unit to the location of the corresponding draining lymph nodes. Results: Lymphoscintigraphy, performed in 54 patients, identified 125 SNs, in the parotid and occipital regions in addition to the 5 standard cervical node levels. A discrepancy between the lymphoscintigram and operative findings was identified in 7 SNs, found in the parotid or level II cervical region. 10 patients (19%) clearly demonstrated trans-aural drainage (drainage from one surface of the ear to the other via the auricular cartilage). Regional recurrences (n=3) occurred only in the patient group (n=22) whose sentinel nodes were observed, not removed, all at sites previously identified as containing the SN. Conclusions: Analysis of ear lymphatic drainage has confirmed the parotid, level II and post-auricular nodes as the commonest sites for SNs. The location of the SN was independent of the position on the auricle of the primary site.

P-084 POTENTIAL BARRIERS TO USE OF LYMPHATIC MAPPING AND SENTINEL LYMPHADENECTOMY FOR PATIENTS WITH INTERMEDIATE THICKNESS MELANOMA

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INTRODUCTION: US national and state registry data show that only about 50% of patients with intermediate thickness melanoma undergo lymphatic mapping and sentinel lymphadenectomy (LM/SL). This study examines potential barriers to LM/SL. METHODS: An IRB-approved questionnaire was administered to all North Carolina (NC) dermatologists and to NC surgeons who billed for e3 melanoma procedures over a 4 year period. Pearson's Chi-Square, Fisher's Exact, and Student's t-test were used to test associations between responses and respondent characteristics. RESULTS: 147 dermatologists and 116 surgeons responded (Response Rate=60.0%). Respondents and non-respondents did not differ significantly. Responding surgeons included general surgeons (60%), plastic surgeons (19%), otolaryngologists (8%), and surgical oncologists (9%). 70.4% of surgeons performed LM/SL. Only 3.7% of surgeons neither performed LM/SL nor referred to others for LM/SL. Surgeons who performed LM/SL differed from other surgeons in practice type (38.9% of solo practitioners, 77.4% of surgeons in surgical groups, 61.5% of surgeons in academic practice, 100% of surgeons in multi-specialty groups, p=0.002), specialty (100% of surgical oncologists, 86.4% of general surgeons, 27.3% of plastic surgeons, 14.3% of otolaryngologists, p<0.001), and years of practice in NC (p=0.040). 86.0% of dermatologists reported referring >90% of patients with intermediate thickness melanoma for LM/SL. Most common reasons for not referring were patient age/comorbidity and location of primary tumor. None of the respondents felt cost or travel distance were barriers to LM/SL. >75% of dermatologists referred to a surgeon <1 hour away for LM/SL. CONCLUSION: The majority of melanoma providers in NC report that they perform LM/SL or refer to a nearby surgeon for the procedure. Patient age/comorbidity and primary tumor site were the only consistent reasons for not utilizing LM/SL. It is unclear why only 50% of patients with intermediate thickness melanoma undergo LM/SL, when almost all providers report referring >90% of their patients for the procedure.

P-085 NSC 631570 IN TREATMENT OF MALIGNANT MELANOMA IN XERODERMA PIGMENTOSUM PATIENTS.

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OBJECTIVE. Xeroderma Pigmentosum (XP) is a rare disorder transmitted in an autosomal recessive manner characterized by photosensitivity, pigmentary changes, premature skin aging, and malignancies development. These symptoms are due to a cellular hypersensitivity to ultraviolet radiation resulting from a defect in DNA repair. The aim of therapy is to protect the patient from sunlight and early detection and treatment of frequently occurring neoplasms. Oral retinoids are the only systemic modality and can decrease the incidence of skin cancer in patients with XP, but this therapy is limited by dose-related irreversible calcification of ligaments and tendons. NSC 631570 is a product that results from a reaction of alkaloids from greater celandine with thiotepa in the presence of hydrochloric acid. The drug acts directly on cancer cells inhibiting cell cycle passing and modulating immunity; after intravenously administration NSC 631570 is selectively accumulated in malignancies that in case of skin tumors could be confirmed by observing drug-specific fluorescence under UV-light. PATIENTS & METHODS. Two male patients (12 and 7 years old at the beginning of the therapy) with verified XP diagnosis were treated with NSC 631570 at the average dose 10 mg per week intravenously. In both cases permanent occurrence of malignant melanomas was the major indication of NSC 631570 administration. RESULTS. Administration of NSC 631570 led to: (1) dramatically decrease of malignant skin neoplasms (including melanoma) development that resulted in diminution of surgery performance, (2) decrease of photosensitivity and photophobia and (3) surprising improvement of the quality of life of these patients. CONCLUSION. NSC 631570 was effective in the treatment of 2 XP cases. In spite of the existence of the data concerning the possible molecular mechanisms of action of NSC 631570, future investigations, especially on detecting the the site-specific chromosomal binding of NSC 631570 and its consequences are necessary.

P-086 RADIOTHERAPY OF BRAIN METASTASES FROM CUTANEOUS MELANOMA

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Background: The aim of the present retrospective study was to evaluate the efficacy of radiotherapy in the treatment of brain metastases from cutaneous melanoma. Patients and Methods: Between September 1991 and June 2004, 15 patients (pts), 8 males and 7 females, mean age 55 (range 24-74) years, with brain metastases from cutaneous melanoma were irradiated at our department. Location of primary tumour was as follows: 2 pts on the head and neck, 10 pts on the trunk, 1 pts on the upper extremity and 2 pts on the lower extremity. Pathological TNM stage was as follows: 5 pts stage I, 7 pts stage II and 3 pts stage III. Clark level was as follows: 5 pts Clark II, 3 pts Clark III and 7 pts Clark IV. Breslow index was as follows: < 1.0 mm in 6 pts, 1.01-2 mm in 3 pts, 2.01-4 mm in 6 pts. Thirteen pts also had extracranial metastases at the time brain metastases were detected. In 2 pts the brain involvement was solitary. Three pts had surgery before start of radiotherapy. Eleven (73%) pts were treated with whole brain radiotherapy and 4 (27%) pts with partial brain radiotherapy. Fourteen pts received dose 30 Gy in 10 fractions (3 Gy daily) and 1 pt 20 Gy in 5 fractions (4 Gy daily). Results: The median interval between diagnosis of the primary tumour and brain metastases was 1113 days (95% CI: 252-1588 days). The median of survival from the time of brain metastases was 158 days (95% CI: 102-179 days). One-year survival from the time of brain metastases was 16.7Ç% (95% CI: 0-37.8%) At the date of evaluation (June 30th, 2004) all pts died. Conclusions: The prognosis of patients with brain metastases from cutaneous melanoma is poor. Radiotherapy has only short term palliative effect.

NOTES:

P-087 INTRON A HEALTH MANAGEMENT PROGRAM (HMP) IN HIGH RISK MALIGNANT MELANOMA SHOWED THE POSITIVE IMPACT OF HYDRATION: AN ASSESSMENT OF ONCOLOGY NURSING SUPPORT TO IMPROVE COMPLIANCE.

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Background: Skin cancer affects more then 75,000 Canadians each year. Malignant melanoma is rare but accounts for >75% of all skin cancer deaths and the incidence is increasing at an annual rate of 2%. Intron A is the only adjuvant therapy that has increased survival in high-risk melanoma patients. However side effects may lead to early discontinuation of therapy or sub-optimal drug exposure. Methods: Patients were educated on the benefit of therapy and were given comprehensive patient education materials. Oncology nurses provided support to help patients better manage and control adverse events. Results: A total of 251 patients were scheduled to receive 20 MIU/m2 5 days a week for 4 weeks followed by 10 MIU/m2 3 times a week for 48 weeks. Twenty-nine percent of patients progressed before completion of therapy. Of the remaining patients, 52% completed a full year of therapy, with 93% of those patients being compliant more than 80% of the time. The majority of discontinuations occurred during the induction phase (57%) vs during the maintenance phase (43%). Males were more likely to be compliant than females ($p<0.05$), especially during the first few months of the maintenance phase. The two most common reasons for discontinuation were adverse events (58%), and disease progression (29%). Patients with fluid intake >1.5 liter/day were more likely to complete therapy (64%) compared to those drinking a smaller volume (32%, $p<0.0001$). The impact of hydration could be seen both during the induction and maintenance phase. Conclusion: A significant proportion of melanoma patients who receive high dose Intron A therapy discontinue early due to adverse events. The importance of fluid intake was clearly established by the Intron Health Management Program, since it was the most favorable predictor for completion of therapy.

P-088 WHEN IS THE CORRECT TIME TO REFER MELANOMA PATIENTS TO PALLIATIVE CARE SERVICES?

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OBJECTIVE. Trace what is understood to constitute correct timing to refer patients to Palliative Care services, examine the unique features of metastatic melanoma patients and to describe the support services that have been developed to care for these patients. METHOD. Retrospective review of the case management of over 200 metastatic melanoma patients using the WHO definition of referral to Palliative Care services. These are illustrated by 3 case histories showing the support services involved and the timing of these referrals. RESULT. The unique features of metastatic melanoma spread and morbidity of the patient is apparent through these case histories Services that are required to support these patients are different to services required in other oncology conditions. DISCUSSION. Case management of patients with metastatic melanoma demonstrates that there is a real need to individualize the care offered to each one. There is a need not to be limited by definitions and to approach each patient as unique.

P-089 ADJUVANT IFN ALPHA THERAPY STIMULATES TRANSPORTER PROTEINS ASSOCIATED WITH ANTIGEN PROCESSING AND PROTEASOME ACTIVATOR 28 IN PATIENTS WITH MALIGNANT MELANOMA

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The use of cytokines, especially of interferon alpha (IFN alpha) for the treatment of metastatic melanoma was evaluated in several clinical trials. Although IFN alpha shows a broad spectrum of immunmodulatory and antiproliferative effects in a variety of malignancies the mechanisms of its antitumor effect and its action in adjuvant melanoma therapy remained unclear. In this clinical study we showed that expression of transport proteins associated with antigen processing (TAP1 and TAP2) and proteasome activator 28 (11S REG) was significantly upregulated by i.v. adjuvant treatment with 10 million IU/m2 IFN alpha in 20 patients with malignant melanoma (stage III, UICC). This strong stimulatory effect was seen in blood mononuclear cells (PBMC) both on the RNA level using RT-, Real time- PCR and on the protein level using immunohistochemistry and immunoblotting. Depending on the patient analyzed, 2- to 5-fold upregulation of TAP1 mRNA expression could be detected by Real time- PCR. The finding that IFN alpha stimulates the cytotoxic effector functions in PBMC of patients receiving intermediate high dose immunotherapy by enhancing TAP expression and proteasome activity contributes to the understanding of the immunoregulatory role of type 1 interferons and may help to explain the efficacy of IFN alpha in the treatment of tumors. These parameters will also provide a new clinical tool for measuring the efficiency of INF alpha therapy in vivo and will give further information about the most effective dose and the duration of IFN alpha administration.

P-090 5-AZA-2'-DEOXYCYTIDINE (5-AZA-DC) OVERCOMES RESISTANCE OF CELLS TO INTERFERON(IFN)-INDUCED APOPTOSIS BY INCREASE IN TRAIL R1 IN SK-MEL28 MELANOMA CELLS AND AN INCREASE IN XAF-1 IN A375 MELANOMA CELLS

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Postulating that silencing of interferon-stimulated genes (ISGs) by methylation of 5 CpG islands mediates melanoma resistance to IFNs, we assessed the DNA demethylating agent 5-AZA-dC in SK MEL 28 and A375 human melanoma cells. Neither SK-MEL28 nor A375 underwent apoptosis upon TUNEL assay (<2% apopototic cells) in response to even high doses of IFN-ß or IFN-±2. 5-AZA-dC at 100 nM in SK MEL 28 cells or 200 nM in A375 cells restored IFN responsiveness resulting in up to 30 % apoptotic cells with 100 U/ml of IFN-±2 and 80% with IFN-ß, without significant apoptosis from 5-AZA-dC alone (<5% apoptotic cells). Immunoblots for caspase 3 and PARP cleavage confirmed results. Western blot confirmed significant decrease in DNMT1 with 5-AZA-dC. Genes essential for IFN-induced apoptosis and potentially silenced by DNA methylation, Stat1, Stat2, TRAIL, IRF1, TRAIL R1, TRAIL R2, XAF-1, were then assessed by qPCR in 5-AZA-dC treated cells. In A375 treated cells 5-AZA-dC expressed XAF-1 about 70x while other genes were only minimally increased. In SK-MEL28 TRAIL R1 was increased by 5-AZA-dC (>25x), an effect further increased with IFNs. Methylation specific PCR further confirmed the role of 5-AZA-dC and DNMT-1 in TRAIL R1 in SK MEL28 and XAF 1 increases in A375 cells. We have previously shown that TRAIL/Apo2L and XAF1 are ISGs in melanoma cells, particularly in response to IFN-ß. The augmentation by 5-AZA-dC of apoptosis by IFNs in SK MEL 28 cells likely results from an increase in TRAIL R1 and in A375 cells from an increase in the pro-apoptotic XAF1. Data suggest that reactivation of silenced pro-apoptotic genes by DNA demethylators such as 5-AZA-dC may restore IFNantitumor responses in melanoma.

P-091 GERANYLGERANIOL (GGOH) INDUCES APOPTOSIS IN HUMAN LATE PRIMARY AND METASTATIC MELANOMA CELL LINES IN VITRO

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Objective: The mevalonate pathway is regulated by HMG-CoA reductase and is important in the biosynthesis of sterol and nonsterol isoprenoids. Geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) are two nonsterol isoprenoids that are crucial for proper cell function. These isoprenoids are required for prenylation, which posttranslationally modifies and activates select proteins involved in cell proliferation. HMG-CoA reductase inhibitors (statins) inhibit cell growth and metastasis of melanoma in vitro by inducing apoptosis via a geranylgeranylation specific mechanism. Recent investigation has focused on more specific inhibitors of GGPP such as geranylgeraniol (GGOH). GGOH decreases the amount of GGPP available for isoprenylation, a modification that occurs to approximately 0.05% of cellular proteins. This study evaluated the effects of GGOH on human melanoma cell lines and an epidermoid-derived cell line. Materials and Methods: Cell lines included human metastatic melanoma cell lines (WM1617, HS294T, A375), human early primary melanoma cell line (WM35), human later primary melanoma cell line (WM278) and epidermoid-derived carcinoma line (A431). Growth inhibition of cells treated with GGOH was determined using an MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethozyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium,innersault] viability assay. Ethidium Bromide/Acridine Orange fluorescent staining was used to measure apoptosis. Results: GGOH (40(M) decreased cell growth more than 60% compared with the solvent control after 48 hours in 4 cell lines (A375, A431, HS294T, WM278) (Graph). Fluorescent microscopy revealed that GGOH induced tumor cell apoptosis. Conclusions: GGOH triggered apoptosis in several late growth melanoma, metastatic melanoma and epidermal-derived carcinoma cell lines. However, GGOH did not induce cell death in 1 metastatic melanoma cell line (WM1617) nor in the early primary melanoma cell line (WM35). Work in our lab now focuses on explaining variable melanoma cell line sensitivity to GGOH-induced apoptosis and potentially exploiting these differences to improve melanoma therapy.

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P-092 ULTRAVIOLET B RADIATION OF HUMAN MELANOCYTES LEADS TO CDC2 MIGRATION IN NUCLEAR SPECKLES WITH GADD45A AND P21WAF1 AND G2/M ARREST

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OBJECTIF: Epidemiological and biological studies indicate that, in addition to host factors, exposure to solar UVB radiation is involved in cutaneous malignant melanoma aetiology. However the mechanisms of melanocyte transformation remain unclear. We have previously shown that, when exposed to UVB radiation, melanocytes and melanoma cells use a p53-independent pathway involving the activation of GADD45A gene expression by N-oct3. The goal of the present study was to investigate the effects this activation on the specific cellular response of melanoma cells to UVB. METHODS: Following UVB irradiation of two melanoma cell lines with distinct p53 status, we analysed the cell cycle progression using flow cytometry. The localisation of Gadd45a and its partners involved in the cell cycle regulation, p21Waf1 and p34Cdk1/Cdc2was determined using immunofluorescence and confocal microscopy. Finally, the interactions between proteins were further analysed through co-immunoprecipitation. RESULTS: We observed that UVB radiation induced a G2/M arrest in both cell lines. In these cells we showed that Gadd45a and p21Waf1 were mainly localised in Nuclear Speckles and interacted before and after treatment. Importantly, we observed that UVB induced the migration of p34Cdk1/Cdc2 to Nuclear Speckles and that this relocalisation was associated with increased Gadd45a-p34Cdk1/Cdc2 interaction. CONCLUSION: These observations suggest that: 1-in melanoma cells, Gadd45a and p21Waf1 are mainly located in Nuclear Speckles. 2- following UVB, Gadd45a overexpression induces the sequestration of p34Cdk1/Cdc2 in Nuclear Speckles. 3-this sequestration results in the dissociation of the cyclin B1- p34Cdk1/Cdc2 complex and G2/M arrest Finally, whereas Nuclear Speckles were so far only linked to RNA splicing, our results contribute to define a new role for these nuclear bodies.

P-093 METASTATIC MELANOMA MIGRATES TOWARDS LYMPHATICS DUE TO RECOGNITION OF LYMPHATIC ENDOTHELIAL CELL SECRETED CHEMOKINES

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Melanoma morbidity results from metastatic deposits rather than the primary lesions. It is not clear whether the mechanisms by which lymphatic metastases occur is by active migration of melanoma towards the lymphatics or vice versa. The aim of this project was to determine in vitro and in vivo, whether metastatic but not non-metastatic melanoma cells will actively migrate towards lymphatics. A375 human melanoma cells, two subclones of this line metastatic (A375SM) and non metastatic (A375P) lines, human dermal endothelial cells (HDEC), immunomagnetically separated into lymphatic endothelial cells (LEC) and Blood endothelial cells (BEC) were grown to confluence in vitro. 1x105 LECs or BECs in 100µl PBS were injected subcutaneously into 6 nude mice. 1x106 A375 or A375P cells were then injected ~10mm rostral to the EC injection site (marked with Monastral blue). Tumours were excised up to 12mm in diameter. Macroscopic measurements were made from prosections of the tumour area relative to the endothelial cell injection site. A375 (83±3% of tumours on EC side of injection), but not A375P (16±15%) melanoma grew significantly towards the LEC cells ($p<0.05$) but not the BEC (A375, 27±7%, A375P, 43±4% on EC injection side). To investigate potential molecular mediators of the migration, metastatic (A375SM) and non-metastatic clones (A375P) of the A375 melanoma cells migrating towards HDEC conditioned media (CM) was measured in modified Boyden Chambers in response to inhibition of candidate chemokines. A375SMs (9.6±0.4 fold), but not A375P (2.7±0.1), migrated towards HDEC CM. This was significantly inhibited by neutralising antibody to CCL21 (5.0±0.5 A375SM, $P<0.01$, t test). These results suggest that lymphatic metastatic melanoma can specifically grow towards lymphatic endothelial cells in vitro and in vivo, supporting the hypothesis that melanoma metastasises by actively migrating towards lymphatics due to recognition of secreted chemokines such as CCL21. Supported by SCaRF and Luff funds.

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P-094 INFREQUENT METHYLATION AND CONSTANT EXPRESSION OF TWIST DURING TUMOR PROGRESSION IN MELANOMA

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Twist is suggested to be a key regulator of metastasis through repression of E-cadherin in breast carcinoma and expression level and methylation status of Twist correlate with disease histopathologic characteristics. However, the role of Twist in melanoma progression is unknown, whereas E-cadherin expression level inversely correlates with the progression stage of melanoma. To examine whether Twist influenced progression of melanoma, we surveyed the methylation status and the expression level of Twist in melanoma. Nineteen paraffin-embedded melanoma samples (6 primary and 13 metastatic melanomas) were microdissected and tumor DNA was isolated for methylation-specific PCR (MSP) assay to determine the methylation status of Twist promoter region. MSP assay revealed no methylation of Twist in nineteen melanomas suggesting that epigenetic silencing of Twist was infrequent in melanoma. Next, twenty-seven melanomas (15 primary and 12 metastatic melanomas) were examined by immunohistochemical study for Twist using two polyclonal anti-twist antibodies (twist c-17, twist n-19). All of 27 melanomas showed Twist expression in a higher or lower degree, but there was no significant difference in expression level of Twist between primary and metastatic tumors. Twist expression increased at invasive lesions in vertical growth phase melanomas. These results suggest that Twist is constantly expressed during melanoma progression and may play an important role in melanoma. Further functional study is needed to understand the role of Twist in melanoma progression.

P-095 THE STUDY OF NEVI IN CHILDREN (SONIC): DESIGN, METHODS, AND RECRUITMENT

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Introduction:. Nevi are important precursors and risk markers for melanoma. Cross-sectional studies suggest that adolescence is a critical period for the evolution of nevi; however, there is limited research on common nevi during pre-adolescence. Methods: We describe the design and methods of a population-based, prospective cohort study of the prevalence and evolution of moles in Framingham Massachusetts. The study entitled Study of Nevi in Children (SONIC) is a joint collaboration between the Memorial-Sloan Kettering Cancer Center, Boston University, and the Framingham Schools System. Framingham is a racially and ethnically diverse town (population 60,000+) with 10 public and parochial elementary schools. Families of children ages 10-11 (Grade 5) were recruited through multiple channels. Multiple mailings and more than 4,000 calls were required to consent 64% (443/691) of eligible families to participate in parent and child survey collection (conducted in 5th grade through 8th grade), photographic/dermoscopic examination of the back of children during the school's scoliosis examinations (5th grade and 8th grade), and testing for multiple pigmentation genes (5th grade). Results: Seventy percent of the children were white, 38% were girls, median age was 10.7, 63% had very fair or fair skin, and 71% tanned when exposed to the sun. Thirty-four percent of children reported having at least one sunburn during the previous summer. Twenty-three percent of parents reported a personal or family history of skin cancer. Embarrassment about the examination and concern about genetic testing were the major reasons for non-participation. Children whose parents did not consent were 58% white, 52% girls, and 38% fair/very fair skin color. Conclusion: Anchoring skin cancer studies within the school's existing health educational framework is promising; however there are numerous challenges required to recruit families to such studies, particularly with young girls who are reluctant to participate in the screening examination.

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P-096 CONSTITUTIVE ACTIVATION OF THE MAPK SIGNALING PATHWAY IN ACRAL MELANOMAS

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One of the most attractive clinical targets for melanoma is the mitogen activated protein kinase (MAPK) signaling pathway, which plays a central role in regulating the growth and survival of neoplastic cells. In most superficial spreading melanomas, this pathway was reported to be constitutively activated most likely a consequence of either NRAS or BRAF mutations. In acral melanomas, however, the reported frequency of NRAS/BRAF mutations was low, and whether or not MAPK signaling is constitutively activated is unknown. In this study, we examined NRAS/BRAF mutations and MAPK signaling activation in clinical samples of acral melanomas. We also examined the amplifications of the cyclin D1 gene (CCND1), which is an important down-stream effector of the MAPK pathway and is known to be frequently amplified in acral melanomas. In a total of 28 acral melanoma samples, consisting of 13 primary tumors and 15 metastases, NRAS/BRAF mutations were rare; only one primary tumor had a NRAS E61R mutation, and one primary tumor and 2 metastases harbored BRAF V599E mutations. Western blot analyses, however, revealed phosphorylated ERK1/2 proteins in 11/14 (78.5%) of the acral melanoma tumors, indicating that the MAPK signaling pathway is constitutively activated and plays an important role in acral melanomas. FISH analyses revealed prominent amplification of the CCND1 in 5 of 21 (23.8%) tumors examined. Interestingly, three of four tumors that were negative for phosphorylated ERK proteins according to Western blot harbored CCND1 amplifications, suggesting that the increased gene dosage of CCND1 may exert similar effects to phosphorylated ERK proteins in cell growth. We conclude that, despite the low frequency of BRAF/NRAS mutations, the MAPK signaling pathway is constitutively activated in the majority of acral melanomas. This provides a rational basis to include acral melanomas into the clinical trials with MAPK inhibitors.

P-097 P14ARF INTERACTS WITH THE SUMO-CONJUGATING ENZYME UBC9 AND PROMOTES THE SUMOYLATION OF ITS BINDING PARTNERS

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The p14ARF tumour suppressor regulates a series of cell cycle regulatory proteins to promote cell cycle arrest in response to abnormal hyperproliferative growth stimuli. p14ARF alterations are common in human cancers and, when inherited, confer susceptibility to cutaneous melanoma. We now propose that the mechanism of p14ARF action may involve the covalent modification of its binding partners with the small ubiquitin-related protein SUMO-1. In particular, we demonstrate that p14ARF interacts with the SUMO E2 conjugating enzyme, Ubc9 and enhances the sumoylation of its binding partners, hdm2, E2F-1, HIF-1±, TBP-1 and p120E4F. Furthermore, p14ARF-induced sumoylation is abrogated by a subset of melanoma-associated p14ARF mutations. These results provide a mechanism for p14ARF action through a common modification of diverse binding partners.

P-098 ENFORCED EXPRESSION OF P14ARF INDUCES P53-DEPENDENT CELL CYCLE ARREST BUT NOT APOPTOSIS

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Expression of the p14ARF tumour suppressor is induced by hyperproliferative signals produced by RAS, MYC and other oncogenes. p14ARF quenches inappropriate mitogenic signaling by activating the p53 pathway, and the frequent loss of p14ARF in human cancer diminishes the duration and level of the p53 response. Consistent with this role, p14ARF accumulation can induce potent cell cycle arrest, but its role in promoting apoptosis has not been well established. Therefore we investigated the effects of p14ARF on the survival and growth of several human cell types. To avoid the toxicity associated with adenoviral-based vectors, we established inducible expression of p14ARF in p53-intact and p53-deficient human cell lines. As expected, transient and inducible expression of p14ARF induced rapid cell cycle arrest only in tumour cells expressing intact p53. Further, p14ARF expression did not promote apoptosis in primary human fibroblasts, or in any human tumour cell line tested, irrespective of p53 status. Instead, p14ARF expression sensitized cells to apoptosis in the presence of inhibitors of topoisomerase II (adriamycin) and transcription (DRB). Thus, loss of p14ARF would be an important step in the selection of apoptotic resistant tumour cells.

P-099 AVB3 INTEGRIN EXPRESSION IN MELANOCYTIC NEVUS AND CUTANEOUS MELANOMA

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Alpha-v-beta3 integrin (v 3) is a ligand to vitronectin and increased expression of subunit beta 3 appears to be related to melanoma progression, a possible indication aggressive behavior. In melanoma cell cultures, v 3 expression is inhibited by heath shock protein 27. Activation of metalloproteinase-2 (MMP-2) is simultaneous to v 3 expression, either in primary invasive melanoma and metastases, both in vivo and in vitro. Furthermore, VEGF appears to modulate v 3 expression. OBJECTIVES: We aimed to evaluate the expression of v 3 in melanocytic nevi, superficial spreading cutaneous in situ melanoma, invasive and metastatic, both in conventional and tissue array (TMA) paraffin embedded tissue specimens. We also analyzed the relationship between v 3 expression to histopathological variables and patient survival. MATERIAL AND METHODS: A total of 159 tissue samples from compound nevi (n=19), in situ melanoma (n=5), thin melanoma (n=34), thick melanoma (n=72), metastatic melanoma (n=29). Thick (≥1.01 mm) primary cutaneous and metastatic melanoma were included in a TMA with 111 samples in duplicate. The remaining samples were stained in conventional sections. Anti- v 3 integrin (Abcam, UK), was diluted at 1:50, and the results scored from 0 to 12. RESULTS: Compound nevus epithelioid cells had a mild expression of v 3. In situ melanoma cells had a significantly higher score among all specimens, when compared to nevi staining (p=0.0000) and to invasive melanoma (p=0.0003). Expression of v 3 did not differ according to depth of invasion. Lung metastases had the lowest median score among all. No increased expression of v 3 was found in metastatases. CONCLUSION: Our series have shown no differential expression in primary invasive melanoma, regardless of depth, nor between primary and metastatic disease, indicating that v 3 integrin might have no impact on melanoma behavior. However, high levels of v 3 integrin expression for in situ melanoma may be related to pre-invasive phenotype preparation to invasion.

P-100 CYCLIN D1 EXPRESSION IN SUPERFICIAL SPREADING CUTANEOUS MELANOMA

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Cyclin D1 gene encodes regulatory enzymes that promotes progression through the G1-S phase of the cell cycle. Overexpression of its encoded protein (CD1) has been found in many tumors. However, in melanoma, the actual role of CD1 is not completely understood. OBJECTIVES: We aimed to evaluate the expression of CD1 in nevi, superficial spreading cutaneous in situ, invasive and metastatic melanoma, both in conventional and tissue array (TMA) paraffin embedded tissue specimens. We also analyzed the relationship between CD1 expression and tumor thickness. PATIENTS AND METHODS: A total of 175 tissue samples from compound nevi (n=10), in situ melanoma (n=20), thin melanoma (n=44), thick melanoma (n=72), metastatic melanoma (n=29). Thick (≥1.01 mm) primary cutaneous and metastatic melanoma, and nevi were included in a TMA with 121 samples in duplicate. The remaining samples were stained in conventional sections (n=64). Skin, lymph node and lung metastases were included. Immunostaining using mouse anti-Human Cyclin D1 (Santa Cruz SC-839), diluted at 1:320 was performed. Only nuclear staining was scored as positive. Positivity cut off was > 8.66 % (percentile 90). RESULTS: None of the nevi expressed CD1. Overall positivity in melanoma was 9.8 %. In situ and thin melanomas had a significantly higher expression than thick melanomas (p=0,004). Expression of CD1 in metastasis did not differ according to localization and none of them expressed CD1 above 8,66%. CONCLUSION: Our series have shown differential expression between thin and thick and metastatic melanomas and nevi. This may indicate that expression of CD1 may be an early event in the malignant transformation. However, other factors may influence this expression, and will be evaluated in the same series in the next future.

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P-101 THE C-KIT EXPRESSION IN PRIMARY AND METASTASIC ACRAL LENTIGINOUS MELANOMA. IS IMATINIB AN OPTION FOR THOSE PATIENTS?

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Objective: The expression of c-Kit is crucial in some malignancies like leukemia and gastrointestinal sarcomas. The c-Kit gene encodes a transmembrane receptor that has tyrosine kinase activity and plays an important role in gametogenesis, hematopoiesis and melanogenesis. The expression of this receptor in melanoma is conserved in the radial growth phase and lost in metastasic tissues. The expression is maximum in acral lentiginous melanomas, type most frequent in mexican population. Material and Methods: 88 samples of primary and metastasic melanoma tissues, formalin fixed and paraffin-embedded were examined inmunohistochemically for expression of c-Kit oncogene product using polyclonal antibody antihuman c-kit (Dako®). The expression was reported as positive or negative and assigns a value of expression using a objective index. The variables analyzed were: growth pattern, Breslow thickness, and primary and metastasic melanoma. Results: We found and overall expression of 63%. The primary, lymph node and skin metastases expressed the receptor in 92%, 31% and 30% respectively. There is no difference between the growth pattern: nodular (100%), acral lentiginous (82%) or superficial spreading melanomas (100%). The expression was strongest in the superficially lesions (Breslow thickness <1mm vs 1-4 mm vs > 4 mm) as well the acral lentiginous growth pattern. The lymph node and cutaneous metastases express the same level of intensity. Conclusion: The primary and metastasic melanoma express the c-Kit receptor, but more studies are necessary to provide accurate information about the activity of this protein and their ligands.

P-102 USE OF SUBTRACTIVE CDNA LIBRARIES TO ANALYZE MOLECULAR EVENTS INVOLVED IN REGRESSION OF CUTANEOUS MELANOMA IN THE MELIM MODEL

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Spontaneous regression is a well-defined phenomenon commonly observed in human melanoma in their partial histopathological form but sporadically in their complete expression. In the MeLiM (Melanoblastoma-bearing Libechov Minipig) model, swine exhibit cutaneous melanomas which appear around birth. These lesions are clinically and histologically comparable to their human counterparts. Nevertheless, in swine most of the highly invasive melanoma undergo complete spontaneous regression. Induction of regression is expressed by clinical features including changes in color, drying aspect and decrease in size; histologically we observed infiltration of histiocyte-like cells, fibrosis and lymphocytic infiltrate later. So, this model offers the opportunity to study the molecular events which conduct from an invasive tumor to a regressive one. We excised samples of growing and regressive tumors in the first days of induction and choose the best couple by clinical and histological parameters. Additionally, cell culture of progressive and regressive melanoma was performed to specifically study the role of melanoma cells. Therefore, we used Suppression Subtractive Hybridization to isolate differentially expressed transcripts between regressive and growing tumor tissue and cell extracts from the two tumor states. In order to characterize differentially expressed genes in tumor growth and spontaneous regression, sequencing of SSH clones was realized. Subsequently, isolated transcripts that showed sequence homology with genes of known function were classified according to their biological pathways to identify major functional classes of genes involved in spontaneous tumor regression. The validation of differential expression was conducted by RT-PCR. The generation of SSH libraries is a useful tool to approach molecular events that could lead to tumor regression in the MeLiM model. A large-scale validation of this work will be pursued by microarray analysis.

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P-104 THE CANCER-RETINA ANTIGEN RECOVERIN IS EXPRESSED IN MELANOMA AND RECOGNIZED BY SERA OF MELANOMA PATIENTS

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Recoverin is a Ca2+-binding protein of vertebrate photoreceptors involved in regulation of the visual transduction, which can be a paraneoplastic antigen in lung carcinomas and some other kinds of cancer. Recoverin aberrant expression in tumors can lead to the development of paraneoplastic retina degeneration associated with cancer. In this study we have addressed the expression of recoverin in malignant melanoma, a tumor originating from neuroectodermal melanocytes. We showed that recoverin can be expressed frequently in melanoma tissues and cell lines, and serum autoantibodies against recoverin can be detected in melanoma patients. Using two mouse melanoma models we confirmed that recoverin can be expressed in spontaneous developing melanoma and can induce an autoantibody response. Besides, we were the first to detect expression of mRNA for recoverin in healthy skin, which was revealed only in melanocytes. At the same time, expression of recoverin protein has not been detected in these cells. Thus, we suggest recoverin to be the first member of a new class of tumor antigens which can be named cancer-retina antigens. We hypothesize molecular mechanisms underlying transition from expression of mRNA for recoverin in normal melanocytes to recoverin protein expression in melanoma.

P-105 RND3/RHOE RHO GTPASE UPREGULATION AND FUNCTION IN MELANOMA

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OBJECTIVE: Approximately 90% of melanomas harbor mutations in NRAS or BRAF highlighting the importance of the RAF>MEK>ERK mitogen-activated protein kinase cascade in melanoma development. A critical outcome of RAS-RAF effector signaling is the regulation of transcription factor activity, resulting in changes in gene expression. Presently, little is known regarding the key gene targets of RAS and RAF important for oncogenesis. The aim of this study was to identify and validate novel targets for melanoma therapeutic intervention. MATERIALS & METHODS: Agilent 44K (G4112A) 60mer oligonucleotide microarrays were annealed with cDNA synthesized from 15 human melanoma cell lines and two cultures of human primary melanocytes and analyses supervised to elevations in phosphorylated and activated ERK levels. RESULTS: A microarray clustering of 114 genes upregulated by the RAF>MEK>ERK pathway were identified and upregulation of protein expression verified by western blot analyses of several genes including the Rnd3/RhoE small GTPase. Rnd3 is a member of the Rho GTPase family and aberrant Rho GTPase function has been implicated in oncogenesis. We show that RhoE/Rnd3 protein expression is upregulated in many of the melanoma tumor cell lines and treatment with the MEK inhibitor, U0126, diminished Rnd3 expression. Rnd3 has been shown to alter actin organization, cell-cell interactions and cell cycle progression. The importance of Rnd3 in melanoma tumorigenesis is currently being investigated by inhibition of Rnd3 expression using interfering RNA and pharmacologic inhibitors. For example, we determined that Rnd3 function can be blocked by farnesyltransferase inhibitors. Whether inhibition of Rnd3 activation can suppress melanoma tumorigenesis is also currently being studied. CONCLUSION: Validation of Rnd3 upregulation in many of the melanoma tumor cell lines demonstrates the reliability of the microarray analyses performed and suggests that the upregulated expression of Rnd3 is important for the aberrant growth of melanomas.

P-106 SUNLIGHT - IMMUNOSUPPRESSION AND CANCER

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In the last twenty years there has been a dramatic increase in the appreciation of the role of chronic sun exposure in the development of skin malignancies. A clear dose response relationship is established for squamous cell carcinoma but not for basal cell carcinoma and melanoma. Observations of photoinduced immunosuppression conceptually can be seen as offering a potentially unifying hypothesis to explain the inability to statistically prove the association between chronic sun exposure and the development of melanoma and basal cell carcinoma. The hyposthesis is explored.

P-107 EXPRESSION PROFILES OF ID1 AND P16 PROTEINS IN ALL-TRANS-RETINOIC ACID-INDUCED APOPTOSIS AND CELL CYCLE RE-DISTRIBUTION IN MELANOMA

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All-trans-retinoic acid (atRA) exerts its effects via apoptosis and cell cycle re-distribution. However, the mechanisms behind the effects have not been fully understood. In this study, we used a model system of matched primary and metastatic melanoma cells to investigate whether expression of Id1 and p16 proteins were involved in atRA-induced apoptosis and cell cycle re-distribution. Melanoma cells were exposed to 0.1 or 10 μM atRA for 1-96 hours. Apoptosis and cell cycle were measured by flow cytometry. Expression of Id1 and p16 proteins was examined by Western blotting and immunocytochemistry. After exposure to atRA we found a marked increase in apoptosis and cell cycle re-distribution in both primary and metastatic melanoma cells. Expression level of Id1 protein was decreased and the p16 was increased a dose- and time-dependent ($p<0.05$) manner after treatment with atRA. Alterations of these proteins were more pronounced in the primary melanoma cells than the matched metastases ($p<0.05$). These data suggested that the alterations of Id1 and/or p16 proteins were involved in atRA-induced apoptosis and cell cycle re-distribution in melanoma. These expression profiles of Id1 and p16 proteins may provide molecular evidence for better chemotherapy primarily for early stages of melanoma.

P-108 MELANOMA IN COLOMBIA

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Although there are no precise statistics available on melanoma in Colombia, for women, the annual incidence per 100.000 was 3.0 for the period 1978 to 1982; for men, it was 3.3 per 100.000 for the same period. The National Cancer Institute of Colombia (NCI) in Bogota, which is an institutional cancer research and treatment referral center for ambulatory and hospitalized care, provides a reliable data on this type of skin cancer. Most patients treated at the NCI are from low income backgrounds and reside throughout the entire country. One hundred and ten melanoma patients a year, equally divided among men and women, seek treatment at the NCI. Their age range is: <41years = 18%; 41-70 years = 66% and >71years = 16%. These patients have acral lentiginous melanoma (63%), lentigo maligna melanoma (18%) and other types (19%). The main symptoms which patients observe include ulceration (42%), itching (36%) and bleeding (34%). Upon examination at the NCI, the thickness of patientstumors ranges from: <1mm = 7%, 1-4mm = 72% and > 4mm = 21%. To sum up, melanoma in Colombia differs from melanoma in countries with predominantly white populations because: it occurs less frequently, the age at presentation is higher, acral lentiginous is the most frequent subtype and it is treated in its advanced stages.

