# Gliwice Scientific Meetings 2005

# The 80$^{th}$ Anniversary of Professor Mieczysław Chorąży

## Gliwice, 18-19 XI 2005
## http://gsn.io.gliwice.pl

## Organizers:

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch

Association for the Support for Cancer Research

The Silesian University of Technology

## Supported by:

Ministry of Scientific Research and Information Technology

Committee for Human Genetics and Molecular Pathology, Polish Academy of Sciences

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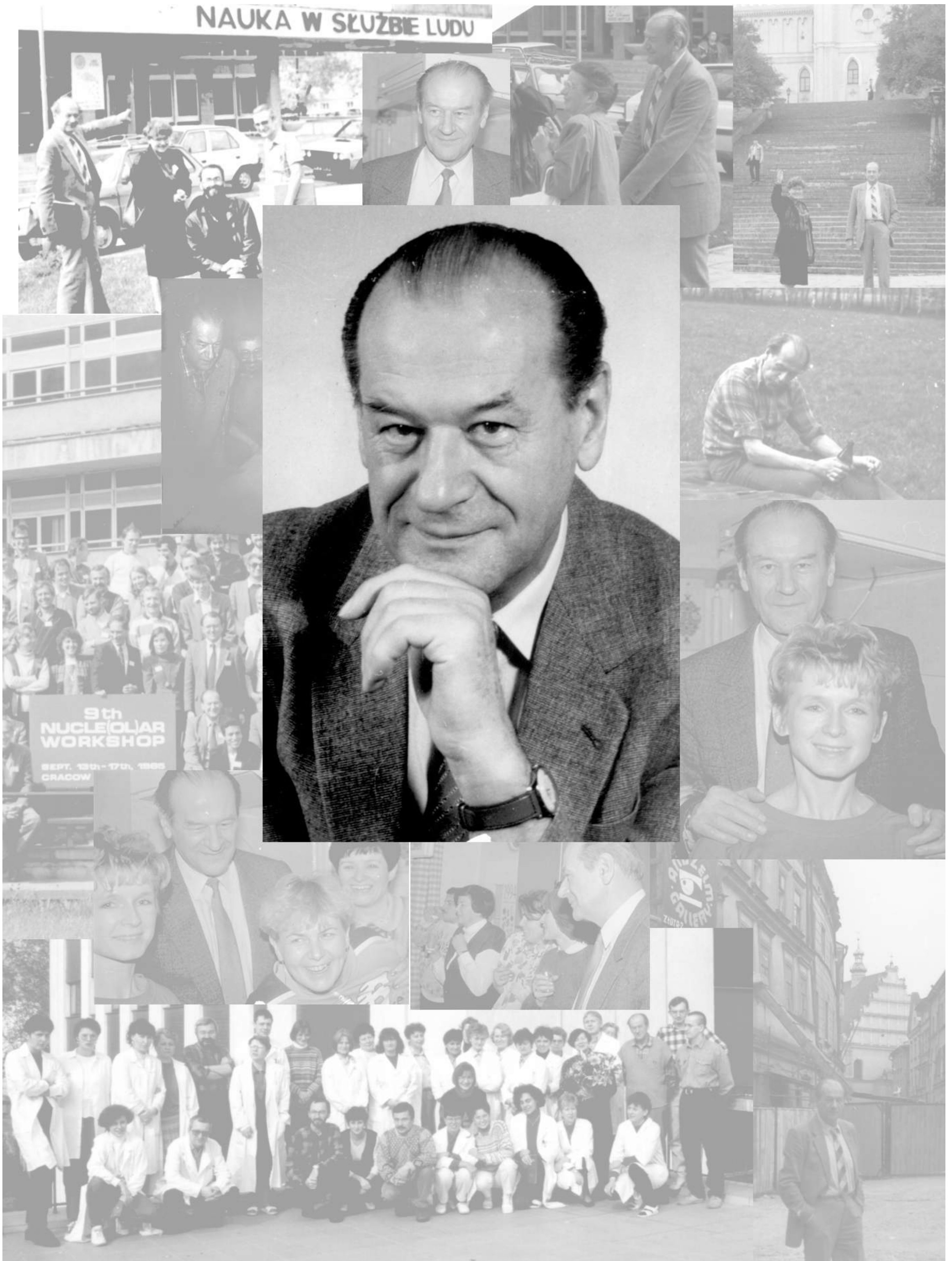
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### The 80th Anniversary of Professor Chorąży

Mieczysław Rajmund Chorąży was born on August 31, 1925 in Janówka near Biała Podlaska in Eastern Poland. During the 2nd World War years he attended an underground school and completed his secondary education. Meanwhile he became a member of the clandestine Polish Home Army in the ranks of which he later fought in the Warsaw Uprising of 1944. As a war prisoner he was deported and detained in Altengrabow, Germany. The Uprising had left him with a token: paresis of his right hand, an obvious handicap in the laboratory. Between 1945 and 1951 he pursued graduate education at the Medical Academy in Warsaw. These were the darkest years of the Stalinist regime in Poland. No big wonder then that Mieczysław Chorąży, for having belonged to the Home Army, having fought in the Warsaw Uprising and generally lacking a politically correct "law-abiding" attitude had to relocate in 1951 from the capital city of Warsaw to Gliwice. Once there, he started work, under the direction of Professor Kazimierz Dux, in the recently formed State Anticancer Institute (currently: Comprehensive Cancer Center and Maria Skłodowska-Curie Memorial Institute of Oncology). He was awarded the M.D. degree in 1958 with specialty in Biochemistry. Mieczysław Chorąży obtained the subsequent titles of Associate, Assistant and Full Professor in 1961, 1970 and 1984, respectively. From 1961 until 1995 (that is until his retirement) Mieczysław Chorąży had been the Head of the Tumor Biology Department (ZBN) at the Oncology Institute in Gliwice.