P-109 EPIDEMIOLOGY AND SURVIVAL OF CUTANEOUS MELANOMA IN SPAIN: A 552 CASES REPORT (1994-2003)

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Introduction: Incidence of cutaneous melanoma is increasing worldwide, including not fair-skin subjects as Spanish population. There aren't trustworthy studies in this country about the epidemiology and survival of patients diagnosed of melanoma during last years. Material and methods: We collected data from all patients diagnosed of cutaneous melanoma between January 1st, 1994 and December 31th, 2003, and that were attended for this reason in in the General Universitary Hospital 'Gregorio Marañón'of Madrid. A descriptive and analytical epidemiologic study was performed Results: We described the epidemiological, clinical and histopathologic characteristics of these melanomas, as well as the evolution of these patient, beside analyzing the variation of these factors throughout the duration of the study. We also analyze which are the possible factors with influence in the overall survival (OS) and in the appearance of metastases or disease survival (DFS). Discussion: The incidence of melanoma in our population has doubled in the last decade. This effect seems to have become stable during the last years. The majority of melanomas is in stage I at the moment of the diagnosis (45 %). Nevertheless an important proportion of melanomas continues being diagnosed in advanced stages (III-IV; 14,5 %). The following factors were associated with a poor global survival: advanced age; masculine sex; multiple melanoma; metastatic melanomas; stages III-IV; nodular type; thickness superior to 4 mm (or Clark V); presence of ulceration and melanoma in sentinel node biopsy.

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P-110 ANALYSIS OF A PROSPECTIVE COHORT OF 1498 FRENCH MELANOMA CASES

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We present herein a prospective cohort study of 1 498 primitive melanoma patients followed up for a two years period. 33 French dermatological centers have included during a 12 months period all newly diagnosed melanomas. A two years follow up was performed for all patients with data collected at 12 and 24 months or at the relapse time. 1 498 patients with a medium age of 55 years have beenincluded. Our findings confirm that localization,confirm that localization, melanoma thickness and staging, age or gender are prognostic factors melanoma . Stage of melanoma at diagnosis is lower in women The average age was 57 years in men versus 53 years in women. After one year, 121 (8%) of patients have relapsed, 11 (1 %) died (in 5 cases due to melanoma), 1 198 (80%) were free of relapse and 95 (6 %) could not be followed up. Characteristics of relapsing melanoma are tumour ulceration (50 %), Breslow thickness (40% higher than 4mm), histological subtype (32 % of nodular melanoma), vascular embols (17 %). The localization is also a predictive factor of relapse. During the second year 72 patients (5%) have relapsed corresponding to a total percentage of relapse of 13%. These results suggest that high risk patients should be particularly monitored during the first year after the surgical treatment of melanoma

P-111 ANALYSIS OF BIASES ASSOCIATED WITH AWARENESS OF RISK FACTORS AND CONTROL SELECTION IN A CASE-CONTROL STUDY OF MELANOMA AND SUNBED USE.

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Objectives. We analysed misclassification and selection biases that could have nullified a potential association between sunbed use and melanoma risk in a large European case-control study in which naevi were also assessed. Results. Surprisingly, in this study, none of the sun exposure parameters were associated with risk of melanoma nor naevi. In multivariate analysis, sunbathing during hot hours even showed a non-significant protective effect (OR= 0.78 CI95%[0.60-1.01]). In a multivariate Poisson regression estimating the number of naevi on arms, sunbathing was not a predictor of naevus number for controls (0.3% increase in naevus number for sunbathing CI95%[-0.9% - 8.1%]); and sunbathing was almost significantly negatively correlated with the number of naevi in cases (-7.8% CI95% [-18.7 - 0.9]). We tried to estimate the potential impact of biases on the measurement of melanoma risk associated with sunbed use. Using exposure rates from a recent cohort study as an unbiased reference for sunbed use in Northern Europe, we showed that if only 25% of cases tend to underreport two fold their exposure to sunbeds, then a 'true' OR of melanoma risk associated with sunbed use of 1.29 is biased towards 1. In order to estimate specificity in cases and sensitivity in controls, we used data on sunbathing from two recent case-control studies and applied these estimations to our data set. This enabled us to calculate that, in the absence of biases, the actual OR of melanoma risk associated with sunbed use in our study could have been 1.35 (p=0.01) or 1.39 (p=0.005). Conclusion. Misclassification and selection biases are likely to have occurred in our case-control study. Small differential changes in the reporting of exposure could have masked or even reversed the risk of melanoma associated with sun and sunbed exposure.

P-112 MELANOMA RISK FACTORS REVISITED.

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Background. Melanoma is influenced by independent host factors and sun exposure. However relation between sun exposure and melanoma is complex. Anatomical distribution of melanomas suggest that intermittent and cumultative sun exposure play a different role according to age. Interactions between sun exposure and host factors are yet poorly understood, and it has recently been hypothetised that melanoma development may follow different pathways in sun sensitive and less sun sensitive patients. Objectives. To define the respective roles of host factors and solar exposure in melanoma risk according to age. Methods and Results. We re-analysed a case-control study of 412 cases and 445 controls conducted in Europe in 1991-92, and unrestricted by age and histological type. After stratification on age, risk associated with skin phototype and mean duration of exposure varies with age: the risk associated with skin phototypes I-II vs skin phototypes III-IV was 1.81 (CI 95% [1.23-2.68]) before the age of 50 and 1.35 (CI 95% [0.91-1.99]) after the age of 50. On the contrary, cumulative sun exposure significantly impacts melanoma risk after the age of 50. The joint effect of childhood and adulthood intermittent exposure on melanoma risk was analysed according to age: all categories of exposure are significantly associated with an increased risk of melanoma before the age of 50 (OR = 7.11 (CI 95% [1.74-29.12]) for the highest category), whereas all but one categories were not significantly associated with an increased melanoma risk after the age of 50. Conclusion. Melanomas in younger populations, are driven by intermittent exposure and skin sensitivity, and observed more frequently on unexposed body site (trunk, legs), while in older populations melanomas are associated with cumulative sun exposure and are more frequently localised to chronically exposed body sites (head and neck).

P-113 INDIVIDUAL SUN EXPOSURE CAN BE ASSESSED USING METEOROLOGICAL SATELLITE MEASUREMENTS.

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Objective: To compare measurements of UVA and UVB exposure of children recorded with personal dosimeters with assessment through a detailed questionnaire and satellite measurements. Methods: 25 families with one index child participated in the study. Participants filled a questionnaire detailing daily activities, geographical location and circumstances of sun exposure. Corresponding satellite measurements of local UVA and UVB irradiation were obtained from the European database SoDa. Results: Out of 353 days of sun exposure, 437 episodes were recorded with a dosimeter. Median duration of each session was 2 hours, 62% of exposures occurring between 11 a.m. and 3 p.m. There was a good correlation between measurements from dosimeter and satellite (r=0.48 for UVA; r=0.40 for UVB, Spearman correlation p<0.0001). Dosimeter records tend to underestimate the total exposure measured from the dosimeter (difference per session: 40Wh/m2 UVA, 1.5 Wh/m2 UVB). The correlation was better for exposure in the sun (r=0.5 and 0.43 for UVA and UVB respectively), on the beach (r=0.57 and 0.42), at the seashore (r=0.64 and 0.40). Multivariate analysis adjusting for weather, exposure duration, horizontal or vertical use of dosimeter, shade, environment and activity showed that Satellite measurements were only significantly influenced by the weather. Dosimeter records were essentially influenced by the type of use (81% in UVA and 73% in UVB decrease of measure when dosimeter was worn on the belt), exposures in the shade (54% decrease for both UVA and UVB), environment (60% decrease for UVA and 34% for UVB for exposure in the country side). When adjusting for all variables, there was a significant independent correlation between dosimeter and satellite measurements (p<0.0001 for UVA and UVB). Conclusion: Satellite measurements give a good estimate of individual UVA and UVB exposure, independently of exposure conditions and could be used to estimate actual exposure.

P-114 ETHNIC DIFFERENCES AND SURVIVAL IN MELANOMA PATIENTS

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Objectives: Cutaneous melanoma is rare among minority populations. Surveillance, Epidemiology, and End Results (SEER) data were used to examine the disparities with respect to race/ethnicity in melanoma patients in terms of incidence, histologic subtypes, disease stage at presentation, and disease-specific survival (DSS). Materials & Methods: A total of 37,376 patients were identified from 12 population-based SEER registries as having primary melanoma (1994-2001), including 36,183 Caucasians, 204 African Americans (AA), 45 American Indians (AI), 284 Asians, and 660 Hispanics. Multivariate analyses were performed to identify factors associated with tumor stage. Associations between race/ethnicity and DSS were estimated using the Cox proportional hazards model. Results: The average annual age-adjusted melanoma incidence per 100,000 population were 22.3 for Caucasians compared to 1.1, 1.9, 1.4 and 4.1 for AA, AI, Asians and Hispanics, respectively. Caucasians developed primary melanoma most commonly on the truncal region (34%), while minority populations were more likely to develop lower extremity melanoma (29% to 50% versus 19%). Acral lentiginous melanoma was more common among minority populations: AA (odds ratio (OR) = 21.8), AI (OR = 4.9), Asians (OR = 12.5), and Hispanics (OR = 5.9) compared to Caucasians. Minority patients were more likely to have stage IV disease at presentation: 17.2% AA, 15.6% of AI, 8.5% Asians, and 9.1% of Hispanics, compared to 3.8% of Caucasians. Minority populations had 190% to 270% greater risk of disease-specific mortality after adjusting for age, sex and tumor registry (p<0.001). Stage-specific differences in DSS were not identified among the groups. Conclusion: Cutaneous melanoma among AA, AI, Asians and Hispanics differs in incidence, histologic subtype, anatomic distribution, and stage at presentation compared to Caucasians. Although uncommon, melanoma occurring in minority populations is associated with an increased mortality likely related to delay in diagnosis. Measures to improve public awareness, screening, and health care access are needed.

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P-115 DISTRIBUTION OF CUTANEOUS MELANOMA ON THE HEAD AND NECK AND ULTRAVIOLET EXPOSURE

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OBJECTIVE: The aim of this study was to determine the site distribution of primary invasive cutaneous melanoma on subsites of the head and neck in Australian patients and to correlate this distribution with ultraviolet (UV) exposure at these subsites. MATERIALS & METHODS: This retrospective descriptive study involved 1282 patients from the Sydney Melanoma Unit treated between 1990 and 2000. Full clinical and histologic details were available in each patient and the head and neck subsite was subdivided into 9 subsitess; scalp, forehead, eyelids, nose, lips, chin, ears, cheeks and neck. The relative tumor density (RTD) at each subsite was calculated and results correlated with the estimated UV exposure at this subsite. RESULTS: In general, the highest levels of UV exposure are, in decreasing order, on the nose, cheeks, ears and forehead. These subsites also had the highest RTDs ranging from 7.0-2.5 respectively. When the data were analyzed by gender, a similar trend was observed which was in keeping with differences in UV exposures between the sexes. Superficial spreading or nodular melanoma was the most common histologic subtype, the notable exception being the lips, where desmoplastic melanoma predominated. CONCLUSIONS: These results support the hypothesis that chronic sun exposure has a role in the development of melanoma. However, chronic sun exposure may not be the sole UV radiation related risk factor for melanoma which may be multifactorial or dependent on different human phenotypes.

P-116 MC1R GENE ACTS AS A MODIFIER GENE IN MELANOMA RISK OF CDKN2A SPANISH CARRIERS

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Introduction: Mutations in the exons of the cyclin-dependent kinase inhibitor gene CDKN2A are melanoma-predisposition alleles which have high penetrance, although they have low population frequencies. In contrast, variants of the melanocortin-1 receptor gene, MC1R, confer much lower melanoma risk but are common in European populations. There are no studies about this issue in Spain. Objective: To test the possible modifier effect on melanoma risk of MC1R variants in CDKN2A mutation-carrying Spanish patients. Material and methods: From sixteen pedigrees, a total of 69 CDKN2A mutation carrying patients were assessed for MC1R genotype, 30 of them had melanoma. Results: Only seventeen patients had consensus MC1R wild-type. Nineteen patients had red-hair variants (Arg151Cys, Arg160Trp, and Asp294His), two of them had 2 variants. The mean age of melanoma diagnosis was significantly older in non-carriers of MC1R red-hair polymorphism compared with MC1R red-hair variants (40.1±14.9 years-old vs 30.7±8.8 p=0.04). Risk relative to develop melanoma in carriers of MC1R red-hair variants is 1.37 (95%CI 0.986-1.986). Fourteen patients with melanoma were carriers of other variants no related with red-hair while only 4 melanomas were wild-type, but this difference is not statistically significant. Conclusion: The impact of MC1R variants on risk of melanoma in CDKN2A mutation-carrying Spanish patients was mediated largely through the action of three common alleles, Arg151Cys, Arg160Trp, and Asp294His, that have previously been associated with red hair, fair skin, and skin sensitivity to ultraviolet radiation.

P-117 EPIDEMIOLOGIC STUDY OF CUTANEOUS MALIGNANT MELANOMA IN MALLORCA, SPAIN (1998-2004)

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OBJECTIVE: descriptive retrospective study of cutaneous malignant melanoma diagnosed in the Spanish island of Mallorca in the period 1998 to 2004. MATERIALS & METHODS: A database with relevant information was created with data obtained from pathologic reports and clinical history of the Hospitals in our area. The SPSS statistical package was used. RESULTS: A total of 426 cases in 408 patients (224 women and 184 men) were included. Mean age was 57 yr. (range = 93). Fourteen patients had multiple melanoma. Trunk was the most frequent localization in men (59%) and limbs in women (67.7%), followed by head/neck in both sexes. Mean size of the tumor was 11.2 mm (range = 47 mm). 22% of cases were "in situ". Superficial spreading was the most frequent type of invasive melanoma (56.5%) followed by nodular (20,1%), lentigo malignant (11.2%), acral (3.6%), and others (5.8%). There were two cases without primary. A nevus was present in the 35% of biopsies, primarily in the trunk. Most melanomas were thin (Breslow's thickness <1mm; 51.8%); 43% intermediate (1-4 mm) and 12.9% thick. Ulceration was present in 78.1% of thick melanomas (and in 20.7% of all invasive melanomas). Regression was mentioned in 63.2% of non-invasive melanomas (v.s. in 24% of the invasive), and in 55% of cases with regression there was an inflammatory response rated as intense. Sentinel node biopsy was performed in 73 cases and resulted positive in 19. CONCLUSION: Incidence of melanoma and Breslow's thickness did not changed significantly over the period of study. When comparing thin and thick melanoma, histological regression, inflammatory response and presence of nevus were associated with the former, whereas ulceration, vascular invasion or neurotropism were observed in the later ($p < 0.0001$).

P-118 NODULAR MELANOMA- PATIENT FACTORS

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Scotland has seen large increase in melanoma incidence rates in the last 25 years reported previously by Scottish Melanoma Group (SMG). This has occurred alongside continuing educational ventures which are credited with contributing to diagnosis of increasing numbers of early lesions. Most public and porfessional education has targetted the superfical spreading melanoma , currently the most frequent type in Scotland. Recognition of nodular melanomas which constitute almost 50% of thick(>3.5mm) lesions in Scotland has not been helped thus far . We have looked at nodular melanomas (NM) in East of Scotland (SMG) database between 1979 and 2003 to identify potential patient recognition factors.Of a total of 3353 melanomas 447 were nodular with male to female ratio of 1:1.24 overall. Over time the proportion of women has increased .Head and neck was the commonest site(24.4%)followed by distal limb(excluding hand /foot) 23%,. Chest, trunk below waist and hand/ foot were least frequent.More than 53% of lesions had Breslows of over 4mm and only 1.8% Breslow<1mm.Over the decades studied the mean age of NM patients increased from 54.7 to 65.9 years . In almost a quater of cases these often poor prognosis lesions were < 1cm in diameter. Thus nodular melanoma remains largely untouched by current educational ventures . Compared to 20 years ago the typical Scottish patient with nodular melanoma is likely to be older, have a lesion on head/ neck or distal limbs and is less often male. These factors should be bourne in mind along with lesion specific recognition points when targeted education is being considered

P-119 EPIDEMIOLOGY AND PROGNOSTIC FACTORS OF HEAD AND NECK MELANOMA

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Objective: Epidemiology and factors affecting mortality in head and neck (H&N) melanoma are poorly understood. The aim of this study is to describe the epidemiology of H&N melanoma, and to identify factors associated with mortality from this disease. Method: This is a population-based study. Patients treated for H&N melanoma in Ontario between 1990 and 2002 were identified through a prospectively-generated Cancer Care Ontario database. Information collected included patients demographics, cancer characteristics, treatment modalities and vital status. The data were analyzed using logistic regression model. The main outcome of the study was vital status. Results: 2448 patients with cutaneous and mucosal melanoma of the H&N were identified, comprising 16% of all melanomas in Ontario. The average age of the cohort was 63 years, 61% (1496) were males. The incidence of H&N melanoma increased from 1.7/100,000 in 1995 to 3/100,000 in 2000, while mortality remained stable. Of all H&N cases, 3% (72) were mucosal melanomas with a predominance of nasosinus cavities. The most common site for cutaneous lesions was face 52% (1292). Eyelid and face melanomas were more frequent in females (p<0.001) where ears, scalp and neck were predominant in men. Multivariate analysis showed increased age (OR 1.05 CI 1.04; 1.05, p<0.001) and male gender (OR 1.41 CI 1.16; 1.72, p=0.001) were significant predictors of mortality. Mucosal melanomas of the pharynx (OR 19.95 CI 1.98; 201.07, p=0.011), oral cavity (OR 5.28 CI 1.77; 15.74, p=0.003) and nasosinus cavities (OR 6.54 CI 3.29; 13.1, p<0.001) were also strong predictors of mortality. Of all cutaneous sites only facial melanomas were significant and associated with improved survival (OR 0.73 CI 0.60; 0.89, p=0.002). Conclusion: Our study demonstrated an increase in incidence of H&N melanoma in Ontario. Advanced age, male gender and mucosal site were strongly associated with higher mortality whereas facial melanomas had favourable prognosis.

P-120 IS LENTIGO MALIGNA A DISTINCT EPIDEMIOLOGICAL TYPE AMONG MELANOMA OF THE ELDERLY? A DOUBLE CROSS- SECTIONAL STUDY

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IS LENTIGO MALIGNA A DISTINCT EPIDEMIOLOGICAL TYPE AMONG MELANOMA OF THE ELDERLY ? A DOUBLE CROSS-SECTIONAL STUDY Background: Incidence of melanomas (MM) over 65 is increasing with a poor prognosis. Among MM of elderly, 'lentigo maligna' (LM) is a frequent subtype characterized by anatomo-clinic criteria, which is linked to cumulative sun exposure. However, other subtypes of MM (OMM) can also develop in the elderly and may have different epidemiological profile. No specific precursor or risk marker to develop MM late in life, have yet been identified. We have shown that a high density of senile lentigos is associated with an excess of intermittent sun exposure during the life, and it may thus be an interesting epidemiological marker to identify different MM subtypes in the elderly. Objectives: 1/ To test whether the risk factors for LM are different from other MM of the elderly, and 2/to investigate whether the density of senile lentigos could be a useful marker for the risk of MM late in life Methods: Cross-sectional study comparing LM over 65, and 2 groups matched for age (5 years) and sex: other types of MM (OMM) over 65, and controls. Skintype, life sun exposure by 10 year-periods, skin ageing marks, keratoses and density of senile lentigos were recorded. Three case-control analyses were conducted: LM vs controls, OMM vs controls LM, and LM vs OMM . Results: 80 LM (51 F, 29 H), 80 OMM (51 F, 29 H) and 160 controls (102 F, 58 H) have been enrolled. Conclusions: Results of the analysis will be presented at the meeting.

P-121 THE EPIDEMIOLOGY OF CUTANEOUS MALIGANT MELANOMA IN NOVA SCOTIA, CANADA

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Background: Since 1993 the annual increase in melanoma incidence has been one of the highest for all cancers registered in Canada. Provincially, Nova Scotia has the highest incidence rate of melanoma in males and the second highest rate in females. The purpose of this study was to comprehensively document the pathologic and epidemiologic data on cases of melanoma in NS. Methods: The NS Cancer Registry (NSCR) records all incident cancer cases in the province according to nationally-recognized standards. All malignant melanoma cases identified by the NSCR during 1998-2002 were evaluated for patient and tumor characteristics. Recent survival analyses along with incidence and mortality trends over the last 30 years were also computed. Results: Between 1998 and 2002, 925 melanoma cases were recorded. The age-standardized incidence rate for this period was 19.2 and 16.1 per 100,000 males and females, respectively. Age-specific rates demonstrated differences between the two genders. In the age-group 65 and older the incidence per 100,000 was 80.0 in males and 44.9 in females. The most common melanoma had a Breslow's index less than 1.0 mm (61.9%) with a Clark's level of II (34.9%) and localized to the trunk (32.3%). There was no significant seasonal variation in time of diagnosis. Survival analyses indicate that sex, age, tumor location and thickness are significant prognostic factors (P<0.001). Despite the increase in incidence there has been no significant change in annual mortality. Conclusion: Melanoma is a disease diagnosed at any time of the year. In NS, the incidence rate increases with age and is nearly doubled for males compared to females in the age-group 65 and older. Thin melanomas on the extremities of young females have the best prognosis in NS as they do for other parts of the world. Public health interventions are necessary to reduce the burden of this disease.

P-122 BRAF MUTATION RATES IN ACQUIRED MELANOCYTIC NEVI ARE DIFFERENT DEPENDING ON THE ANATOMIC SITES

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BACKGROUND: Recently, a high frequency of BRAF mutations at a single site (V599E) in the kinase domain of the exon 15 has been reported in melanomas and melanocytic nevi, suggesting that the mutational activation of the RAS/RAF/MAPK pathway is a critical step in the development of melanocytic neoplasia. Although sun exposure is implicated in the pathogenesis of both melanomas and nevi, the relationship between BRAF mutations and sun exposure is unknown as the mutations do not have the standard UV signature. To investigate whether BRAF V599E mutation rates are associated with sun exposure patterns, we extracted DNA from paraffin-embedded tissues of 69 acquired melanocytic nevi (either compound or dermal nevi) excised from various anatomic sites, and directly sequenced the exon 15 of the BRAF gene. RESULTS: BRAF V599E mutation rates were 9/23 (39%) in nevi on intermittently sun exposed sites (mostly trunk), 4/21 (17%) in nevi on constantly sun exposed sites (mostly face) and 2/25 (8%) in nevi on non-exposed sites (mostly soles of the foot). The difference in the mutation rates between intermittently exposed sites and non-exposed sites was statistically significant (P=0.016, by Fisher's exact test). The differences were not significant between constantly exposed sites and intermittently exposed sites, and between constantly exposed sites and non-exposed sites (P=0.19 and 0.39, respectively). Overall BRAF V599E mutation rate in nevi on constantly or intermittently exposed sites was 29% (13/44), the frequency much lower than the reported frequencies of 70~80% in acquired nevi from Caucasian patients. Lower mutation frequency of nevi on sun exposed sites in Japanese than in Caucasians may be explained by an increased melanin load in Oriental skin CONCLUSION: Our results suggest that sun exposure may be an important causative factor in the acquisition of BRAF mutations in acquired melanocytic nevi.

P-123 STATISTICS ON MALIGNANT MELANOMA (1975-2001): EPIDEMIOLOGY, PROGNOSTIC FACTORS, AND SURVIVAL IN JAPAN

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A total of 2,639 patients with malignant melanoma were registered between 1975 and 2001. The data subjected to analysis included the age, sex, anatomical distribution, clinical features of the primary tumor (ulceration), Clark's subtype, tumor thickness (Breslow), Clark's level, disease stage, and treatment. We analyzed the annual trends and survival rates according to prognostic factors. Malignant melanoma is gradually becoming more common in Japan, particularly among women. The anatomical sites involved and the Clark's subtypes have also changed. More lesions are now occurring on the face, trunk, and legs, and superficial spreading melanoma is becoming more frequent. The annual survival rate has improved, and the outcome of treatment for advanced disease has also improved due to the combined use of chemotherapy with biological response modifier therapy.

P-124 UNIQUE DNA MICROARRAY FEATURES OF JAPANESE MELANOMA PATIENTS AND CO-EXPRESSION OF CYCLIN D1 AND PHOSPHORYLATED RB BY IMMUNOHISTOCHEMISTRY AS A POORER PROGNOSTIC MARKER FOR OVERALL SURVIVAL

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Cutaneous malignant melanomas have four major subtypes that are affected uniquely by racial, genetic background. However, Japanese melanomas are unique, as they being mostly acral lentiginous type (ALM) and having a poor prognosis compared Caucasian melanomas of superficial spreading type. In this study we carried out DNA microarray comparison of primary and metastatic ALM lesions, in order to identify new prognostic markers uniquely expressed in Japanese melanoma patients. We found that melanoma inhibitory activity (MIA), S-100 Ca2+ binding protein A, S-100 Ca2+ binding protein 2, Wnt inducible signaling pathway protein 2, transcription factor AP-2±, and cyclin D1 are amplified higher in primary melanoma than in metastatic one. Small inducible cytokine subfamily A (Cys-Cys), #21 (#19), stromal cell derived factor 1, transgelin, and frizzled-related protein are amplified higher in metastatic melanoma than in primary one. Among these expressions, our further study was focused on Wnt signaling pathway. Cyclin D1 is one of the target genes of Wnt signaling pathway and promotes G1/S transition of cell cycle by the phosphorylation of Rb protein. 2-Catenin forms a complex with Tcf/Lef and APC in Wnt signaling pathway, is translocated into nuclei and activates transcription of target genes such as cyclin D1. We found the parallel relationship in gene expressions between cyclin D1 and 2-catenin, and a high nuclear expression of cyclin D1 and phosphorylated Rb to be a poorer prognostic marker for overall survival (Logrank test $p<0.1$). Furthermore this trend was more significant in male patients than females.

P-125 A POPULATION-BASED STUDY OF MELANOCORTIN-1 RECEPTOR VARIANTS AND MELANOMA

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Objective: Few studies of melanocortin-1 receptor gene (MC1R) variants and melanoma etiology have used population-based recruitment of study participants; some have limited sample size or have genotyped selected variants. To address these potential methodological issues, we investigated melanoma risk associated with MC1R variants using a large, international population-based study design. Methods: Direct sequencing was used to characterize the complete MC1R coding region for 3264 individuals from Australia, Canada, Italy, and the United States. Case subjects (n=1130) had two or more incident primary melanomas, and control subjects (n=2219) had one incident primary melanoma. Results: After adjustment for age at diagnosis, sex, age-sex interaction, and study center, an increased risk of melanoma was observed for several MC1R variants that we denote as 'high risk' (H), including D84E [OR=1.5 (0.99-2.2)], R142H [OR=1.4 (0.81-2.4)], R151C [OR=1.3 (1.1-1.6)], R160W [OR=1.2 (0.99-1.5)], and D294H [OR=1.4 (0.98-1.9)]. This was not the case for 'low risk' (l) variants: V60L, V92M, I155T, R163Q, and an aggregate category indicating carriage of rare variants. Compared to MC1R consensus carriers (wt/wt), elevated risk of melanoma was associated with carriage of a high risk variant in trans with a consensus allele (H/wt; OR=1.5 (1.2, 1.9), low risk variant (H/l; OR=1.7 (1.3, 2.2), or high risk variant (H/H; OR=1.6 (1.2, 2.2); whereas carriage of a low risk variant in consort with a consensus allele (l/wt) or a low risk variant (l/l) was not statistically associated with risk [OR=0.95 (0.73-1.2); OR=1.3 (0.93-1.7), respectively]. Conclusions: Our results confirm that carriage of MC1R high risk variants increase risk of melanoma. Point estimates in our study were smaller than previously observed. This may be due, in part, to differences in our study design compared with other studies. We conclude that MC1R is causally involved in melanoma development, but the magnitude of risk conferred by MC1R variants is moderate.

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P-126 INTERPLAY OF MC1R, ASIP, AND DNA REPAIR IN SPORADIC AND FAMILIAL MELANOMA RISK IN A MEDITERRANEAN POPULATION

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OBJECTIVE. To explore possible pathways to melanoma development by investigating the interplay of the melanocortin-1 receptor (MC1R) and Agouti Signaling Protein (ASIP) genes, sun exposure, and DNA Repair Capacity (DRC) in relation to sporadic and familial melanoma risk, and to melanoma progression in a Mediterranean population. METHODS. We studied 649 subjects, 267 melanoma cases and 382 controls, mostly CDKN2A/CDK4 mutation negative, from a case-control and family study in Northeastern Italy. A single dermatologist assessed subjects' host factors by skin examination, questionnaire, spectrophotometer, and minimal erythema dose measurement. MC1R was sequenced, the ASIP g.8818A>G polymorphism was genotyped, and DRC was measured by the host-cell reactivation assay. Odds ratios were estimated by logistic regression models. RESULTS. Overall, we found 29 and 15 different non-synonymous variants in subjects from the case-control and family study, respectively. Carrying MC1R variants increased both sporadic and familial melanoma risk 2-4 fold, particularly in subjects with multiple variants (OR=3.9, 95%CI=3.3-4.6) or fewer additional risk factors. Similarly, MC1R carriers were 2-4 times more likely to have thicker melanomas than non-carriers. The "red hair color" (RHC) variants of MC1R were significantly associated with light pigmentation, freckling, and nevi count ($p<0.03$, overall). The association between RHC and freckling and between RHC and nevi were stronger in subjects with high intermittent sun exposure and low DNA repair capacity. The association between RHC and melanoma was stronger in subjects carrying the ASIP polymorphism ($p<0.02$, test for interaction). ASIP was not associated with pigmentation, nevi or melanoma risk overall. CONCLUSIONS. MC1R was significantly associated with melanoma risk and progression in a Mediterranean population. We are now studying whether MC1R and BRAF oncogene interact in the association with melanoma risk in this population. Molecular distinctions in melanoma development may allow tailoring of clinical approaches and public-health messages to the specific populations at risk.

P-127 CHILDHOOD MELANOMA: DEMOGRAPHICS AND CLINICAL PRESENTATION

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Objective: Melanoma in children is rare. This study examines demographics and clinical presentation of pediatric melanoma in a large United States national database. Materials and Methods: The National Cancer Data Base, a function of the American College of Surgeons, collects information on hospital-based incident cancer cases. Information on age, race, sex, site of disease, location of cutaneous melanoma, and extent of disease was examined for patients diagnosed with melanoma at age 18 or younger from 1985 through 2002. Group differences were evaluated with chi square tests. Results: A total of 2679 cases were identified: 96.5% cutaneous, 2.9% ocular, and 0.6% with unknown primary. There were no reported cases of mucosal melanoma. Overall, melanoma was more common in girls (54%). Only 332 cases were in children under 10 years. Younger children were more likely to have a racial designation other than non-hispanic white, accounting for as much as 13.3% of cases in children 1-4 years old. Demographics and presentation of the 2585 cases of cutaneous melanoma were different in children under 10 than in older children. As compared to children 10-18 years old, those < 10 years at diagnosis (n=317) were: 1. more likely to be male (57% vs. 44%), 2. more likely to be non-white (11% vs. 4.2%), 3. more likely to have a head/neck primary (34.4% vs. 22.0%), 4. less likely to have a truncal primary (19.6% vs. 36.9%), and 5. more likely to present with regional metastases (26.2% vs. 11.8%). All comparisons $p<0.001$. Conclusion: Melanoma is much less frequent in young children than in older children. Compared to older children, those with cutaneous melanoma under 10 years are more likely to be male and non-white. Younger children have a different distribution of cutaneous primaries and are more likely to have regional metastases at presentation.

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P-128 THE RISK OF MELANOMA IS INCREASED BY SUNBED USE-RESULTS FROM A PROSPECTIVE POPULATION BASED COHORT

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Objective: Case-control studies by us (1,2) and others have indicated that sunbed use increase melanoma risk. There is a need for complimentary data from prospective studies. Methods: From a prospective population based cohort in South Sweden of 40 000 invited women aged 25-65 melanoma incidence was related to use of sunbeds and other melanoma risk factors. The cohort, established 1990-92, was matched with the Swedish Cancer Registry and vital status and cancer incidence were followed up until Dec 2004. Results: During follow up 101 melanoma cases had developed. After adjustment for hair colour, number of nevi, sunburns and family history use of sunbeds increased melanoma hazard ratios in women born 1951 and younger, while no significant increased risk was seen for older women (see table). Conclusion: In this population based cohort use of sunbeds increased melanoma risk for women born after 1951. This was especially evident after sunbed use of more than 10 times per years.

P-129 THE IMPORTENCE OF SNPS IN MC1R, CDKN2A AND EGF FOR MELANOMA DEVELOPMENT

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This project aims to identify low-susceptibility alleles of importance for melanoma risk at the population level. Defining key genetic alterations will improve the possibility to identify individuals at high risk for developing malignant melanoma and lead to better strategies for prevention and early diagnosis. The genetic basis for malignant melanoma is to a large extent still unknown. In the general population, mutations in high-penetrant genes like the CDKN2A gene are extremely rare and account for probably less than 1 % of all cases. Instead, more common, functional genetic polymorphisms with weak penetrance are more likely to account for most of the genetic risk in the general population. We are investigating the distribution of several polymorphisms in the candidate susceptibility gene MC1R, as well as single polymorphisms in the CDKN2A and EGF genes in healthy control individuals and in different subgroups of melanoma patients of Swedish origin. We have at present genotyped over 1,200 patients and 500 control individuals using a micro array-based SNP-typing platform called PrASE (proteinase K allele-specific extension). Preliminary data indicates that the carrier frequency of MC1R variant alleles is high in the Swedish population and that the proportion carrier tends to be higher among melanoma patients compared to control individuals. Several variants of the MC1R gene seem to be of importance in melanoma risk. For example, the R151C and R160W gene variants, strongly associated to the RHC-phenotype (red hair, fair skin-phenotype) and also to melanoma development in other populations, almost doubled the risk for melanoma in the Swedish populations (OR: 1.7-2.0, 95 % CI:1.4-2.4). More thoroughly analyses of the data, including haplotype analyses, gene-gene interactions and correlations between gene variants and different clinical parameters will be presented.

P-130 DEVELOPMENT OF A CANADIAN SUN SURVEY: BUILDING ON THE 1996 NATIONAL SURVEY OF SUN EXPOSURE AND PROTECTIVE BEHAVIOURS

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(On behalf of the National Sun Safety Committee) OBJECTIVE: To design and secure funding for a national sun survey that will permit comparison with the previous survey (1996); provide improved information for planning; and have adequate power for regional analyses. MATERIALS AND METHODS: A priority of the National Sun Safety Committee (NSSC) of the Canadian Strategy for Cancer Control is to conduct a national sun survey (NSS). A Subcommittee reviewed the instrument and protocol for the 1996 NSS to identify areas requiring change. A survey specialist then critically reviewed 1996 question wording and survey methods; evaluated sample size requirements; developed new question modules (specifically around knowledge and attitudes); and pilot and focus-group tested these. RESULTS: In 1996, 4,023 Canadians aged 15+ participated in the first NSS on behalf of themselves and (collectively) their children under age 12. Results have been used to inform public health actions in at least some jurisdictions (e.g., Ontario). The new survey, to be implemented in 2006, will comprise two parts: a relatively small "comparison sample" (n=2000) who will be asked key questions from the 1996 survey in the identical way, thereby permitting comparison over time, and a larger "base sample" (n=6000) who will be asked the complete set of new and improved questions. The base sample will include at least 900 respondents aged 15+ from each region (not including the territories), permitting basic regional analyses. Parents of children under 12 will be asked a series of questions about one randomly selected child only. New question modules have been developed and tested, and methods related to timing of interview in relation to the summer, question order and comparability of questions with other surveys have been reviewed. CONCLUSIONS: A protocol is being prepared by the NSSC to submit for funding in the fall.

P-131 EPIDEMIOLOGY OF CUTANEOUS MELANOMA IN MÉXICO.

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Objective: The epidemiology of malignant melanoma in Latin-American population is quite different from North American, European and Australian people. We report the mean epidemiologic features from a Mexican database from twenty-two years Methods: A retrospectively review was carried out and includes a 1,241 patients with histologically confirmed malignant melanoma from January 1980 to august 2002. We analyze the presence of ulceration, positive nodes or metastases, Breslow Thickness, Clark Level, growth pattern, gender and age. Concomitantly we revised the impact of each variable in our population. Results: There rate male-female were 1:1.5 (F 60.1%, M:39.9%). The median age was 55 years (14-98). The median time between first manifestation and medical assistance were 13 months (0-60). Only in 572 were possible to obtain all the variables needed to stage. In the rest were no feasible to know Breslow thickness, presence of ulceration, or in earlier stages the Clark level. Median Breslow thickness was 4.78 mm, almost two thirds of patients (59.3%) had ulcerated melanomas. The growth pattern distribution was acral lentiginous (50.5%), nodular (33.5%), superficial spreading melanoma (14%) and lentigo maligna melanoma (1.4%). The localization were: Extremity (68.8%), Head and Neck (14%), trunk (16%), primary unknown (1.2%). The distribution according to Breslow thickness was <1mm (22.1%), 1-2 mm (19%), 2-4 mm (15.6%) and > 4 mm (43.3%). 34.9% of patients had positives nodes. Conclusion: The majority of patients was female, midage, and high risk ulcerated melanoma (IIC-IIIC). The main type of melanoma in Mexican population is acral lentiginous.

P-132 A POPULATION BASED STUDY OF CUTANEOUS MELANOMA IN ALBERTA (1993-2002)

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Objective Some evidence suggests that melanoma incidence rates in Canada continue to rise. The comparison of results from our study (1993-2002) to previously published analyses in Alberta (1967-1976), will permit an understanding of cutaneous melanoma trends in Alberta. Further epidemiologic evidence of cutaneous melanoma in other Canadian provinces will also be discussed. Methods A retrospective study of 3,479 patients with cutaneous melanoma diagnosed in Alberta between 1993 and 2002 was conducted. The information obtained included age, gender, diagnosis date, site, morphology, and tumor characteristics, including Clark level of invasion, Breslow thickkness, stage, and date of death where relevant. Estimates of relative survival (using the Ederer II method) compare the survival of melanoma patients with the Alberta population to describe the likelihood of surviving melanoma in the absence of other causes of death. Results and Conclusions For the period of 1993-2002, annual melanoma age-standardized incidence rates per 100,000 person-years ranged between 11.1 and 15.9 (males) and 9.8 and 14.1 (females). These rates are greater than the highest previously reported Alberta incidence rates of 4.1 (males) and 4.8 (females) in 1976. The analysis of the trend of melanoma incidence rates in Alberta between 1993 and 2002 did not show a significant increase nor decrease for either males or females, suggesting relatively uniform rates over that decade. The predominant site for melanoma was the trunk in men and the lower limb/hip in women, as expected. Pathologic analysis revealed 69% of tumors were d 1.0 mm thick in males, with a corresponding value of 72% in females. Less than 0.1% of tumors were > 4.0 mm thick in males and females. The 3 and 5 year relative survival rates over the study period were 93.6% and 90.3 %, respectively. Presently, univariate/multivariate analysis of the impact of these parameters on patient survival is being elucidated.

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P-133 NAEVOID MELANOMA

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Background: Naevoid melanoma (NM) is an uncommon type of melanoma that pathologically resembles a benign naevus. It is controversial whether NM has a better or worse prognosis compared with non-NM. In the largest published study on NM, 8 out of 33 (24%) patients with 5 or more years FU died of melanoma. Subsequent smaller studies appear to show better survival rates. Aims: The aims of this study were to determine the relative prevalence of NM; to review the histopathological criteria for diagnosis for NM; and to determine its prognosis. Methods: The database of the Sydney Melanoma Unit (SMU) and the archival files of the Department of Anatomical Pathology at the Royal Prince Alfred Hospital (RPAH) were searched for cases of NM diagnosed from 1994-2003. Preset criteria based on previously published papers on NM were established, and the diagnosis was confirmed in each case by review of histopathology by two investigators. Follow-up (FU) information was obtained for each case Results: Twenty-six cases of NM were identified. During the same time period 9800 patients were treated at the SMU for newly diagnosed primary melanoma. Of the 26 NM cases, 21 were Clark level IV and five were Clark level III. Breslow thickness ranged from 0.50 to 6.3 mm, median 1.5 mm and mean 1.84 mm. Ulceration was present in six cases. The overall rates of local recurrence and distant metastasis were 13% and 21%, respectively at a median FU period of 1.75 years. Time-related survival during a median FU of 2.5 years was 94% for 21 patients with at least a years FU. The five-year survival was 84%. Only two patients died of melanoma. Conclusions: NM is a rare sub-type of melanoma, which appears to have a better prognosis compared with non-NM.

P-134 TRENDS IN CUTANEOUS MELANOMA THICKENESS AND SURVIVAL IN INSTITUTO PORTUGUÊS DE ONCOLOGIA DE LISBOA - A 20'YEARS REVIEW

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Educational campaigns concerning sun exposure and skin cancer took place in our country over the last 15 years. Monitoring the impact of these campaigns in the incidence and mortality rates of malignant melanoma (MM) is an important but difficult task. The purpose of our study was to assess the evolution of thickness of MM over the last 20 years in our Institution, since it correlates well with early diagnosis. A retrospective study was made concerning all cutaneous MM cases from 1985 to 2004 seen in our Institution. Age, sex, type, thickness, ulceration of the tumour as well as and stage and survival of patients were studied. Survival was calculated by Kaplan-Meier method and compared with log-rank tests; mean thickness was analysed with Student s t test. 1753 patients (727 men and 1026 women) were included in the study: 519 in the first 10 years and 1234 in the second decade. The median tumour thickness varied insignificantly when comparing the first decade (mean thickness: 3,98 mm) with the second one (mean thickness: 3,31mm) ($p>0.05$). Thinner tumours (<1mm) were more frequent in women (35,2% vs 30,6% in men). Despite the thickness of the tumours, the overall survival of the patients improved significantly in the second decade ($p<0,005$). The number of patients in the last decade almost tripled. In our study thickness reduction was insignificant over the last 20 years. However, the fact that our Hospital is a major referral Institution for advanced tumours introduces a significant bias in our sample. Nevertheless, the overall survival of MM patients has been improved.

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P-135 CHANGES IN THE SITE DISTRIBUTION OF MALIGNANT MELANOMA IN SOUTH EAST (SE) SCOTLAND (1979-2002)

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In 2002 the Scottish Melanoma Group (SMG) reported increasing incidences of invasive malignant melanoma (MM), over a 19 year time period. This mirrors the increase which has been reported worldwide, which may reflect changes in sun-exposure behaviour, and thus a change in melanoma site distribution might be anticipated as a reflection of variation of clothing over time. We present a retrospective study of changes in site distribution of invasive MM in SE Scotland over a 24year time period, 1979-2002. All data was collected as part of the SMG population based data base of clinical and pathological features and follow up data. Sites were grouped into frequently exposed (9 sites), usually covered (9 sites) and unknown. Differences in proportions were tested using the chi-square statistic. Changes in incidence rates over time were assessed using Poisson regression. 2,790 patients were diagnosed with MM over the time period, 1,759(63%) females and 1,031(37%) males. The commonest sites were the leg (29%), head (20%) and posterior trunk (19%). For both sexes the proportion of posterior trunk and arm tumours increased over time, decreasing proportions were seen for head, leg and unknown sites, the anterior trunk showing no change. There was a decreasing proportion of leg tumours in males and of head tumours in females over time. There was a significant decrease in mean Breslow thickness and Clark's level over time for all sites except head. There was a significant increase in the proportion of MM at usually covered sites vs. frequently exposed sites in females only, the proportion occurring at usually covered sites increasing from 44% to 56% with a corresponding drop in those at frequently exposed sites from 54% to 42% (p=0.001). This change in site distribution over time would support a suggestion that intermittently exposed skin may be more susceptible to development of melanoma.

P-136 CDKN2A IN SPANISH SPORADIC MELANOMA

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Background: Sporadic melanoma accounts for the vast majority of patients and genetic factors that mediate susceptibility to this form of melanoma are not well understood. Objectives: To describe the prevalence of germ line mutations in CDKN2A and CDK4 in sporadic melanoma, in a Hospital setting, in a reference Melanoma Unit in Spain and to evaluate clinical differences between carrier and non-carrier patients. Subjects and Methods: 696 Spanish melanoma patients (292 males and 404 females) without familial history of melanoma, attended at our Melanoma Unit, consented to be enrolled in a genetic study. Most patients (614) had only one primary melanoma but 82 had at least two primaries (64 patients 2, 14 patients 3, 3 patients 4 and one patient 6). Exon 1alfa, 1beta, 2, 3 and IVS2-105 of CDKN2A, -34G>T at the CDKN2A promoter region, and exon 2 of CDK4, were studied by PCR-SSCP and sequencing. Results: CDKN2A mutations were detected in 16 patients (2,3%) and these mutations were more frequent in patients with multiple primaries (MPM) compared with non-MPM (12.2% vs 1% p=0.000). The age of melanoma diagnosis in carriers of mutations in CDKN2A was younger compared with non-carriers (36,63 y-o SD 14,96 vs : 50,63 y-o SD 16,60; p=0,001). The polymorphism A148T was present in 14.6% of MPM but only in 7.2% of non-MPM (p=0.02). Conclusion: CDKN2A mutations are responsible of melanoma susceptibility in at least some Sporadic melanoma patients in Spain. MPM had more mutations than non-MPM. The increased prevalence of A148T in MPM compared with non-MPM suggests a possible role of this polymorphism in melanoma susceptibility actuating as low-penetrance gene.

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P-137 EVALUATION OF THE ASSOCIATION OF PARKINSON'S DISEASE WITH MALIGNANT MELANOMA

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Objective: To assess the prevalence of Parkinson's disease (PD) in malignant melanoma (MM) patients compared with age- and gender-matched controls. Materials and Methods: Data for age and gender were determined for patients with MM enrolled at 10 participating study sites in the United States. Each investigational site also identified age- and gender-matched controls without a history of MM. Prevalence of PD was determined in the MM cases and compared with the prevalence of PD among the controls. Since PD typically presents after age 60, a subgroup analysis compared PD prevalence rates among MM cases and controls older than 60. Results: The study enrolled 862 MM patients (451 men, 411 women) and 862 age- and gender-matched controls. Among the MM cases, 25 (2.9%) had PD. All PD patients were age 64 or older. In contrast, only 11 (1.3%) of the controls were noted to have PD. The age and gender demographics of this study group were consistent with multiple large series of MM patients and the age and gender demographic profile of PD. A chi-square analysis demonstrated that the prevalence of PD among the MM patients was significantly elevated compared with controls (P = 0.014). In the analysis limited to subjects older than 60, PD was also present in a significantly higher proportion of MM patients (P = 0.016). Conclusion: The prevalence of PD among this series of patients with MM was significantly higher than in age- and gender-matched controls. These results are consistent with prior studies demonstrating an increased prevalence of MM in PD patients. To our knowledge, this is the first report demonstrating an increased risk of a nonneoplastic disease with MM. PD and MM share several cellular, biochemical, and embryological features and further research is warranted to better understand the clinical significance, mechanisms, and implications for the association.

P-138 COMMON MELANOCYTIC NEVI ON CHRONICALLY, INTERMITTENTLY AND RARELY UV-EXPOSED BODY SITES IN SCHOOLCHILDREN RESIDING AT DIFFERENT LATITUDES IN SWEDEN. A FOLLOW-UP STUDY

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BACKGROUND: Studies of the epidemiology of common melanocytic nevi (CMN) have the potential to shed light on the aetiology of melanoma. The incidence of melanoma in the north of Sweden is half of that in the south. OBJECTIVE: The aim was to investigate the number of CMN in relation to body sites among children and to compare residing at different latitudes. MATERIAL & METHODS: Municipalities were chosen at latitude 65-68N in the north of Sweden and at latitude 57N in the south. Children born in 1994 and registered in the municipalities were to be included (N=1676). A questionnaire was sent to their parents, asking about the child's tanning habits. Of the children, 1380(82.3%) had a full body examination for CMN 2mm or larger excluding the genital area and the scalp. RESULTS: For all examined body sites CMN was significantly less prevalent among children living at latitude 65N(inland), with mean number of nevi per square meter body surface area of 7.0(95%CI:6.0-8.2), than among those living at latitude 57N(coastal), with mean values 13.0(CI:11.1-15.2). For intermittently exposed body sites the mean values were 9.2(CI:7.7-10.9) at latitude 65N and 17.9 (CI:15.2-21.1) at latitude 57N. Also blond hair, blue/green eyes, holidays at seaside resorts abroad, more frequent sunburns, and parents fancying tanning, were significant predictors of large numbers of CMN. CONCLUSION: These findings support previous evidence that the development of CMN is related to the level of sun exposure in childhood expressed as latitude of residence. In intermittently exposed body sites the difference between north and south was more pronounced compared to all examined sites. This relation remained statistically significant after adjustment for the effects of other factors that were related to CMN numbers.

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P-139 SURVIVAL IN QUEENSLAND FROM CUTANEOUS INVASIVE MELANOMA 1982-99 - A POPULATION STUDY

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Objective: We aimed to assess the factors that influence the survival of patients with cutaneous melanoma documented on a compulsory state based cancer registry (QCR) from 1982-1999. Methods: Patients with a primary cutaneous melanoma reported to the QCR from 1982 - 99 were analyzed for date of diagnosis, sex, age, site, tumour morphology, level and thickness with this data matched to the State and Australian death registries up to 1999, with a minimum of two years follow up to 2001 from primary treatment. The cancer specific survival estimates were generated. Multivariate Cox proportional hazard models were fitted to the data to assess the interaction of the variables. Results: There were 22,671 patients with a single primary invasive cutaneous melanoma with a male to female ratio of 1.2:1. The overall five and 10 year survivals were 94% and 91.5% respectively. Allowing for the interaction each of the variables there was no difference in the survival over the time period. There was an adverse effect on survival for patients over 30, males and lesions on the scalp (for all thickness groups). Thickness and level were confirmed as important prognostic indicators with an interaction suggesting a poorer outcome for deeper levels in each thickness group most notable in patients with melanoma <1mm (lesions level II better than level III and IV) or >4mm (level III better than level IV and V). Histologic morphology was not an independent prognostic factor. Conclusions: The five year survival from cutaneous melanoma in Queensland was 81.7% in 1966-69. There was a stable survival from cutaneous melanoma over the study period, with a five year survival of 94.5% for the 1995-99 period. Factors independently influencing survival were the patients sex, age over 30years, scalp lesions and the histologic factors of thickness and Clark's level.

P-140 HEALTH CARE UTILIZATION AND COST FOR THE TREATMENT OF MELANOMA IN THE SIX MONTHS FOLLOWING INITIAL DIAGNOSIS IN THE US

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OBJECTIVE: There is a need to better understand the impact of melanoma on healthcare resource use and cost of care. The purpose of this study is to estimate the resource use and costs associated with melanoma after diagnosis. METHODS: Data for this study were obtained from the Medstat MarketScan database. Adult patients (without any other prior cancer diagnosis) newly diagnosed with melanoma between July 1, 1999 and June 30, 2001, with at-least six months of continuous follow-up data were included in this study. Patients with Stage III-IV melanoma were identified based on medical claims of lymph node dissection, chemotherapy, and/or radiation. Study measures included the type, cost, and duration of treatment. RESULTS: A total of 996 patients were identified, including 317 with stage III-IV melanoma. The mean age was 58.4 years, 49% were female, and 54.7% resided in Southern USA. Of those patients diagnosed with melanoma, 95.4% underwent a surgical procedure, 19.7% underwent chemotherapy, 7.4% underwent immunotherapy, 2.8% had inpatient treatment, and 1.9% received radiation; corresponding percentages for Stage III-IV patients were 85.9%, 57.6%, 23.3%, 9.2%, and 5.2%, respectively. The average total 6-month cost of care for patients with melanoma was $5,174 ($2,265 surgical, $1,360 chemotherapy, $618 immunotherapy, $466 other outpatient services, $246 inpatient services, $56 pharmacy); costs of care for those with Stage III-IV disease averaged $12,608 ($4,251 surgical, $4,274 chemotherapy, $1,908 immunotherapy $1,009 other outpatient, $651 inpatient, $79 pharmacy). CONCLUSION: Melanoma strikes during the prime of life with survival rates of 7-9 months in those with Stage III/IV disease. Treatment costs for melanoma are substantial in the first six months following diagnosis, especially for those with Stage III-IV disease. More studies are needed to better understand the health economic impact of the current standard of care and the impact of emerging immonotherapies for melanoma.

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P-141 EPIDEMIOLOGY OF MELANOMA IN IRAN

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Background: Primary cutaneous malignant melanoma is an uncommon tumor in Iran compared with other countries. There are no previous comprehensive Iranian studies on this tumor and proper statistical data are nonexistent. The aim of this study was to determine the epidemiological aspects of cutaneous malignant melanoma among Iranian inpatients, their demographic status and length of stay involved in hospitals. Methods: Records of 1041 inpatients who were hospitalized with malignant tumors in Iranian public hospitals during 2000-2002 were studied. The neoplasms had been coded and classified according to International classification of Diseases, 10th Revision (ICD-10). The frequency distribution of cancer patients was evaluated by age, sex, and place of residence and the length of stay at hospital. Results: There were 655 male patients and 386 females. The mean ± sd age was 57.6 ± 17.6 with a median of 61 years. The average for females (55.5 ±17.9 yrs) was significantly lower than that for males (58.9 ± 17.3 yrs) ($p<0.001$). Malignant melanoma was found to be more common in male than women, in the ratio of approximately 1.7, due to higher exposure of men to sunlight. Rural patients with melanoma accounted for 32.1%. The majority of cases were of the superficial spreading variety, followed by the nodular, lentigo maligna, and acrolentignous melanoma, repectivley. Conclusion: The incidence of cutaneous malignant melanoma, formerly considered a rare tumor in Iran, is now recognized to be on the increase. Exposure to sunlight seems to be the most prominent risk factor in the development of this tumor among Iranians. Therefore, rise of mortality and incidence rates for melanoma requires special attention in control and prevention programs.

P-142 MELANOMA IN YEMEN

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Introduction: Melanoma is one of the major killers among skin tumours which was neglected for many decades in Yemen due to bad health services, ingorance and low socio-economic status of the majority of Yemeni peoples. Most of malignant melanoma in Yemen arised from congenital or acquired nevi due to late detection and management and many racial and environmental factors.Aim of this paper: is to study the epidemiological aspects of cutaneous melanoma among Yemeni population.Method: Population-Based study. Clinical data and histo-pathological findings of 53 melanoma patients (28 men and 25 women), seen in the period from January 2004 to Dec. 2004 in tow major hospitals and my clinic. The analysed data included age, sex, site, skin type, work, residence, sosio-economic status, sun-exposure and burns and family hisory.Results: Malignant melanoma was found to be a common neglected tumour in Yemen. More in females than males. More frequently between faired skin (type I, II, II) farmer with low socio-economic status patients than dark skin with high socio-economic patients. The most common types are superficial varieties, lentigo ligna, nodular than acrolentiginous melanoma respectively. More on head then trrunk. Mean age 51-58 years old. The prevalence rate of malignant melanoma in Yemeni population is 6.8/100,000/year. Therefor, around 1360 patients suffering from malignant melanoma in Yemen in year 2004. Mortality rate is 2.6/100.000/yaer. There's no previous Yemeni study on this tumour. Formal statistical data on mortality and incidence are not available in Yemen. Conclusion:Melanoma is a neglected growing dangerous tumour in Yemen. Increasing by time with high mortality rate. Ingorance, bad health services, misconception of peoples and chronic sun-exposure are the mean risk factors in the development of melanoma in Yemen.

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P-143 LEVEL OF 18-FLUORODEOXYGLUCOSE UPTAKE PREDICTS RISK FOR RECURRENCE IN MELANOMA PATIENTS

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Objective. The incidence of melanoma has increased worldwide. Identification of additional prognostic factors besides ulceration and Breslow (among others) may allow the development of individualized treatment strategies. A multivariate analyses was undertaken to evaluate the potential role of the standard uptake value (SUV) in FDG-PET to predict disease-free survival (DFS) and overall survival (OS) in melanoma patients with lymph node metastases. Methods. All melanoma patients with palpable lymph node metastases who where referred for an FDG-PET scan were eligible. The SUV in the lymph node metastasis was calculated. Data were analyzed (Kaplan-Meier) and differences in cumulative DFS and OS were assessed (log-rank test). Univariate and multivariate analysis (Cox proportional hazard model) were performed to determine independent prognostic factors. Results. Thirty-eight patients were included in the study. Ulceration of the primary melanoma (p=0.023) was an independent predictor of OS. No statistical difference in OS between high or low SUVmean (p=0.11) was found. However, a significant difference was found in DFS (p=0.03). Multivariate Cox regression showed adjuvant radiation (p=0.001), localization of the primary melanoma (p=0.017) and a high SUVmean (p=0.009) as independent prognostic factors for the DFS. Conclusion. DFS of melanoma patients was prolonged in those with a low SUVmean value (p=0.03) in their lymph node metastasis, as compared to those with a high SUVmean. However, this difference was not found for OS. In multivariate analysis high SUVmean was an independent prognostic factor (p=0.009) for DFS. If these results are confirmed by prospective research, adjuvant treatment should be considered for patients with high FDG uptake in melanoma lymph node metastases.

P-144 PERCEPTION OF DIAGNOSTIC TESTS BY MELANOMA PATIENTS WITH LYMPH NODE METASTASES

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Introduction. Studies have suggested that the period of uncertainty prior to diagnosis and treatment is one of the hardest periods for the cancer patient. Those patients who experience embarrassment, discomfort or anxiety during diagnostic tests may disrupt these tests. The aim of this study was to describe the perception of the diagnostic tests (X-ray, CT and PET) by melanoma patients with lymph node metastases. Methods. After finishing all diagnostic tests, patients were requested to complete a self-administrated questionnaire containing questions concerning the levels of burden. Experienced levels of embarrassment, discomfort and anxiety for the different tests were calculated, as well as (total) scores for each burden. The non-parametric Friedman test for related samples was used to see if there was a difference in burden. Results. The questionnaire was completed by 25 patients; response rate was 86%. In total 27% felt mild to extreme embarrassment (x-ray 17%, CT 44%, PET 39%), 24% mild to extreme discomfort (x-ray 0%, CT 25%, PET 75%) and 29% mild to extreme anxiety (x-ray 16%, CT 42%, PET 42%) during the diagnostic tests. Overall, 27% felt some kind of burden. There was no difference in embarrassment or anxiety between the three tests. However, patients experienced more discomfort during the PET scan than during the other tests (p=0.005). Discomfort during the PET scan was significantly correlated with discomfort during the CT scan (r=0.45, p=0.024). Discussion. Overall levels of embarrassment, discomfort and anxiety were low. However, patients experience more discomfort during the PET scan, possibly as a result of lying immobile for a long time. The accuracy, costs and percentage of patients upstaged will probably be the most important outcomes to determine the additional value of FDG-PET and CT, but it is reassuring to know that the overall burden of these diagnostic tests is low.

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P-145 ELUTRIATION OF MONOCYTES WITHIN A CLOSED SYSTEM FOR CLINICAL SCALE GENERATION OF DENDRITIC CELLS TO BE USED IN MELANOMA VACCINATION TRIALS

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Dendritic cells (DC) are promising tools for the immunotherapy of cancer. The induction of tumor-specific T cells and clinical regressions have already been observed in early phase I/II vaccination trials. As DC-vaccination is now facing trials with larger patient collectives it becomes increasingly important to obtain large numbers of cells suitable for therapeutic applications under labor- and cost-effective conditions. We describe here a procedure that uses a novel cell separator (Elutra, Gambro BCT) to enrich monocytes from an entire apheresis product within one hour. Cells are separated on the basis of size and to a lesser extent density, by elutriation in a 40 ml conical chamber. The total monocyte recovery following elutriation (n= 6) was 98,53 % (+/- 8,07 %), the recovery in the monocyte-rich fraction 75,45 % (+/- 11,31 %) and the mean purity 82,95 % (+/- 6,01 %). These monocytes can be cultured either in conventional culture dishes or in closed cell culture bags and differentiated, by using GM-CSF + IL-4 followed by a maturation cocktail composed of IL-1beta + IL-6 + TNF-alpha + PGE2, into fully mature DC. The Elutra-separator allows for fast and easy enrichment of monocytes within a closed system. Subsequently, elutriated monocytes can be successfully cultured into phenotypically and functionally mature DC for immunotherapeutic approaches. The method neither requires a density gradient step to enrich PBMC from leucapheresis products nor does it apply (xenogeneic) antibodies to target monocytes. Isolation of monocytes with Elutra may greatly facilitate future DC-based vaccination approaches.

P-146 TREATMENT OF PATIENTS WITH METASTATIC MELANOMA OR OTHER ADVANCED SOLID TUMOURS WITH INTRALESIONAL INJECTIONS OF ADENOVIRUS-INTERLEUKINE 2 (TG1024)

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Objective : To assess feasibility, tolerance and clinical activity of intralesional injections of adenovirus-IL-2 (TG1024). Materials and Methods: 35 patients (25 with metastatic melanoma and 10 with other advanced solid tumours) have entered this phase I/II study with TG1024. Twenty patients have been treated in successive cohorts of escalating doses given every three weeks: 3.108, 3.10E9, 3.10E10, 8.10E10 and 3.10E11 vp (viral particles). At this point the study was extended to 15 additional patients in three cohorts in which treatment was performed every other week, every other week in combination with dacarbazine, and finally every week. Response and tolerance were assessed according to respectively the WHO and CTC criteria. Results: Below 3.10E11 vp no objective response has been observed. Among the 18 patients with metastatic melanoma treated at a dose of 3.10E11 vp, 5 have presented an objective response at the injection site (3 CR and 2 PR) while 4 have been stable for more than 12 weeks. Two patients have presented a distant effect: 1 partial and 1 minor response. The responses were neither correlated with dosing schedule nor with addition of dacarbazine. One patient with a spinocellular carcinoma presented a complete response of an injected lesion. The most commonly observed adverse events were: injection site reactions, flu-like syndrome, asthenia and transient lymphopenia. Conclusion: The intralesional injection of TG1024 is feasible and well tolerated. TG1024 shows clinical activity in metastatic melanoma at 3.10E11vp and warrants further investigation.

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P-147 MODULATION O6-ALKYLGUANINE-DNA-ALKYLTRANSFERASE BY METHIONINE-FREE DIET IN ASSOCIATION WITH NITROSOUREA TREATMENT OF METASTATIC MELANOMAS: PRELIMINARY RESULTS

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In Vitro and In vivo experiments, Methionine (MET) depletion used in association with cytostatic drugs has been shown to improve of their therapeutic index. One hypothesis by which MET deprivation sensitized these tumors to nitrosoureas (CENUs), could be attributed to down-regulation of the repair protein O6-alkylguanine DNA alkyltransferase (AGT), one of the main mechanisms of resistance to CENUs. On the basis, we initiated a phase I clinical trial associating dietary MET restriction with nitrosourea treatment (cystemustine) for metastatic melanomas. we evaluated the impact of this association on AGT activity in peripheral blood mononuclear cells (PBMCs) during treatment. Ten patients received 2 months of treatment, i.e. 4 cycles every two weeks of the association of MET-free diet and Cystemustine (60 mg/m2).. Every cycle, AGT activity level was measured by HPLC in PBMCs isolated on ficoll from blood samplings before and after diet period. Daily concentrations of plasma methionine, nutritional status and toxicity were also evaluated. Dietary MET restriction reduced MET concentrations from 21.2±1.3 1⁄4M before diet to 12.0±1.0 1⁄4M from only one day of diet, with a mean optimal decline of 41%. This MET free diet did not have a deleterious effect on nutritionnal status and did not exert any clinicaly toxicity. Actually, analysis of AGT activity was performed for 3 patients. AGT activity level ranged from 95 to 552 fmol per mg of protein with a mean level of 362 ± 154 fmol/mg. Comparing before and after diet period, the AGT activity in PBMC of these patients was not affected by MET free diet. Individual interpatient variability was very important. However, plasma MET variation (before and after diet) seemed to be correlated with the AGT activity variation. These preliminary results might be confirmed with analysis of AGT activity of the other patients of this trial.

P-148 METHIONINE-FREE DIET IN ASSOCIATION WITH NITROSOUREAS TREATMENT A PHASE I CLINICAL TRIAL IN MELANOMA : DETERMINATION OF THE OPTIMAL METHIONINE-FREE DIET DURATION

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The growth of human and animal tumors has been shown to be inhibited by dietary methionine (MET) restriction. Cytostatic drugs in association with MET restriction presented with a substantial improvement of their therapeutic indexes. We initiated a phase I clinical trial of dietary MET restriction in association with nitrosoureas treatment, to determine the optimal MET-free diet duration and the feasibility of this schedule associated to cystemustine delivery. Ten patients with recurrent gliomas or metastatic melanomas received 4 biweekly cycles of chemotherapy. During each cycle, patients received standard diet the 1rst day and then MET-free synthetic diet, which allowed testing randomly 4 periods of 1, 2, 3 or 4 regime days. Cystemustine (60 mg/m2) was given the last day of MET-free diet. Nutritional status was evaluated by BMI and PINI determinations before and after diet. Daily fasting (8 AM) and feeding (12 AM) concentrations of plasma MET were measured. MET-free diet have not deleterious effect on nutritional status. Dietary MET restriction reduced feeding MET concentrations from 21.2±1.3 1⁄4M at D0 to 12.0±1.0 1⁄4M after only one day of diet, with a median optimal decline of 45%. No cumulative effect have been observed despite the lengthening of MET-free diet duration. The toxicity OMS grade 3-4 remained moderated (3/10 thrombopenia and 3/10 neutropenia). Hence, a 1 day MET-free diet will be adopted to realize a phase II clinical trial aimed at evaluating the therapeutic efficacy of the association MET restriction diet with cystemustine treatment.

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P-149 MULTICENTER PHASE I/II STUDY ASSESSING MAXIMAL TOLERATED DOSAGE AND CLINICAL RESULTS OF TEMOZOLOMIDE ASSOCIATED WITH PEG-INTRON IN PATIENTS WITH METASTATIC MELANOMA.

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Objective : Only 12 to 20% of patients with metastatic melanoma (MM) display an objective response with usual treatments but a combined approach with chemo-immunotherapy might increase the clinical efficiency. A trial using an association of Temozolomide (TMZ) with Peg-interferon a2b (Peg) in patients with MM was set up to assess this hypothesis. The first aim of this study was to evaluate the maximal tolerated dosage of both drugs, with the assessment of clinical results as a secondary objective. Methods : Patients with MM in first or second line of treatment and without cerebral metastasis were enrolled in a multicenter, prospective, open-label study using a dose escalation protocol according to the MCRM scale with four different cohorts : TMZ 150mg/m2 5 d/w every 4 weeks and Peg 0.5 mg/w, TMZ 150 mg/ m2 5d/w and Peg 1.0 mg/w, TMZ 200 mg/ m2 5 d/w and Peg 0.5 mg/w, TMZ 200 mg/ m2 5d/w and Peg 1.0 mg/w. Patients received a maximum of 6 cycles. Results : 31 patients completed the study. One patient was enrolled in the first dose level, 1 in the second one, 18 in the third one and 11 in the fourth one. In this latter group, 4/11 patients displayed a dose-limiting and 4 a non dose-limiting toxicity, mainly hematological (grade IV thrompopenia) and leading to a dose reduction. An objective response was observed in 4 patients (2 complete responses and 2 partial responses). The disease remained stable in 4 patients and 7 patients were alive 21 to 31 months after enrollment. Only patients receiving dosage level 3 or 4 displayed an objective response. Conclusion : The association of TMZ with Peg in MM showed an unacceptable hematological toxicity with the maximal dosage but was effective at lower dose levels with 23% of non-progressive disease.

P-150 SENTINEL LYMPH NODE BIOPSY FOR MELANOMA: ADVANTAGES AND DISADVANTAGES

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Objectives: The aim of the study was to evaluate advantages and disadvantages of sentinel lymph node biopsy (SLNB) in patients with cutaneous melanoma (CM). Patients and methods: Between 1995-2004 300 patients with CM (>1.0 mm) underwent SLNB. Results of the SLNB procedure, postoperative complications, incidence of lymphedema, follow up, recurrences, DFS and OS were evaluated. Results:
The SLNB detection rate was 99%. 85 patients had a tumorpositive SLNB (28%) and underwent completion regional lymph node dissection; 215 patients underwent SLNB alone. Postoperative complications after SLNB were 7%. The incidence of slight lymphedema was 11% and 6% for respectively axillary and inguinal SLNB. However, when completion lymph node dissection was performed, the incidence of slight lymphedema was 7% and 64% for respectively axilla and groin. With a median follow up of 51 months, the false negative rate of the SLNB procedure was 11%. There were 23% recurrences (SLNB neg: 19%; SLNB pos: 34%; p=.005). In-transit metastases (ITM) were found 4% in the SLNB neg group and in 20% in the SLNB pos group (p<.001). The 5-year DFS and OS were respectively 79% and 86% in SLNB neg patients; in SLNB pos patients 57% and 71%. Multivariate analysis showed that independent prognostic factors for DFS were presence of ulceration (p<.001) and SLNB positivity (p<.01). Prognostic factors for OS were presence of ulceration (p<.001) and male sex (p<.05). Also multivariate analysis showed that SLNB positivity (p<.001) and presence of ulceration (p<.01) were the significant prognostic factors for developing ITM. Conclusion: SLNB in patients with CM is still only of prognostic value since survival benefit is not proven. Disadvantages of SLNB are false negative rates, possibility of an increased risk of ITM in SLNB pos patients, postoperative complications and slight lymphedema. These have to kept in mind when offering patients SLNB.

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P-151 SERUM TNF-ALPHA LEVEL IS A PREDICTOR OF CLINICAL OUTCOME IN ADJUVANT IFN-ALPHA2B TREATMENT FOR MALIGNANT MELANOMA

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OBJECTIVE: In adjuvant IFN-(treatment for melanoma there are no established factors which may help to predict response to therapy. Clinical activity of IFN-(is not only mediated by direct anti-proliferative effects, but also by modulation of secondary cytokines. We examined whether cytokine levels might serve as predictors for outcome or the extent of side effects. MATERIALS & METHODS: In a randomized, prospective clinical study 66 patients in stage II or III were treated with adjuvant IFN-(2b. All patients received induction treatment of 10 MU IFN (2b s.c. 5x/week, followed by either 10 MU or 5 MU IFN (2b s.c. 3x/week for a total of 2 years. Before treatment, at 4 weeks, 12 weeks, thereafter every 3 months serum levels of IL-1(, IL-2, IL-2R, IL-6, IL-10, TNF-(and beta-2 microglobulin (b2m) were measured by ELISA. RESULTS: The most interesting results were found in serum TNF-(levels. The median baseline levels for TNF-(were 5,8 pg/ml in the whole study group. In patients with relapsing disease under IFN-treatment significant lower levels of TNF-(were observed at baseline as well as under therapy (p=0,017). In patients discontinuing treatment because of side effects significant higher levels of TNF-(could be detected. B2m and IL-2R showed a significant increase throughout the therapy phase (p<0,0001) and could be identified as new biological markers of adjuvant IFN-(2b treatment. IL-2 and IL-6 showed lower levels in patients with relapse compared to patients without relapse. For IL-10 no significant group differences could be detected. CONCLUSION: Patients with low TNF-(baseline levels do not mount TNF-(increases upon IFN-therapy and are prone to relapse. Vice versa, patients with severe constitutional side effects exhibit increased TNF-(levels. Elevated IL-2R and b2m levels can serve as biological markers for ongoing IFN-(2b therapy.

P-152 A PHASE II STUDY OF THE DUAL ENDOTHELIN RECEPTOR ANTAGONIST BOSENTAN (TRACLEER(R)) AS FIRST-OR SECOND-LINE THERAPY IN STAGE IV MELANOMA

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Thirty-five patients with stage IV metastatic melanoma were enrolled in this open-label, single-arm, prospective, multicentre trial. The objective was to assess the effects of bosentan monotherapy (500 mg oral tablets, twice daily) given as first- or second-line therapy on tumour response. Patients were followed up until disease progression, death or drug toxicity occurred. Tumour response was assessed at 6-weekly intervals using the Response Evaluation Criteria in Solid Tumours (RECIST). Results: The study population (N=35) had a median age of 61.0 years (32 to 79) and an American Joint Committee on Cancer (AJCC) class of M1C (N=21, 60.0%), M1B (N=10, 28.6%) and M1A (N=4, 11.4%). Nine patients (25.7%) had prior therapy for stage IV melanoma. Stable disease (SD) was observed in 6/35 patients at week 12, 5 of whom (M1A=2, M1C=2, M1B=1) were stable at >24 weeks. Five of the 6 patients with SD received bosentan as first-line therapy. Bosentan was well tolerated; it was discontinued due to adverse events in only 7/35 patients, one of whom had progressive disease at the same time. The most frequent adverse events were headache (N=15, 42.9%), fatigue (N=12, 34.3%), nausea (N=11, 31.4%), back pain and abnormal hepatic function (both N=8, 22.9%). Conclusions: Bosentan is well tolerated and may have benefit in disease stabilization in patients with metastatic melanoma. Further studies to evaluate bosentan as first-line treatment may be warranted.

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P-153 PROSPECTIVE ASSESSMENT OF COMPLICATIONS FOR MELANOMA PATIENTS FOLLOWING SELECTIVE SENTINEL LYMPHADENECTOMY

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Objective: Selective sentinel lymphadenectomy (SSL) has been established as a reliable procedure to stage early melanoma patients. The aim of our study is to collect prospective follow-up data on postoperative complications of primary melanoma (PM) patients undergoing wide local excision (WLE) and SSL. Methods: Prospective data on PM patients undergoing WLE and SSL from 2/2003 to 10/2004 include pain, numbness, wound separation, skin graft failure, seroma, cellulitis, limitation of limb range of motion, and lymphedema by measurement (2cm increment) were collected during the preoperative, initial postoperative and 3 months interval visits. All patients signed informed consent approved by the UCSF Committee on Human Research. Results: Ninety nine patients were included in this study (upper extremity=44, trunk=31, lower extremity=24). The initial postoperative complications from the PM sites were numbness (47.5%), pain (16.2%), cellulitis (6.1%), and wound separation (3%). For the SSL sites (123 SSL procedures performed), complications consisted of numbness (37.4%), seroma (24.4%), limb motion restriction (16.3%), pain (15.4%), lymphedema (12.5%), and cellulitis (4.9%). Sixty six patients were followed three months post-operatively (upper extremity=31, trunk=18, lower extremity=17). In comparison to PM site complications of the initial postoperative visit, by three months postoperatively the numbness rate decreased by 0.5%. Pain decreased by 14.7%. Cellulitis and wound separation were no longer present. For the 88 SSL sites being followed three months postoperatively, numbness increased by 2.6%. Seroma and cellulitis were no longer present. Limb motion restriction decreased by 13.3%. Pain decreased by 9.1%. Lymphedema increased marginally by 0.9%. No patients were hospitalized for any severe complications. Conclusions: The prospective assessment of complications of SSL provides a detailed account of complications in a real time fashion, thus, giving the PM patients a reliable guide as what to expect from the SSL procedure.

P-154 AUTOLOGOUS MELANOMA CELL VACCINE RESULTS IN IMPROVED SURVIVAL FOR PATIENTS THAT DEVELOP STRONG DTH RESPONSE TO UNMODIFIED AUTOLOGOUS MELANOMA CELLS.