Scientific career of Mieczysław Chorąży has been reflecting his life-long interest in various aspects of tumor metabolism. His initial investigations concerned nitrogen metabolism in tumor-bearing rats. Later, he focused his interests on the studies of genetic material: transformation of cells using foreign DNA, DNA sequence organization in different species (repetitive sequences), biological activity of compounds interacting with genetic material and processes accompanying liver regeneration and human tumors' etiology. Lastly, scientific interests of Professor Chorąży became focused on mutagenesis and environmental carcinogenesis. Since 1955 until 2005 he wrote 105 original papers, 14 reviews, 3 monographs, translated 5 books and published a wealth of articles in popular science magazines and social periodicals.

Professor Mieczysław Chorąży himself values the best his early studies concerning DNA transfer into model eukaryotic cells [1]. Its results have demonstrated that cells are capable of taking up large DNA fragments. Another much appraised study concerned the methods of metaphase chromosome isolation [2]. In 1972, 1973 and 1985 Professor Chorąży published a series of pioneering monographs that dealt with basics of tumor biology and revealed links between genetic instability and carcinogenesis [3-5]. In the late eighties and in the nineties these research interests were the mainstay of investigations carried out in the Tumor Biology Department at the Institute in Gliwice. During the most recent years research interests of Professor Chorąży involved also links between polymorphism of detoxification enzyme-encoding genes and the neoplastic processes [6-8].

Professor Mieczysław Chorąży pursued most of his scientific career in the difficult and obscure era of Communist-ruled Poland. Despite that he has always shown an unbending attitude in the vital matters of scientific research in Poland. He fought hard administrative battles with various bureaucrats in central state offices in Warsaw in order to gain modern equipment as well as reagents needed for research in his Tumor Biology Department. At that time the Department could probably boast one of the best molecular biology-oriented library resources in the Upper

Silesia and perhaps in whole southern Poland. In the seventies, he organized, as the first in Poland, the DNA Structure and Function School.

Scientific merits of Professor Chorąży have always been of great importance to his students and successors. But even more important were perhaps his personality, moral attitude and, beyond any doubt, courage. In times full of cowardice and hypocrisy the Department he headed was for decades an island of relative liberty in the Communist sea. It is perhaps hard to comprehend such things today for those who had not been then and there. No wonder work at the Department attracted many talented people as they found there a conducive atmosphere for research (among them: Jan Filipski, Ewa Grzybowska, Małgorzata Hanausek-Wałaszek, Jerzy Jurka, Paweł Kisielow, Stefania Krzyżowska-Gruca, Józef Mendecki, Ewa Minc-Ninio, Czesław Radzikowski, Lidia Sadzińska-Matter, Zenon Stęplewski, Andrzej Vorbrodt, Zbigniew Wałaszek and the current Heads of the three emerged research departments: Zdzisław Krawczyk, Joanna Rzeszowska and Stanisław Szala). This is why we – the students of Professor Mieczysław Chorąży - are thankful to him for this. Not to be forgotten are his perennial battles with security service bureaucrats and local communist party officials to allow fellow co-workers travel abroad to improve skills. Professor Chorąży was advisor of eighteen Ph.D. theses; several of his co-workers became associate professors and five are full professors.

Mieczysław Chorąży holds the title of full Professor of Medical Sciences. He is a member of the Polish Academy of Sciences (PAN), Polish Academy of Arts and Sciences (PAU), an honorary member of the Polish Society of Oncology (between 1974-78 its Chairman),an honorary member of the Hungarian Oncological Society, member of the Polish Biochemical Society, European Association for Cancer Research (EACR, and its vice-president between 1985 and 1989), European Cell Biology Organization (ECBO), American Association for Cancer Research (AACR) and many other Polish and foreign scientific societies. For his military conduct and lifetime civil courage he was awarded with highest national distinctions including the Gold Cross of Merit and the Order of Polonia Restituta. Professor Mieczysław Chorąży maintains live contacts with many outstanding leaders of science, politics, art and culture.

Recently, Professor Mieczysław Chorąży wrote and published his autobiography ”Z Janówki w świat” (“From Janówka into the big world”). It spans seventy years of his productive life and is full of lively accounts from Professor’s childhood and youth, work and scientific career as well as memories from trips abroad. Painting vividly bygone battles for truth and human dignity, this book makes one also reflect deeply upon human life, ways and destiny. Last but not least, the reader can share Professor’s afterthoughts concerning the shape of Polish science at the time of freedom and integration with the Western half of our continent.

List of selected publications:

[1] M. Chorąży (1962) Badania nad wnikaniem kwasu dezoksyrybonukleinowego w komórkach raka wysiękowego Ehrlicha. (Studies on the penetration of DNA into Ehrlich ascites carcinoma cells). *Post. Hig. Med Dośw.* 16, 37-83.

[2] M. Chorąży, A. Bendich, E. Borenfreund, D.J. Hutchinson (1963) Studies on the isolation of metaphase chromosomes. *J. Cell. Biol*. 19, 59-60.

[3] S. Szala, M. Chorąży (1972) Powtarzające się sekwencje DNA w genomie wyższych organizmów, (Repetitive DNA sequences in the genome of higher organisms) Monografie Biochemiczne, PWN, Warszawa, pp. 50.
[4] K. Dux, M. Chorąży (1973) Wstęp do biologii nowotworów (Tumor biology, Introduction), PWN, Warszawa, pp. 408.
[5] M. Chorąży (1985) Sequence rearrangements and genome instability. A possible step in carcinogenesis. *J. Cancer Res. Clin. Oncol.* 109, 159-172.
[6] K. Hemminki, E. Grzybowska, M. Chorąży, K. Twardowska-Saucha, J.W. Sroczyński, K.L. Putman, K. Randerath, D.H. Phillips, A. Hewer, R.M. Santella, F.P. Perera (1990) DNA adducts in humans related to occupational and environmental exposure to aromatic compounds. In: IARC Scientific Publications vol. 104, Complex mixtures and cancer risk, ed. H. Vainio, M. Sorsa, A.J. McMichael, Lyon, 181-192.
[7] F.P. Perera, K. Hemminki, E. Grzybowska, G. Motykiewicz, J. Michalska, R.M. Santella, T.-L. Young, C. Dickey, P. Brandt-Rauf, I. deVivo, W. Blaner, W.-Y. Tsai, M. Chorąży, (1992) Molecular and genetic damage in humans from environmental pollution in Poland. *Nature*, 360, 256-258.
[8] D. Butkiewicz, E. Grzybowska, K. Hemminki, S. Øvrebø, A. Haugen, G. Motykiewicz, M. Chorąży (1998) Modulation of DNA adduct levels in human mononuclear white blood cells and granulocytes by *CYP1A1*, *CYP2D6* and *GSTM1* genetic polymorphism. *Mutat. Res.*, 415, 97-108.