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Objectives: This study evaluates the overall survival (OS) and disease free survival (DFS) of melanoma patients that were treated with an autologous melanoma cell vaccine, administered as a post-operative adjuvant. Patients and methods: Included are 82 patients with totally resected metastatic melanoma to regional lymph nodes, AJCC IIIB and IIIC (N1-3b) with a median follow up of 46.5 months (6-97). The treatment consisted of eight doses of a vaccine made of 10-25x106 autologous melanoma cells. Tumor cells were conjugated with hapten dinitrophenyl (DNP), mixed with Bacille Calmette Guérin (BCG) and irradiated to 170 Gy. A subgroup of 32 patients was analyzed for emergence of antibody reactivity to livin, an inhibitor of apoptosis over expressed in melanoma, and to gp100, a melanocytic differentiation antigen. Results: The median survival time for the whole group was 32 months (95% CI 14-51). Thirty six patients (44%) had no disease recurrence, 34 (41.5%) died of disease at the time of analysis. Patients' OS was found to be correlated with intensity of evolving delayed type hypersensitivity (DTH) to subcutaneous injection of unmodified melanoma cells. Patients with a DTH reaction of e10 mm (59%) had a median OS of 46 months. In contrast, patients with a negative or weak DTH (34%) had a median OS of only 19 months (p=0.009). The overall DFS was 12 months. The difference in DFS as a correlate of DTH status was not statistically significant (14 versus 10 months). Twenty nine out of 32 patients experienced an increase in antibody level to livin. The relative increase was found to be correlated with OS (p=0.05). Antibody reactivity to gp100 did not change consistently and did not correlate with outcome. Conclusions: The adjuvant administration of autologous melanoma vaccine was associated with improved overall survival to selected patients that successfully attained anti-melanoma reactivity

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P-155 A PHASE I STUDY OF ARSENIC TRIOXIDE PLUS DACARBAZINE IN MALIGNANT MELANOMA

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Background: Arsenic trioxide has an apoptotic effect on human melanoma cells lines in vitro. Sensitivity to arsenic trioxide is increased by lowering cellular glutathione (GSH) levels by either ascorbic acid or buthionine sulfoximine. Dacarbazine (DTIC) also depletes intracellular GSH; thus, treatment with DTIC followed by arsenic trioxide may increase apoptosis in melanoma cells. The primary objectives of this study were to assess the safety and establish the appropriate dose of arsenic trioxide in combination with DTIC in patients with malignant melanoma. Patients and Methods: Nine patients with stage IV malignant melanoma, previously untreated or refractory to initial therapy, were enrolled. DTIC was given at a fixed dose of 200mg/m2 immediately before dosing with arsenic trioxide. Arsenic trioxide was escalated from 0.10mg/kg to 0.18mg/kg to 0.25mg/kg in three-patient cohorts. Patients were treated with DTIC and arsenic trioxide for 5 consecutive days per week for 1 week of each 3-week cycle. Results: Nine patients with a median age of 63 years received a total of 20 cycles of therapy; the median number of cycles was 2, one patient received 7 cycles. One patient treated at the 0.18mg/kg dose level was taken off study because of hypotension and tachycardia related to dehydration. No dose limiting toxicities were reported. The most common toxicities were grade I fatigue, cough, chest pain, nausea, leukopenia, dyspnea. No responses were seen; there was one stable disease for 4.8 months. Conclusions: Arsenic trioxide following DTIC was well tolerated; the maximal tolerated dose for this combination in patients with melanoma was not reached. No activity was reported at these doses and schedule. Administering DTIC before arsenic trioxide may have contributed to a DTIC-induced elevation in NFkB-levels, resulting in resistance to arsenic trioxide.Thus, alternative schedules and/or doses may be explored, possibly in preclinical studies, to establish a more optimal dosing schedule.

P-156 TREATMENT OF SUBMICROSCOPIC METASTASIS IN SENTINEL LYMPH NODES OF MELANOMA PATIENTS: A PILOT STUDY WITH EMPHASIS ON DISEASE EVOLUTION.

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Introduction: The use of molecular techniques to study sentinel lymph nodes (SLNs) of melanoma patients identifies metastatic implants that are invisible to conventional pathologic analysis. The biologic significance of those submicroscopic detection remains unknown. We present the preliminary results of a pilot prospective study on the survival impact of the early treatment of that metastasis. Methods: Melanoma patients whose SLNs contained submicroscopic metastasis (pathologically negative / tyrosinase mRNA RT-PCR positive) were prospectively recluted for the study. Patients with nevus aggregates in SLN were excluded. Patients were randomized in 3 therapeutic groups: A: observation, B: lymph node dissection (LND), and C: LND + high doses (20MU ev 5d/w x 4 w, 10MU sc 3d/w x 48w) of alfa2b interferon (IFN). Patients were followed-up to evaluate disease free survival (DFS) and overall survival (OS). Results: 59 patients were included (groups A: 27, B:16, C 16). After a median follow-up period of 28.7 months (min 13.9, max 44), no exitus was observed and only 3 patients relapsed (all in group A). DFS could only be established in the group with recurrences (group A), and it was 40 months (CI 95%). Differences between groups did not reach statistical significance (log-rank test). Conclusions: Early treatment of melanoma SLN submicroscopic metastasis, either by LND or LND+IFN, improves DFS. This observation reinforces the hypothesis of the real biologic significance of submicroscopic metastasis detection, and points out the need of treating them. The limited number of patients in this single institution pilot study difficults the statistical evaluation of our results, which should be confirmed in multicentric studies, as the ongoing Sunbelt Melanoma Trial.

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P-157 A RANDOMIZED PHASE I/II VACCINATION TRIAL USING THE RECOMBINANT MAGE-A3 PROTEIN LOADED ON MYELOID DC OR MIXED WITH ADJUVANT ASO2B IN MELANOMA PATIENTS.

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Objective: The MAGE-A3 gene codes for tumor-specific antigens recognized by CD4+ and CD8+ T-cells. We evaluated the potential advantage of the concurrent presentation of CD4+ and CD8+ epitopes on the recombinant (rec) MAGE-A3 protein loaded on myeloid DC, as compared to the protein combined with adjuvant AS02B. Methods: 19 patients with evaluable stage III or IV MAGE-A3 expressing melanoma received 4 vaccines of recMAGE-A3 protein either loaded on autologous DC (40-100 x 10E6 i.d./s.c.), or mixed with AS02B adjuvant (adj.) (300 É g of protein i.m.). DC were generated from adherent PBMC for 7 days with GM-CSF + IL-4, then pulsed with the protein for 1h.T-cell response was analyzed by Elispot after in vitro restimulation, and Ab response by ELISA. Results: 18 patients were eligible and, among them, 14 received all 4 vaccines (3 rapid progressions and 1 technical problem). In both arms, no serious toxicity was reported. We observed in the adj. arm (9 patients): one partial response (7 mo), one mixed response (>34mo, with vitiligo surrounding a responsive lesion), and one stable disease (>26mo); in the DC arm (5 patients): one mixed response (>23mo). Patients showed a strong Ab response against the protein in the adj. arm, while no Ab was detected in the DC arm. Moreover, increase in anti-MAGE-A3 T cell frequency was detected in 4 out of 9 pts in adj. arm, and in 1 out of 5 pts in DC arm (responders: n=5, mean fold increase baseline = 9.7). All but one pt with clinical responses were in the T cell responder group. Conclusions: Vaccines with recMAGE-A3 protein given with AS02B adjuvant or loaded on DC were safe. Although the cohorts are small, clinical and immune responses against MAGE-A3 protein seem to be more frequent in the AS02B adjuvant arm than in the DC arm.

P-158 ULTRASOUND DIFFERENTIATION OF BENIGN AND MALIGNANT LYMPHADENOPATHY IN MELANOMA PATIENTS - PRELIMINARY REPORT

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Follow-up programs in melanoma patients are directed towards early detection of tumor recurrence. The first expected site of disease relapse are regional lymph nodes. Identification of involved lymph nodes and their removal are significant in patient treatment. We analized ultrasound characteristics of sonography detected regional lymph nodes in sight of presence of melanoma metastasis: echogenicity of lymph node center, type of lymph node vascularization, intranodal lymph node vascularization and resistance index, their sensitivity and specificity, respectively. Twenty one patients in clinical stage III melanoma were subjected to this study in our clinic (12 men, 9 women, average age 46,7 years). Ultrasound examination was performed with ACUSON SEQOIA 2000 ultrasound device. Multifrequent probe, with frequency level from 5 to 8 Hz was used. All lymph nodes were examined in B-real time mode, pulse Doppler and power modem settings. Sensitivity and specificity were determined comparing methods with histopathological examinations. Sensitivity of the ultrasound lymph nodes characteristics was shown with this data: echogenicity of lymph node 90.9%, type of lymph node vascularization 90.9%, intranodal lymph node vascularization - 90.9 with resistance index sensitivity of 99.9%. The data shown are obtained in the early phase of our study. This research is still being performed in our institution, but it requires further clinical confirmation.

NOTES:

P-159 A PHASE II TRIAL OF VACCINATION WITH AUTOLOGOUS, TUMOR-DERIVED HEAT-SHOCK PROTEIN PEPTIDE COMPLEXES GP96, IN COMBINATION WITH GM-CSF AND INTERFERON-A IN METASTATIC MELANOMA PATIENTS

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Background: We have previously demonstrated the feasibility of vaccination with tumor-derived HSPPC-96 in metastatic melanoma patients. Moreover, a melanoma-specific T-cell response could be achieved in 50% and a clinical response in 18% of the treated patients. To investigate whether immune reaction to HSPPC-96 can be enhanced by cytokines, we administered HSPPC-96 together with GM-CSF s.c. and IFN-± systemically. Methods: 38 patients with AJCC stage IV melanoma underwent surgery to harvest tumor tissue for vaccine production. Subsequent treatment consisted of: HSPPC-96 (25 1⁄4g s.c. on day 2); GM-CSF (75 1⁄4g s.c. on days 1, 2, 3); IFN-± (3 MU i.v., twice weekly). If no tumor progression occurred and vaccine was available a 2nd cycle of 4 bi-weekly injections was given. Antigen-specific anti-melanoma T and NK lymphocyte response was assessed by enzyme-linked immunospot (ELISPOT) assay on peripheral blood mononuclear cells obtained before and after vaccination. Results: Treatment was well tolerated. 27 patients were vaccinated and 20 were considered assessable having received at least one cycle of vaccination. Of these, 2 were free of disease (DF) and 18 had residual melanoma post surgery. Of the DF subjects, 1 remained DF for 414 days. Of the 18 patients with residual tumor, 11 showed SD lasting from 130 to 320 days. ELISPOT assay revealed an increased class I HLA-restricted T and NK cell-mediated post-vaccination response in 5 out of 17 and 12 out of the 18 patients tested. Conclusion: These results suggest that GM-CSF, at this dose and schedule followed by systemic IFN-±, do not increase T-cell responses post HSPPC-96 vaccination as compared to our previous study. However, both immunological and clinical responses were not increased as compared with those recorded in a previous similar study of vaccination with HSPPC-96 only.

P-160 A PHASE I STUDY OF TEMOZOLOMIDE IN COMBINATION WITH THE NOVEL POLY(ADP-RIBOSE)POLYMERASE (PARP) INHIBITOR AG014699 SHOWING ENCOURAGING ACTIVITY IN MALIGNANT MELANOMA.

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Introduction: The methylating agents temozolomide and dacarbazine are standard treatments for metastatic melanoma, but response rates are low. Most DNA damage caused by these is repaired by the base excision repair pathway (BER). PARP-1 is a nuclear enzyme involved in BER, whose inhibition enhances temozolomide cytotoxicity in pre-clinical models. We report safety, efficacy, and tumour PARP activity of the novel PARP inhibitor, AG-014699 combined with temozolomide. Methods: In part 1, patients with solid tumours received AG-014699 + temozolomide daily x 5 every 28 days. Temozolomide dose was half standard (100 mg/m2 po) and AG-014699 (30 min infusion) escalated to the PARP-inhibitory dose (PID), defined as maximal (>50%) reduction in PARP activity in peripheral blood lymphocytes (PBLs) 24 hr after AG-014699. In part 2, AG-014699 dose was fixed at PID and temozolomide escalated to maximum tolerated dose or 200 mg/m2 in metastatic melanoma patients. The overall objective based on xenograft data was to achieve > 40% tumour PARP inhibition. Results: 33 patients were enrolled. In part 1 (n=18), AG-014699 dose levels were 1, 2, 4, 8 and 12 mg/m2. No dose-limiting toxicity (DLT) was observed. PID was 12 mg/m2 based on 74 -97% inhibition of PBL PARP activity. In part 2 (n=9) there was one DLT with temozolomide doses up to 200 mg/m2. Median tumour PARP inhibition at 5 hours was 90% (range 50 - 98%). An additional dose level (AG-014699 18 mg/m2, TMZ 200 mg/m2) was tested to maximize tumour PARP inhibition (n=6). DLT was observed in 1/6 patients (grade 4 febrile neutropaenia and thrombocytopaenia). 2 patients required dose delays for myelosuppression. Discussion : Encouraging activity occurred with 2 partial responses, 2 prolonged disease stabilisation (>6 months) and 3 early responses in 18 patients with metastatic melanoma. The phase II study in this indication opened in March 2005.

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P-161 MONTHLY DOSING OF THE HUMAN ANTI-CTLA4 MONOCLONAL ANTIBODY CP-675,206 (TICILIMUMAB) IN PATIENTS WITH ADVANCED MELANOMA: PHASE 1 TRIAL

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Objective: Single doses of ticilimumab demonstrated antitumor activity and a manageable safety profile. This phase 1 trial was conducted to further assess safety and tolerability of ticilimumab and the recommended monthly dose for a phase 2 trial. Materials & Methods: Inclusion: Stage IIIc-IV melanoma, measurable disease, no active brain metastasis. Results: Enrolled 14 pts (9 M, 5 F), mean age 55 (range 42-67): 3 at 3.0 mg/kg, 3 at 6.0 mg/kg, 8 at 10.0 mg/kg. 57% had stage IVc disease. In all, 51 doses were administered: pts at 3 and 6 mg/kg received 2 doses, at 10 mg/kg a median of 4.5 (range 2-8) monthly doses. No G4 treatment-related toxicities were observed. G3 diarrhea in 1 pt at 3 mg/kg, none at 6 mg/kg, 2 pts at 10 mg/kg. G2 treatment-related adverse events included 4 dermatitis and 1 each of diarrhea, pruritus, hypothyroidism, urticaria, and myalgias. Preliminary PK analysis suggests little accumulation of ticilimumab with multiple dosing and no evidence of HAHA. 1 pt at 10 mg/kg has an ongoing response in multiple in-transit metastases and continues on therapy after 10 doses. Despite 10 mo of CTLA4 blockade, toxicities in this pt have been limited. Four biopsies of residual lesions revealed no melanoma in most areas, with a dermo-epidermal CD3/CD8 lymphocytic infiltrate. An area with viable melanoma cells had a prominent intratumoral infiltrate by CD3/CD8 lymphocytes. Another pt at 10 mg/kg developed new brain metastasis at 2 mo. After XRT, no new metastases were noted for 7 mo. Five of 8 pts treated with 10 mg/kg are alive at a median of 14+ months. Conclusion: Ticilimumab can be administered safely and has antitumor activity in melanoma at monthly doses consistent with prolonged CTLA4 blockade. The recommended monthly dose for further exploration in a Phase 2 trial is 10 mg/kg.

P-162 EX VIVO TRACKING OF MONOCLONAL T-CELL RESPONSES IN MELANOMA PATIENTS FOLLOWING SERIAL PEPTIDE VACCINATION

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CD8+ T lymphocytes recognize and destroy diseased cells which have been altered, e.g. by transformation into cancer cells. Although tumor-specific cytolytic T-cells can be detected in patients suffering of cancer, these immune responses often fail to control or eliminate the disease. The relative lack of cells bearing high avidity T-cell receptors (TCR) within the tumor-specific pool of T lymphocytes may be a major reason why immune responses towards tumor antigens are usually non-protective. To test this hypothesis there is an urgent need to characterize tumor-specific T-cell responses at the clonal level. Here, we used a novel approach that combines FACS technology, gene expression profiling and TCR BV spectratyping for ex vivo molecular characterization of the Melan-A/MART-1 specific CD8+ T cell response in a melanoma patient with a favorable clinical evolution. We found a strong dominant monoclonal response that prevailed in the primed effector-memory compartment. TCR-Vb CDR3 sequencing revealed that the dominant T-cell clone arose before immunotherapy and persisted for longer than three years. Moreover, a dramatic increase in its frequency was observed following serial peptide vaccinations, reaching up to 2% of the circulating CD8 pool. Interestingly, this clone was also dominant among tumor infiltrating lymphocytes (TILs), highly expressed IFN-g and Granzyme B ex vivo. Representative T cell clones generated in vitro efficiently killed autologous melanoma cells. Finally, this monoclonal T-cell subset showed a high turnover in vivo as estimated from relatively rapid telomere shortening (500 bp/yr). Similar observations were made in three additional patients. Overall, our molecular-based approach allows for the first time to track and characterize dominant monoclonal tumor-specific T-cell responses ex vivo over extended periods of time. Identification of such potent anti-tumor TCRs is a key step for TCR gene transfer into polyclonal, unselected T cells as a strategy to induce defined tumor-specific immunity.

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P-163 IL-3 AND IFN2 DCS MATURED WITH POLYI:C ELICIT ANTI-NA17.A2 CTLS IN MELANOMA PATIENTS.

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Objective: DC generated with type-I interferon have high expression of MHC and costimulatory molecules, of migration-enhancing CCR7 chemokine receptor, of Th-1 response-promoting IL-15 cytokine, and of IFN-Éø in response to poly I:C. They were shown to induce efficient T cell responses against tumor antigens in vitro. We investigated the ability of DC generated in interferon (IFN)-É¿ and IL-3 (DC I3) and matured by poly I:C to migrate and induce CD8+ T cell responses against the NA17-A2 tumor peptide in patients (pts) with stage III or IV melanoma. Methods: DC were generated from adherent PBMC by a 6-day culture with IL-3 and IFN-É¿ followed by overnight maturation with poly I:C and loading with NA17. A2 peptide. 8 HLA-A2 pts with NA17+ melanoma received 3 DC I3 vaccines (22.5 millions DC/cycle injected ID, SC and IN). Anti-NA17-A2 CD8+ T cell responses were measured using mixed lymphocyte-peptide culture under limiting dilution conditions followed by enumeration of cells stained with NA17-HLA-A2 tetramers. DC labeling with In-111 enables to study cell migration after injection. Results: The DC I3 vaccine was well tolerated with only minor and transient flu-like symptoms and local inflammatory reaction at the injection site. In 7 out of the 8 pts, isotopic imaging documented DC migration from the injection site to draining lymph nodes. Anti-NA17-A2 CD8+ T cell responses were found in 4 out of 8 pts: from <0.6 (min pre-vaccine) to 21 (max post-vaccine) specific T cells / 10E6 CD8+ lymphocytes. Among 4 pts with no evidence of disease (NED), 3 remained free of disease at >9, >11 and >13 months, and among 4 pts with evaluable lesions, one had stable disease (SD) (>6mo). Conclusions: DC generated in type-I IFN might represent an interesting alternative to DC generated in IL-4 and GM-CSF for the development of more efficient cellular vaccines against melanoma.

P-164 SENTINEL NODE BIOPSY WITH COMBINATION METHOD OF DYE AND RADIOISOTOPES FOR MALIGNANT MELANOMA IN JAPANESE PATIENTS.

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OBJECTIVE: Cutaneous malignant melanoma is relatively rare in Japan. Dye-guided sentinel node biopsy was performed in many institutions on patients with cutaneous malignant melanoma around 1997 in Japan, and at that time, we also initiated the procedure primarily on patients with inguinal lymph nodes as a regional lymph node. From April 2002 onward, we performed preoperative lymphoscintigraphy and intraoperative gamma probe detection in combination with the dye method. MATERIAL and METHODS: 62 Japanese patients of malignant melanoma underwent operations in National Cancer Center Hospital between April 2002 and January 2005.We performed preoperative lymphoscintigraphy and intraoperative gamma probe detection in combination with the dye method. Approximately 98.2NBq technetium sulphur colloid was injected intradermally in four equal parts around the primary lesion. For intraoperative lymphatic mapping , 1ml of 1 or 2% patent blue V was injected intradermally at about ten sites around the primary lesion. In addition a hand-held gamma probe was used to identify hot node(s). RESULTS: The identification rates of individual regional lymph nodes were 97% for inguinal lymph nodes, 96% for axillary lymph nodes, and 89% for cervical lymph nodes. In all lymph nodes, total identification rates were 95% (59/62 cases), which was significantly higher than those achieved by the dye method alone. CONCLUSION: Intraoperative gamma probe detection has been shown to improve the rate of sentinel node identification compared to the use of blue dye alone. The combination method of dye and RI is usefulness and essential for accurate identification of sentinel nodes.

P-165 TOPICAL ANTIOXIDANT

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In the last twenty-five years there has been a dramatic increase in the appreciation of the role of chronic sun exposure and the development of photoageing and skin cancer. Animal models clearly identify three exogenous vitamins (A,C and E) as able to influence some aspects of photoageing when presented in a topical formulation. The effects would appear to be additive and include immediate enhanced and persistent photoprotection; prevention of sun induced immunosuppression and in the longer term reversal of some aspects of photoageing as well as a probable reduction in the frequency of new skin cancers, including melanomas. The basic science concepts and global clinical evidence will be reviewed. New results from a five year office based clinical trial will be presented

P-166 CIRCULATING MELANOMA CELLS DISPLAYING MULTIPLE CHROMOSOMAL CHANGES ARE ASSOCIATED WITH A REDUCED SURVIVAL IN PATIENTS WITH METASTATIC MELANOMA.

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Purpose: The aim of our study was to quantitatively detect circulating melanoma cells, to assess their prognostic significance and to describe their genomic characteristics. Materials and Methods: In a prospective study blood samples were taken from 164 patients with cutaneous or uveal melanoma and from 50 controls. Circulating melanoma cells were detected using the murine monoclonal antibody 9.2.27 combined with immunomagnetic enrichment. We carried out a genetic characterisation of antigen positive cells using single-cell comparative genomic hybridisation on the DNA of 15 individually isolated antigen positive cells from 7 patients. Results: Detection of circulating melanoma cells was associated with the clinical stage of the patient and with the tumor load. All controls were negative. The finding of two or more cells correlated significantly with a reduced survival of patients with metastatic melanoma. All cells that were analyzed by comparative genomic hybridization displayed multiple chromosomal changes. Chromosomal changes associated with a poor prognosis in primary tumors such as gains of 6p in cutaneous melanoma and monosomy three in uveal melanoma were among the most frequent chromosomal changes detected in individually isolated circulating melanoma cells. Conclusion: Circulating melanoma cells displaying multiple genomic changes may be detected in peripheral blood of melanoma patients. The prognostic impact on survival of metastatic patients apparently reflects the aggressiveness of an ongoing hematogenous tumour spread. Direct genomic analysis of the isolated cells will help to further clarify the molecular-genetic basis of the establishment of generalized melanoma.

P-167 ISOLATED LIMB PERFUSION WITH HIPERTERMIA AND CHEMOTHERAPY (ILP): 28 CASES OF INITIAL EXPERIENCE, HOW YOU DIMINISH SIDE EFFECTS DURING LEARNING CURVE

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OBJECTIVE: To analyze the casuistry of 28 initial cases of Hospital do Cancer focusing the tax of completes response, complications associates with the procedure and how you minimize them. MATERIAL AND METHODS: We retrospectively analyze the 28 initial perfusions carried through the Department, in the period of 4/01 you the 12/04. The perfusions had been indicated mainly in patients with in transit melanoma metastases. It was analyzed histology; response, Relapses, complications, CPK. Statistical analysis used was carried using the program Statistical Package will be the Social Sciences. RESULTS: Complete response was 42,90 % (12); 17.9 % partial (5); 17.90 % without response (5); and in 6 cases was not possible to evaluate the response (3 amputations caused by complications and 3 recent procedures). Maximum CPK in the postoperative varied from 14 to 45610. Of the 12 patients who had presented completes response, 5 had presented local relapse. The relapses had occurred in average 10.60 months. Of the 28 procedures of perfusion, 17 evolved with some type of complication. 4 patients had evolved with necessity of amputation caused by the tumor relapses. One died caused by sepsis. Beside the fact, that it was to never used temperature higher than 40 oC, in two cases of amputation caused by complication it was possible to notice retrospectively, that hyperthermia was common causes. In one patient it was kept 1 hour in 37 oC prior to the perfusion waiting for the drug. The second patient was very fat and probably the probe reach only the subcutaneous tissue, the temperature in circuit was 42 oC and probably the muscle had temperature to higher than the measured one. Discussion: The ILP learning curve can be better if we can show some steps of the procedure.

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P-168 COMPARISON OF DIFFERENT S-100 B PROTEIN CONCENTRATION CUT-OFF IN THE FOLLOW-UP OF MALIGNANT MELANOMA: A 5 YEARS EXPERIENCE

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Objective. Serum S-100 B protein (S100) is the most widely applied among the several biomarkers proposed for malignant melanoma (MM). We previously demonstrated (Dorizzi et al. Clin Biochem 2005; 38:197-8) the overall reliability of the 250 ng/L cut-off in the clinical follow-up of patients suffering from MM. The aim of the present study was to compare the diagnostic power of 175, 250 and 300 ng/L S100 concentration cut-offs. Materials and methods. S100 concentration was measured in 1457 blood samples obtained from 254 patients consecutively admitted for malignant melanoma with histologically confirmed stage I and II disease (Breslow > 1 mm) using an automated chemiluminescence analyzer (Liaison, Diasorin, Saluggia, Italy). Blood samples were collected at time 0 and in the same day of the periodical (3-4/year) clinical examinations; all the samples were collected between 11.00 and 12.00 a.m., and results were sent to the plastic surgeon in charge of the patient within 2.00 p.m. just before the clinical examination. Mean follow-up was 28 months and all patients had no evidence of disease after their primary surgery and received adjuvant therapies during the study only when necessary. Results. Sensitivity, specificity, positive and negative likelihood ratios (LR) calculated at 175, 250 and 300 ng/L yielded respectively the following results: 175 ng/L= 0.84, 0.88, 7.19, 0.18; 250 ng/L: 0.74, 0.97, 33.1, 0.26; 300 ng/L: 0.55, 0.99, 40.8, 0.45. Conclusions. The present study confirms our previous results; the lower specificity (0.97 vs 0.99) is counterbalanced by a higher sensitivity (0.74 vs 0.55). According to Evidence Based Medicine a positive LR of 33.1 is quite effective for any diagnostic tool. On the basis of these data patients with values higher than 250 ng/L should be carefully re-examined looking for metastasis since high risk patients who might benefit from appropriate chemo/ immunotherapy could be earlier identified.

P-169 PROGNOSTIC FACTORS AFTER CERVICAL LYMPH NODE DISSECTION FOR CUTANEOUS MELANOMA METASTASES

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Objective Cervical lymph node dissection (CLND) is the surgical therapy for local control of regionally metastasized cutaneous head and neck melanoma. This study evaluated the outcome of patients undergoing a CLND in our institution in order to determine prognostic factors for recurrence free survival and overall survival after this procedure. Materials and Methods Hospital records of 66 patients with histologically proven lymph node metastases who underwent a curative or palliative CLND from 1982-2004 were analyzed. Characteristics of the patients, the primary tumor, and the surgical procedure were recorded. During follow-up, the incidence of local or distant recurrences was recorded and survival was determined. Results Of the 66 patients, a (modified) radical neck dissection was performed in 20 and a selective procedure in 46 patients. Five-year actuarial overall survival was 26%; recurrence-free survival was 22%. Neither primary tumor characteristics nor the extent of surgery was of prognostic value; the number of positive nodes affected both overall survival ($p=0.046$) and overall recurrence-free survival ($p<0.001$). Conclusion A selective CLND is the recommended procedure for patients with cervical metastases of cutaneous melanoma. The number of positive lymph nodes significantly affects the outcome of the patients.

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P-170 PALLIATIVE VALUE OF TNF-BASED ISOLATED LIMB PERFUSION IN METASTATIC MELANOMA PATIENTS.

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Objective Melanoma is a disease with limited treatment possibilities, once the tumor has metastasized systemically. In patients with bulky melanoma in-transit metastases, the local tumor burden may be so problematic that even in patients with systemically metastasized disease an amputation may be inevitable. Isolated Limb Perfusion (ILP) with Tumor Necrosis Factor-± (TNF) and Melphalan has proven to be an excellent limb-saving local treatment option in locally advanced extremity in-transit metastases. We investigate here the palliative value of the procedure in stage IV melanoma patients. Materials and Methods From 1991 to 2003, out of 100 TNF-based ILPs performed on melanoma patients, 14 procedures were performed for stage IV melanoma. All patients had large and multiple tumors in the limb causing severe discomfort and making primary palliative surgery impossible. Patients underwent an ILP with TNF and Melphalan of the lower limb with 50-140 mg Melphalan and 2-4 mg TNF. In two patients, interferon was added to the perfusate according to the then valid trial prescriptions. Results In the stage IV melanoma patients, complete response on ILP was 43%, partial response was 50% and in 1 patient (7%), no response was recorded. Three patients experienced local progression 2, 2 and 3 months after partial response on ILP respectively. Thus, local control could be obtained in 10 patients. Local and systemic toxicity was mild to moderate in all cases, which is reflected in the median hospital stay of 9.5 days (range 4-17). All melanoma patients preserved their limb during a median survival time of 7 months. Conclusions TNF-based ILP is an excellent procedure to provide tumor control and limb salvage for the short survival time of metastasized patients with very bulky limb-threatening extremity tumors.

P-171 RECURRENT ACRAL LENTIGINOUS MALIGNANT MELANOMA

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OBJETIVE: we report a 67-year-old man affected of an acral lentiginous malignant melanoma in the foot. The lesion is excised using wide margins, but ten years follow-up of patient shows four recurrences of lentiginous malignant melanoma close to primary scar. CASE REPORT: a 67-year-old man has a pigmented lesion in his right heel in 1994. The lesion suspicious for melanoma is removed with narrow margins, and pathology report describes an acral lentiginous malignant melanoma (Breslow thickness 2mm, Clark level IV). More than ten years follow-up evidence four recurrences consisting of small pigmented lesions appearing close to primary scar (1998, 1999, 2004, 2005). These lesions are removed with histologic confirmation of acral lentiginous malignant melanoma in situ. Interval history and physical exam, laboratoty tests and imaging studies... donÂ t evidence systemic disease. CONCLUSION: we discuss this case report, the atypical melanoma behaviour and the few similar cases published in the literature. REFERENCES: Kuchelmeister C, Schaumburg-Lever G, Garbe C. Acral cutaneous melanoma in caucasians: clinical features, histopathology and prognosis in 112 patients. Br J Dermatol. 2000 Aug;143(2):275-80.

P-172 RECURRENT MELANOMA IN OLDER PATIENTS

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OBJECTIVE: The incidence of melanoma in patients (65 years has nearly tripled and the mortality rate more than doubled over the past two decades. In the United States, more than half of new melanomas are diagnosed in patients over age 60. While early stage melanoma is often easily treated, recurrent disease may require more complex and potentially morbid therapy. We undertook this study to evaluate outcome after treatment of recurrent disease in melanoma patients (65 years. MATERIALS & METHODS: We studied 161 consecutive patients (65 years diagnosed with melanoma, entered prospectively into our Melanoma Registry and followed for a mean of 46 months or until death. Statistical analysis was done with SAS software. RESULTS: Patient age ranged from 65 to 94 years (median 76 years). Of the 161 patients, 20 died of melanoma and 28 died of other causes. Nine patients (6%) were diagnosed with a second primary melanoma. Recurrence was diagnosed in 32 patients (20%) at a mean of 25(4 months after the initial diagnosis. The site of first recurrence was local in 28%, regional in 31% and distant in 41%. Mean survival after recurrence by treatment modality was 4(9 months for observation, 7(11 months for medical therapy (chemotherapy, biologics, hormonal agents) and 38(6 months for surgery, $p<0.01$. Surgical treatment included thoracoscopic resection of lung metastases, craniotomy, parotidectomy, radical lymphadenectomy, ear amputation and wide excision with skin grafting. There was no 30-day postoperative mortality and morbidity was minimal. CONCLUSION: These data suggest that elderly melanoma patients should be followed and treated for both recurrent and new primary melanoma much as younger patients are. While treatment should be individualized based on life expectancy from comorbid illness, standard treatment that may prolong life and well being should not be denied solely on the basis of age.

P-173 MELANOMA XENOGRAFTS ARE SENSITIZED TO MELPHALAN BY MIBG AND HYPERGLYCEMIA SUGGESTING THERAPEUTIC GAIN

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The respiratory inhibitor meta-iodobenzylguanidine (MIBG) in combination with excess glucose leads to inhibition of oxygen consumption and excess lactate production in human melanoma cells. Furthermore, melanoma xenografts are sensitized to radiation and hyperthermia by hyperglycemia + MIBG by virtue of tumor oxygenation and acute acidification, respectively. Magnetic resonance spectroscopy (MRS) demonstrated that hyperglycemia and MIBG reduced intracellular pH (pHi) and extracellular pH (pHe), and coincidentally, reduced the nucleoside triphosphate: Pi ratio in tumors; wheras, in liver there were only minimal changes in pHi, pHe and the nucleoside triphosphate:Pi ratio. These results suggest the synergetic effects of hyperglycemia and MIBG are largely selective for melanoma in comparison with normal tissues (Zhou et al., Cancer Res. 60:3532-6, 2000). Axial bone marrow in some mice may have areas that are hypoxic (Allalunis-Turner and Chapman, Int. J. Radiat. Biol. 49:415-22, 1986) and may be chronically acidotic. Thus, it is possible that bone marrow may be a limiting normal tissue for the techniques that enhance tumor acidification during thermoradiotherapy. We now show that human melanoma xenografts growing in the hind limb of nude rats are sensitized to melphalan by MIBG and hyperglycemia during isolated limb perfusion. By contrast irradiation or heating tibias of Balb/c male mice in the presence or absence of hyperglycemia with or without MIBG at the same concentrations used for sensitization of tumor response does not sensitize bone marrow to radiation or hyperthermia. These results suggest that MIBG plus hyperglycemia do not sensitize this normal tissue, and suggest a potential increase in therapeutic gain by selective acute acidification and oxygenation of melanomas with MIBG + hyperglycemia. Results and a proposed mechanism involving the expression of the glycolytic enzyme 6-Phosphofructo-2-kinase/Fructose-2,6-bisphosphatase-isoform 3 (PFKFB3) in melanomas will be presented. (Supported by P01 CA56690).

P-174 PROLONGED SURVIVAL FOLLOWING COMPLETE SURGICAL RESECTION OF STAGE IV MELANOMA AT THE SYDNEY MELANOMA UNIT

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OBJECTIVE: The median survival once melanoma has metastasized to distant sites is only 6-8 months. The one treatment factor that has consistently been noted to predict survival is complete resection, and many retrospective studies have demonstrated increased survival in a selected group of patients following surgical resection. The aim of this study was to document the median survival after aggressive surgical resection of metastatic melanoma and to evaluate survival following surgical removal of metastases at multiple sites. MATERIALS & METHODS: Patients with AJCC Stage I or II disease that progressed to Stage IV who had undergone complete surgical resection and were in remission were identified from the computer database of the Sydney Melanoma Unit for the period 1990 to 1994. The primary objective to evaluate overall survival after complete surgical resection and review disease free survival from the time of complete resection to either the next metastatic recurrence or last clinical follow up. RESULTS: A total of 84 patients met the study criteria. Of those that had undergone complete resection a total of 69 patients had systemic metastases, 10 patients had non-regional lymph node metastases and 5 patients had skin and soft tissue metastases. The median overall survival of the 69 patients presenting with systemic metastases was 40 months (range 1.5-164 months). These patients included 25 with resected lung metastases, 14 with resected cerebral metastases, 17 at other systemic sites and 13 with multiple metastatic sites. There was decreasing survival outcomes within these subgroups. CONCLUSIONS: This retrospective study highlights the benefits of aggressive surgical resection of distant metastases, demonstrating good survival outcomes. Keywords: Melanoma, Surgery, and Survival Word Count: 263 words

NOTES:

P-175 LACK OF STABILITY OF MELPHALAN IN NORMAL SALINE - IMPLICATIONS FOR ISOLATED LIMB INFUSION

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Isolated limb infusion (ILI) is used in the palliation of multiple inoperable melanoma metastases confined to a limb. ILI requires high dose melphalan to be administered after dilution in normal saline at 38oC. It has previously been shown that melphalan in saline at 38oC is unstable over 4-6 hours. However current UK practice requires that the melphalan/saline mixture is made up in pharmacy and subsequently distributed to the ILI team. In our institution this can take up to 100 minutes, and is distinct from the published method of adding melphalan directly to the infusate at the time of the procedure. Therefore we have examined the stability of melphalan in normal saline, with and without actinomycin-D and heparin, normal constituents of the ILI infusate. Melphalan in conventional non-saline diluent was further diluted in normal saline at 38oC at a concentration of 0.1mg/ml. Melphalan levels were measured at 60 minute intervals over 5 hours by High Performance Liquid Chromatography (HPLC), using established methods. The procedure was repeated using melphalan in combination with actinomycin D and heparin. Melphalan in saline at 38oC degrades with second-order kinetics at a rate of approximately 10% h −1 to hydroxymelphalan, the principle in vivo metabolite, and one other degradation product. This rate is not affected by actinomycin D and heparin. These results confirm and extend previous reports of the instability of melphalan in saline and need to be taken into account when interpreting ILI pharmacokinetics. Addition of melphalan to saline immediately prior to ILI is essential to avoid loss of efficacy due to drug degradation, and is the method that should be followed by institutions which provide this procedure.

P-176 THE RESULTS OF ISOLATED LIMB PERFUSION (ILP) WITH MELPHALAN AND MILD HYPERTHERMIA FOR IN TRANSIT MELANOMA METASTASES IN MÉXICO.

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Objective: In transit metastases occurs in 5%-8% of high risk melanoma patients and in 23% of sentinel node positive patients. The overall response rate is about 62%-100% with isolated limb perfusion with melphalan and regional hyperthermia. We report the experience with ILP in México. Materials and Methods: We conducted a prospective program of Isolated Limb Perfusion and include in seventeen patients with histologically proved in transit melanoma metastases with no other site of disease, since november 2002 to august 2004. All perfusions were done with melphalan (10 mg/L in leg and 13 mg/L in the arm) and mild hyperthermia (39-40°C). The leakage monitoring was performed with Tc99 radiolabelled erythrocytes with a hand held gammaprobe. Results: There were nine patients (10 women, 7 men) with a median age of 56 years (range 38-75). The level of perfusion was iliac (6), femoral (9) and brachial (2). The response rate was complete in two patients and partial in twelve, for an overall response rate of 82%. The median leakage was 3.1% (0-6%). Regional toxicity was recorded according to Wieberdink scale, eight patients had grade II and one patient had to be disarticulated (grade V). With a median follow up of 18 months (1-24 months, median 4.8) nine patients are alive with no evidence of disease and two are alive with systemic disease (lung and skin outside the perfunded leg or arm). The mean time to progression was 21 weeks. No recurrence or progresion in perfunded area was observed. Conclusions: Isolated Limb Perfusion with melphalan and hyperthermia is efficient therapy for melanoma in transit metastases. The poor results with other therapies and the overall response rate with ILP support the development of the program in our country.

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P-177 RADIOFREQUENCY THERMAL ABLATION OF LIVER MELANOMA METASTASES. A STUDY OF 19 CASES

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Melanoma with liver metastases represents a disease stage with severe prognosis and few conventional therapeutic options. Percutaneous Radiofrequency Thermal Ablation (PRTA) is a kind of energy formed by electromagnetic waves with frequency between 300 and 800 KHz. We treated with PRTA selected cases of liver melanoma metastases in order to evaluate necrosis of treated lesions, local recurrence rate, overall survival, local or general toxicity and quality of life for patients. Nineteen patients (eleven males and eight females) with recorded diagnosis of liver melanoma metastases were treated at our Institution between 01.01.2000 and 31.12.2004. The cases were included independently from characteristics of primary melanoma. Patients were treated under local anesthetistic attendance, in regimen of shelter for one night. Determined by the limits of the technique, our protocol includes patients with at least three lesions and a maximum diameter of three cm each one. Fourteen patients had only one epatic lesion at the diagnosis; four patients had two distant spread and one case presented three liver metastases. Complete necrosis of tumor was obtained in all cases. The average lenght of overall survival for patients was 9,7 months. Eleven of them died for progression of disease; five are alive with evidence of distant spread; three patients are alive without clinical and radiological evidence of disease. There were no recurrences in the sites were liver metastases underwent to a complete necrosis. PRTA didn't determine local or general toxicity and was well tolerated by patients.The most frequent syntoms were moderated abdominal pain, nausea and asthenia for some hours after treatment; the morning after the patients were discharged. RPTA is a low invasive treatment which can be performed in combination with other therapies in some selective cases of melanoma liver metastases, to obtain a good local control of disease with minimal pain for patients.

P-178 TWENTY YEARS EXPERIENCE OF MULTIPLE ISOLATED LIMB PERFUSIONS IN THE MANAGEMENT OF RECURRENT CUTANEOUS MELANOMA

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OBJECTIVE Cutaneous limb recurrence of melanoma remains a complex therapeutic problem with isolated limb perfusion (ILP) being the mainstay of treatment. Our aim was to determine if repeat ILP offers effective palliation of this condition. METHODS Case notes of 29 patients treated with multiple ILP for recurrent cutaneous melanoma were reviewed to determine if response rate or recurrence varied from the initial ILP. Follow-up data was obtained from the Scottish Melanoma Group database. RESULTS 29 patients underwent multiple ILP (2-4) using melphalan alone or melphalan with tumour necrosis factor - alpha. Repeat ILP achieved a response rate of 97% (complete response 35%, partial response 62%). This is comparable to the response rate after initial ILP. Disease control was maintained for a mean of 11.8 months (range 2-90 months). CONCLUSION Repeat ILP, though technically demanding, remains an effective therapy in the palliation of recurrent melanoma and should be considered for appropriate patients.

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P-179 BIOCHEMOTHERAPY FOR METASTATIC MELANOMA: THE IMPORTANCE OF DOSE INTENSITY

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Background. Despite a decade of study, the utility of biochemotherapy for metastatic melanoma remains controversial, as the favorable single-institution phase II results have not been confirmed by phase III studies. Dose intensity has been recognized as an important determinant of response and survival in the chemotherapy of several malignancies, but has not been studied in biochemotherapy. In this study we describe the relationship between achieved dose intensity and response rate of inpatient decrescendo biochemotherapy at our center. Methods. A retrospective study of 38 consecutive patients with metastatic melanoma treated between September 2002 and July 2004 was undertaken. The planned doses were dacarbazine 800 mg/m2 on day 1 or temozolomide 150 mg/m2 d 1-4, cisplatin 20 mg/m2 d 1-4, vinblastine 1.5 mg/m2 d 1-4, interferon-alpha-2b 5 million IU (MIU)/m2 d 1-5, and interleukin-2 36 MIU on d 1, 18 MIU on d 2, and 9 MIU on days 3 and 4. Six cycles at 21-day intervals were planned. Results. Of 38 patients that received a total of 204 cycles of therapy, 8 (21%) complete response and 14 (37%) partial responses were observed for an overall objective response rate of 58%. The number of responses at 18 weeks (22/38, 58%) was higher than the number of responses at 9 weeks (14/38, 37%) suggesting that six cycles of therapy may be necessary for maximum response. The median overall survival was 16 months. Eleven patients received less than six cycles of therapy, three because of toxicity and eight because of disease progression. 85% of cycles 2-6 began on time. Achieved dose intensity (actual dose/planned dose) was high with patients receiving 98.7% interleukin-2, 87.1% interferon, 90.7% DTIC, 94% temozolomide, 87.2% cisplatin, and 89.7% vinblastine. Conclusion. Six cycles of inpatient decrescendo biochemotherapy can be given with a high administered dose intensity and acceptable toxicity. This is the first study, to our knowledge, of biochemotherapy to report detailed data on the dose intensity achieved for all administered drugs for each cycle and the number of cycles administered on time. High response rates with biochemotherapy for melanoma may correlate with dose intensity, dose density, and the number of cycles given on time.

P-180 THE RADIOTHERAPY IN THE CURE OF THE CUTANEOUS MALIGNANT MELANOMA: INDICATIONS

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OBJECTIVE: Esteem that annually, to world-wide level, is taken place approximately 100.000 new cases of cutaneous melanoma (approximately 15% in more than in the previous decade). That corresponds to approximately 1% of the total of the malignant tumors. MATERIALS & METHODS: The use of the radiotherapy as adiuvante therapy in the patients with the cutaneous malignant melanoma has been hindered from the sideboard that the cells of melanoma were radioresistent. In demonstrated literature it has been hour that the native place-regional recidivism of the melanoma is common after the surgery when present they are determined characteristic clinician-pathological. Between the factors that more frequently are associated to elevated risk of recidivism of the primitive tumor are the subtypes desmoplastico, the positivism of the microscopically margins, the presence of thick primitive lesions and with the ulceration. An elevated risk of recidivism is associated also to the linfonodale involvement with the extension to extracapsulare, a four or more lymph nodes interest and dimension > 3 centimetres. CONCLUSION: Numerous studies support the effectiveness of the radiotherapy in these clinical situations. They give to you in the literature indicate that the x-ray is effective in the eradicazione of the lymph nodes micro metastases and those loco regional after removal of the primitive melanoma. Consequently, there is the possibility to integrate the x-ray in the treatment of multimodale of the patients to high risk of recidivism and subclinico lymph nodes interest, especially in positive case of lymph nodes lookout. In such patients it could be opted for the use of the x-ray makes us external in substitution of the regional lymph nodes dissection.

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P-181 COMPARATIVE STUDY TO EVALUATE THE BENEFITS OF POSITRON EMISSION TOMOGRAPHY VERSUS COMPUTORIZED TOMOGRAPHY IN MALIGNANT MELANOMA

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Positron emission tomography with Fluorodesoxyglucose (FDG-PET) is a new method for staging and follow-up of melanoma patients (pts). The cost of this technique implies a criterious selection of the patients. Our aim was to compare the results obtained by computerized tomography (CT) and FDG-PET in restaging pts with bad prognosis melanoma (thick, ulcerated or with vascular or lymphatic invasion). We studied retrospectively 40 pts, 20 male and 20 female, with a mean age of 54,7 years (20-86 y), for restaging. Localization of primary tumours was: skin - 31 pts; vulva - 1 pt; anus - 2 pts; eye - 4 pts and unknown - 2 pts. In all the pts, except 3, histological samples of the primary lesion were reviewed in our Institution. At the time PET was required, staging of the pts was: stage I - 1 pt; stage II - 9 pts; stage III - 10 pts and IV - 20 pts. FDG-PET and CT had a mean interval of 3 months. Pts were divided in four groups: A) CT negative/FDG-PET negative - 7 pts; B) CT positive/FDG-PET negative - 9 pts; C) CT positive / FDG-PET positive - 18 pts; D) CT negative / PET positive - 6 pts. PET and CT had a concordance of 62,5%. PET understaged 9 pts (22,5%) and detected unknown metastasis in 6 pts (15%). In group C we analysed also the number of lesion/organ in the two techniques. In group B, the suspected lesions were mainly pulmonary and all the patients are disease-free (mean follow-up - 1 year). CONCLUSIONS: PET altered patient staging in 37,5% of our group and was more reliable in cutaneous, lymph node, pulmonary and bone lesions. In advanced disease, PET is a good method for staging and therapy evaluation.

P-182 REGIONAL PERFUSION FOR LIMBS' IN TRANSIT METASTASES FROM MELANOMA: RESULTS IN A SINGLE INSTITUTION

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The efficacy of Isolated Limb Perfusion (ILP) for the treatment of locally recurrent melanoma and in-transit metastases (ITM) is well established and is currently considered the treatment modality of choice. However, the optimal drug regimen to be used as well as powerful prognostic factors that could help select favourable patients have yet to be defined. 260 consecutive patients with stage IIIB/IIIC melanoma of the extremities underwent 296 ILP procedures at the National Cancer Institute of Milan. The ILPs were carried out with 5 different drug regimens. A second ILP was performed in 33 patients, and a third perfusion in 3 patients. Treatment-related mortality was observed in 2 (0.8%) patients. Regional toxicity was graded according to Wieberdink scale. Severe or grade V toxicity occurred in 4 (1.5%) patients only. Median follow up was 83 months. Melphalan with mild or true hyperthermia and the Melphalan, g-Interferon and r-TNFa (MIT) regimen had higher response rates than the other two regimens. Five-year overall survival (OS) was 40% and median survival was 42 months. Stage IIIB and IIIC patients had a 5-year OS rate of 49% and 27%, respectively (p=0.004). Complete, partial and minor responders had a 55%, 33% and 20% 5-year OS, respectively (p<0.001). ILP performed with L-PAM is an effective treatment modality with acceptable morbidity. A second and third ILP did not cause increased morbidity and provided improved local control. Stage of disease and the extent of immediate response are two significant prognostic factors.

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P-183 METRONOMIC CHEMOTHERAPY PLUS/MINUS ANTIINFLAMMATORY TREATMENT IN FAR-ADVANCED MELANOMA: A RANDOMIZED MULTI-INSTITUTIONAL PHASE II TRIAL.

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Objective: Recently we have shown that control of tumor-associated inflammatory processes may result in improved progression-free (PFS) and overall survival (OS) independent of tumor type (Cancer 2003, 2004, Br J Haematol 2005). Methods: A randomized multi-institutional phase II trial was designed to select metronomic chemotherapy (Arm A: trofosfamide 50 mg po three times daily, day 1+) or combined anti-inflammatory/ metronomic treatment (Arm B: trofosfamide as abovementioned plus rofecoxib 25 mg po, day 1+, and pioglitazone 60 mg po, day 1+) for further evaluation in a phase III trial. Following disease progression cross-over from Arm A to B was allowed. Results: Patient characteristics: Eligible patients (pts) Arm A: 32, Arm B 35. Treatment characteristics were equally balanced for age, AJCC stage, performance status, number of prior chemotherapies (A: 62%, B: 60% of pts), and C-reactive protein (CRP). Two pts achieved objective response (Arm A: 1 PR, Arm B: 1 CR). Median PFS of pts in Arm A was 1.2 months (0.9 to 1.5), in Arm B 2.0 mos (1.2 to 2.8), p = 0.002. Median PFS and OS for CRP responders (Arm A 6%, Arm B 69% of pts) was 2.0 vs 1.4 mos, p = 0.0098, and 18.8 months vs 5.3 months, p = 0.0092, respectively. The median OS of pts in Arm A was 8.2 mos (range 6.6 to 9.8), in Arm B 18.5 mos (5.9 to 31.1), p = 0.16. WHO Grade 3 (no Grade 4) toxicities were reported in 6 patients (19%) in Arm A and 10 patients (28%) in Arm B, due to higher rates of edema. Conclusion: The current phase II trial has shown for the first time that control of tumor-associated inflammatory processes may improve PFS beyond that achieved with metronomic chemotherapy, and that CRP response is a valid indicator for favorable PFS and OS.

P-184 "N-RATIO" AS A NOVEL PROGNOSTIC FACTOR FOR PATIENTS WITH STAGE III CUTANEOUS MELANOMA

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Objective Among stage III melanoma patients the 5-year overall survival can range between 27 and 70%. There is no consensus regarding the efficacy of adjuvant treatments for stage III, and the conflicting results might depend on the fact that only a subset of patients would deserve adjuvant treatment after radical surgery. We already demonstrated that the ratio between positive and excised lymph nodes ("N-ratio") can independently predict survival in patients with gastric carcinoma. Here, we tested the prognostic value of this parameter in patients with stage III cutaneous melanoma. Methods 214 consecutive patients underwent radical lymph node dissection for stage III melanoma. There were 95 males and 119 females, the mean age being 59 years. According to the last AJCC TNM classification, there were 51 stage IIIA, 72 stage IIIB, and 92 stage IIIC. Univariate and multivariate survival analysis was performed using the log-rank test and the Cox proportional hazard model, respectively. Independent variables were the following: patient age/sex, primary tumor site, Breslow thickness, Clark level, ulceration, absolute number of positive lymph nodes, total number of excised lymph nodes, TNM stage, and N-ratio. Results The median number of metastatic and excised lymph nodes was 2 (range 1-18) and 17 (range 14-36), respectively. Patients were categorized into three N-ratio classes according to the percentage of positive lymph nodes (less than 10%, N=91; 10-25%, N=62; more than 25%, N=61). At univariate analysis, all variables but sex and primary tumor site were significantly related to overall survival (P <0.05). Interestingly, multivariate analysis showed that the only N-ratio (P=0.006) and number of lymph nodes excised (P=0.01) independently predict survival. Conclusions This study shows that N-ratio is a novel independent prognostic factor for melanoma patients with lymph node metastasis. Moreover, is suggests that extended lymphadenectomy might have a therapeutic role in patients with cutaneous melanoma.

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P-185 IS ULTRASONOGRAPHY USEFUL TO DETECT LYMPH-NODE INVASION IN MELANOMA PATIENTS? RESULTS OF A META-ANALYSIS USING TECHNIQUES ADAPTED TO THE EVALUATION OF DIAGNOSTIC TESTS

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Objectives: Because treatment of distant melanoma metastases is poorly effective, attempts to diagnose nodal spread early are warranted to initiate earlier therapeutic lymphadenectomy. Physical examination alone often fails to detect nodal metastases or cannot unambiguously classify palpable nodes. The usefulness of lymph node ultrasonography, an inexpensive procedure, to improving nodal invasion detection during initial staging and follow-up of melanoma patients is debated. Our aim was to assess, using for the first time in this field meta-analysis techniques for diagnostic tests, the respective value of US and palpation examinations to detect nodal invasion in AJCC stage I-III melanoma patients. Methods: Five computerized databases were screened to December 2003. Original studies exploring the scope of our study were selected when they compared palpation and ultrasonography and provided calculated or calculable sensitivity and specificity values. Results: We selected 12 studies representing 6642 patients and at least 18,610 paired palpation and US examinations which were performed by trained physicians in 5 and 7 studies, respectively. Main limitations of selected studies were variations in the definition of false-negatives, and verification bias. Ultrasonography had a higher discriminatory power than palpation, with respective estimated odds ratios of 1755 [95% confidence interval (CI) 726 to 4238] and 21 [95%CI 4 to 111](p=0.0001), respective estimated positive-likelihood ratios of 41.9 [95%CI 29 to 75] and 4.55 [95%CI 2 to 18], and respective estimated negative-likelihood ratios of 0.024 [95%CI 0.01 to 0.03] and 0.22 [95%CI 0.06 to 0.31] Conclusion: Ultrasonography clearly more accurately detects lymph-node invasion than palpation and should probably be used routinely in melanoma patients.

P-186 TEMOZOLOMIDE FOR THE TREATMENT OF BRAIN AND SYSTEMIC DISEASE IN PATIENTS WITH METASTATIC MELANOMA. NATIONAL CANCER INSTITUTE SLOVAKIA-THE FIRST

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Temozolomide is a novel oral alkylating agent that is active against metastatic melanoma. Temozolomide crosses the blood-brain barrier and may be therefore effective in patients with brain metastases. Purpose of the study: To evaluate the activity and toxicity profile of temozolomide for the treatment of extracranial disease and brain metastases in patients with metastatic melanoma. Methods and patients characteristics: From 08/2002 to 03/2005 8 consecutive patients (pts) received oral temozolomide 200mg/m2, day 1-5, every 4 weeks. 7 patients were assessable for efficacy. Patients characteristics: 5 men, 3 woman. 4 patients had multiple brain metastases, 4 had one solitary lesion. 1 patient presented only with brain involvement. 4 patients with brain metastases were treated with whole brain external radiotherapy, 1 patient underwent surgical resection of solitary brain metastasis followed by external radiotherapy boost and 3 patients had stereotactic radiosurgery, before starting chemotherapy with temozolomide. Median age: 42 ys (26-55), median Karnofsky PS: 91% (80-100), median number of metastatic sites: 2,9 (1-4). 5 patients received temozolomide up front, 3 were pretreated. Median number of administered lines of chemotherapy: 1,5 (1-3). Dose intensity of temozolomide: 89% (72-100). Median number of given cycles: 3,63 (2 - 7+). Findings: We observed partial response in 2 patients/7 (28,6%), disease stabilisation in 2 pts/7 (28,6%), 3/7 pts progressed on the treatment (42,9%) in extracranial sites. Brain response was evaluable in 5 patients. Regression was seen in 3 patients, stable disease in 1 patient and progression in 1 patient. Median overall survival is 27 + weeks (13-35 +). Toxicity was seen in 2 pts/8: anaemia Gr. 4 - 1 pt, trombocytopenia Gr. 4 - 1pt, Gr. 2 - 1 pt, neutropenia Gr. 2 - 1 pt. Interpretation: Our first results show, that temozolomide is promising and safe treatment in patients with metastatic melanoma.

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P-187 HIGH DOSE INTRA-ARTERIAL CISPLATIN FOR ADVANCED RECURRENT DESMOPLASTIC MELANOMA IN THE MANDIBLE

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Introduction: Desmoplastic neurotrophic melanoma (DNM) is a rare variant of malignant melanoma associated with a aggressive behaviour dissimilar to other cutaneous melanomas. Treatment of advanced recurrent melanoma is limited with systemic chemotherapy and radiotherapy providing few durable responses. This case demonstrates a dramatic response to high dose intra-arterial chemotherapy in a gentleman with an advanced recurrent DNM which had failed previous multimodality treatment. Details: A 59 year old man presented with recurrent DNM in the left mandible with perineural spread to the skull base, 3 years after treatment of a lip primary with wide excision and postoperative radiotherapy. The disease progressed despite DTIC and further radiotherapy, his tumour presented through the mouth and was deemed unresectable due to the skull base disease. High dose intra-arterial (IA) of cisplatin with systemic thiosulphate rescue was offered as palliation. Result: Three weekly IA infusions of cisplatin initially resulted in near complete resolution of the intra-oral tumour mass for four months. The mass recurred and the patient represented requesting retreatment. Four further infusions were given resulting in complete disappearance of the oral tumour for 6 months. No complications resulted from any of the 7 infusions. The patient resumed normal work and family life during that period. The skull base disease inevitably progressed and the patient died from his melanoma more than 15 months after presenting with unresectable recurrence. Conclusions: High dose intra-arterial cisplatin proved to be effective palliation in this case of advanced, recurrent desmoplastic melanoma, previously resistant to standard systemic therapy. The dramatic response to this treatment suggests that its use as definitive therapy in locally advanced melanoma warrants further investigation.

P-188 TREATMENT OF UVEAL MELANOMA METASTATIC TO THE LIVER BY HEPATIC INTRAARTERIAL CHEMOEMBOLIZATION WITH CISPLATIN AND GELATIN SPONGE: A SINGLE-CENTER EXPERIENCE WITH SEVENTEEN JAPANESE PATIENTS

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OBJECTIVE: Uveal melanoma has a remarkable propensity for only hepatic metastasis and this pattern of dissemination differs from that of cutaneous melanoma. For Japanese patients with liver metastases, who have a median survival of 5 to 7 months, surgery and systemic conventional chemotherapy have little to offer. The objective of this study was to establish the efficacy and toxicity of hepatic intraarterial chemoembolization with cisplatin and gelatin sponge, in patients with liver metastases from uveal melanoma. MATERIAL and METHODS: Between May 1997 and December 2003, seventeen Japanese patients with isolated hepatic metastases from uveal melanoma were treated with hepatic arterial chemoembolization using cisplatin and gelatin sponge. The dose of cisplatin was set at 70mg/m2. The therapy was repeated every 3-4 months until progression or unacceptable toxicity. RESULTS: All patients were evaluated for the trial. Partial response (PR) of the tumor was observed in four patients, the response rate was 24%, eight had stable disease (SD), and tumor progressed in five patients (PD). The median survival time from diagnosis of liver metastasis was 24 months (range, 2 to 39+ months). Six patients survived for more than 2 years and six patients are still alive. Upper abdominal pain, appetite loss, nausea, and elevation of liver enzymes were observed as treatment related morbidity factors, but lasted briefly. CONCLUSION: Intraarterial chemoembolization treatment of uveal melanoma metastatic to the liver using cisplatin and gelatin sponge, is well tolerable by the patients and prolonged their survival time.

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P-189 ISOLATED LIMB INFUSION FOR RECURRENT EXTREMITY MELANOMA: MINIMALLY INVASIVE BUT REQUIRING A SIGNIFICANT HOSPITAL STAY

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Objective: Isolated limb infusion (ILI) has been recently developed as a minimally invasive alternative for the administration of regional chemotherapy to treat extremity in-transit melanoma. This technique is distinguished from the traditional surgical-based hyperthermic isolated limb perfusion (HILP) approach: percutaneous catheter placement, low-flow, hypoxia, and mild hyperthermia. We sought to compare the post-operative toxicities and length of stay (LOS) of these two modalities. Methods: All patients (N=19) who underwent ILI with melphalan and actinomycin-D were analyzed. Intra-operative arterial blood gases of the perfused limb were recorded in the ILI group. Post-operative creatine kinase (CPK) levels, signs of CPK-related toxicity (i.e., renal failure or compartment syndrome), and length of stay (LOS), were compared to those of 19 consecutive patients who underwent HILP (melphalan +/- TNF). Results: The median limb PaO2 and pH after 30 minutes of ILI were 16 mm Hg and 7.18, respectively. Serum CPK peaked significantly later in ILI (post-operative day [POD] 4) vs. HILP (POD 1) ($p<0.0001$). CPK levels also peaked significantly higher in patients undergoing ILI (3738u/l) vs. HILP (2319u/l) ($p<0.012$) (figure 1). There were no cases of myoglobin-induced renal failure or compartment syndrome seen in either group. The median LOS in both groups was 7 days. LOS in patients undergoing ILI was directly related to delay in, and resolution of, peak CPK levels and concomitant monitoring for signs of CPK-related toxicity. Conversely, LOS in patients undergoing HILP was associated with the surgical approach: post-operative pain, wound and drain care management, and return to an ambulatory state. Conclusions: Although ILI is minimally invasive, in-hospital monitoring is required as long as for patients undergoing HILP because of the delayed development of high CPK peaks, likely secondary to the hypoxic perfusion conditions. A multi-center efficacy and toxicity study of ILI is currently underway.

P-190 QUANTITATIVE ANALYSIS OF PROTEIN BIOMARKERS IN MALIGNANT MELANOMA PROVIDES MOLECULAR MODEL FOR OUTCOME PREDICTION.

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OBJECTIVE: To define prognostically-relevant molecular classifications of melanoma by in situ assessment of protein expression, toward the goal of developing a molecular assay to augment the prognostic value of Breslow depth and Clark level. METHODS: A 521 case tissue microarray, including 214 primary melanomas, 293 metastases, 14 local recurrences, 22 nevi, and 15 cell block controls (including melanoma cell lines and normal melanocytes) was constructed with complete clinical annotation. Candidate biomarkers were selected from previous studies evaluating prognostic potential of markers, gene expression profiling studies (cDNA, oligonucleotide, etc.), and functional studies on individual genes and gene products. The novel AQUA" (Automated Quantitative Analysis) technology was used to determine exact expression levels of protein biomarkers within nuclear, non-nuclear (i.e., cytoplasmic) and total melanocytic compartments. RESULTS: Sixty-six variables (derived from 48 independent protein assays) have been analyzed. Cox univariate analysis demonstrates that 32 are associated with a better outcome, while 34 are associated with a poor prognosis, however, only 16 are significantly ($p<0.05$) associated with outcome. The base markers for the significant analyses include: ATF2, CD44, p16, N-cadherin, E-cadherin, 2-catenin, Fibronectin, AP2, Osteonectin, PCNA, p21, Ki-67. Unsupervised clustering analysis of the significant markers demonstrates groups of patients with significant differences in melanoma-specific survival. For example, clustering as few as 3 markers defines a group of primary melanomas with 20% 5-year survival compared to other groups in the 70-90% range. Optimization studies are underway to define the smallest group with the most prognostic value. Preliminary analysis of misclassification error (for Breslow alone versus Breslow + one/multiple markers) for survival at 5 years reveals that the addition of 4-6 markers to Breslow reduces misclassification error by approximately 6%. CONCLUSION: We have demonstrated that it is possible to define molecular subsets of melanoma with clinical relevance, based on in situ measurement of protein expression.

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P-191 MORBIDITY AND MORTALITY OF MALIGNANT MELANOMA, CIENFUEGOS, CUBA, 1987 - 1998

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The objective of this study was to characterize the morbidity and mortality of malignant melanoma incidents in Cienfuegos, Cuba We carried out a retrospective series of case studies involving 73 patients with a hystologic diagnosis of malignant melanoma. The patients were identified through the National Cancer Registry and the Mortality Registry from the Provincial Department of Statistics and their clinical history reviewed between January 1, 1987 and December 31, 1998. Observed were a decrease incidence of malignant melanoma and a minimal increase of mortality over the final 5 years of the study. There were minimal differences related to the patients' sex, with a slight predominance of cases involving males (57.5%). White skin (97.28%) and extremities (localized areas on the body's 49.2%) were important factors. Furthermore, during the clinical and hystological diagnosis, primary stages 1 (59.4%) and 2 (33.3%) were predominant. The time between diagnosis and death was an average of 2.4years, with a total survival rate of 3 years at 84%.

P-192 APPLICATION OF SURFACE-ENHANCED LASER DESORPTION/IONIZATION (SELDI) IN MELANOMA BIOMARKER DISCOVERY

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The overall goal of this project is to test the feasibility of using of Surface-Enhanced Laser Desorption/Ionization (SELDI) in melanoma biomarker discovery. The ultimate goal of the project is to develop proteomic approaches to the early detection of melanoma that can be utilized for screening, prognostication, selection of therapy, identification of potential targets for immunotherapy and the measurements of responses to therapy. The approach involves making no a priori assumptions regarding the nature of the proteins that may be useful as markers of melanoma but rather provides a high throughput system for analyzing the entire sample, in this project, the serum. Serum was obtained from 25 patients with stage III or IV melanoma and 25 healthy volunteers. The serum was collected under similar conditions for both the patients and the volunteers. The samples were fractionated and processed for SELDI. Three major peaks were identified that had differential expression in the two populations. Two of the peaks had increased serum quantities in melanoma patients and one peak had a decreased quantity in melanoma patients as compared with the controls. Protein identification by mass spectroscopy to date has identified the proteins for two of the three major peaks. Transthyretin is diminished in the melanoma patient's sera. Similar results have been reported in patients with ovarian carcinoma. Human platelet factor 4 is increased in melanoma patients. This finding is interesting considering that this molecule has been shown to have anti-angiogenic properties. These findings show promise for this approach to biomarker discovery and the results are being validated on additional patients and other control populations.

P-193 EXPRESSION OF HLA-G IN THIN MALIGNANT MELANOMAS AS A POTENTIAL DETERMINANT OF METASTATIC POTENTIAL

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Background: Although the key prognostic indicator in malignant melanoma, Breslow depth alone cannot predict disease progression, as up to 5% of thin melanomas (d 1.00 mm) go on to metastasize. Increasing evidence suggests that melanoma is able to spread by expressing cell surface molecules that allow it to avoid immunodetection. The objective of the current study was to correlate expression of one such molecule, HLA-G, in thin melanomas with progression to metastatic disease. Methods: In the first phase of the retrospective case-control study, thin melanoma cases were identified through the Nova Scotia Cancer Registry. This group was divided into patients that had developed metastatic disease and those that had not. The second phase involved the immunohistological examination of the identified paraffin-fixed specimens. The primary lesions in the metastatic case group and a matched non-metastatic group underwent staining for HLA-G expression. Results: Nine-hundred thin melanoma cases were identified; 20 had documented progression to metastatic disease. Matched groups of non-metastatic thin melanomas as well as benign melanocytic nevi were also identified. Although the small number of case specimens limited quantitative analysis, herein we present preliminary qualitative results of our immunohistochemical analysis. Conclusion: Although initial staining findings are promising, efforts are ongoing to identify further metastatic cases to allow for quantitative analysis. If HLA-G is indeed expressed to a higher degree in thin melanomas that go on to metastasize, the molecule may prove to be a marker to identify that subset of patients that require closer monitoring.

P-194 IMMUNOMAGNETIC BEAD EXTRACTION OF CIRCULATING MALIGNANT MELANOMA CELLS: OPTIMISATION OF TECHNIQUE AND PROGNOSTIC SIGNIFICANCE OF A POSITIVE RESULT

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Two main dissemination pathways exist for metastatic spread of malignant melanoma, the blood stream and the lymphatic system. The biological mechanisms which determine lymphatic or haematogenous spread are unknown. Investigation of circulating cells may elucidate the specific mechanisms involved. A novel approach to identify circulating cancer cells has been the use of immunomagnetic bead extraction from blood samples. The advantage of this technique is that it retrieves potentially 'viable' cells for further immuno-, cytological and genomic investigation. Further, a positive finding of Melanoma cells in the blood by RT-PCR/ immunomagnetic bead extraction is reportedly associated with rapid disease progression and poor survival. An optimised immunomagnetic bead retrieval method based on the melanoma associated chondroitin sulphate proteoglycan antibody 9.2.27 and an anti-s100 antibody was used. Following ethics committee approval and written informed consent 20 ml of blood were drawn upon diagnosis of stage III/IV disease. 23 patients with malignant melanoma were included in this study, nineteen male and four female. The mean age was 62.5yrs (range 37 - 89). Six healthy volunteers acted as negative controls. 7 of 15 (47%) patients with stage III disease and 5 of 8 (62.5%) patients with stage IV disease had detectable circulating melanoma cells. A positive finding was associated with a reduced survival time, 5.2 months following a positive finding versus 9.5 months for negative patients, and poor overall survival, 9 of 12 positive patients (75%) have died of their disease, whereas only 2 of 14 (14.3%) negative patients have died of their disease, with an additional 3 patients (21.4%) alive with clinically detectable disease. Immunomagnetic bead retrieval of circulating cancer cells is a simple, yet effective method of identifying circulating melanoma cells in patients with stage III/IV malignant melanoma. A positive finding is associated with a reduced survival time and poor overall survival.

P-195 BETA-1,6-N-ACETYLGLUCOSAMINYLTRANSFERASE V AND PITUITARY-TUMOR TRANSFORMING GENE EXPRESSION IN MELANOMA.

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Background: Deregulated adhesion, aberrant glycosylation patterns and angiogenesis are characteristic of metastatic phenotype in melanoma. Beta-1,6-N-acetylglucosaminyltransferase V (GNTV) catalyzes the addition of beta-1,6-GlcNAc to N-glycan on glycoproteins. GNTV seems to be involved in focal adhesion turnover and signaling through PI3K/Akt. Pituitary-tumor transforming gene (PTTG1) functions through activation of growth factors, including bFGF mediated-angiogenesis. Methods: In silico expression for GNTV and PTTG1 in skin cancer were analyzed using representation profiles obtained with virtual northern blot (VNB). Gene-expression for these metastasis related markers were examined using reverse transcriptase (RT)-PCR in melanoma cells lines. Results: VNB data showed a significant (p=0.01) higher expression for GNTV in skin cancer compared with normal skin. There was a trend toward higher serial analysis of gene expression (SAGE) tags in skin cancer than in normal skin libraries for PTTG1. GNTV and PTTG1 mRNA expression were analyzed in FEM, A375 and CTL-OJC2 human melanoma cell-lines. CTL-OJC2 was developed in our laboratory from metastatic melanoma derived pleural effusion. Specific amplicons for GNTV and PTTG1 were demonstrated by RT-PCR in all the melanoma CL tested. Conclusions: Our data confirms that GNTV and PTTG1 are expressed in human melanomas. They could be further investigated as surrogate molecular markers for angiogenic phenotype and invasive properties in melanoma

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P-196 INCREASED GENE EXPRESSION LEVELS OF COLLAGEN RECEPTOR INTEGRINS ARE ASSOCIATED WITH DECREASED SURVIVAL PARAMETERS IN PATIENTS WITH METASTATIC MELANOMA

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Expression of collagen receptor integrins alpha1/beta1 and alpha2/beta1 has been associated with progression and metastatic potential of malignant melanoma. Integrin alpha2/beta1 was originally characterized as a melanoma progression antigen, but to our knowledge, the expression of alpha10 or alpha11 integrins has not been described in melanoma before. OBJECTIVE: We wanted study, whether collagen receptor integrins might have prognostic value in metastatic melanoma. MATERIALS & METHODS: We used real-time quantitative RT-PCR to study the mRNA expression levels of all putative collagen receptor integrin chains, i.e. alpha1, alpha2, alpha10 and alpha11 in 30 patients melanoma metastases. RESULTS: We found that high expression levels of alpha10 mRNA in these tumors (n=13) was associated to poorer disease-free survival (DFS; mean 11 months) when compared to patients (n=17) with lower alpha10 mRNA expression levels (mean 27 months, p=0.029). Similarly, survival after initiation of biochemotherapy was significantly decreased in all patients, whose tumors expressed high mRNA levels of alpha1 integrin, alpha2 integrin or alpha11 integrin when compared to lower tumor expression levels. Furthermore, high mRNA expression levels of integrin alpha2 and alpha10 were found to be associated with poorer overall survival. High alpha2 mRNA levels (n=9) predicted median survival of 35 months and low alpha2 mRNA levels (n=21) median survival of 53 months (p=0.021). Patients with low alpha10 mRNA expressing tumors (n=13) survived 112 months, but patients with high alpha10 expressing tumors (n=17) survived only 35 months (p=0.010). CONCLUSION: We conclude that collagen receptor integrins are important in the progression and prognosis of metastatic melanoma and their measurements might be used as predictive markers when assessing disease progression.

P-197 GAGE PROTEIN EXPRESSION IN MALIGNANT MELANOMA

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The GAGE family of tumor-associated antigens is present in a wide spectrum of human tumors but its expression in normal tissues is restricted to testis. So far, it has been reported that GAGEs expression on mRNA level implies poor prognosis in malignant melanoma. We have generated a rabbit polyclonal antibody for characterization of GAGE-7b protein expression. GAGE-7b protein was cleaved from recombinant GST-fusion protein by thrombin and purified by anion exchange chromatography using a HPLC-operated MonoQ column. Purified GAGE-7b was used for rabbit immunization and subsequent purification of the monospecific antibody from the rabbit hyperimmune serum. The antibody was used in both Western blotting and immunocytochemistry. 50% of all melanoma cell lines tested (n=14) showed expression of GAGE-7b in Western blotting. GAGE-7b immunostaining could be abolished by preincubation of the antibody with antigen. Moreover, 6% of melanoma patients (n=72) showed detectable autoantibodies against the recombinantly expressed GAGE-7b protein in comparison to 0% recognition in controls (n=36). In conclusion, we describe for the first time the generation of a rabbit polyclonal antibody against GAGE-7b which can be used in Western blotting and immunocytochemistry. Most likely, the antibody recognizes not only GAGE-7b but all 8 different GAGE proteins since they mainly differ by single amino acid substitutions. Using our GAGE-specific antibody we can now investigate functional aspects of GAGE expression in malignant melanoma.

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P-198 POSTTRANSLATIONAL REGULATION OF CD44 MEDIATED FUNCTIONS IN CELLS OF MELANOCYTIC ORIGIN

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Adhesion between CD44 receptor and hyaluronic acid plays an important role in cell migration, tumor growth and progression. Although the alternative splicing of CD44 variant exons represents the most important regulatory mechanism of the CD44-mediated functions, we provide evidence that CD44 spliced variants are scantily expressed on melanoma cells. This means that melanoma cells preferentially express the standard CD44 receptor (CD44s 90Kd or CD44H), the putative receptor for hyaluronic acid, and may likely regulate its function at posttranslational level. By using metabolic strategies to inhibit N- and O- glycosylation in vitro, as well as melanoma transfectants expressing CD44s O-glycosylation site-specific mutants, we performed structural and functional analysis of N- and O-deglycosylated CD44s molecules expressed in melanoma cells. We discovered that complete N-and O-glycosylation is not required by CD44s to be correctly expressed on melanoma cell surface. Indeed, variably glycosylated and functionally different CD44s molecules are constitutively expressed in primary and metastatic melanomas. Furthermore, we demonstrate that CD44s cleavage was regulated at post-translational level also. In fact, spontaneous CD44s shedding was dependent on the presence of partial or complete O-glycosylation of four serine-glycine motifs localized in the membrane-proximal CD44s ectodomain. Mutation of these serine residues, as well as an extensive metabolic O-deglycosylation in vitro, strongly impaired CD44-shedding. Moreover, an O-glycosylation-independent mechanism of CD44 cleavage has been identified. This alternative mechanism of CD44 cleavage is PMA inducible, is influenced by the activity of metalloproteinase and requires the presence of N-linked sugar residues. Thus, we conclude that posttranslational modifications of CD44s (i.e. different degree of glycosylation) are crucial for modulating CD44s-mediated functions in cells of melanocytic origin.

P-199 TUMOR REGRESSION AND ANTITUMOR IMMUNITY BY COMBINED CRYOTHERAPY AND TOPICAL IMIQUIMOD IN A SUBCUTANEOUS MURINE MELANOMA

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OBJECTIVE: Cryotherapy has become an accepted therapeutic modality. Primary melanoma destruction with cryotherapy also can provide the immune system with an antigen source for the induction of antitumor immunity. Combinig this treatment with immune-potentiating strategies may improve the immune response induction. MATERIAL AND METHODS: We used the murine B16-OVA melanoma cell line to develop a tumor model (female C57BL/6 mice). We explore the efficacy of a combination approach of melanoma cryotherapy and immunostimulation with imiquimod. Mice were divided into four groups: control, cryotherapy, imiquimod, and cryotherapy + imiquimod. Tumors were treated with cryotherapy when their diameter measured 5-7 mm. Complete regression of tumors was observed in mice after 1 or 2 sessions of cryotherapy. After cryotherapy, during 16 days, a subgroup of mice received topical treatment with 75 mg of imiquimod. Forty days after cryotherapy of primary melanomas, mice were challenged by subcutaneous injection on the contralateral back with the same cell line. RESULTS: We demonstrate that following cryotherapy a tumor-specific immune response is induced, resulting in protection against a tumor rechallenge in 15-25% of the mice. This response was significantly potenciated by administration of imiquimod. Imiquimod treatment without cryotherapy was not sufficient to eradicate either the primary tumor or a tumor challenge given 25 days after topical application. CONCLUSION: This study support the combined use of cryotherapy and imiquimod for the induction of specific antitumor immunity.

P-200 DIFFERENCES IN THE MICRO-RAMAN SPECTRA OF NORMAL AND MALIGNANT MELANOCYTES

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Micro-Raman spectroscopy is a tool that gives information on the abundance and structure of molecules present in a sample on a microscopic scale. It works by focusing high intensity monochromatic light onto the sample and measuring the small frequency changes between incident and scattered light. These frequency changes can be correlated to molecular vibrations. The method is used routinely for research into semi conductors and super conductors, however it can be just as easily be applied to biological tissue where it can give important information on biological processes including the pathophysiology of disease. It is non-destructive and non-invasive and thus can be used in vitro or in vivo and used repeatedly at the same location. We used micro-Raman spectroscopy with an excitation spot size of about a micron in diameter to obtain spectra from normal and malignant melanocytes. We found that there were significant changes between the spectra from the two cell types. In order to try and quantify the observed changes we fitted the spectra with a linear combination of reference spectra using a maximum likelihood routine. The fits showed that there were changes in the contribution of nucleic acids, histones, actin like proteins, and other unidentified components to the spectra. These results show that micro-Raman spectroscopy has great potential to give information on the pathophysiology of melanomas and may be helpful in the development of a Raman based diagnostic method.