**Gliwice Scientific Meetings 2005**
**18-19 November**

**Leśny Hotel, 137 Toszecka Str.**

***Friday, 18th November***
***Framework program***

| | |
|---|---|
| 10.00 - 10.15 | Opening ceremony and congratulations to Professor Mieczysław Chorąży |
| 10.15 – 11.15 | Session I |
| 11.15 - 11.30 | Coffee break |
| 11.30 - 13.00 | Session I cont. |
| 13.00 - 14.00 | Lunch break |
| 14.00 – 16.00 | Session II |
| 16.00 – 17.00 | Poster session I: poster viewing and coffee break |
| 17.00 – 19.00 | Session III |
| 20.00 - | Social events/party |

***Program details***

10.00 - 10.15 Opening ceremony and congratulations to Professor Mieczysław Chorąży
10.15 – 11.15 Session I

Session I: **Structure and Function of the Genome, Epigenetic Mechanisms**

**William T. Garrard** – UT Southwestern Medical Center, Dallas: ***Mechanisms of activation and silencing of the immunoglobulin kappa locus***
**Andrzej Jerzmanowski –** Institute of Biochemistry and Biophysics, Polish Academy of Science, Warszawa: ***Chromatin remodeling and linker histones in plant development***

11.15 - 11.30 Coffee break
11.30 - 13.00 Session I cont.

**Sergey Razin** – Institute of Molecular Biology, RAS, Moscow: ***Assembly of nuclear matrix - bound protein complexes involved in non-homologous end joining is induced by inhibition of DNA topoisomerase II***
**Harry Sherthan –** Institute of Radiobiology (Bundeswehr), Muenchen: ***Satellite DNA, meiotic telomeres and chromosome evolution***
**Jan Barciszewski** - Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań: ***Diagnosis and therapy of brain tumors***

13.00 - 14.00 Lunch break/poster viewing
14.00 – 16.00 Session II

Session II: **Response to Stress and Alterations of Genome and Transcriptome in Cellular Pathology**

**Lise Lotte Hansen –** University of Aarhus, Aarhus: ***Genomic instability of the HCP region in somatic breast cancer***
**Ryszard Oliński** - Nicolaus Copernicus University, Bydgoszcz: ***Clinical significance of oxidative DNA damage***
**Krzysztof Szyfter -** Institute of Human Genetics, Polish Academy of Sciences, Poznań: ***Progression of head and neck cancer studied by molecular cytogenetics***
**Stanisław Cebrat –** University of Wroclaw, Wrocław: ***The role of crossing-over in the computer simulated populations***

16.00 – 17.00 Poster session I: poster viewing and coffee break
17.00 – 19.00 Session III

Session III: **Protein Sciences in Biology and Medicine**

**Alfred Pingoud -** Justus-Liebig-Universitaet, Giessen, Germany: ***Engineering of meganucleases***
**Janusz M. Bujnicki –** International Institute of Molecular and Cell Biology, Warszawa: ***Protein structure prediction by combination of fold-recognition and de novo holding***
**Jerzy Ostrowski –** MSC Cancer Center and Institute of Oncology, Warszawa: ***Strengths and limitations of microarray and mass spectrometry-based methods in clinical research***

| | |
|---|---|
| 20.00 - | Social events/party |

***<u>Saturday, 19th November</u>***
***Framework program***

| | |
|---|---|
| 9.00 – 10.10 | Session III cont. |
| 10.10 - 10.20 | Coffee break |
| 10.20 –12.15 | Session IV |
| 12.15 – 13.15 | Lunch break |
| 13.15 – 14.15 | Poster session II: reports and discussion |
| 14.15– 14.30 | Closing remarks |

*<u>Parallel Events (in Polish):</u>*
*in Institute of Oncology (15, Wybrzeze Armii Krajowej):*

- Sympozjum satelitarne: **Nowe leki i strategie terapeutyczne we współczesnej onkologii**

*in Silesian University of Technology (16, Akademicka Str.):*
- Warsztaty **- Biostatystyka i Bioinformatyka**

***Program details***

| | |
|---|---|
| 9.00 – 10.10 | Session III cont. |

**Michał Dadlez –** Institute of Biochemistry and Biophysics, Polish Academy of Science, Warszawa: ***Application of mass spectrometry for the studies of proteomes***
**Krzysztof Puszyński** - Silesian University of Technology, Gliwice: ***Logical analysis of proteomic data***
**Marcin Pacholczyk** - Silesian University of Technology, Gliwice: ***Some algorithms of molecular docking***

| | |
|---|---|
| 10.10 - 10.20 | Coffee break |
| 10.20 –12.15 | Session IV |

Session IV: **Therapeutic Strategies and Mathematical Models in Contemporary Oncology**

**Pedro de Campos-Lima –** Laval University, Quebec: ***New strategies in therapy of virus- caused tumors***
**Marek Łoś** - University of Manitoba, Winnipeg*:* ***Apoptin - a novel, cancer-selective killer***
**Andrzej Świerniak,** Grzegorz Gala - Silesian University of Technology, Gliwice: ***Antiangiogenic*** *therapy as a control problem*
**Philippe Getto,** Silesian University of Technology, Gliwice: ***A simple mathematical model for cell signaling in radiation experiment***
**Piotr Czerski** IBB PAN -OLIGO.PL*:* ***Sekwencjonowanie i synteza DNA*** *(7 min.)*

| | |
|---|---|
| 12.15 – 13.15 | Lunch break |
| 13.15 – 14.30 | Poster session II (reports, discussion) and closing remarks |