P-201 THE UNIQUE RAMAN SCATTERING AND NIR FLUORESCENCE PROPERTIES OF MELANIN IN VITRO AND IN VIVO

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BACKGROUND Optical properties of melanin are of great interests for skin photobiology and cancer biology researches. However, most studies of melanin optical properties, especially in vivo studies are focused on its absorption characteristics. Using a rapid Raman spectroscopy (RRS) system built in house, we observed unique Raman scattering signatures and near infrared (NIR) fluorescence emission of melanin from in vivo skin. This has led us to perform a detailed study on the Raman scattering and NIR autofluorescence properties of melanin in vitro and in vivo. MATERIALS AND METHODS Multiple laser wavelengths (457.9, 514.5, 632.8, 785 nm) and two conventional laboratory Raman systems as well as the RRS system were used to study melanins obtained from three different sources: synthetic DOPA-melanin, melanin extracted from cuttlefish (Sepia officinalis), and melanin within human and feline black hair. In vivo studies were performed with the RRS system and 785-nm laser on human skin including normal dark skin, benign melanocytic compound nevus, and malignant melanoma. The NIR fluorescence spectra were obtained as a background signal from the 785-nm excited Raman measurements and were extracted using polynomial data fitting. RESULTS The Raman signals of in vivo cutaneous melanin are similar to those observed from natural and synthetic eumelanins. The melanin Raman spectrum is dominated by two intense and broad peaks around 1580 1/cm and 1380 1/cm, which can be interpreted as originated from the in-plan stretching of the aromatic rings and the linear stretching of the C-C bonds within the rings, along with some contributions from the C-H vibrations in the methyl and methylene groups. Our results also demonstrated that under NIR excitation melanins exhibit prominent autofluorescence within the skin and its pigmented appendages. These unique Raman scattering and NIR fluorescence properties may have practical applications for melanin quantification and diagnostic evaluation of pigmented skin lesions.

P-202 FAMILIAL MELANOMA - 6 YEARS REVIEWS FROM HOSPITAL DO CÂNCER - SÃO PAULO - BRASIL

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BACKGROUND : It has been estimated that around 10% of cutaneous melanomas are hereditary. Risk families are characterized by: early onset of melanoma, higher risk of second primary frequent association with atypical nevi. The clinical criteria for definition of familial melanoma are not well defined in the literature, so we have defined some clinical criteria to identify familial cases of melanoma. OBJECTIVE : The main objective is to compare, based on clinical predefined criteria, sporadic and familial cases of cutaneous melanoma. METHODS : 407 cases of melanoma treated on the Department of Skin Oncology - Hospital do Cancer - São Paulo - Brasil, from january 1998 to october 2004 where reviewed. Patients with one major criteria or 2 minor ones where considered hereditary (major criteria: first degree relative with melanoma or pancreatic cancer; minor criteria: presence of atypical nevi, second primary melanoma, previous personal history of pancreatic cancer. RESULTS : thirty-three (8,1%) patients where identified as having clinical criteria for familial melanoma. Mean age was 44,7 yo, 45,5% had less than 40 yo. Second primary occurred in 9,1% of patients, atypical nevi histologically confirmed in 45,5% and histological evidence of previous nevi on the melanoma in 33,3%. Only one patient had pancreatic cancer. In sporadic melanoma group, mean age was 52,2 yo, 24,1% having less than 40 yo. Second primary melanoma occurred in 0,5% in this group, atypical nevi histologically confirmed in 4,0% and histological evidence of previous nevi on the melanoma in 16,3%. CONCLUSION : The clinical criteria used in this paper defined two groups with some differences. Clinical identification of familial cases is important in order to identify high-risk patients for cutaneous melanoma.

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P-203 COEXISTING MELANOMA AND RENAL CELL CARCINOMA IN THE SAME PATIENTS

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Background. There is an increased risk of renal cell carcinoma (RCC) in patients with cutaneous melanoma (M)1-2. Objective. To study clinical data in patients with coexisting RCC and M. Methods. A retrospective study was performed in our institution between 1975 and 2004. Thirty four patients had histologically confirmed coexisting M and RCC. Results. The mean age (16 women and 18 men) at diagnosis for both M and RCC was 52 years. The M was the 1st cancer for 28 patients. The mean duration between diagnosis of M and RCC was 7.5 years. Melanoma were located on head and neck (n=4), trunck (n=16), limbs (n=16), other (n=3) and consisted of SSM (n=22), LM (n=4), NM (n=3), ALM (n=1) and unclassified M (n=6). Histological type of RCC were clear-cell RCC (n=24), oncocytoma (n=2), Wilms tumour (n=3), papillary RCC (n=3), urothelial carcinoma (n=2), and chromophobe RCC (n=1). Ten patients (30%) had 3 to 5 tumours. Two patients had 2 RCC and 2 patients had multiple primary M. Others malignancies were lymphoma (n=2), colorectal carcinoma (n=2), bladder papillary carcinoma (n=1), and breast, prostate and cervical cancer (1 case each). Study of the family history showed in 23.5% of patients a familial M and in 5.8% of patients a familial RCC. Others cancers in first degree relative were gastric cancers (2 carcinoma and 1 GIST), head and neck carcinoma (n=3), bladder carcinoma (n=2), pancreatic cancer (n=2), lung and breast carcinoma (2 cases each), thyroid carcinoma (n=1). Conclusion. The increased risk for both M and RCC suggests coMon risk factors. The present study shows that nearly one fourth of the patients had a family history of M. In addition, a high aggregation of other primary cancers may suggest a genetic predisposition. References 1. Tihan T et al. Cancer. 1996;77:2325-31. 2. Schmid-Wendtner et al. Br J Dermatol.2001;145:981-5.

P-204 MALIGNANT MELANOMA AND THYROID CARCINOMA IN THE SAME PATIENT : ANALYSIS OF A SERIES OF 21 PATIENTS AND SEARCH OF BRAF GERMLINE MUTATIONS

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Background: BRAF somatic mutations had been identified in tumor tissues of papillary thyroid carcinoma (TC) and of primary melanoma (M). Objective : To perform a clinical analysis of a series of 21 patients(pts) with coexisting M and TC, and to seek for a BRAF germline mutation. Methods: Mutations of BRAF were sought in 16 pts (DHPLC, exons 11 and 15). Mutations of CDKN2A were sought in pts with multiple M. Results: Mean age of 15 women and 6 men at 1st cancer was 37 years(y). M and TC were respectively the 1st cancer in 8 and 9 cases. Mean age at diagnosis of TC and M were respectively 42 y, and 47 y. Mean interval between TC and M was 11 y. M was located on the trunk (7 pts), on limbs (9 pts) and on head and neck (5 pts). Pathological analysis available in 19 pts showed 1 mucous M , 18 M (11 SSM, 1 ALM, 2LM, 1 desmoplastic, 3 unclassified) with a mean Breslow of 2.06 mm. Pathological types of TC were: papillary (13 pts), follicular (5 pts), and unknown (1 pt). Seven pts had had 3 tumors including 3 pts with a 2nd M, 1 pt with a meningioma, 1 pt with a cardiac myxoma. Four pts had 4 cancers including 1 pt with 3 M. Other cancers were carcinoma of the breast or cervix uteri, Hodgkins disease and cutaneous sarcoma. Family history for M was present in 1 pt and for TC in 3 pts. No germline mutation of BRAF but one of CDKN2 (G101W) was identified. Conclusion: although TC were predominantly of the papillary type, no germline mutation of BRAF was identified. Association of M and TC may be coincidental. However, in 1/3 of these pts, there was an important aggregation of cancers.

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P-205 TUMOUR PATTERNS IN THE FAMILIES OF PATIENTS WITH FOUR OR MORE PRIMARY TUMOURS INCLUDING AT LEAST ONE MALIGNANT MELANOMA.

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Objective: Patients with four or more primary tumours including at least one cutaneous malignant melanoma (CMM) are rare. In a previously published study (1) all these patients in southern Sweden between 1958 and 1999 were investigated and screened for the Swedish CDKN2A/p16 associated founder mutation (113insArg). Four specific tumour patterns were found; multiple melanomas (MCMM) at a younger age associated with non-melanoma skin cancer (NMSC), MCMM associated with neurinomas and meningeomas, and single melanoma associated with adenocarcinomas and NMSC respectively at higher ages. Four p16 mutation carriers were found. The aim of the present study was to investigate the tumour incidence in the families in relation to the index patients tumour pattern. Methods: All first- and second-degree relatives of the previously described 44 patients were traced via the Swedish Population Registry.Tumours occuring among the relatives were identified through the population based Swedish Cancer Registry and vitala status and cancer incidence were studied until December 2004. Results: 624 relatives were found. Some families were large (> 50 relatives) but some contained only few relatives. The p16 associated families also had extensive family histories of melanoma. The diversity in tumour presentation within and among the other families will be presented in relation to subgroup at the meeting. Discussion: This rare group of patients with multiple primary tumours and the tumour patterns of their relatives might give us new clues to genetically associated tumour syndromes including melanomas. As expected a familial melanoma association for p16 associated cases was found. Ref: Nielsen K. et al. Br J Dermatol 2004;150:531-6.

P-206 MODELING FAMILY-BASED RESEARCH IN THE PREVENTION OF MELANOMA

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Genetic diseases are family diseases. Thus a disease diagnosed in one individual has significance for the health of relatives. This underlies physicians belief in the value of providing genetic risk information, even to affected individuals who can provide this risk information to unaffected relatives, potentially leading to enhanced prevention behaviors. Melanoma, as a familial cancer, is a particularly important case in which enhanced family communication could lead to substantial health benefit. Though the disease appears in adulthood, the primary time of risk is during childhood .For example, the melanoma diagnosis given to a man, if shared with his sister, might lead the sister to enact prevention behaviors that could prevent melanoma in the man's niece. This model would be most efficacious if all relatives accessed, discussed and shared the most current information about sun protection and skin screening. In actuality, little is currently known about how families support or interfere with each others' health behavior choices. Most research in cancer genetics investigates the health beliefs and practices of individuals. Overwhelmingly, studies on family communication collect data on family interaction and cohesion from only one individual. Instruments and statistical methods for dealing with data points provided by multiple family members are lacking or underdeveloped. Our study has utilized both traditional and recently developed methods of statistical analysis, along with new survey instruments aimed at considering the family as the unit of analysis. In particular, application of a mixed effects models is adopted to account for the natural heterogeneity across families and for the within-family correlation. A global test of multiple measures (e.g., skin examination practices and sun protection behaviors) from multiple family members is also considered. We present our model of family data collection and analysis and suggest its particular relevance to improving health outcomes in families at risk for melanoma.

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P-207 CDKN2A IN SPANISH FAMILIAL MELANOMA: SURVEILLANCE PROGRAM AND EARLY DETECTION OF MELANOMA

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Objectives: To describe the prevalence of germ line mutations in CDKN2A and CDK4 in familial melanoma in Spain, to evaluate clinical differences between carrier and non-carrier families and to describe a specific surveillance program. Subjects and Methods: 49 Spanish families with at least two well documented melanoma cases were included. One family has 6 cases of melanoma, one 5, two 4, fourteen 3 and thirty-one 2 cases. All families were invited to be included in an specific surveillance program that includes total body photography and dermoscopy for early melanoma diagnosis. Exon 1alfa, 1beta, 2, 3 and IVS2-105 of CDKN2A, -34G>T at the CDKN2A promoter region, and exon 2 of CDK4, were studied by PCR-SSCP and sequencing. Results: 5 different missense mutations (G101W, L65P, D84Y, R87W, V59G) and 2 frame-shift mutations in exon 2 (358delG and 102-106delG) were detected in CDKN2A. G101W was detected in 7 unrelated families. The polymorphism A148T was present in affected melanomas in 9 families (in 3 of them associated to G101W or D84Y). 100% of families with more than 3 cases of melanoma had mutations in CDKN2A but also in 29% and 23% of 3-cases or 2-cases melanoma families. In follow-up, 25 primary melanomas were diagnosed in 16 families (10 of them with CDKN2A mutations) in a mean follow-up of nearly 6 years. Conclusion: The prevalence of CDKN2A mutations in Spanish families is similar to those described for other countries. Interestingly, all families with more than 3 cases are carriers of mutations in our setting and mutations are detected in 44.4% of families with at least 3 cases. The specific surveillance program was useful in the early diagnosis of melanoma either in carrier and non-carrier families.

P-208 THE CHICKEN LEG MODEL

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The management of Clinical Stage I Malignant Melanoma has been improved following the introduction of Lymphatic Mapping and Sentinel Lymphadenectomy, (LM/SL). Sentinel Lymphadenectomy detects the 20% or so patients with occult micro-metastatic disease of the regional node basin before their disease manifests clinically. These patients are then offered an early therapeutic completion lymphadenectomy, adjuvant therapies and provided with a more appropriate prognosis. The "Triple Technique" of pre-operative lymphoscintigraphy followed by intra-operative identification of the Sentinel node/s using blue dye and a hand-held gamma probe is standard in most centres. The "blue dye" phase is user dependant. The dye must be injected intradermally to be effective in identifying the sentinel lymph node draining the Melanoma. Assessing the ability to perform the blue dye technique would be advantageous to any prospective surgeon wishing to offer LM/SL. Initial published reports of the technique used live animal models. We have devised a method of teaching and practising intradermal blue dye injection and lymphatic identification that doesnt require live animal models. The model has recently been utilised as a teaching tool on The Royal College of Surgeons of England Sentinel Node Biopsy Course. It is widely available, inexpensive and accurately reproduces the "in-vivo" surgical setting.

P-209 AGGRESSIVE FAMILIAL UVEAL MELANOMA IN THREE SIBLING CASES IN URUGUAY

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Familial uveal melanoma accounts for only 0.6% of patients with uveal melanoma. Uveal melanoma usually appears sporadically in the absence of clear predisposing genetic factors. Considering the low incidence of uveal melanoma in the general population the possibility of developping this pathology in a familial context is very rare. Three clinical cases (38, 39 and 40 year age at diagnosis) of histopathologically proved intraocular malignant melanoma involving first generation members of the same family at a very young age are reported. All the cases corresponded to mixed uveal melanoma; in one of the cases the patient also had a malignant breast tumor who was diagnosed 2 years before. The three siblings had an extremely aggressive behaviour of their pathology developping liver, bone and breast metastasis (histologically confirmed)dying very shortly thereafter. The genealogic family tree shows many other tumors in the maternal side of same family (lung, breast). Although germ line BRCA2 mutations may be involved, there may be another loci contributing to the family association between ocular melanoma and breast cancer. The clinical cases and their evolution are analysed.

P-210 THE OCCULT PRIMARY MELANOMA

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The incidence of unknown primary melanoma ranges from 2% to 16%. There are many contradictions about the definition and the explanation of this distinct phenomenon. Authors analysed the clinical histories of 120 patients who were referred to the NCI between 1994-2003 with the diagnosis of metastatic melanoma of unknown origin. Each of them had histologically proven melanoma metastasis. The radiologic and endoscopic examinations failed to identify the primary tumour. The most frequently involved site was the different nodal areas (axilla, neck and groin) Surprisingly high was the brain manifestation (19/120). The second most frequent metastatic site was the lung /12/120/ Interestingly, there were patients having metastasis in the uterus, sinus maxillarias and jejunum, respectively. The careful examination of the entire skin surface helped to find a grayish blue or whitish patch in more then half of the patients (62/120). In these cases the histological examination confirmed the final stage of melanoma regression or the late form of it (fibrosis, melanophages). Sometimes even photo taken by a conventional camera could show the pigmented lesion of at a questionable site. There were two cases with whom the suspect primary bluish-whitish region completely disappeared during the follow-up period. Authors conclude that the entity of unknown primary is a sort of perplexing puzzle in oncology. In the clinical practice, however, the subclinical primary is of no prognostic significance, as the survival of the patient depends on the metastatic sites. Instead of time consuming examinations the most important is to scrutinize the entire skin surface searching for the well known subtle signs of melanoma regression.

P-211 INTEGRA AND SSG REPAIR OF DEFECTS FOLLOWING WIDE LOCAL EXCISION OF MELANOMA IN THE FOOT AND ANKLE

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Introduction This presentation describes the novel use of Integra and SSG in reconstruction of melanoma wider excision defects extending to deep fascia, joint capsule and para-tenon in the foot and ankle. Patients and Methods Six patients aged 32-68 years (mean 42) underwent 2 cm margin wider excision of biopsy proven melanomas (Breslow thickness 1- 6 mm) in the foot and ankle. Integra was applied to the defects (ranging from 4 - 8 cm in diameter) and a Vacuum Assisted Closure (VAC) dressing applied for five days. The Integra was then dressed with Betadine soaked gauze until it appeared vascularised when a SSG was applied with VAC support for a further five days. Backslab protection was provided till graft take was stable. Results Integra neovascularisation permitted grafting within three weeks in all cases with good take except for a 68-year-old lady with prior lymphoedema whose defect remains fragile. The remainder have sufficiently stable grafts to allow gradually improving mobilisation in conventional footwear. Conclusion This small series suggests that reconstruction of full thickness defects in the foot and ankle with Integra and SSG can provide durable, non-bulky reconstruction in this functionally demanding region.

P-212 PROGNOSTIC FACTORS OF THIN CUTANEOUS MELANOMA: DATA FROM THE SWEDISH MALIGNANT MELANOMA REGISTRY.

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Objective: To analyse the 10-year melanoma specific survival from the national population based prospective melanoma registry for 1990-2002 and to analyse associated prognostic factors for survival and to compare the results with other recently published data. Material and methods: 6658 patients with clinical stage I cutaneous melanoma d 1.0 mm have been included in Kaplan-Meier analysis of survival and 5764 in Cox multivariate analysis of prognostic factors. Mortality data until 2002 was obtained from the Swedish Cause-of Death Registry and used to calculate melanoma-specific survival. Results: The 10-year survival for all patients was 96.3% (95% C.I. 0.7%). For melanoma 0-0.50 mm survival was 98.7% (95% C.I. 0.9%), for 0.51-0.75 mm 96.2% (95% C.I. 1.4%) and for 0.76-1.0 mm 92.9% (95% C.I. 2.1%) (p.<0.0001). Survival was 96.6% (95% C.I. 0.8%) for melanomas without ulceration and 89.8% (95% C.I. 4.8%) (p.<0.0001) if ulcerated. In a Cox multivariate analysis factors significantly associated to survival were: age, melanoma thickness, Clark level of invasion, ulceration and location but not gender or regression. In the multivariate Cox analysis a HR of 4 was seen for thickness 0.76-1.0 mm followed by 2.9 for thickness 0.51-0.75 mm , 2.7 for ulceration , 2.4 for head and neck and 2.1 for trunk location, 2 for age >50 years , and 1.8 for Clark level III-V. Conclusion: The Swedish 10-year melanoma-specific survival for thin melanoma is better than in the AJCC 2002 database. In contradiction to published data from the Central German Melanoma Registry we still find an influence of ulceration on survival in thin melanoma. The recommended T1 cut-off level of 1.0 mm could be discussed.

P-213 THE ROLE OF A DEDICATED MELANOMA NURSE PRACTITIONER AND SURGICAL ONCOLOGIST IN IDENTIFYING SUSPICIOUS CUTANEOUS LESIONS IN THE MELANOMA PATIENT

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Objective: Cutaneous melanoma patients are at increased risk of developing second primary melanomas and require aggressive dermatologic surveillance. The role of the dermatologist in diagnosis and follow-up is well established. However, the role of the surgery team is not well defined. We hypothesized that a dedicated melanoma nurse practitioner and surgical oncologist can detect and diagnose a significant number of second cutaneous cancers. Methods: At presentation, all melanoma patients had a total body skin and scalp exam by a dedicated melanoma nurse practitioner and surgical oncologist. Following definitive melanoma surgery, all patients had regular exams in the surgical oncology clinic in addition to close dermatologic follow-up. Follow-up was performed on a semi-annual basis for the first two years and annually thereafter. All skin punch biopsies performed during the study period were reviewed. Results: From July 2003-October 2004, 60 skin biopsies were performed in 41 patients. Overall, 17 (28%) were abnormal, including 12 (20%) malignancies and 5 (8%) atypical dysplastic nevi. Of the malignancies 6 (50%) were second primary melanomas: 3 melanomas in situ and 3 invasive melanomas (mean Breslow thickness 0.46mm, range 0.3mm- 0.63mm). Other lesions included 3 (25%) squamous cell carcinomas, 1(8%) basal cell carcinoma and 2 (17%) dermal melanoma metastases. Seven (41%) of the abnormal lesions were biopsied at initial presentation, 7 (41%) were identified at the 6 or 12 month follow-up visits, and the remaining 3 (18%) were detected on annual exams several years after original diagnosis. Discussion: This study demonstrates the value of a dedicated melanoma nurse practitioner and surgical oncologist in identifying suspicious cutaneous lesions in the melanoma patient. Continued follow-up of melanoma patients, not only for second primary melanomas, but also for nonmelanoma skin cancers, is important and is likely to find lesions at an early stage.

P-214 SIDE EFFECTS OF NON-PEGYLATED INTERFERON ALPHA IN THE ADJUVANT TREATMENT OF 72 MELANOMA PATIENTS

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Background: Incremental use of INF-± world-wide emerge awareness of adverse reactions. Side-effects are diversified from laboratory deviations to persistent organ failure. Objective: Quantitative and qualitative evaluation of adverse drug reactions during IFN-± treatment with relevant dosages as they are used for ongoing and future treatment protocols of melanoma. Methods: 72 melanoma patients were treated with IFN-a in the Dermatological Department, Münster. Side-effects were graded either as WHOo I - IV or assessed as mild, moderate or intense. Over 100 relevant symptoms, diagnosis and laboratory values were evaluated and categorized as flu-like, gastro-intestinal, cutaneous, cardio-pulmonal and neuro-psychiatric. Patients were grouped according to dosages of non-pegylated INF-a2a/b. Higher dosages were used in a protocol recommended by Kirkwood: Group I = 3-10 MIO IU s.c. with duration of treatment 7 + 2.3 months, n =55; group II = 11-20 MIO IU s.c. with duration of treatment 3.7 + 0.8 weeks, n = 8; group III > 20 MIO IU i.v. with duration of treatment 3.1 +1.1 weeks, n = 9. Results: In group I (low dose) flu-like symptoms (69%), cutaneous side-effects (40%), gastro-intestinal pain (50%), neuro-psychiatric symptoms (25%) and dyspnoe (16%) are specified. 4 pts. showed an increase of liver enzymes WHOoIII. There was 1 pt. with a reversible hearing loss and 1 with persistent ataxia. In group II (middle dose) 75 % flu-like symptoms, 62% gastro-intestinal pain, 37% neuro-psychiatric symptoms and 37% cutaneous reactions were documented. In group III (high dose) 89% flu-like symptoms, 55 % gastro-intestinal symptoms, 44% cutaneous reactions occurred. In 5 cases increase of liver enzymes WHOoIII was noticed. 1 pt had a reversible vision loss. Conclusions: Adverse reactions under IFN- ± treatment are equally distributed independent of usually used dosages of IFN- ±. Rare but serious complications demand experienced guidance.

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P-215 SYNCHRONOUS PRIMARY MELANOMA

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The incidence of primary cutaneous melanoma has been increasing for decades. Personal or familiar melanoma history and the presence of atypical moles are related to an increased risk of developing melanoma. Recently, genetic predisposition as CDKN2A mutations has already been identified in some individuals with melanomas. The incidence of a second primary range between 1 and 8,5% in the medical literature. In about 30% of these patients with multiple melanomas, the second primary is usually diagnosed within 3 months of the first malignancy and is therefore considered synchronous. In this study we describe seven cases of multiple synchronous primary melanomas with the purpose of review the medical literature about this subject and to warn clinicians about the importance of a total body examination even after diagnosing a first suspicious lesion. In the last two years seven patients with synchronous primary melanomas were observed at our department, corresponding to 1,9% of total cases of melanoma diagnosed at the same period (360) in our institution. One patient had a personal history of melanoma and another had a familial history of melanoma. The other 5 patients didn`t have any relevant risk factor for developing melanoma. Two patients had 3 synchronous primary melanomas and the other had only 2. Two patients had the lesions in the same anatomic area, facilitating the diagnostic but five patients had distant lesions requiring a careful exam. As conclusion, we reinforce the need for careful examination of the whole body surface at the time of detection of a primary cutaneous melanoma because the possibility of synchronous multiple melanoma is real. As 5 patients didn`t have an important risk, we suggest that genetic susceptibility may be involved.

P-216 SURVIVAL OF PATIENTS WITH THIN CUTANEOUS MELANOMA FROM THE ITALIAN NATIONAL CANCER INSTITUTE IN GENOA AND PRESENCE OF CDKN2A MUTATIONS

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We investigated the survival of patients treated at the NCI Melanoma Unit (IMU). The IMU is the main referral center for melanoma in the Italian region of Liguria, an area with a prevalent melanoma susceptibility founder mutation, CDKN2A G101W. Data on all the patients diagnosed or attending follow-up between 1994 and 2000 (n= 563) were provided by the NCI Registry. Cases with metastasis at diagnosis (21) were not included in the study. Five hundreds and forty two cases were available for analysis, 283 consented to DNA testing, and 40 were found to be CDKN2A-positive. Among these, 28 carried the known G101W founder mutation, 4 carried the newly identified E27X founder mutation, 1 the P48T mutation, 1 the R58X mutation, 1 the A68L mutation, 1 the D47Y mutation, 1 the A127P and 2 carried alterations in the 5UTR. Ten-year Kaplan-Meier survival rates were calculated in patients with thin melanoma (breslow <1.5 mm) and differences were established with the log-rank analysis. We found an overall 10-year survival (OS) of 86.1% (SE=4.7%). The median lesion thickness was 0.77 mm. One hundred and thirteen patients had lesion thickness £ 0.75 mm and 114 had lesion thickness >0.75 mm. Patients with lesions measuring £ 0.75 mm had a 95 % (SE=3.7%) ten-year OS compared with 81.8 % (SE=6.4%) for patients with lesions 0.76 -1.5 mm (p=0.01). Interesting patients with breslow 0.76-1.0 mm have a superimposable OS (80.4%) comparing to patients with 1.0-1.5 mm melanoma (82.3%). The 10-year OS of patients with ulcerated melanomas was 45.8% (32.7%), and OS was 83.9% (SE=6.3%) for patients who had non ulcerated melanomas (p=0.0003). These results confirm the very high survival rates of melanoma patients with thin, not ulcerated lesions.

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P-217 THE PHANTOM MODEL

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Every surgical procedure has its Learning Curve reflecting evidence that performing a greater number of procedures leads to better results. There is therefore an increasing need for models upon which to practice surgical techniques, allowing the trainee the opportunity to optimise his/her skill before moving onto patients. Lymphatic mapping and Sentinel Lymphadenopathy is an increasingly utilized procedure in the surgical management of Malignant Melanoma, Squamous Cell, Breast and other cancers. We would like to present a simple, inexpensive and easily constructed device with which the technique of intra-operative hand-held gamma probe localization of the sentinel lymphnode/s can be practiced. The advantages of this simulator are it is a simple, easy to assemble and inexpensive. It effectively simulates both the anatomical aspects and difficulties inherent to the procedure. It is also adjustable to increase/decrease the level of difficulty and importantly it is safe and reliable, giving a realistic representation of intra-operative hand-held gamma probe lymphoscintigraphy in a surgical setting

P-218 KEYSTONE FLAP REPAIR OF MELANOMA RESECTION DEFECTS ON THE LOWER LEG SIGNIFICANTLY REDUCES PATIENT MORBIDITY AND IN-PATIENT CARE

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Purpose: Resection defects for intermediate and thick melanoma are commonly 4cm or larger in diameter and repair of these on the lower limbs can present a significant reconstructive undertaking. Split skin grafting involves significant morbidity including a donor site, prolonged hospitalisation and reduced patient mobility. Skin flaps for larger defects on the lower leg have been not infrequently complicated with delayed healing. This study was undertaken to evaluate the reliability and impact of the recently described Keystone flap (Behan, 2003) on the morbidity and in-patient stay of these patients Methodology: Patients with lower leg melanoma treated at the Sydney Melanoma unit over two consecutive years (2003-2004) were reviewed. The caseload in the 12 month periods prior to and following the introduction of this flap were analysed with regard to in-patient bed days and wound healing. Results: The use of Keystone flaps to repair the resection defects of 4cm or greater was associated with a 74% reduction in the number of hospital inpatient days. All 62 flaps healed without significant complication. Substantial in-patient cost reductions were noted together with markedly reduced peri-operative morbidity. Improved long term wound stability and aesthetics were also observed compared to split skin grafting. Conclusions The Keystone flap, based on both perforating fascial vessels and incontinuity subcutaneous veins, has proved to be a valuable reconstructive option for the management of many lower limb melanoma resection defects and transformed the surgical management of primary melanoma on the lower leg at the Sydney Melanoma Unit.

P-219 MALIGNANT MELANOMA ON BURNING CIGARETTE (A CASE REPORT)

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At age 76 a man was referred by a general practitioner with an enlarging, discharging ulcerated lesion at his right plantar; it had appeared six years earlier after a minor trauma like a corn. He often burned it by cigarette. At the present appearance was suggestive of malignant melanoma and a biopsy was taken. This showed invasive malignant melanoma with a Breslow thickness of 4mm. Histological examination confirmed an acral lentiginous malignant melanoma with a predominant ulcerated nodular component of maximum Breslow thickness 4 mm, melanoma was found in an enlarged right inguinal lymph node and had metastasized to liver. Early detection of malignant melanoma is essential since survival prospects are strongly related to tumor (Breslow) thickness at the time of diagnosis. The Breslow thickness, measured on histological examination, is the distance between the overlying epidermal granular layer and the deepest invasive area of the primary lesion. For lesions of Breslow thickness <1 mm the recommended excision margin is 1 cm and 5-year survival is 95-100%. For Breslow thickness >4 mm the recommended margin is 2-3 cm and 5-year survival is about 50%. The levels of invasion into the dermis introduced by Clark et al are a similar prognostic indicator related to penetration by the primary lesion, level 5 signifying invasion into fat. Conclusion: Any changing or atypical mole or non-healing skin lesion should be referred urgently to a dermatologist or to a surgeon with a special interest in pigmented lesions.

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P-220 SENTINEL LYMPH NODE BIOPSY IN THE MANAGEMENT OF PATIENTS WITH PRIMARY CUTANEOUS MELANOMA: THE EXPERIENCE OF A GENERAL HOSPITAL

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OBJECTIVE: The purpose of this review is to evaluate the value of sentinel lymph node biopsy (SLNB) in accurate staging of patients with primary cutaneous melanoma, choosing those who have indication for ellective regional lymph node dissection (ERLND) and/or adjuvant therapy, and prognostic importance of this technique. MATERIALS & METHODS: We reviewed the records of the patients with primary cutaneous melanoma who underwent lymphatic mapping and SLNB between May 1999 - September 2004. One hundred and fifteen patients were retrieved, 64 from our Dermatology Department and 51 from other Dermatology Departments. SLNB was performed in patients in Stages I or II. A retrospective analysis was conducted with attention paid to clinical and histological variables, pathological SLN status, adjuvant therapy, follow up period, appearance of metastasis and disease-free survival. RESULTS: Forty nine male and 66 female patients were studied. The mean follow up period was 31.5 months. Twenty two patients had positive SLN (PSLN) and were submitted to lymphadenectomy and proposed for interferon alfa therapy. In the PSLN group 27.3% patients metastized in the next two years. These patients had melanomas with 5.3 mm average thickness and only one was ulcerated. In the negative-SLN (NSLN) only 4.4% patients had metastases. In the group with more than 3 years of follow up, two patients died of melanoma, both with PSLN. The three years disease free survival was 75% in PSLN and 95.5% in NSLN. CONCLUSION: - SLNB is important for accurate staging of patients with melanoma - 19.1% change of clinical stage. - SLNB allows early detection of nodal micrometastasis and prompt therapeutic ERLND, providing local control of the disease - 31.7% had nodal metastasis. - Patients with PSLN have significantly increased risk for recurrence (27.3% - PSLN against 4.4% - NSLN)

P-221 AUTOMATIC BORDER DETECTION IN DERMOSCOPY IMAGES

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OBJECTIVE: Automated border detection is the first step in the computerized analysis of pigmented skin lesions. This step is critical to enable accurate extraction of the dermoscopic features. In this study, we propose an automatic border detection method based on a modified version of the JSEG algorithm (color quantization followed by spatial segmentation on local windows in the 'J-image'). MATERIALS & METHODS: The data set consists of 100 dermoscopy images (30 malignant melanoma, 70 benign). The procedure is as follows: 1) smooth the image using a median filter (11x11 kernel) to reduce the influence of skin texture, bubbles, and hairs 2) reduce the number of colors in the image using a color quantization algorithm 3) segment the image into homogeneous color regions by region growing 4) merge the resultant regions based on color similarity 5) determine the healthy skin color by calculating the median color of four patches taken from each corner of the image 6) eliminate the regions that should belong to the healthy skin based on their color similarity to the healthy skin. RESULTS: The detected borders are compared with those determined manually by a dermatologist (Dr. Stoecker). The border detection error is quantified by a metric developed by Umbaugh et al. computed as the number of pixels for which the automatic and manual borders disagree divided by the number of pixels in the manual border. The mean and standard deviation of border errors are found to be 14.91% and 8.40% for the melanoma set and 10.78% and 6.28% for the benign set. CONCLUSION: The results demonstrate that the proposed method achieves acceptable accuracy in detecting the borders of dermoscopy images. The advantages of the JSEG algorithm over alternative methods such as active contour (snake) and watershed methods include rapid processing and robust behavior with noisy images.

P-222 NODULAR MELANOMA - NOT AS SIMPLE AS ABC

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Objectives: The purpose of this study was to identify historical or clinical features that may distinguish nodular melanoma and possibly aid earlier detection. Materials & Methods: We surveyed 125 patients attending the Victorian Melanoma Service between 1998 and 2000 with either superficial spreading or nodular melanoma and compared parameters by tumour type and thickness. Results: Nodular melanomas were more often thicker at diagnosis and occurred in older persons. Patients with nodular melanoma were less likely to notice colour change as a presenting symptom but more likely to notice bleeding, elevation or catching on clothing. Nodular melanomas were more often symmetric, elevated, uniform in colour and non-pigmented. Conclusions: Nodular melanomas often fail to meet the ABCD diagnostic criteria and may be easily confused with inflammatory lesions or benign tumours. Whilst they are more often patient detected, they tend to be elusive at an early stage. Perhaps those at highest risk of developing thick tumours such as older persons and men in particular deserve closer surveillance?

P-223 FEASIBILITY OF A NON-INVASIVE, HANDHELD OPTICAL DEVICE FOR IN VIVO DETECTION OF MELANOMA IN SUSPECT PIGMENTED LESIONS

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OBJECTIVE: Determine the feasibility of a non-invasive, handheld optical device using elastic scattering spectroscopy (ESS) for in vivo detection of melanoma in pigmented skin lesions. MATERIALS & METHODS: A spectrometer unit was built in and connected to a computer running controller software. A pulsed xenon lamp delivers light to the lesion via a 400 mm optical fiber in a handheld probe; the elastically scattered return is collected by a 200 mm fiber bundled into the same cord, and directed to an Ocean Optics S2000 grating spectrometer. For in vivo measurement, scattering spectra were obtained from five specific locations on each selected pigmented lesion (to account for any heterogeneity), in a procedure which takes, at most, 3-4 minutes. Clinical data was gathered from consenting patients referred to the Boston University Medical Center's Dermatology Clinic for a biopsy of suspect pigmented lesions. Following the spectra measurements, the indexed lesions are excised for histological assessment. RESULTS: Extending preliminary work, we have developed spectral analysis algorithms, using a few discrete wavelength regions between 320 and 770 nm, to differentiate benign, dysplastic nevi, and melanoma. Comparing the histological results from each of the five sites for each lesion to the corresponding scattering spectra allows for identification of spectral features uniquely associated with each lesion category. Different pathologies exhibit differences in the wavelength dependence of optical absorption and scattering, making possible spectrometric discrimination via analysis of regional red-to-blue ratios, fits of the detected spectra with second- and third-order polynomials, and other analysis. CONCLUSION: To date the project has shown feasibility. Effective diagnostic algorithm can be programmed into integrated circuit boards, packaged with LED sources, into a compact spectrometer. The development of a handheld optical spectrometer for personal or medical detection of melanoma appears possible.

P-224 TRIAGING SUSPICIOUS PIGMENTED SKIN LESIONS IN PRIMARY CARE USING THE SIASCOPE- A PRELIMINARY REPORT

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Objective: The SIAscope is a non-invasive multispectral scanning technique deriving microarchitectural information regarding the skin within seconds. SIAgraphs map the concentration of dermal and epidermal melanin, blood and collagen thickness across the imaged skin lesion. Previous work in secondary care has demonstrated that SIAscopy is a simple and effective tool in the early diagnosis of cutaneous malignant melanoma. A wealth of data suggests that GPs have low specificity for diagnosing melanoma. To date, studies of the SIAscope have been conducted on referred populations; this study examines its use in primary care. Materials and Methods: Patients attend their GP's practice and are assessed in the usual way with the GP stating their intended action. The patients are separately reviewed by our team by means of clinical examination, photography, dermatoscopy and SIAgraphy. The 'silver standard' for referral is based on expert opinion of the history, photograph and dermatoscopy, and this is compared to the GPs action and the SIAgraph. Results: Preliminary data (78 lesions) shows that whilst GPs are performing well, SIAscopy can increase the positive predictive value (94%) and the specificity (94%) of the diagnosis of pigmented skin lesions.
Conclusion: This is the first study to investigate the diagnostic characteristics of the SIAscope in a primary care population, where the key clinical decision is not to diagnose melanoma but to determine if the lesion is sufficiently suspicious to warrant expert assessment and excision. By comparing SIAgraphs with GP and specialist diagnoses, the role of the SIAscope in the diagnosis of suspicious pigmented skin lesions is being examined. Preliminary data is encouraging, especially showing that the SIAscope may well be useful in triaging benign lesions that do not require referral to already overburdened pigmented skin lesion clinics.