Sympozjum satelitarne: **Nowe leki i strategie terapeutyczne we współczesnej onkologii**

**Moderatorzy: Czesław Radzikowski, Stanisław Szala**

9.00-9.20 **Piotr Wysocki** (Akademia Medyczna, Poznań): ***Efekt przeciwnowotworowy kaptoprilu – druga strona medalu***
9.20-9.40 **Elżbieta Pajtasz-Piasecka** (Instytut Immunologii i Terapii Doświadczalnej PAN, Wrocław): ***Aktywność przeciwnowotworowa szczepionki na bazie komórek dendrytycznych transdukowanych genami mysiej IL-12 lub IL-2 u myszy z zaawansowanym nowotworem MC38***
9.40-10.00 **Dominika Nowis, Marcin Makowski, Tomasz Grzela, Ewa Wilczek, Grzegorz Wilczyński, Magdalena Legat, Józef Dulak, Alicja Józkowicz, Mariusz Adamek, Tomasz Stokłosa, Marek Jakóbisiak i Jakub Gołąb** (Warszawska Akademia Medyczna, Warszawa): ***Badanie mechanizmów niszczenia komórek nowotworowych przez terapię fotodynamiczną oraz próby potęgowania jej efektywności***

10.00-10.20 przerwa

10.20-10.40 **Danuta Duś** (Instytut Immunologii i Terapii Doświadczalnej PAN, Wrocław): ***Białka oporności wielolekowej w komórkach macierzystych krwi***
10.40-11.00 **Maciej Ugorski** (Instytut Immunologii i Terapii Doświadczalnej PAN, Wrocław): ***Wykorzystanie RNAi do hamowania ekspresji glikozylotransferaz***
11.00-11.20 **Joanna Wietrzyk, Magdalena Rybak, Andrzej Kutner, Adam Opolski** (Instytut Immunologii i Terapii Doświadczalnej PAN, Wrocław): ***Toksyczność nowych analogów witaminy D i ich aktywność in vitro w terapii kombinowanej z cytostatykami w modelu raka gardła***
11.20-11.40 **Katarzyna Szczaurska, Krystyna Dąbrowska, Janusz Boratyński, Beata Weber-Dąbrowska, Andrzej Górski i Adam Opolski** (Instytut Immunologii i Terapii Doświadczalnej PAN, Wrocław): ***Aktywność przeciwnowotworowa bakteriofagów w terapii skojarzonej z cytostatykami***

11.40-13.00 przerwa/obiad

13.00-13.20 **Janusz Boratyński** (Instytut Immunologii i Terapii Doświadczalnej PAN, Wrocław): ***Koniugaty metotreksat – nośnik – właściwości chemiczne i biologiczne***
13.20-13.40 **Aleksandra Rusin**, Zbigniew Jedliński (Centrum Onkologii, Gliwice): ***Przeciwnowotworowe i przeciwzapalne właściwości nowych koniugatów niesteroidowych leków przeciwzapalnych i oligomerów 3-hydroksymaślanu***
13.40-14.00 **Tomasz Cichoń**, Ryszard Smolarczyk, Aleksander Sochanik, Stanisław Szala (Centrum Onkologii, Gliwice): ***Kombinacja cyklofosfamidu z cytokinami indukowanymi przez sekwencje CpG DNA w hamowaniu wzrostu doświadczalnych przerzutów czerniaka B16(F10) w płucach myszy***

14.00 - zakończenie sympozjum

**Warsztaty z biostatystyki i bioinformatyki**

**Organizatorzy: Jacek Leluk (ICM, Warszawa) i Andrzej Polański (Politechnika Śląska, Gliwice)**

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|---|---|
| 9.00 – 10.30 | Wykład I: Narzędzia bioinformatyczne i ich stosowanie |
| 10.30 – 11.00 | Przerwa na kawę |
| 11.00 – 12.30 | Wykład II: Genomika białek |
| 12.30 – 13.00 | Przerwa na kawę |
| 13.00 – 14.15 | Warsztaty: Korzystanie z baz danych (prowadzący – dr hab. Jacek Leluk) |

**Program ramowy warsztatów:**
***Problem oszacowania stopnia istotności homologii białek i sekwencji nukleotydowych; Algorytm semihomologii genetycznej;***
***Aplikacje algorytmów porównywania sekwencji układów biologicznych;***
***Zmienność białek a model Markowa;***
***Przegląd metod identyfikacji sekwencji kodujących w genomie;***
***Praktyczna prezentacja podstawowych programów do analizy teoretycznej, przewidywania struktury drugorzędowej oraz modelowania białek.***

*Program szczegółowy warsztatów:*
**1. Problem oszacowania stopnia istotności homologii porównywanych białek i sekwencji nukleotydowych (przegląd stosowanych metod):**

- podstawy analizy stopnia identyczności i podobieństwa sekwencji;
- niezbędne kryteria, uwzględniane przy oszacowaniu istotności podobieństwa porównywanych sekwencji;
- stopień identyczności, podobieństwo i homologia porównywanych sekwencji;
- algorytm oszacowania istotności stopnia identyczności. Procent identyczności, a długość sekwencji. Program SSSS - jego przeznaczenie i obsługa;
- dystrybucja pozycji identycznych wzdłuż porównywanych łańcuchów, jako istotne kryterium analizy podobieństwa sekwencji aminokwasowych i nukleotydowych;
- sekwencje o niskim stopniu identyczności. Kryteria fizykochemiczne i genetyczne jako narzędzia wspomagające w oszacowaniu faktycznej relacji porównywanych sekwencji aminokwasowych;
- porównanie analizy podobieństwa za pomocą matrycy unitarnej, prostego oszacowania procentowej identyczności, narzędzi z grupy BLAST oraz za pomocą programu SSSS.

**2. Algorytm semihomologii genetycznej:**

- podstawowe założenia algorytmu i jego struktura;
- trójwymiarowy diagram tanzycji/transwersji na poziomie nukleotydowym i aminokwasowym;
- aplikacja dot-matrix i program SEMIHOM;
- zastosowanie algorytmu semihomologii genetycznej do analizy porównawczej różnych rodzin białkowych;
- możliwości aplikacyjne algorytmu semihomologii genetycznej i informacje uzyskiwane w wyniku jego zastosowania.