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P-225 INCIDENCE OF NEW AND CHANGED NEVI AND MELANOMAS DETECTED USING BASELINE IMAGES AND DERMOSCOPY IN PATIENTS AT HIGH RISK FOR MELANOMA

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OBJECTIVE: To determine the incidence of new, changed and regressed nevi and melanomas in a cohort of patients at high risk for melanoma using baseline total body photography and dermoscopy. MATERIALS AND METHODS: A cohort study of patients at high risk for melanoma who had baseline cutaneous photography between 1 January 1992 and 31 December 1997 and at least one follow-up visit by 31 December 1998. Study subjects were 309 patients who had at least one of the following risk factors for melanoma: personal history, family history, 100+ nevi, 4+ dysplastic nevi. Outcome measures were: number of new, changed and regressed nevi and melanomas detected during the study interval. RESULTS: The incidence of new, changed and regressed nevi decreased with increasing age ($p<0.01$), while the incidence of melanomas increased ($p=0.05$). The number of dysplastic nevi at baseline was positively associated with the incidence of changed nevi ($p<0.01$) and melanomas ($p=0.03$). The use of baseline photography and dermoscopy was associated with low biopsy rates and early detection of melanomas. The development of melanoma in association with a preexisting nevus was not directly correlated with change in a preexisting lesion rather than appearance as a new lesion. CONCLUSION: Nevi are dynamic and only a small percentage of all new and changed melanocytic lesions were melanomas. Patients aged younger than 50 years had a lower incidence of melanomas and a higher rate of new, changed and regressed nevi when compared to patients older than 50 years. A new or changed pigmented lesion is more likely to be a melanoma in patients older than 50 years.

P-226 ELASTOGRAPHY IN MELANOMA METASTASIS

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Objective Todetermine whether the swelling lymphnodes are metastasis or not-we used elastography (a new technique of the ultrasound equipment). Material) Six nodes with metastases, 6 nodes without metastases and 6 lesions of intransit metastases. Method) Ultrasonic equipment : EUB 8500 (Hitachi Medico)To detect lymph nodes, we examined B-mode sonography before elastography. Results)In elastography, all metastatic nodes changed deep blue and all nodes without metastases were not change. In addition. The intransit metastasis changed deep blue too. Conclusions)Elastography is useful for diagnosis of melanoma metastasis.

P-227 SENTINEL NODE BIOPSY IS A PRECISE METHOD FOR DETECTING OCCULT NODAL METASTASES IN THIN MELANOMA

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OBJECTIVE: Sentinel node biopsy (SNB) has been widely accepted as a highly accurate tool to stage cutaneous melanoma. In the T1-category (Breslow depth ≤ 1mm) the indication of SNB is controversial, since the risk of nodal metastasis is low and the therapeutic benefit is not certain. However, thin melanomas constitute prognostically a heterogeneous group, and a small fraction of these patients will have aggressive disease that carries a considerable risk of metastasis. The aim of this study was to determine, whether SNB can identify nodal disease among patients with thin melanomas. MATERIALS & METHODS: SNB was performed prospectively in 135 patients with cutaneous melanoma. Patients with clinical stage I-II invasive melanoma with any Breslow thickness or Clark level II-V were enrolled in the study. All patients underwent preoperative lymphatic mapping and SNB. All patients with positive sentinel lymph nodes underwent complete lymph node dissection. RESULTS: A total of 269 sentinel lymph nodes were excised. Metastatic disease was detected in 24 patients (18%) by SNB. 56 (41%) were T1-melanomas and in this group SNB revealed nodal involvement in three patients, constituting 5% of all thin melanomas and 12% of all sentinel-positive cases. Histopathologically, there were no factors of primary tumors that would have predicted these metastases. The Breslow depth was 0.5—0.9 mm, the level of invasion Clark II—III and only one tumor was ulcerated. There was no lymphovascular invasion, regression or microsatellitosis present and the mitotic rate was low in all specimens. CONCLUSION: Sentinel node biopsy is a precise method to detect clinically silent nodal metastases in thin invasive melanoma. Advanced melanoma is a lethal disease and accurate staging is essential also in the T1-group. For stage III patients with occult nodal metastases, metastasectomy is a better option for cure than observation.

P-228 ASSESSING BORDER IRREGULARITY WITH IRREGULARITY INDICES

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Objective: Border irregularity is an important clinical feature in the diagnosis of malignant melanomas, which are often described as lesions with irregular borders. We have developed an image-based method to assess border irregularity by dividing a lesion border into indentation and protrusion segments, where a quantitative measure, termed irregularity index, is computed for each individual segment. As a result, the lesion border is represented by a series of indices, which are used to generate 8 derived indices: Overall Irregularity Index (OII), Indentation Irregularity Indices (III), Protrusion Irregularity Indices (PII), Maximum Indentation Irregularity Index (MIII), Maximum Protrusion Irregularity Index (MPII), Average Overall Irregularity Index (AOII), Average Indentation Irregularity Index (AIII), and Average Protrusion Irregularity Index (APII). For this study, we evaluate how well the image-based method correlates with dermatologist's observations and compare the predicting power of the derived indices. Materials & Methods: After implementing all derived indices, we performed two analyses. (1) In a user study with 20 dermatologists, we compared the dermatologists' notion of border irregularity with OII for 40 lesion borders. (2) Using 188 pigmented lesions, including 30 melanomas and 158 benign lesions, we tested the predicting power of all 8 derived indices by ROC analysis. The area under the curve (AUC) is used to determine the predicting power. Results: (1) For the user study, the correlation between dermatologists and OII was 0.88. (2) The AUC for the 8 derived indices ranged from 0.64 to 0.73. The III had the best predicting power, and AOII has the least. If we combine III, PII, MIII, and MPII, the AUC increased to 0.77. Conclusion: The analyses showed that our image-based method capture the expert's notion of border irregularity and the derived indices had a reasonable to good predicting power to differentiate melanoma from benign lesions, especially with combined indices.

P-229 AN 8 YEAR OLD BOY WITH PIGMENTED EPITHELIOID MELANOCYTOMA

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Objective: We present a case of pigmented epithelioid melanocytoma (PEM), a recently described entity encompassing previously described animal-type melanoma and epithelioid blue naevi (EBN) of Carney complex. Materials and Methods: Evaluation of the clinical and histological presentation of a patient at the Massachusetts General Hospital Pigmented Lesion Clinic. Guidelines for clinical treatment were based on the clinical-pathologic analysis of 41 consecutive PEM patients (Zembowicz et al. Am J Surg Pathol 2004). Results: An 8 year old boy of Irish descent had sudden onset of a purple nodule underneath his right eye in April 2004. The patient was otherwise healthy and without family history of dysplastic nevi or malignant melanoma. The lesion slowly increased in size over the next 8 months and was nearly completely excised by shave biopsy in January 2005. Hematoxylin and eosin stained tissue sections revealed an intraepidermal and dermal proliferation of predominantly epithelioid melanocytes. Abundant cytoplasmic melanin obscured the cytomorphology. Bleach treated sections revealed a pleomorphic population of melanocytes that extended along the base of the shave specimen with a solitary mitotic figure. The melanocytic proliferation occupied the entirety of the biopsy specimen; the maximal tissue thickness was 0.77 mm. Conclusion: The histological findings are those of pigmented epithelioid melanocytoma (PEM), a provisional histologic entity encompassing both animal-type melanoma and epithelioid blue nevus. In a published study of 41 patients, regional lymph nodes were sampled in 24 cases (59%). In 11 cases, lymph nodes contained metastases (46%). Based on the prior study, a repeat excision was recommended, but sentinel lymph node biopsy was not recommended given tumor depth <2mm. This tumor is primarily found in a younger population in both sexes of different ethnic backgrounds. This case raises questions with regards to therapeutic options and follow-up for patients with this unusual markedly pigmented melanocytic tumor.

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P-230 DOBUTAMINE-TC-99M MIBI SCINTIGRAPHY IN THE EVALUATION OF MELANOMA PATIENTS: PRELIMINARY RESULTS.

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Tc-99m MIBI scintigraphy has been proposed as a clinically valuable technique for the detection of melanoma metastases. Besides, dobutamine is a synthetic catecholamine that can hyperpolarized plasmatic membranes of certain cells. Therefore, we wanted to determine whether the infusion of low-dose dobutamine could enhance Tc-99m MIBI uptake in melanoma lesions. We included sixteen patients with proven cutaneous melanoma, with clinically localized primary disease (n=5, AJCC stage IIC) and during follow-up, patients with palpable regional lymph nodes (n=11, AJCC stage III). Planar images of regions of interest were acquired 10 min after tracer injection followed by a whole-body scan, using a dose of 740-1110 MBq and a LFOV gamma camera equipped with a LEHR collimator. Three-five days later, the study was repeated after the infusion of low-dose dobutamine (2.0 mg/min for 60 min), using the same acquisition protocol. Tumor-to-normal background tissue uptake ratios (T/B) were calculated for each lesion. Scan findings were confirmed by pathology in all cases. Baseline MIBI scintigraphy identified 16/21 lesions (76%). Dobutamine-modulated MIBI scanning (DMS) detected 21 lesions: primary tumors (n=2), skin metastasis (n=1) and lymph node metastases (n=18). Clinically unsuspected lymph node lesions were detected by both baseline (n=2) and dobutamine-modulated MIBI scans (n=5). No false-positive results were found. Uptake ratios of DMS were significantly higher (2.76±1.48) than those calculated from baseline scans (2.18±1.15), p=0.0371, n=21, (mean±SD). We conclude that low-dose dobutamine infusion could increase Tc-99m MIBI uptake in melanoma lesions. Thus, DMS has the potential of supplying clinically relevant information.

P-231 MELANOMA REFERRAL TRENDS IN THE NATIONAL HEALTH SERVICE (NHS) IN NORFOLK, ENGLAND

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OBJECTIVE: This audit, running from May to December 2003, reviewed patients with a new diagnosis of malignant melanoma referred to the Norfolk & Norwich University and James Paget Hospitals, UK. MATERIALS & METHODS: We evaluated the referral mode of 110 patients diagnosed with melanoma, as well as the transfer times between primary care, secondary care and the definitive surgical procedure within the National Health Service (NHS) The data for the first three months were retrospective and then prospective for the next three months. RESULTS: There were 45 male and 65 female patients, with 93 of these presenting with primary melanoma and 17 with metastases. The mean Breslow thickness was 2.85 mm (range 0.2 to 24 mm). Referrals to secondary care were graded urgent (77), soon (7) or routine (14). Referral targets included dermatology (61), plastic surgery (28) and general surgery (4). The mean wait for all urgent patients (from the date the letter was received by the hospital) was 11.9 days, soon referrals were all seen within 6 weeks and routine referrals were seen within 12 weeks. 10 of the 14 routine referrals had their priority status upgraded immediately by the specialist upon receipt of the letter. The initial procedure for diagnosing the melanoma was as follows; excision (67), biopsy (21), curettage (3) and amputation (2). The mean wait between initial consultation and initial procedure for all suspected diagnoses (including patients not clinically suspected of having a melanoma by the specialist) was 13.6 days. When melanoma was suspected (67 patients) the mean wait was 6.2 days. The average time between initial procedure and any wider excision (65 patients) was 33.2 days. CONCLUSION: Although many melanomas are now receiving the prompt attention they deserve, a significant proportion (over 20%) are still not being graded as urgent referrals by primary care physicians.

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P-232 NEW DIAGNOSTIC TECHNIQUES IN EARLY DIAGNOSIS OF MELANOMA IN SITU

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An accurate and timely diagnosis is important to reduce the morbidity and mortality associated with malignant melanoma. To evaluate the role of digital epiluminescence microscopy (DELM) in the differential diagnosis of cutaneous pigmented lesions and to improve the early diagnosis of melanoma in situ, 8384 pigmented lesions from 3927 consecutive patients were evaluated using MoleMax II (digital epiluminescence microscope), in the period of three years (1999-2002), in a prospective study at the Department of Surgical Oncology of the Belgrade UMC 'Bezanijska Kosa'. After clinical examination and skin-surface microscopy of the pigmented skin lesions, suspicious skin lesions, undergone to further surgical procedures of removing skin lesions, with 'ex tempore' biopsy. From the total number of 3927 patients, we were suspicious about the malignancy of the pigmented skin lesion in about 147 patients. 147 patients underwent to the surgical treatment, from which all of 144 patients (97.6%) had pigmented skin lesion diagnosed by 'ex tempore' biopsy during surgical management as a malignant pigmented skin tumor (malignant melanoma). >From the total number of patients with suspicious pigmented skin tumors (147 patients) on 97.6% patients diagnosis made by DELM was the same as diagnosis got from 'ex tempore' biopsy, which means that this procedure is highly accurate, especially if we know that all 15 patients with melanoma in situ verified with 'ex tempore' biopsy, was diagnosed prior with DELM, what is 100% accuracy. Surgical procedure-excision of the tumor was the only treatment of the patients with melanoma in situ. Digital epiluminescence microscopy is helpful device to the surgeon oncologist in deciding which pigmented lesions need surgical excision, as well as in diagnosing of melanoma in situ which use will increase, with adequate surgical procedure, a 5 year survival period of these patients.

P-233 SMALL DIAMETER MELANOMAS: IS 6 MILLIMETERS STILL A USEFUL REFERENCE IN THE EVALUATION OF PIGMENTED LESIONS OF THE SKIN?

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Background: In 1984, members of our research group published the ABCD acronym for the early diagnosis of cutaneous melanoma. The Diameter criterion of 'greater than 6 mm' was based on a review of over 1100 prospectively accrued melanomas from 1972-1982. In that cohort, greater than 95% of melanomas had diameters greater than 6mm. Since 1990, several published reports have described melanomas with diameters less than 6mm. Many of these publications used measurements from histopathology laboratories for their analyses. Objective: The purpose of this study was to re-examine the usefulness of the 'greater than 6mm' reference used in the ABCD acronym using measurements of skin lesions prior to their removal. Methods: The maximum diameters of pigmented lesions from patients participating in the MelafindTM study, which utilizes a computerized skin imaging system, were determined by computer analysis. Lesion diameter measurements and histopathologic diagnoses were available for 1657 prospectively accrued patients. Overall, 138 melanomas were diagnosed. Results: One hundred-five of 806 (13.0%) biopsied lesions greater than 6mm in diameter were invasive or in-situ melanomas, versus 33/851 (3.9%) biopsied lesions less than or equal to 6mm in diameter. The proportion of melanomas among lesions less than 6.0 mm in diameter did not vary significantly based on lesion diameter, remaining stable at 2.8% - 5%. Notably, a natural breakpoint in the data was seen at the 6 mm diameter with the proportion of melanomas rising to 19/196 (9.7%) among lesions with diameters between 6.01mm and 7.00 mm. Discussion: These data confirm the utility of the 'greater than 6 mm' reference in the ABCDE acronym.

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P-234 DERMOSCOPIC FEATURES OF PIGMENTED LESIONS ON MUCOSA.

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Background: Pigmented lesions on mucosa can clinically mimic mucosal melanoma. Dermoscopy is a diagnostic method that has been demonstrated to enhance the clinical diagnosis of nearly all pigmented skin lesions. Although little is known about its use on mucosa, it may be extremely helpful. Objective: We aimed to analyze dermoscopic features of pigmented mucosal lesions, compare them to their clinical diagnosis and describe them histologically. Methods: A total of 26 patients with oral, genital and perianal lesions on mucosa were selected. All patients had clinical and dermoscopic images stored using a digital epiluminescence microscopy camera system. All lesions were analyzed according to their dermoscopic features. Some of them were excised and then underwent histological examination. Results: A total of 34 lesions (oral=13, genital=17 and perianal=4) were analyzed and the most frequent dermoscopic features identified were homogeneous areas, globular pattern and parallel pattern. The homogeneous areas were dermoscopically characterized by diffuse pigmentation ranging from light-brown to gray-blue color and histologically by hyperpigmentation along the basal cell layer. The globular pattern was characterized by nests of pigmented cells within the epidermis and/or papillary dermis. The parallel pattern showed prominent hyperpigmentation along the basal cell layer with accentuation at the tips of elongated rete ridges. The most frequent dermoscopic feature was homogenous areas (n=19) and most of them was clinically diagnosed as mucosal melanosis (n=17). Discussion: We believe that dermoscopy was useful to differentiate vascular from melanocytic lesions. Nevertheless, among melanocytic lesions this method could not help to identify lesions with focal atypical melanocytes (n=2) and these observations are in agreement with the literature, in which atypical nevus and early melanoma have less specific dermoscopic features. Conclusion: Prospective studies including mucosal melanomas are needed to establish dermoscopic criteria that allow the better diagnosis of pigmented lesions on mucosa.

P-236 SENTINEL NODE BIOPSY FOR MALIGNANT MELANOMA. THE FIRST 250 CASES.

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Regional lymph node status is the most powerful prognostic indicator in malignant melanoma. Morton introduced the concept of preoperative lymphoscintigraphy and selective lymphadenectomy (SNB) to identify the 20-30% of AJCC stage I/II patients who have occult lymph node metastases, based on the fact that cutaneous lymphatic drainage from a primary tumour to the first, "Sentinel", node in the regional lymphatic basin can be predicted by mapping the lymphatic pathway and secondly that the primary malignancy will metastasise to the sentinel node before involving other nodes in that basin. Subsequent studies have shown the tumour status of the sentinel node predicts the status of the regional lymph nodes with 99% accuracy. Using the triple technique of preoperative lymphoscintigraphy with intra-operative blue dye and gamma probe detection we have performed SNB in 250 patients with cutaneous melanoma since 1998. Surgical identification of the sentinel node was successful in greater than 98% of cases. SNB was positive for tumour metastases in 18% (45/250) of patients. 15.5% (7/45) of these patients had further nodes involved in addition to the sentinel node. The false negative rate (recurrence in SNB negative patients) was 7.3% (15/205). We have found SNB to be an accurate staging tool. Early detection of occult nodal disease facilitates prompt surgical treatment and instigation of adjuvant therapies.

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P-237 SOCIODEMOGRAPHIC FACTORS INFLUENCING DISTANCE TO DIAGNOSING PROVIDER

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BACKGROUND: Breslow depth at diagnosis has been shown to be independently associated with travel distance between patient and diagnosing provider; as distance increases, Breslow depth increases. Distance to provider is not simply a measure of geographic isolation, as most patients are not diagnosed by the nearest provider. The aim of this study is to explore sociodemographic factors that may influence distance to provider. METHODS: A secondary data analysis was performed, using the North Carolina cohort from the Genes, Environment, and Melanoma study. The study included all incident cases of invasive cutaneous melanoma in 2000 from a 42 county area. Euclidian distance between patients and diagnosing providers were calculated. Linear and robust regression analyses were performed to test associations between distance and sociodemographic variables. RESULTS: Complete data were available for 94% of cases (616/655). Distance to diagnosing provider ranged from 0-386 miles (median 8 miles; IQ range 4-16 miles). On multivariate analysis, distance was not significantly associated with primary site, age, gender, or census tract poverty rate ($p>0.05$), and patients from rural counties traveled on average only 2.4 miles further than patients from non-rural counties ($p=0.001$). Patients from counties with >=1 dermatologist traveled on average 9.7 miles less than patients from counties without a dermatologist ($p<0.001$). However, patients who were actually diagnosed by dermatologists did not travel significantly different distances than patients who were diagnosed by other physicians ($p=0.439$). CONCLUSION: The greatest predictor of distance to diagnosing provider is the presence of a dermatologist in the county. This association is independent of the specialty of the actual diagnosing provider. This suggests that presence of a dermatologist does not directly impact distance, but rather is a marker of a high density of available healthcare services, which does impact distance to diagnosing provider.

P-238 SOFTWARE IMPROVEMENTS IN HAIR DETECTION USING DULLRAZOR

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OBJECTIVE: The Dullrazor program of Lee et al provides a software solution to removing hair with good precision from dermoscopy images of pigmented lesions. The main problem associated with Dullrazor processing is the misclassification of pigment network and other areas as hair, resulting in subsequent removal of some significant portions of the pigment network within lesions. An improved hair detection mask was developed for Dullrazor. MATERIALS & METHODS: Twenty pigmented lesion dermoscopy images, all with at least some hair within the image, were processed with Dullrazor and then processed with Dullrazor successively modified by a) removal of small noise with optimized threshold b) removal of noise with a binary threshold for internal/external lesion location c) removal of noise followed by binary noise thresholds for internal/external location d) post processing. Success of the method was judged by the hair finding metric HAIR ERROR TOTAL = (FALSE POSITIVE PIXELS (non-hair pixels found as hair) + FALSE NEGATIVE (hair pixels not found))/ (TOTAL HAIR PIXELS (found by dermatologist)) RESULTS: The Dullrazor technique was found to be subjectively accurate, with an intermediate hair mask error exceeding 50%. It was found that each hair removal step decreased the HAIR ERROR TOTAL. Problems solved with code modifications included 1) omission of one step in original Dullrazor mathematical morphology processing 2) false detection of narrow black boundaries, removed with lower noise limits 3) difficulties in following hairs inward from lesion borders. CONCLUSIONS: The Dullrazor technique may be made more robust by the software steps above. The majority of Dullrazor hair mask errors, which included narrow noise near the hairs, false positive hair-like noise from the pigment network and narrow dark borders, as well as blob noise, may be removed with the steps outlined above. The new modified Dullrazor is made available on the web.

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P-239 AUTOMATIC MELANOMA DISCRIMINATION BY SALIENT POINT DETECTION

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OBJECTIVE: The pigment network and border of dermoscopy images of pigmented lesions provide considerable information used by dermatologists in diagnosing malignant melanoma. Salient points, defined as those points on a line which have a high second derivative and a zero first derivative in a direction normal to a line, can give a measure of the number of sharp network and border points in an image. METHODS: Salient point detection using the intensity plane pre-processed with a Gaussian blurring of sigma 1.02 was applied to a set of 455 pigmented lesion dermoscopy images, including 137 malignant melanomas and 318 benign nevi, with about 2/3 of the benign set dysplastic nevi. Post-processing removed hairs, telangiectases, and edges of blotches and globules. Salient point statistics were calculated including mean, median and variance and the ratio of each of these to the mean block intensity, computed over block sizes ranging from 11x11 to 27x27. RESULTS: After comparing discrimination of benign vs malignant lesions, the best results were found for the 27x27 block size. For this block size, all six features were used as input to a standard back-propagation neural network for lesion discrimination. The 6 input features were computed for the outer 10%, 20%, 30% and 40% of each lesion. Results for these four cases were compared. Using a leave-one-out method for separation of training and test sets, the best discrimination of melanoma occurred using the outer 10% of each lesion, which produced a ROC curve demonstrating approximately 80% correct classification of malignant melanomas vs benign lesions using this feature alone. DISCUSSION: Salient points in the outer 10% of pigmented lesions appear to carry considerable diagnostic information, when statistics are computed over moderately sized (27x27) areas. Salient points may provide a significant feature for automatic discrimination of benign lesions vs malignant melanomas.

P-240 COLOR AND STRUCTURAL FEATURES FOR AUTOMATIC SKIN LESION DISCRIMINATION IN DERMOSCOPY IMAGES

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OBJECTIVE: Color and structural features provide useful information for discriminating malignant melanoma from benign simulators in digital dermoscopy skin lesion images. Color indices include the percentage of the lesion that is melanoma colored (PMC), the clustering of melanoma colors within the lesion (CCR), and the fuzzy ratio based on the moderate confidence of benign colors in the lesion. Structural features including size, distribution and shape characteristics of blotches and globules and statistical features of peripheral salient points are useful in melanoma diagnosis. MATERIALS & METHODS: 22 skin lesion features were extracted for lesion discrimination from 457 pigmented lesion dermoscopy images, including 137 malignant melanomas and 320 benign nevi, with about 2/3 of the benign set dysplastic nevi. For lesion discrimination, three color indices (PMC,CCR,FR), seven salient point size and distribution features, five globule size and distribution features, four blotch distribution size and shape features, average lesion intensity, and chromaticity and variance in red were investigated. Using a standard back propagation neural network for lesion discrimination, the image data set was partitioned into 10 randomly generated training/test sets, with disjoint test sets containing 10% of the melanoma and 10% of the nevi images. The neural network training procedure lasted 5-35 epochs or until the training RMSE error was less than or equal to 0.001. RESULTS: The average neural network discrimination results over the 10 training/test sets were true positive and true negative rates of 90.8% and 93.3%, respectively, with an overall discrimination rate of 92.5%. FR, salient point, CCR and globule features yielded better discrimination information than the PMC, intensity, red chromaticity and red features. CONCLUSIONS: The malignant melanoma discrimination results achieved in this study are promising, demonstrating the utility of integrating color and structural features for automatic discrimination.

P-241 "SUBLUMINISCENCE", "TRANSLUMINISCENCE" - A NEW METHOD TO DETECT THE SEVERITY OF DUBIOUS BENIGN ATYPIC OR DYSPLASTIC NEVI

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Often the differential diagnosis of benign nevi whether or not they are atypic or dysplastic is not easy. Everybody knows the problem that the clinical view with naked eye is not always topped by epiluminiscence-microscopy. In our study we first have taken digital photographs of nevi with the "MoleMax" computer microscope in normal manner by illuminating the skin from above out of the "chemney" where the digital camera is placed . In the second step we took photographs in the new way by "Subluminiscence" or "Transluminiscence" with a light source in the skin beyond the lesion. This light source is brought in a thin fibre to the top of cannulas of different gauges. In local anaesthesia just immediately before the lesion was being excised, we punctured the skin adjacent to the lesion and put the cannula with the light-fibre straight below the nevus. In this way the nevus shines through, it is "transluminated". This action is taken as a digital photograph on the screen of the Molemax Computer System. In magnification on the screen the pigmented structure of the nevus can be shown in this way either a normal network without any suspicious criteria or there can be shown globules, dots and irregular networks as in normal Epiluminiscence-Microscopy. We show typical cases of suspicious nevi as clinical lesion and as lesion seen as epiluminiscence photographs and as transluminiscence photographs and the histologic patterns. We show that some nevi can be better identified as not suspicious with the new method so that the excision can be done routinely without safety margins.

P-242 SPECIFIC DERMOSCOPY PATTERNS AND AMPLIFICATIONS OF CYCLIN D1 GENE DEFINE HISTOLOGICALLY UNRECOGNIZABLE EARLY LESIONS OF ACRAL MELANOMA IN SITU

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Early lesions of acral melanoma pose diagnostic problems because they show minimal histological changes despite their atypical clinical features. Amplification of the cyclin D1 gene (CCND1) is one of the characteristic genetic aberrations recently found in acral melanomas that may help to define these lesions. We carried out fluorescence in situ hybridization (FISH) analyses to examine the CCND1 amplification in a total of 33 pigmented lesions on aclar volar skin which were clinically suspected of early melanoma. Histologically, 17 of them were either melanoma in situ (8 lesions) or benign melanocytic nevi (9 lesions). Amplification of CCND1 was observed in 2 of 8 (25%) melanoma in situ. None of the 9 nevi showed amplification. The remaining 16 lesions were, however, difficult to classify histologically because most of them only showed a slight increase of non-atypical melanocytes in the basal cell layer of the epidermis. Nine of these 16 lesions exhibited a parallel ridge pattern on dermoscopy, which has been reported to be highly specific to melanoma in situ, and 4 (44%) of them had amplifications of CCND1. Amplifications were not found in any of the remaining 7 lesions that showed dermoscopic patterns suggestive of melanocytic nevi. CCND1 amplification detected by FISH identifies a very early progression phase of acral melanoma that precedes histologically defined melanoma in situ. The present study also indicates the specificity of the parallel ridge pattern on dermoscopy in detecting melanomas on acral volar skin at such a very early developmental phase.

P-243 SKIN ASSESSMENT BY SPECKLE

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OBJECTIVE: Skin surface characteristics are the subject of intensive investigation in dermatology. One of the main physical characteristics of surface is roughness, so it is reasonable to use this parameter as an objective quantification criterion for assessing and diagnosing skin diseases such as cutaneous malignant melanomas and seborrheic keratoses. The aim of this research is to develop a noninvasive, precise, rapid, and reliable method for skin surface roughness measurements. The basic hypothesis is that an optical technique can be developed to achieve our goal. However, traditional optical imaging methods are either incapable of measuring surface roughness at the micro-scale of our interests (tens to hundreds of microns) or are too costly for skin disease detection and evaluation. We choose speckle optics and speckle imaging based approach to test our hypothesis. MATERIALS & METHODS: A low coherent speckle technique is applied for the characterization of human skin surface micro texture, particularly, root mean square roughness. Being illuminated by low coherent light a rough surface creates speckle pattern, which are captured by a CCD camera and analyzed quantitatively. The contrast of the speckle pattern is used to derive the surface roughness parameters. RESULTS: The system is verified using reference objects (metal surface standards, abrasive papers, and human skin replicas) and comparing roughness determined by speckle contrast with the independent precise profilometry results. The difference in roughness value for different skin lesions including melanomas and seborrheic keratoses is estimated. CONCLUSION: We have successful developed a low coherent optical speckle imaging system and data analysis method, which can provide information about skin roughness useful for diagnostic and research purposes. The system shows potential to be a reliable, rapid, noninvasive, and simple method for differentiating melanomas and seborrheic keratoses.

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P-244 DETECTION AND GENOMIC CHARACTERIZATION OF LATENT DISSEMINATED MELANOMA CELLS IN SENTINEL LYMPH NODES

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Objective: Current histopathological methods may fail to detect small numbers of melanoma cells in sentinel lymph nodes (SLNs). In our prospective study we tested a novel immunocyotochemical assay for detection of melanoma cells in the SLN and describe genomic changes found in single disseminated tumour cells. Materials and methods: 358 consecutive melanoma patients underwent sentinel lymph node biopsy. Their 494 SLNs were examined by immunocytochemistry and by standard histopathology. For immunocytochemistry the lymphatic tissue was disaggregated mechanically and stained with the antibodies HMB-45 and Melan A. Microscopic detection of antigen positive cells was correlated with the result of standard histopathology which included haematoxylin and eosin staining and immunohistochemistry. 30 individually isolated antigen positive cells were analyzed by single-cell comparative genomic hybridization. Results: 43 of 358 patients (12%) were positive by standard histopathology while HMB45 immunocytochemistry detected 159 (44%) positive patients. None of the 59 control samples from nonmelanoma patients reacted with the HMB45 antibody. Twenty-four of 30 isolated immunocytochemically positive cells (80%) displayed chromosomal aberrations indicative of their malignant origin. Interestingly some of these cells harbored the almost complete set of genomic aberrations characteristic for fully metatastatic cells although they were isolated from nodes that contained only very few tumor cells. Both the number of immunocytochemically positive samples and the number of positive cells in the sentinel node correlated with the thickness of the primary tumor ($p=0.001$ and $p<0.0001$, respectively) strongly arguing for the clinical relevance of the method. Conclusion: We conclude that findings of immunocytochemistry more accurately reflect the presence of disseminated melanoma cells in the SLN than standard histopathology. Further studies will show whether this quantitative approach in combination with a whole genome analysis of the isolated cells might enable us to define individual risk factors for disease progression in patients with clinically localized melanoma.

P-245 A META-ANALYSIS OF MELANOMA AND NEVI

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OBJECTIVE: The goal of this study is to quantify the association of nevi with the occurrence of non-familial cutaneous malignant melanoma (CMM). We examined the strength and consistency of the associations of nevus counts with CMM in the published literature. MATERIALS & METHODS: We abstracted information on risk of CMM related to the number, location, and type of nevi from a comprehensive MEDLINE search of articles published from 1966-2004. Odds ratios and variances were pooled across studies, stratifying by method of assessment and locale of nevus counts. Pooled odds rations and 95% confidence intervals were calculated for each nevus type and location. RESULTS: According to the pooled analyses, nevus counts are strong predictors of CMM regardless of body site. However, the size and location of nevi denote marked differences in calculated risk associated with CMM. While clinically atypical nevi are associated with the highest risk of CMM, common nevi also denote a substantial risk. As expected, CMM risk associated with nevus counts of any body site decreases when analyses are restricted to studies adjusting for sun-sensitivity. Alternatively, this risk increases when restricting analyses to studies where clinicians assessed and counted nevi. Consistently, the association of number of common nevi and CMM remains significant even after adjusting for all other factors assessed. CONCLUSION: Results from this meta-analysis indicate that the presence of common nevi denotes a substantial risk of CMM independent of other known risk factors (constitutional factors and sun exposure). Publications vary widely on the method of assessing and counting nevi. Consequently, the degree of risk for non-familial CMM differs according to the number, type and location of common nevi. While the presence of clinically atypical nevi is an important risk factor for CMM, clinicians must be aware of the substantial CMM risk associated with the presence of common nevi.

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P-246 WHAT IS THE EVIDENCE IN MELANOMA THAT ALL MICROSCOPICALLY INVOLVED SENTINEL NODES, IF LEFT IN SITU, WOULD PROGRESS TO OVERT NODAL METASTASES?

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What is the evidence in melanoma that all microscopically involved sentinel nodes, if left in situ, would progress to overt nodal metastases? An assumption has been made that all sentinel node (SN) -positive patients are prognostically equivalent to patients who develop overt nodal metastases when survival is calculated from the date of wide local excision. That is to say, all positive SNs would have progressed to overt nodal disease had these nodes not been removed. Morton's "matched pair" analysis (Ann Surg 2003;238(4):538-49) and others make this assumption but is it accurate? Do the tiniest, earliest traces of melanoma, so avidly sought by multiple sections, immunohistochemistry and RT-PCR necessarily have clinical and prognostic significance? There is evidence that not all microdeposits are equivalent in prognosis. We know that 2/3 of metastases in sentinel nodes are less than 1mm in maximum diameter, and 1/3 greater. Several authors (Starz, Ann Surg Oncol 2004;11(3S):162S-8S, Carlson, Ann Surg Oncol 2003;10(5):575-81, Ranieri, Ann Surg Oncol 2002;9(10);975-81) have shown that within the sentinel node, geographical distribution of micrometastases as well as tumour burden significantly affect overall survival. It appears that deposits >1mm (Starz) have a prognostic significance similar to overt palpable nodal disease while deposits <1mm behave no differently from cases with no discernable melanoma deposits. Therefore, small deposits may be associated with dormancy, latency, low proliferative rates, inactivation and cell demise depending on a balance of microbiological molecular factors such as VEGF or immunosuppression. The developmental pathway taken by melanoma micrometastases within sentinel lymph nodes is uncertain. Metastases of different size have different prognostic significance. Therefore it cannot be assumed that SN-positivity is prognostically equivalent to overt nodal metastases.

P-247 LONG STANDING MELANOMA REGRESSION WITH AN ANNULAR SHAPE

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We present a case of a melanoma regression in a 59 year-old man, 12 year evolution, with a slowly progressive grow. The lesion was located on his back, had 4.5 x 6 cm, an annular shape, whitish hue and a spared central area. Initial clinical and dermatoscopical diagnosis was a non melanocytic lesion, before a diagnostic cutaneous biopsy was performed. The histopathological examination revealed atypical melanocytes widely scattered throughout the epidermis and a band-like infiltrate of lymphocytes, fibroplasia and scattered melanophages in papillary dermis. A diagnosis of melanoma in situ with regression was made. The lesion was removed with a margin of 1 cm. The patient is currently completely asymptomatic.

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P-248 CORRELATION WITH DIGITAL DERMOSCOPIC IMAGES CAN HELP DERMATOPATHOLOGISTS TO DIAGNOSE DIFFICULT PIGMENTED SKIN TUMORS

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Objective: To investigate, if the inter-observer agreement between histopathologic diagnoses of difficult pigmented tumors made by two referral centers can be improved by additional use of dermoscopic images. Retrospective study using tumors excised in two pigmented skin lesions clinics and primarily diagnosed in the referring centers based on clinical data, histopathology, and if required immunohistochemistry. Slides were exchanged between the participating centers. There, diagnoses were made 1) based solely on dermatopathology and clinical information, and 2) after a washout phase with the additional use of dermoscopic images. Materials & Methods: 160 pigmented tumors were selected in Graz and 141 in Tübingen. H&E sections, patient s age and sex, tumor localization, and digital dermoscopic images were sent to the other center. The 301 tumors studied were primarily diagnosed as 74 melanomas, 218 melanocytic nevi, and 9 non-melanocytic tumors. The main outcome measure was the Cohens kappa coefficient of the primary diagnoses of the center submitting the cases and the diagnoses of the other center without and with dermoscopy. Results: The kappa coefficient between the primary diagnoses and those made by the second center without dermoscopy was 0.90 in Graz, 0.73 in Tübingen, and 0.81 overall. With the additional use of dermoscopy kappa was constantly high with 0.89 in Graz, and improved to 0.87 in Tübingen, and to 0.88 overall. Conclusion: The advances of telemedicine offer means to easily store and transfer dermoscopic images. In the near future more and more dermoscopic images will be sent to pathologists with excised tumors. In our study the additional use of digital dermoscopic images could further improve the overall very good agreement of histopathological diagnoses between two referral centers of dermoscopy and dermatopathology. Studies to show the benefit of dermoscopic images for dermatopathologists who are non-experts in dermoscopy including formal training in dermoscopy are required.

P-249 THREE INDUCTORS OF APOPTOSIS: TNF ALPHA, FASL AND TRAIL, IN MALIGNANT MELANOMA

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We have investigated the presence in peripheral blood of malignant melanoma patients of three inductors of apoptosis, members of TNF family : TNF alpha, FASL and TRAIL. Each of these, represent the ligand for a distinct apoptosis pathway : the FAS/FASL pathway being the main way in normal and tumoral cells, TRAIL pathway, activated especially in tumoral cells and TNF alpha also connected with inflammatory processes. Melanocytes express receptors on their surface for each of these ligands. Twenty patients with melanoma were investigated into two lots lot A: 8 patients with brain metastases of malignant melanoma and lot B: 12 patients with cutaneous melanoma, unique tumor (more 1,5 cm3). Serological determinations performed before surgery were: TNF alpha (normal values:< 15 pg/ml); FASL (40-145 pg/ml) and TRAIL (28-135 pg/ml) all by ELISA (R&D System). Results. Lot A: TNF alpha, all cases with increased values (media 240 pg/ml), FASL 3 cases with increased values (210 pg/ml), sFAS 6 cases with increased values (21 000 pg/ml), TRAIL 7 cases with increased values (400 pg/ml). Lot B, TNF alpha presented elevated values in all cases (media 240 pg/ml) FASL with increased values in 4 cases (180 pg/ml), sFAS increased values in 4 cases (24 000 pg/ml), TRAIL elevated values in 9 cases (450 pg/m;l) Conclusions. TRAIL values were elevated in 16 cases suggesting an active mechanism in both clinical forms of melanoma. FASL values were decreased in 13 cases and sFAS increased in 10. All cases with decreased FASL presented increased values of sFAS suggesting a serological blocking of FASL by sFAS. TNF alpha increased values in all cases does not seem to be apoptosis connected. The lower levels of TRAIL in lot A may be interpreted as an active but little efficient pathway for apoptosis in malignant melanoma

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P-250 S100, A MARKER OF MELANOMA AND DENDRITIC CELLS

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Objective: Characterization of S100 in melanoma and dendritic cells (DC). Introduction: Serum S100B is a valuable marker for monitoring response to chemotherapy in stage IV melanoma. Patients with stage IV melanoma and normal or rapidly normalized serum S100B have a better survival than those with increased S100B (median 14 versus 5 months) and may be candidates for more intensive treatment. DC can be recognized by S100B expression and may be responsible for (low) serum S100B levels. Materials and methods: DC preparations were made from PB monocytes by stimulation with GM-CSF and IL-4. Maturation was induced by TNFa. S100B was measured in supernatant by an immunolumino-metric assay (Elecsys S100 Roche). Individual dimers S100A1B and S100BB were measured by ELISA (CanAg S100 EIA). Expression of S100A1 and S100B in immunohistochemistry was studied with monoclonal antibodies. Results: In 17 supernatants of DC a higher S100B was measured in mature DC (median 0.67 mg/l) than in immature DCs (0.33 mg/l). Comparison of S100B with the maturation markers IL-12 and % CD83 in 7 cases showed no correlation. Nine samples of DC showed no A1B, but only BB dimers. After loading DCs with melanoma cell lysate, high S100B values were found in the supernatant (29.3 mg/l) with both A1B and BB dimers, similar to melanoma cell lines. In immunohistochemistry melanoma showed expression of S100A1 and S100B, whereas DCs in lymph nodes (sinus, paracortical area) showed only S100B. In contrast follicular DCs showed only S100A1. Conclusion: S100 in melanoma cells differs from S100 in DC: A1B and BB dimers in melanoma, versus only BB dimers in DC from PB and in LNs. Levels of S100B in supernatants of DCs are lower than in melanoma cells. Normal levels of serum S100B in patients/controls may be derived from DCs, but requires further study.