**3. Aplikacje algorytmów porównywania sekwencji układów biologicznych - użyteczność, stosowalność, zgodność, ilość informacji możliwej do uzyskania. Statystyczna i niestatystyczna analiza porównawcza sekwencji białkowych - UM, GCM, MDM, PAM, BLOSUM, FASTA, BLAST, SEMIHOM:**

- rozwój algorytmów porównania teoretycznego sekwencji - rys historyczny;
- charakterystyka podstawowych typów algorytmów - macierz unitarna (UM), macierz kodu genetycznego (GCM), algorytmy statystyczne i programy (ClustalW, Multalign, FASTA) oraz statystyczne matryce (PAM i BLOSUM); algorytm semihomologii genetycznej jako przykład niestatystycznego podejścia do analizy porównawczej sekwencji białkowych;
- porównanie różnych algorytmów pod względem użyteczności, zgodności i możliwości aplikacyjnych.

**4. Zmienność białek a model Markowa substytucji aminokwasów w białkach:**

- interpretacja wymiany mutacyjnej nukleotydów i aminokwasów jako odwzorowanie łańcuchów Markowa (w czasie mierzonym liczbą zachodzących zdarzeń);
- zastępowanie aminokwasów na drodze pojedynczej tranzycji/translacji jest procesem niemarkowowskim - dowód teoretyczny i empiryczny;
- przegląd stosowanych metod porównawczych sekwencji w świetle ich odwoływania się do markowowskiego procesu wymiany; konsekwencje zastosowania obu rodzajów podejścia.

**5. Przegląd metod identyfikacji sekwencji kodujących w genomie:**

- metody oparte na wzorcowym DNA kodującym;
- metody niewymagające wzorcowego kodującego DNA.

**6. Praktyczna prezentacja podstawowych programów do analizy teoretycznej, przewidywania struktury drugorzędowej oraz modelowania białek. (Predict7, ProtSA, Antheprot, SSSS, GEISHA, Consensus Constructor).**

# Lecture abstracts

**DIAGNOSIS AND THERAPY OF BRAIN TUMORS**

Jan Barciszewski[1], Stanislaw Nowak[2] and Ryszard Zukiel[2]

[1]*Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland and*
[2]*Department of Neurosurgery and Neurotraumatology, Karol Marcinkowski University of Medical Sciences, Poznan, Poland*

Neoplasms arising from glial cells make up the most common group of primary brain tumors. The clinical outcome, especially the survival rates of the patients with tumors depend on tumor grade expressing their malignancy grade. A prognosis for glioblastomas (WHO IV) is very poor, but for astrocytomas (WHO I and II) it is relatively favourable. There are evidence that oxidative stress and reactive oxygen species (ROS) are crucial in the etiology and progression of a number of human diseases, also neoplasms. An oxidative damage of DNA, lipids and proteins is caused mainly with hydroxyl radical ($^{\bullet}OH$), the most reactive ROS species and may be seriously deleterious. In the reaction with hydroxyl radical all DNA components can be modified. 5-methylcytosine ($m^5C$) a rare but normal component of cellular DNA is relatively easily deaminated to thymine, which pairs with adenine and after a round of replication, CG to TA transition occurs. Because thymine is a normal DNA base, therefore the product of spontaneous deamination of $m^5C$ is not so easily detected by a cell's DNA repair system. Thus, 5-methylcytosine residue constitutes a mutational hotspot and DNA methylation pattern in patients might be useful as a primary diagnosis tool or as a marker for early detection of relapse of the disease.

In recent years a new mechanism of posttranscriptional gene silencing called also RNA interference (RNAi) has been discovered. This phenomenon is based on specific mRNA degradation mediated by double-stranded RNA molecules, approximately 19-28 nucleotides in length, called short interfering or siRNAs. These molecules are produced from long dsRNAs by a dsRNA-specific endonuclease (DICER) and form 300 kD multi-enzyme complex (RISC) which by Watson-Crick base-pairing of noncoding strand with their mRNA-targets induce the specific cleavage. The high sequence-specificity of RNAi shows a new, promising tool in gene-function analysis as well as in potential therapeutics development.

**PROTEIN STRUCTURE PREDICTION BY COMBINATION OF FOLD-RECOGNITION WITH *DE NOVO* FOLDING**

Janusz M. Bujnicki[1,*], Marcin Feder[1], Michal J. Gajda[1], Jan Kosinski[1], Marcin Pawlowski[1], Michal Boniecki[1,2], Dominik Gront[2], Andrzej Kolinski[2]

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A new method for protein structure prediction was developed, which allows modeling regardless of the potential homology with any known protein structure. This method is a combination of the "FRankenstein's Monster" approach for comparative modeling by recombination of Fold-Recognition (FR) models [1], and a new implementation of a Replica Exchange Monte Carlo (REMC) method for protein folding de novo or with restraints [2,3]. The sequence of a modeled protein is submitted to the GeneSilico structure prediction meta server, which is a gateway to a variety of third-party methods for secondary structure prediction and FR analysis (http://genesilico.pl/meta/ [4]). FR alignments are compared and ranked and up to 5 most frequently reported folds are selected for further analysis. For each fold, the target-template alignments are used as a starting point for modeling using the "FRankenstein's monster" approach [1]. Best models obtained (1-15 models for each fold) are evaluated and fragments with best scores are used to derive spatial restraints [5]. Additional restraints can be derived from methods for de novo structure prediction, such as ROSETTA. Secondary structure restraints are derived from the consensus of methods implemented in the GeneSilico meta server [4]. Seconday and tertiary restraints are used to guide the REMC folding simulation using a new high-resolution reduced lattice model CABS [2,3]. The conformations obtained in the course of CABS simulations are subject to the average linkage hierarchical clustering. For a represetnative structure from each cluster a full-atom representation is rebuilt. The performance of the new method will be discussed in the context of our successful predictions in the recent CASP-6 experiment.

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**THE ROLE OF CROSSING-OVER IN THE COMPUTER SIMULATED POPULATIONS**

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We have used the Penna ageing model, based on the Monte Carlo method to study evolutionary significance of the linkage disequilibrium as well as the inside intron recombination.In our computer simulations we have implemented internal structure of genes by declaring the probability of recombination between exons. There are two trivial situations when organisms could profit from the inside intron recombination. The first one is generation of new genes by exons reshuffling. The second one is recovering functional genes from exons dispersed in the genetic pool of the population after a long period without selection for the function of this gene. Populations have to pass through the bottleneck, then. These events are rather rare and we have expected that there should be other phenomena giving profits from the inside intron recombination. In fact we have found that inside intron recombination is advantageous only in the case when after recombination, besides the recombinant forms, parental haplotypes are available and selection is set already on gametes. Nevertheless, parameters of simulations when inside introns recombination is advantageous are very rigorous. That is why we tried to use simpler models of population evolution to study the conditions when the inside introns recombination brings direct profits for evolving populations.

**EXAMPLES OF APPLICATION OF MASS SPECTROMETRY IN PROTEOMICS**

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Proteomics became an integral part of systems biology, providing a necessary complement for genomic approach. The aim of proteomics is defined as enumeration of protein complement of the sample of interest along with their posttranslational modifications. Moreover protein-protein interaction nets can also be elucidated. The lecture will introduce to proteomics technology and mass spectrometry as a basic proteomic tool. Examples of application of proteomics technology will be given.

Mass spectrometry (MS) has emerged in the recent years as the main proteomic tool due to its sensitivity. At present subfemtomole ($10^{-15}$ M) of a protein are sufficient for identification. In addition MS analyses are fast and do not require homogeneity of the sample. On the contrary, in MS technology complex mixtures of proteins can be studied in a single experiment. Proteomic technology application will be illustrated by several results originating from our Laboratory. Successful examples of protein identification along with their posttranslational modifications will be presented. The application of differential proteomics for identification of a new protein involved in pathogenesis of cystic fibrosis and identification of partners of human helicase protein complex will shortly be outlined. Finally, the application of multidimensional liquid chromatography prefractionation coupled to MS (MudPIT) will be illustrated by the study of the contents of *Arabidopsis* nuclear proteome and the human blood plasma peptidome mapping.

**A SIMPLE MATHEMATICAL MODEL FOR CELL SIGNALING IN RADIATION EXPERIMENTS**

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As a reaction to irradiation cells send signaling molecules/ligands/chemicals, which influence the functioning of the other cells (bystander effect). In the ongoing work we are interested whether and how the sending and receiving of signals is dependent on the phase of the cell cycle. The mathematical model which will help to plan the experimental part of the work will be presented.

## MECHANISMS OF ACTIVATION AND SILENCING OF THE IMMUNOGLOBULIN KAPPA LOCUS THROUGH DNA LOOPING AND NUCLEAR REPOSITIONING TO HETEROCHROMATIN

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We have investigated the higher-order chromatin structure of the mouse immunoglobulin (Ig) kappa locus as a function of activation and silencing during B cell development using the techniques of chromosome conformation capture (3C) and 3D 3-color FISH. We find that in the fully transcriptionally active state that each of the three enhancers form complexes with themselves and rearranged gene promoters, while the resulting intervening DNA is looped out. These results fit the looping model for the mechanism of enhancer action. Using chromatin immunoprecipitation in combination with 3C (e.g., ChIP-3C), we demonstrate that the transcription factor E47 is present in such looped complexes. Two of these enhancers also form complexes with a 3' boundary sequence that has CTCF sites. In addition, the 5' region of the active transcription unit exhibits a continuum of interactions with downstream chromatin segments. All of these interactions are B cell specific (Liu and Garrard, Mol. Cell. Biol. 25, 3220 [2005]). Previous studies have shown that allelic exclusion of the mouse Igkappa locus occurs by the combination of monoallelic silencing and a low level of monoallelic activation for rearrangement combined with a negative feedback loop blocking additional functional rearrangements. Using yeast artificial chromosome-based single-copy isotransgenic mice, we have identified a cis-acting element that negatively regulates rearrangement in this locus, specifically in B cells. The element resides in the V-J intervening sequence, and is termed ***Sis*** (silencer in the intervening sequence). ***Sis*** specifies the targeting of germline Igkappa transgenes in B cells to centromeric heterochromatin and their association with Ikaros, a repressor protein that also co-localizes with centromeric heterochromatin. Significantly, these are hallmarks of silenced endogenous germline Igkappa genes in B cells. These results provide new insights into the molecular mechanisms of allelic exclusion.

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**GENOMIC INSTABILITY OF THE HEREDITARY PROSTATE CANCER 1 (HPC1) REGION IN SOMATIC BREAST CANCER**

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In a prospective study with 10 years of follow-up, we have established a significant correlation between allelic imbalance (AI) affecting 16q23.2 -24.2.3 and favourable prognosis by univariate and multivariate analysis of 36 highly polymorphic microsatellite markers along chromosome 16q. No association was found to other clinical parameters such as menopausal status, tumour size, lymph node status, histopathology, and lymph node capsule invasion. This makes allelic loss of 16q23.2-24.2 an independent marker of favorable prognosis for primary breast cancer. A number of cytogenetic studies published over the past two decades have shown that a complex rearrangement resulting in the gain of 1q in combination with allelic deletion of 16q is an early, perhaps the primary, event in breast cancer development.

In the search for a possible link between the gain of 1q, loss of 16q and prognosis for breast cancer patients, we concentrated on the HPC1 region at 1q25.3.

Our initial hypothesis was that activation of ribonuclease L (RNase L) at 1q25.3 may lead tumour cells, with a certain level of genomic instability, into apoptosis. Mutations in the gene have recently been found to segregate in prostate cancer families, and homo- and heterozygosity for the mutation R462Q is a strong predictive marker for inherited prostate cancer. RNase L is part of the interferon-induced pathway against viral infection, it is involved in stress-induced apoptosis in metastatic prostate cancer cell lines and in termination of the protein synthesis.