P-251 ASSESSMENT OF THE ROLE OF SENTINEL LYMPH NODE BIOPSY FOR PRIMARY CUTANEOUS DESMOPLASTIC MELANOMA

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Background: Desmoplastic melanoma (DM) is an uncommon variant of cutaneous melanoma. The role of sentinel lymph node biopsy (SLNB) in the treatment of DM remains undefined. The purpose of this study was to evaluate the use of SLNB for DM in a large academic cancer center. Method: Between November 1992 and October 2003, 1,850 patients with cutaneous melanoma underwent wide local excision and SLNB. Patients with DM were identified and stratified as "pure" DM or "mixed" DM (i.e., DM associated with at least 1 other common histologic subtype). Statistical analyses were performed to examine the association between clinicopathologic factors, pathologic status of the SLN, and disease-free survival. Results: Of the 1,850 patients, 65 (3.5%) had DM. Of these, 46 (70.8%) had pure DM and 19 (29.2%) had mixed DM. Patients with pure DM had a median tumor thickness of 3.5 mm and 6.5% were ulcerated. Compared to patients with pure DM, patients with either mixed DM or non-DM (n=1,785) had thinner primary tumors (1.7 mm vs. 1.5 mm, p<.001) that were more likely to be ulcerated (27.7% vs. 21.3%, p< .05). Although the incidence of a positive SLN was similar in patients with mixed DM (15.8%) and non-DM (17.5%), patients with pure DM were less likely to have a positive SLN (2.2%) (P = .007 vs non-DM). At a median follow-up of 2.9 years, no patient with pure DM had recurred. Conclusion: Despite having thicker primary tumors, patients with pure DM have a lower incidence of positive SLNs compared with patients with non-DM. Whereas patients with mixed DM should be treated like all other melanoma patients, patients with pure DM are less likely to benefit from SLNB.

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P-252 ON THE IMPORTANCE OF PREDICTION AND MONITORING OF MELANOMA PATIENTS.

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Objective: Biochemotherapy has shown a high response rate and durable remissions, but no overall survival benefit in randomised trials. Bcl-2 anti-sense therapy has shown some efficacy but no improved overall survival. Subsets of patients get durable remissions, but this cannot be demonstrated in unselected groups of stage IV patients. We have shown the importance of CD4+ tumour-infiltrating lymphocytes for response to interferon-alpha and to biochemotherapy (A. Hakansson et al., Br J Ca, 74:670,1996. A. Hakansson et al., Br J Ca, 85(12):1871,2001). The treatment outcome can be monitored by analysing regressive changes (A. Hakansson et al., Melanoma Res, 13:401,2003) and increased Bcl-2 positivity after biochemotherapy points to the value of anti-sense therapy (A. Hakansson et al., Ca Immunol Immunother, 52:249,2003). The present study focus on the need for monitoring Bcl-2 both at the protein and mRNA level. Methods: Real-time PCR was used to analyse Bcl-2 mRNA in 15 patients treated with biochemotherapy (Cisplatinum/DTIC/IFN-a). The Bcl-2 protein expression was analysed using immunohistochemistry and comparisons were made with 13 untreated patients. The number of CD4+ lymphocytes were determined in fine needle aspirates pre-treatment. Results: A comparison of the Bcl-2 mRNA in biopsies from untreated and treated patients, scored to have a high protein expression, showed significantly lower mRNA expression in biopsies from treated patients compared to untreated, p=0.004. Conclusions: Bcl-2 has to be determined both at the protein and mRNA level in order to optimally monitor anti-sense therapy. Our updated results on the importance of tumour infiltrating CD4+ lymphocytes to make immunotherapy/biochemotherapy successful will also be presented. Taken together this kind of tests will be of importance as new markers in future trials, increasing the cost-benefit considerably both in terms of patient adverse reactions and health care costs.

P-253 COMBINATION OF SERUM MELANOMA INHIBITORY ACTIVITY (MIA) AND 18F-FDG PET IS USEFUL IN THE FOLLOW UP OF MELANOMA PATIENTS

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BACKGROUND: 18F-FDG PET is currently the most sensitive imaging method for detecting melanoma metastasis. However, the instrument of PET has not been available at nationwide hospital yet, and repeated performance of PET scan is not financially recommended. We recently reported that elevated serum MIA level suggested the presence of occult melanoma metastases in the follow up period after resection of primary melanoma. We conducted this study to know the usefulness of serum MIA screening to detect high-risk patients who should be further studied by PET. METHODS: Twenty patients who had undergone surgical excision of melanoma and were under follow up without clinical evidence of recurrence were included to this study (1-238 months after surgery, median 14.5). All 20 patients underwent whole body 18F-FDG PET imaging and serum MIA measurement by commercial ELISA kit. RESULTS: In 11 of 20 patients, serum MIA level was elevated. In 4 of these 11 patients (36%), 18F-FDG PET imaging was positive. One patient had lymph node metastases in iliac lesion. In the other patient, occipital lymph node metastasis detected by PET was 2 mm in diameter. In the rest of 2 patients, small intestinal metastasis and multiple bone metastases were detected, respectively. On the other hand, in all nine patients with normal serum MIA level, PET imaging showed no evidence of metastatic disease.
CONCLUSION: This preliminary study suggests that serum MIA measurement is helpful in screening the patients who need further assessment by 18F-FDG PET imaging.

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P-254 TENASCIN-C AND EZRIN IN PRIMARY CUTANEOUS MELANOMA

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Objectives: In vivo confocal laser scanning microscopy (CLSM) allows for non-invasive high resolution imaging of cellular and architectural details of human skin. This imaging modality is particularly useful for melanocytic lesions, where melanin and melanosomes provide image contrast, permitting the evaluation of melanocyte distribution and morphology. These characteristics can be helpful in margin assessment of lentigo maligna melanomas and amelanotic melanomas, where clinical assessment may be equivocal and multiple biopsies may be required for precise margin delimitation prior to surgery. Methods: We present a case series of challenging melanomas where confocal microscopy was compared to routine histology for margin mapping. Results: We found overall good correlation between margins assessed by in vivo confocal microscopy and pathology. CLSM detected margins accurately in comparison to histology in the majority of cases, but factors such as severe sun damage and use of topical medications appear to influence cellular distribution, making margin definition less accurate. The presence of pagetoid melanocytes greatly facilitates the recognition of melanoma on CLSM. A limitation of this technique with the technology utilized in this study was the need to reapply the tissue stabilizing ring at multiple sites for large lesions. Conclusion: Newer generation CLSM instruments may significantly improve presurgical margin assessment for large indistinct and amelanotic melanomas.

P-255 CLINICAL SIGNIFICANCE OF CXCR3 AND CXCR4 EXPRESSION IN PRIMARY MELANOMA

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Objective: Analyze the expression of chemokine receptors CXCR3 and CXCR4 in primary melanomas and to correlate it with clinical outcome. Materials & Methods: Punch biopsies were obtained from primary melanomas and nevi. The specimens were frozen for immunohistochemical study. Serial sections were stained with monoclonal anti-human CXCR3, CXCR4, HMB-45, polyclonal rabbit anti -S-100 serum, and isotype matched control antibodies. The expression of chemokine receptors was correlated with the development of metastases and the occurrence of death. Statistical analysis was performed using Chi-Square test, Kaplan-Meier analysis and Cox regression. Results: Forty primary melanomas and seven nevi were available for analysis. More than 50% of the primary tumours expressed CXCR3, whereas roughly one third (35%) expressed CXCR4. None of the cases of melanoma in situ or nevi expressed either chemokine receptor. Patient population included 20 women and 20 men with a mean age of 61 years. The mean Breslow thickness was 1.45 mm (0-6.50). The median follow-up time was 32 months. The expression of CXCR4, but not of CXCR3, was significantly associated with the development of regional lymph node metastases (p=0.04), distant metastases (p=0.04), and an increased mortality rate (p=0.009). Also expression of CXCR4, but not of CXCR3, was significantly associated with the presence of ulceration and increased tumour thickness. Conclusion: Expression of chemokine receptors not only facilitate migration of tumour cells but also enhance their invasiveness, growth and survival. The expression of certain chemokine receptors in primary melanomas, particularly CXCR4, might predict a highly aggressive biologic behaviour and thus correlate with prognosis. Likewise chemokine receptors might prove to be a useful target for biologic therapy in the management of patients at risk for disseminated disease.

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P-256 PROGNOSTIC SIGNIFICANCE ANALYSIS OF MICROSCOPIC AND SUBMICROSCOPIC METASTASES IN SENTINEL LYMPH NODES FROM PRIMARY CUTANEOUS MALIGNANT MELANOMA PATIENTS.

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Background. We studied the frequency and clinical impact of microscopic and submicroscopic metastases in sentinel lymph nodes (SLNs) from early-stage melanoma (AJCC stages I and II). Methods. All patients underwent selective lymphadenectomy and their SLNs were studied with routine H-E staining, immunostaining (S100, HMB45) and reverse transcription-nested PCR (RT-PCR) to detect tyrosinase mRNA. Patients were classified in four groups according the presence of microscopic/molecular metastases in SLNs): A(+/+), B(-/+), C(-/-) and D(-/discordant results). Group A patients were offered completion lymphadenectomy and adjuvant treatment with high-dose interferon-alpha2b. Patterns of recurrence, disease-free survival (DFS) and overall survival (OS) were compared into the four groups. Results. 46 out of 192 patients (23.9%)had at least one histologically positive SLN, 78(40.6%)had only submicroscopic metastases and 51 (26.5%)had no evidence of microcopic either submicroscopic disease. After a median follow-up time of 35 months, 19(9.8%)of the patients recurred: 11 in group A (23.9%), 6 in group B (7.7%) and 2 in group C (3.9%). No recurrences were observed in group D (17 patients). Most of the recurrences in group A were systemic while loco-regional recurrences were the most common in group B or C. Statistical significant differences in DFS were observed only between group A and the rest of the groups (p=0.0001). Conclusions. Detection of submicroscopic disease by tyrosinase RT-PCR does not offer a better prognostic stratification of patients with primary cutaneous melanoma than conventional histologic methods. A multimarker molecular analysis more sensitive and predictive is now in progress.

P-257 PROGNOSTICAL SIGNIFICANCE OF TUMORAL GANGLIOSIDES LEVELS IN MELANOMA

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This study analyse glycosphigolipids expression in cutaneous melanoma and their relationships with invasion, metastasis and overall survivall of patients with cancer. The study was performed in a period of 20 years on 83 cases with malignant melanoma clinical stage I, histopathologically confirmed and previously untreated. The malignant phenotype is associated with gangliosides like: GM3, GM2, GD3, GD2, and O-acetyl-GD3 (results published before). It was observed a positive correlation between gangliosides levels and the increase of invasive ability and metastasis. GM3 is associated with a low rate of tumor growth and a reduced risk of survival and it can be associated with an early recurrence in patients with malignant melanoma. GD3 and GD2 expressions are correlated with malignant melanocytes proliferative ability. O-acetyl-GD3 is associated with later stages of disease and a low rate of survival in patients with melanoma. The decrease of gangliosides synthesis in tumor cells can be realised by glucosylceramide synthase inactivation wich leads to ceramide accumulation in tumor cells. Ceramides mediates antiproliferative responses (growth inhibition, apoptosis, differentiation, telomerase activity up-regulation, senescence). De novo ceramides synthesis stimulation, the increase of sphingomyelinase activity, glucosylceramide synthase inactivation, disfunctions in ceramides catabolism, are promising therapeutic strategies in malignant melanoma.

P-258 PROGNOSIS VALUE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN CUTANEOUS MALIGNANT MELANOMA

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Background: Neoplastic angiogenesis is the process of forming new blood vessels by tumors. This process plays an important role in progression of cancer and metastasis. Vascular endothelial growth factor (VEGF) is a crucial angiogenic factor in stimulation of the vascular endothelial cells and their proliferation and migration. Then, density of VEGF expression in tumors may influence the clinical behavior of cancer. Objetive: To determinate whether density of VEGF expression in invasive cutaneous malignant melanoma influence their clinical prognosis (relapse free survival and overall survival). Methods: A total of 56 patients with invasive cutaneous malignant melanoma (Breslow less than 4 mm) were included in this pilot study. Tissue microarray blocks of melanoma specimens were evaluated by immunohistochemistry for the expression of VEGF. We reviewed each patient s characteristics (age, sex, localization of primary tumor, Breslow, Clark, ulceration, stage (TNM), treatment, localization of relapse, number of relapse, relapse free survival and overall survival). We compared the VEGF tissue expression with these clinical and pathological parameters in order to establish if density of VEGF has prognosis value in invasive malignant melanoma.

P-259 MORPHOLOGIC EVALUATION OF THE SENTINEL NODE DOES NOT CORRELATE WITH SURVIVAL OF MELANOMA PATIENTS

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The aim of this study is to determine whether the characteristics of microscopic involvement of the sentinel node correlate with possible OS and DFS. Between 1999 and 2004 at National Cancer Institute of Milan 1191 had SNB. Out of this 852 had adequate follow up and all histological parameters recorded; 135 had a positive sentinel node and underwent CLND. OS and DFS were considered according to the number of positive sentinel nodes (1, 2 or 3 nodes), to the number of metastatic foci in the sentinel node, to the diameter of the major metastatic foci (single cells, diameter < .5 mm,, diameter between .5 and .9 mm, diameter 1 to 1.9 mm, diameter > 2 mm) and to metastasis localization. OS and DFS in patients (any Breslow thickness) with a negative sentinel node were statistically better (P 10-5) in the group With SNB negative. Even the number of SNB positive (1 vs 2) showed a better prognosis in the group of 1 SNB positive (P 0,0012). We recorded one patient with 3 positive sentinel nodes with a prolonged survival, but we think this isolated case shouldn't be considered. When we considered others morphological features such as number of metastatic foci, even if related to the number of SNB positive, or the dimension of major metastatic focus and the anatomical localization of metastatic deposit in the lymph node, none of these parameters was significant relevant to assess prognosis of these subgroups of patients. SNB provides excellent regional disease control. Our results indicate that the morphologic involvement of the sentinel node does not correlate with OS of melanoma patients. To obtain a biologic significance of micrometastatic melanoma, the morphological characteristics of sentinel nodes probably need to be correlated to the molecular profile of primary tumour and of SNB positive.

P-260 DEVELOPMENT OF A HIGHLY SENSITIVE AND SPECIFIC ASSAY TO DETECT MUTANT BRAF ALLELES IN TUMORS AND BLOOD

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Background: Mutations in the BRAF oncogene at amino acid 600 have been reported in 40% to 70% of human metastatic melanoma tumor tissues, and the critical role of BRAF in the aggressive biology of metastatic melanoma has been established. Clinical trials with several BRAF inhibitors are underway. Objective: To develop a robust method to detect mutant BRAF alleles in clinical specimens that could be used to help select patients for anti-BRAF therapy and to monitor their responses to that therapy. Methods: A mutation-specific PCR assay was optimized to specifically amplify the mutant BRAF allele without amplifying the wild-type allele. Experiments mixing DNA from a BRAF mutant melanoma cell line with wild-type, human placental DNA in varying proportions were performed to determine the overall sensitivity of this assay, and to compare its sensitivity to routine DNA sequencing, the current gold standard for detection. The assay was also applied to human blood and tumor specimens. Results: The mutation-specific PCR was able to reliably detect 0.1 ng of mutant DNA when mixed with 100 ng of wild-type DNA. DNA sequencing could not reliably detect the mutation until the mixture contained 50 ng of mutant DNA mixed with 50 ng of wild-type DNA, making the mutation-specific assay 500-fold more sensitive than DNA sequencing. The mutation-specific PCR was unable to amplify the wild-type allele despite varying the annealing temperature, demonstrating is specificity. The mutation-specific assay was able to detect mutant BRAF alleles in 7/30 (23%) primary melanomas that were negative using DNA sequencing. The assay also detected mutant BRAF alleles in plasma samples from 8/14 (57%) patients with metastatic melanoma. Discussion: Our data demonstrate that the mutation-specific PCR assay is much more sensitive than DNA sequencing, and that it is well suited to detect mutant BRAF alleles in human blood and tumor specimens.

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P-261 CHARACTERIZATION OF DENDRITIC CELLS IN SLN OF MELANOMA PATIENTS

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Objective: Antigen-draining lymph nodes (LN) play a crucial role in the development of an immune response. Studies on interdigitating dendritic cells (DC) present in sentinel LN (SLN) from melanoma patients have suggested an immuno-suppressed status of SLN compared to non SLN. The purpose of this study was to characterize, both quantitatively and qualitatively, the DC present in metastatic versus non-metastatic SLN as well as in non-SLN. Methods: Formalin-fixed, paraffin embedded sections from 37 metastatic SLN, 48 non-metastatic SLN, and 37 non-sentinel LN were stained with antibodies to the DC markers langerin, CD1a, and DC-Lamp (a marker of mature DC). For quantitative analysis, the density of immuno-labeled DC was determined by counting stained cells in areas with highest densities. Double immunofluorescence stainings (anti-langerin/DC-Lamp and anti-CD1a/DC-Lamp) followed by confocal microscopy were performed to assess the maturation state of DC. Results: A comparison of sentinel and non-sentinel LN showed a trend toward a reduction in DC density in the former group. Interesting differences were found between metastasis-free and metastasis-containing SLN. DC-Lamp positive cells (indicative of a mature phenotype) were present at significantly higher densities in metastatic SLN compared to non-metastatic SLN. In contrast, langerin positive cells were present at higher densities in the non-metastatic SLN. Furthermore, irrespective of the type of LN analyzed, the majority of langerin and CD1a positive cells displayed a DC-Lamp-positive mature phenotype. Finally, within the group of patients with a metastatic SLN, a correlation was found between high DC-Lamp densities and a better prognosis. Discussion: These results suggest that early metastatic disease in SLN may be conducive of an immune response and that mature DC may participate in limiting the further spread of melanoma. A large multicenter study on the prognostic value of the density of mature DC cells is underway. This abstract is presented on behalf of the EORTC Melanoma Group

P-262 DIFFERENTIAL EXPRESSION OF CHEMOKINE RECEPTORS IN MELANOCYTIC LESIONS

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It has been suggested that the selective expression of certain chemokine receptors by melanoma cells and the presence of their ligands in specific tissues is part of site specific metastasis. Indeed, expression of CXCR4, CCR7, CCR9 and CCR10 in melanoma cell lines has been found to facilitate metastases to lung, lymph nodes, small bowel and skin, respectively. Because the expression profile of chemokine receptors in tissues of melanocytic lesions is unknown, we performed a comprehensive study on paraffin embedded tissue and investigated the expression on the mRNA level by real-time PCR, employing a semiquantitative approach. A melanocyte cell line, melanoma cell lines (n= 7), congenital nevi (n=5), primary melanoma (n=12), cutaneous/subcutaneous metastasis (n=10), lymph node metastasis (n=12), and visceral metastases (n=12) of malignant melanoma as well as normal tissue (n=4) were studied for the expression of all currently known chemokine receptors. Consistent, but low expression of CXCR4 and CCR1 was found in all lesions. CCR9 was expressed in most lesions and most abundantly in lymph nodes. Surprisingly, CCR10 was not expressed in any of the lesions, but expression was observed in melanoma cell lines. Expression of CCR7 was commonly found in primary melanomas, but was more restricted in lymph node and cutaneous metastases. CCR5 was only expressed in primary melanomas and some cutaneous metastases. De novo expression of CXCR5 and CXCR6 was observed in some primary melanomas and metastases, but not in any of the melanoma cell lines and congenital nevi. Positive staining of CCR9 by immunohistochemistry confirmed the presence of this chemokine receptor in melanocytic tissue. Results revealed a restricted and differential pattern of chemokine receptor expression and warrant functional studies on some receptors.

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P-263 INACTIVATION OF DNA-REPAIR GENE MGMT BY PROMOTER METHYLATION IN MELANOMA METASTASES IS MORE FREQUENT AMONG PATIENTS DEMONSTRATING A RESPONSE TO BIOCHEMOTHERAPY

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Silencing of the gene encoding the DNA-repair enzyme 06-methylguanine-DNA methyltransferase (MGMT) via hypermethylation has been shown to be associated with tumor cell killing by alkylating agents. One mechanism by which metastatic melanoma is associated with a poor prognosis is the development of resistance to the DNA damaging effects of chemotherapy. We sought to determine whether the methylation status of MGMT in tumors from stage IV melanoma patients was associated with response to biochemotherapy (BC). Previously we reported our results of a concurrent BC regimen containing DTIC, Cisplatin, Vinblastine, Interferon, IL-2, and Tamoxifen. In this study we assessed metastatic tumor tissues from 14 patients prior to the start of BC (14 specimens) and 12 patients (35 specimens) who underwent surgical resection of all clinically apparent disease following BC. Paraffin-embedded tumor specimens were subjected to methylation specific PCR for MGMT and the presence of methylated/ unmethylated alleles were validated using CEQ capillary array electrophoresis. MGMT hypermethylation was detected in 10 (20%) of 49 tumors. Eight (31%) of 26 patients had at least one metastasis positive for MGMT methylation and was most frequent among partial responders (6 of 8 patients, 75%) to BC as compared to those with stable (0 of 4) or progressive (2 of 13, 15%) disease In the 8 patients who had more than one tumor resected following BC, 5 (63%) had metastasis that all displayed the same methylation status demonstrating relative homogeneity among each individual's tumors. Methylated DNA for MGMT can be identified, albeit infrequently, in metastasis from patients with advanced melanoma. However, in this pilot study its frequency was greater among patient tumors demonstrating a response to BC. These findings provide molecular evidence which may account for the relative resistance of this disease to treatment, yet when present may identify select patients who respond to BC.

P-264 CRITICAL ASSESSMENT OF THE MELANOMA PARADIGM

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OBJECTIVE: A paradigm is important for interpretation of data and communication of ideas within a scientific community. The current melanoma paradigm is carcinoma-like. It originated with pathologists in the 1950's and is in need of reassessment. MATERIALS & METHODS: Review of selected literature from 1894 to the present. Personal communication with pathologists, clinicians and researchers. Daily examination and interpretation of pigmented lesions. RESULTS: The current paradigm was created at a time of debate about the origin of the melanocyte - transformed keratinocyte versus migrant neural crest. Those in favor of a keratinocyte origin dominated and the result is a carcinoma-like paradigm with in situ and invasion. It originated before description of the dysplastic nevus and before discussion of the dysplasia-melanoma sequence (DMS). The result is that the DMS now begins as nevo-melanocytic tissue and changes mid-sequence to carcinoma-like tissue. As melanoma arises from dysplasia it is not carcinoma-like but rather arises as a series of steps of melanomatous vertical growth. The early steps of melanoma are nevoid rather than carcinoma-like and are commonly layered or accretive as the melanocytes are delivered to the dermis at an increased rate. Less commonly they are migratory or infiltrative. Later steps are expansile and composed of high-grade tumor cells. Large primary melanomas commonly have multiple patterns and cell types representing the clonal steps of evolving melanomatous vertical growth. This has been referred to as polyclonism. DISCUSSION: The melanocyte is a unique tissue type derived from the migrant neural crest. Molecular based diagnosis, treatment and research may require that the DMS remain nevo-melanocytic throughout. It is time to consider upgrading the melanoma paradigm from a carcinoma-like one to one that is compatible with the DMS. The interpretation of molecular data may benefit from using a new melanoma paradigm.

P-265 A CD-ROM TO AID CLINICOPATHOLOGICAL ANALYSIS OF CUTANEOUS MELANOCYTIC LESIONS.

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Melanocytic lesions may be difficult to analyze and a simple and readily accessible reference system would be of value. We propose a CD-Rom-based resource that uses three algorithms: the first based on "clinical information", the second on "lesional silhouettes" (evaluating slides without magnification against good light) and the third on "microscopic examination" (emphasizing lesional location in the skin: epidermis alone, epidermis and dermis, dermis alone or dermis and subcutis). These algorithms assist pathologists to sharpen their diagnostic focus. We have chosen to prepare a CD-Rom because this is currently the most adaptable medium and it permits the use of algorithms with numerous readily accessible critical links to the full range of melanocytic tumors. This CD-Rom does not replace available books on the subject, but provides an accessible tool for education (including self evaluation on the basis of 40 exemplary cases) and to facilitate day to day evaluation of slides from patients with melanocytic lesions. The CD also includes more traditional chapters: - the relative significance of different criteria in determining "benign versus malignant". - technical aspects of the management and evaluation of melanocytic tumors (including immunohistochemistry and sentinel lymph node analysis) - preparation of optimized reports that include macroscopic and microscopic information essential for clinical management and accurate assessment of likely clinical outcome (including the TNM classification). An encyclopedic section of the CD-Rom comprises 127 pages detailing different melanocytic lesions, more than 2000 color photomicrographs, 350 clinical pictures and 200 line diagrams.

P-266 MALIGNANT BLUE NAEVI -- CLINICAL FEATURES AND PROGNOSIS

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OBJECTIVE: Malignant blue naevus (MBN) is a rare cutaneous tumour, the natural history and prognosis of which are poorly documented in the literature. We sought to examine the clinical features, treatment outcomes and prognosis of MBN. PATIENTS & METHODS: Information was obtained from the records of 21 patients diagnosed with MBN at the Sydney Melanoma Unit (SMU) since 1978. RESULTS: Contrary to most reports describing such lesions as occurring predominantly on the scalp, we found that 33% of them were on an extremity, 33% on the trunk, only 24% on the scalp, and 9.5% in the head and neck area other than scalp. Of those whose pathology had been reported at another institution, only half had been diagnosed as MBN. The SMU pathology reports indicated that the tumours had an average Breslow thickness of 6.0 mm, were mostly Clark level 4 or 5 (43% and 48% respectively), were ulcerated in 9.5% of the cases, and had a mean mitotic rate of 3.9 /mm2. The recurrence pattern after initial definitive treatment was: 29% to liver (median time to recurrence 4.5mos), 24% to lung (22mos), 19% in transit (42mos), 19% to regional nodes (11mos), 19% to other sites (39.5mos), 14% local recurrence (7mos), 9.5% to bone (32mos), and 5% to brain (1mo). The median follow-up time was 30 months, the mortality rate was 38%, and survival was 81% at 1 year and 50% at 5 years. CONCLUSION: >From this, the largest series of MBN reported by a single institution to date, we conclude that MBN occurs not only on the scalp, but also quite frequently on the extremities and trunk. It tends to present at a later stage than melanoma but has a comparable metastatic pattern and is not necessarily more aggressive in its behavior.

P-267 EXPRESSION OF TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND (TRAIL) AND ITS DEATH RECEPTORS (DR4 AND DR5) IN HUMAN MELANOMA

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Introduction: TNF related apoptosis inducing ligand (TRAIL) expression on immune cells is believed to be important in immune destruction of melanoma. It induces apoptosis by interaction with death receptors TRAIL- R1 (DR4) or –R2 (DR5) on melanoma cells. Studies on cell lines suggested that there was wide variation in TRAIL death receptor expression with fresh isolates having low death receptor expression. In view of this we studied death receptor expression on formalin-fixed sections of melanoma with monoclonal antibodies (Mabs) specific for these receptors.
Material & Methods: Immunohistochemical staining for TRAIL, DR5 and DR4 were performed on paraffin sections of 100 cases of primary melanoma, metastatic melanoma and benign naevi. Results: DR5 was expressed in most cases of benign nevi and melanoma. DR5 was not detected in 1/42 of primary and 13/38 of metastases. Expression of TRAIL, DR4 and DR5 tended to be focal. The mean percentage of DR5 positive cells showed highly significant differences between different melanocytic tumors. In melanomas <1.0mm thickness, the mean percentage of DR5 (88.9%) ($p<0.0005$) was higher than in compound nevi (49%), dysplastic nevi (57%), subcutaneous metastases (49%) and lymph node metastases (30.6%). The mean percentage of DR5 in melanomas >1.0mm thickness (66.9%) was also significantly higher than that in lymph node metastases ($p<0.001$). In contrast, DR4 expression was not detectable in 42% of all 100 melanocytic tumors and expression was low (<40%). TRAIL expression was found in 32/42 of primary cutaneous melanoma. The mean percentages of positive cells in melanoma <1.0mm and >1.0mm were 50.8% and 47.1% respectively. Conclusions: DR5 but not DR4 was expressed in most primary melanoma. Expression was, however, less in metastases and in many was focal in distribution. These findings suggest that many melanoma cells may not be responsive to therapy based on TRAIL or TRAIL expression unless given with agents that may increase TRAIL death receptor expression.

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P-268 SENTINEL LYMPH NODE BIOPSY (SLNB) IN CUTANEOUS MELANOMA: ANALYSIS OF 240 CONSECUTIVE CASES

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Objective: Evaluate practical rules for SLNB for melanoma and discuss indication, and outcome of 240 patients. The study was done to evaluate efficacy in finding the lymph node, the practical ways to reach better results and the pattern of metastases. Methods: The analysis was performed prospectively and non-randomized in a referral cancer center. The thickness was greater or equal to 1mm. Median thickness was of 1.60 mm, ulceration was found in 30.4%. The median follow-up 27.81 months. The surgery was done with pre operatory Lymphocintigraphy, and pos operatory immunohistochemistry. A statistical analysis was done comparing the need of gamma probe in which location, the value of experience, the need of immunohistochemistry, positivity comparing with Breslow, causes of success in the lymph node localization and evolution. Results: In every lymph node basin the percentage of localization success was directly related to use of the probe, ($p = 0.002$). The rate of success for finding the SL underwent successive, year-by-year, increases. Analysis of lymph nodes disclosed positivity of 12.5%, according to the HE and 17.5% with the immunohistochemistry (excluding the SLN not found disclosed 13,2% according to the HE and 18,5% for HMB45). Immunohistochemistry increased positivity by 40%. Positivity was directly related to the Breslow thickness ($p< 0.001$). Regional lymph node recurrence took place in four cases, all rescued by surgery. Two are dead of disease and two are alive without disease. In this group there was two cases that it wasn't possible to find the sentinel lymph node. Conclusions: The study shows the importance of the gamma probe in all lymph node basins but mainly in the axilla and non usual basins as the importance of experience and immunohistochemistry. As a new procedure, it was possible to recognize the pattern of recurrence in the follow up.

P-269 THE DETECTION OF SECOND PRIMARY CUTANEOUS MELANOMAS

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OBJECTIVES Patients with a history of cutaneous melanoma are at risk not only of melanoma recurrence but also of subsequent primary melanoma (SPM) development. Careful patient follow-up is therefore important. Reliable self-surveillance could justify a reduction in the frequency of routine follow-up by a physician. This study focused on recognition of SPM by self-surveillance. METHODS Sydney Melanoma Unit (SMU) patients with an initial primary melanoma (IPM) and a recently diagnosed SPM were interviewed about the detection of both their IPM and their SPM. The association between clinical and pathological factors and the person who detected the IPM and the SPM was examined. RESULTS In 112 patients, the SPM tended to be thinner than the IPM. Forty-nine percent of IPMs were patient discovered, in contrast to only 37% of SPMs. Patients who found their own IPM were more likely to detect their SPM ($p=0.001$). American Joint Committee on Cancer Stage 0, I and II IPMs were most likely detected by others (e.g. partner, friends, relatives), doctor and patient respectively ($p=0.001$). IPMs and SPMs on the trunk and on other sites poorly visible to the patient were mostly detected by a doctor (all $p< 0.05$). Women and younger patients identified their IPM more often than men and elderly patients ($p=0.003$ and $p=0.03$ respectively). CONCLUSIONS A history of melanoma does not increase the likelihood of patients detecting a SPM. To improve the proportion of patient-detected SPMs, better education in self-examination should focus on those who did not detect their IPM. Partners of patients should be encouraged to assist in the detection of SPMs in anatomical sites poorly visible to the patient.

P-270 GIGANT (GIANT) PRIMARY CUTANEOUS MELANOMA OF THE SCALP

Lj. Panajotovic (Medical Military Academy, Serbia and Montenegro)*

Identification of early and thin cutaneous melanoma has a significant impact on overall survival. Unfortunately, a great number of our patients, at initial operation, have primary tumor thicker then 4 mm. We report a case of gigantic primary melanoma of the scalp. The patient was 57 years old agricultural worker inhabited some kilometers from our capital. The tumor began to rise on the skin of the parietal part of the scalp three years ago. Its growth was progressive to the dimensions: 120 mm in width and 100 mm in height. In the moment of operation it was cauliflower like, exotic, exulcerated, sanguinant, partly necrotic tumor on 50 mm wide pedicle. We didn't know real nature of the tumor, but it was clear that it was malignant, so had done radical excision: horizontal margin was 3 cm from the tumor base and vertical margin was to the skull bones, specimen was 453 g weight. Defect was immediately cowered with the large transposition scalp flap. Secondary defect required split skin grafting. Histopathology study reported that was nodular ulcerated melanoma, epithelioid cell type, Clark V, Breslow 100 mm. A careful preoperative and immediate postoperative physical examination, laboratory studies and extensive radiological examinations did not indicate any sign of metastatic disease. The patient had high performance status after operation and left hospital at forth postoperative day. Unfortunately, three weeks later he suddenly died at the marketplace. Until now, this is the biggest primary melanoma what we have seen in our practice and the biggest seen in the current literature related to melanoma, as well.

P-271 SURGICAL MANAGEMENT OF PRIMARY MELANOMA IN SPECIFIC ANATOMIC REGIONS

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Surgery is still the most effective treatment modality of melanoma. Prompt and radical excision gives the best chance to cure. Biologic characteristics of primary tumor, its malignancy and diameter determine extensivety of excision. Regions of the face are specific in view of localization and complexity of structure. All of them have great functional and cosmetic importance. Oncological safety is important in radical decision, but functional, aesthetic and economic criteria in reconstructive decision. Our tendency is to close the defect directly or by local flap. Last three years we treated 76 newly detected cases with face melanoma. Resection margins of 1 cm, which we preferred in melanoma of various regions of the fa7e, left a big defect according to the region and with consideration of reconstruction. Aesthetic reasons demanded primary closure of those defects with various types of flaps. In the cheek we made rotation, bilobar or transposition flaps, and for temporal defects transposition bilobar flaps. For defects of medial canthal region our choice was supraorbital island flap. Defects of the lower lid were closed with transposition flaps, and in upper lid with bipedicular flaps. We had two similar cases with melanoma of the eyebrow. After excision the defect was closed and eyebrow reconstructed. Reconstruction of the nose integrity was versatile: in one case we used frontal flap, and in the others, various types of local flaps. Ear's helix melanomas we excised including cartilage and reconstructed with composite chondrocutaneous flaps. Up to now no one of our patients had local recurrences.

P-272 LOCAL-REGIONAL DISEASE - WHAT TO DO?

Lj. Panajotovic (Medical Military Academy, Serbia and Montenegro)*

At the time of presentation most patients with melanoma of an extremity are in stage I disease and are cured with wide local excision alone. Melanoma can be an indolent disease with an unpredictable clinical course. The potential for delayed recurrence is often cited as a reason for prolonged surveillance of melanoma patients. Melanoma patients with local-regional metastases (local recurrence, in transit, nodal) have a great risk of systemic recurrence. Despite this risk clinical evaluations detect relatively few distant metastases in asymptomatic patients. We report a case of 65 years old woman who underwent radical excision of melanoma on the dorsal part of the foot. Almost two years after, she got three sanguinant in transit metastases in the same lower leg and we operated them. Three or four months after that operation she got similar local metastases in the lower leg, without distant spread. One year after we made regional lymphadenectomy by reason of inguinal metastasis. In transit metastases continued toappear. We have no possibility to use isolated limb perfusion and we excised whole skin from the lover leg and defect was covered with split skin graft (donor region - another thigh). On our surprises about three months later we beheld a large number of in transit metastases on the scar of the lower leg, and now in the thigh. Five months later disease spread and she died three month later with brain and liver metastases.

P-273 A NEW COMPOSITE TREE-BASED CLASSIFICATION APPROACH FOR PROGNOSTIC GROUPING OF MELANOMA DATA

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OBJECTIVE Tree classification models are becoming increasingly popular to classify survival data in melanoma patients. However, the terminal subgroups resulting from these models often require further manual classification to regroup similar survival curves. Therefore, we develop an effective composite method to determine appropriate prognostic patient subgroups. To evaluate the goodness of fit and reproducibility of our proposed approach, we employ the Brier score and develop a cross-validated log-rank test in a validation study. MATERIALS AND METHODS The AJCC Melanoma Database was used to demonstrate our approach. The data set consists of 17,600 patients from 13 cancer centers and organizations, of which 13,268 patients with localized melanoma are considered for data analysis. We propose a composite classification approach to identify prognostic groups with significant difference in survival profiles. The proposed approach integrates the tree method with the agglomerative hierarchical clustering. We apply the Brier score to assess predictive models and derive a modified log-rank test with 5-fold cross-validation to evaluate reproducibility for our approach. RESULTS The seven prognostic groups defined by tumor thickness, ulceration, primary lesion site, and level of invasion for localized melanoma patients were identified with statistically significant difference ($p<0.0001$). In comparison with the current AJCC staging system, our approach was able to further split out AJCC's prognostic groups (e.g. splitting AJCC stage IB into two significant subgroups). Moreover, the Brier score showed a greater improvement of predictive model by our proposed approach. In addition, the results of cross-validation and modified log-rank test showed high reproducibility of our classification approach. CONCLUSIONS This new approach yields a more informative prognostic classification scheme with high reproducibility and presents interpretable summaries providing statistics and p-values of the log-rank test among terminal subgroups. Therefore, this strategy of risk classification would be useful in the reporting and staging of melanoma patients.

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