We found no significant correlation between AI or mutations in the gene *RNASEL* and any prognostic parameter for breast cancer. We are currently analyzing 300 breast tumours for the expression level of RNase L, since overexpression due to gain of 1q can be related to increased apoptosis, depending on the degree of genomic instability of the tumour cell.

We focused on the region flanking RNASEL, the HPC1 region, and analyzed a 740 Kb spanning from *RGSL2* to *LAMC2* at chromosome 1q25.3 for allelic imbalance with new inter- and intragenic markers, already identified and characterized. We found a high rate of AI across the entire region and numerous chromosomal breakpoints. Breaks affecting three genes have been mapped in detail, and the resulting microdeletions of various sizes are positioned in the coding region of *RGS8, RGS16, and RGSL2.*

This leads to the hypothesis that genomic instability affecting one or more genes at chromosome 1q25.3 is blocking metastasis formation in breast cancer patients. Additional genomic lesions may promote metastases formation.

**LOGICAL ANALYSIS OF PROTEOMIC DATA**

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A parallel implementation of proteomic ovarian cancer diagnosis system based on logical analysis of data is shown. The implementation is based on computational cluster elaborated by the System Engineering Group at the Silesian University of Technology. For verification of algorithm and software Ovarian Dataset 8-7-02 was used. This mass spectrometry data contains intensity levels of 15 154 peptides defined by their mass/charge ratios (m/z) in serum of 162 ovarian cancer and 91 control cases. An OpenMosix with MPI (Message Passing Interface) cluster technology was used to construct in LAD a fully reproducible models (1) using full range and (2) using only 700-12000 of m/z values of peptides and proved in multiple cross-validation leave-one-out tests to guarantee sensitivities and specificities of up to 100 %.

**CHROMATIN REMODELING AND LINKER HISTONES IN PLANT DEVELOPMENT**

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Eukaryotic genomes are organised into chromatin, a structure that enables packaging of very long DNA molecules into a microscopic-size nucleus, while ensuring efficient use of the information contained in the DNA sequence. In flowering plants and mammals the regulatory mechanisms at the level of chromatin show a high degree of evolutionary conservation. In mammals, the mutations in genes encoding proteins involved in post-translational modifications of core histones, methylation of DNA, ATP-dependent chromatin remodeling and in stabilizing heterochromatic state often result in embryo lethality or in serious developmental defects. Plants are more tolerant to such mutations and allow the analyses of their effects in successive generations.

Chromatin remodeling requires specialized ATPases that utilize the energy of ATP hydrolysis to alter chromatin state. All such enzymes characterized so far appear to function within multi-subunit complexes, with the number of subunits ranging from two to more than ten. The prototype yeast SWI/SNF (SWItch/Sucrose-Non-Fermenting) complex is built around the founding member of the remodeling ATPase family, the SWI2/SNF2. All SWI/SNF-type complexes studied so far contain a minimal structural and functional core composed of four evolutionarily conserved subunits, homologues of yeast proteins SWI2/SNF2, SNF5, SWI3 and SWP73. We have recently identified the *Arabidopsis* lines with T-DNA insertions in each of the four genes encoding members of the AtSWI3 family. The analysis of these lines documents an astonishing functional diversity of plant SWI3 proteins.

An important part of our research concerns identification of the possible molecular mechanisms underlying the phenotypic changes in *Arabidopsis thaliana* observed upon RNAi-mediated decrease of the chromatin content of linker histones. We have recently shown that plants with a >90% reduction in *H1* expression exhibit pleiotropic phenotypic defects which segregate independently of the anti-*H1* dsRNA transgene. The phenotypic defects are correlated with minor but statistically significant changes in the methylation patterns of repetitive and single-copy sequences

**APOPTIN - A NOVEL, CANCER-SELECTIVE KILLER**

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Apoptin, a chicken anemia virus derived protein, selectively induces apoptosis in cancer cells but not in primary, non-transformed cells, thus making it a promising candidate as a novel anti-cancer therapeutic. The mechanism of apoptin induced apoptosis is largely unknown. Apoptin likely kills cancer cells by an unknown mechanism that has not been previously found in case of known anticancer drugs. In transformed cells apoptin is known to mainly localize in the nucleus and kill the cells *via* apoptosis. Nuclear localization of apoptin protein is important for its selective toxicity that is executed by the activation of the intrinsic (mitochondria - dependent) apoptotic pathway. However, the role of nuclear localization on apoptin activity and its subsequent signaling to the mitochondria is not known. My presentation will focus on the signaling molecules and pathways that are involved in apoptin signaling from the nucleus to mitochondria. It has been recently shown that some signaling molecules translocate from nucleus to mitochondria upon various apoptotic stimuli. Nur77/TR3/NGFI-B, an immediate early response gene and an orphan member of the steroid/thyroid receptor superfamily, translocates from nucleus to the cytoplasm, and targets mitochondria to induce cytochrome c release and apoptosis in response to apoptotic stimuli. By using different methodologies like siRNA technology, co-imunorprecipitation, confocal microscopy, apoptotic assays etc, we are exposing the signaling pathway employed by apoptin to kill cancer cells.

**CLINICAL SIGNIFICANCE OF OXIDATIVE DNA DAMAGE**

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Oxidative DNA damage is an inevitable consequence of cellular metabolism, with a propensity for increased levels following toxic insult. Whilst over twenty base lesions have been identified, only a fraction of these have received any appreciable study, most notably 8-hydroxy-2'deoxyguanosine. This lesion has been the focus of intense research interest, and been ascribed much importance, largely to the detriment of other lesions. The present work reviews the basis for the biological significance of oxidative DNA damage, drawing attention to the multiplicity of proteins with repair activities, along with a number of poorly considered effects of damage. Given the plethora of, often contradictory, literature reports describing pathological conditions in which levels of oxidative DNA damage have been measured, this review critically addresses the extent to which the *in vitro* significance of such damage has relevance for the pathogenesis of disease. It is suggested that a number of short-comings associated with biomarkers, along with gaps in our knowledge may be responsible for the failure to produce consistent and definitive results when applied to understanding the role of DNA damage in disease, highlighting the need for further studies.

## GENE EXPRESSION SIGNATURES OF NORMAL SQUAMOUS EPITHELIUM CAN DISTINGUISH AMONG GASTROESOPHAGEAL REFLUX DISEASE VARIANTS

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**Background and Aims:** It has been proposed recently that gastroesophageal reflux disease patients may be categorized into three distinct groups: nonerosive reflux disease, erosive reflux disease, and Barrett's esophagus. Thus, different molecular mechanisms may underlie the development of each clinical variant. Among other predisposing factors, sex influences clinical outcomes of gastroesophageal reflux. The aim of this study was to identify distinct molecular subclasses in different variants of gastroesophageal disease.

**Methods:** The measurements were done using the oligonucleotide DNA Affymetrix U133A 2.0 microarray and RNA isolated from mucosal samples of normal squamous esophageal epithelium in 29, 32, and 29 patients with nonerosive reflux disease, erosive reflux disease, and Barrett's esophagus, respectively.

**Results:** Gene expression patterns successfully distinguished mucosal samples from Barrett's esophagus and nonerosive reflux disease patients, but not from erosive reflux disease. In addition, females developing nonerosive, but not erosive, reflux disease showed molecular changes that differed from those of males with Barrett's esophagus. Thus, both clinically and molecularly, erosive is clearly localized between nonerosive reflux disease and Barrett's esophagus.

**Conclusions:** For the first time we have demonstrated different molecular makeup among gastroesophageal reflux disease patients. Our study suggests that this disease is a monophyletic disease developing on the basis of gastroesophageal reflux and that its clinical form is a result of process within epithelium modulated by sexual dimorphism. Differentially expressed genes in esophageal normal epithelium may depend on genetic predispositions but also may reflect different molecular responses of esophageal epithelium to damaging components of gastroesophageal reflux.

**SOME ALGORITHMS OF MOLECULAR DOCKING**

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The study of geometric aspects of molecular docking is performed. Molecular docking can be regarded as a potential method for computer aided drug design and optimization. A solution based on LUDI interaction surfaces and computer vision algorithm pose clustering adapted to the specific needs of molecular docking is shown. As an illustration a reconstruction of the native pose of SO4 ligand in trypanasomal isomerase complex 5TIM is presented. The 3D structure of the complex was retrieved from ProteinDataBank (PDB) database. As a measure of binding affinity, RMS deviation from the reference pose was used.

**ENGINEERING OF MEGANUCLEASES**

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Homologous gene targeting is potentially the ultimate tool for gene targeting /gene therapy, but its use is limited by low efficiency and reproducibility. Site-specific DNA double-strand breaks can induce efficient and selective gene targeting. Engineered, highly specific DNA endonucleases, which are programmable according to the desired specificity are the key to a wider use of this technology in gene therapy. We are in the process of developing programmable "meganucleases" designed to cleave only positions of interest in a genomic context with the aim - based on homologous recombination - to solve the main problem in reverse genetics and gene therapy: to replace genes, efficiently, selectively and reproducibly. Two types of meganucleases are being engineered by us: (i) programmed restriction endonucleases, and (ii) redesigned homing endonucleases.

The first approach depends on the high specificity of restriction enzymes and of triple helix formation; the target site of a restriction enzyme - TFO conjugate is a composite of the recognition site of the restriction enzyme and the triple helix forming site. We have fused a triple helix forming oligonucleotide to single chain PvuII (Simonscits *et al.* 2001) using a bifunctionasl crosslinker. This scPvuII-TFO heteroconjugate cleaves only the composite site, when preincubated with DNA in the absence of Mg-ions to allow triple helix formation, prior to addition of Mg-ions to initiate cleavage. With such programmable restriction enzymes any gene can in principle be targeted given the combinatorial flexibility this fusion offers in addressing a short, yet precisely recognized restriction site next to a defined triple-helix forming site. A drawback of this approach is that triple-helix formation is slow, which means that after delivery to the cell, the restriction enzyme part of the conjugate would cleave DNA at multiple sites before triple-helix formation has targeted the enzyme to the site of interest. A solution to this problem developed by us is to use a "caged" version of the enzyme that requires photoactivation to become active.

For the second approach of meganuclease generation, homing endonucleases are particularly suitable. Homing endonucleases are a large class of proteins (several hundreds of members) found in bacteria, archaea, fungi and algae. They are encoded by mobile genetic elements such as group I introns and inteins, which promote their proper dissemination through their endonuclease activity. They recognize DNA sequences of up to 35 base pairs in length and cleave large genomes only at few positions. Different from restriction endonucleases, they are tolerant to base substitutions. Homing endonucleases of the LAGLIDADG family have a modular architecture, which in principle allows generating homing endonucleases of new (desired) specificity by domain swapping approaches (Chevalier *et al*. 2002; Epinat *et al*. 2003). We have engineered a version of I-DmoI, composed of two copies of domain A of the monomeric wildtype I-DmoI. This homodimeric $A_2$ variant recognizes and cleaves a palindromic sequence composed of two inverted repeats of the half-site 'A' of the I-DmoI recognition sequence; it does not cleave the recognition sequence of wild type I-DmoI ('A-B'). Given the large number of known LAGLIDADG enzymes it should be possible to generate a huge number of variants by domain swapping, which should allow addressing an even larger number of recognition sequences, considering the sequence tolerance of homing endonucleases.

**ASSEMBLY OF NUCLEAR MATRIX - BOUND PROTEIN COMPLEXES INVOLVED IN NON-HOMOLOGOUS END JOINING IS INDUCED BY INHIBITION OF DNA TOPOISOMERASE II.**

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Topoisomerases maintain the DNA structure by relieving the torsional stress and alleviating other topological problems occurring in DNA during transcription and replication. Topoisomerase II appears to have a close association with the family of proteins involved in the organization of chromatin in a series of loops on the proteinaceous chromosomal matrix. Beyond its physiological functions, topoisomerase II is the target for some of the most active anticancer drugs. Inhibition of the topoisomerase II function can result in DNA double-strand breaks (DSBs) and, thus, lead to chromosomal translocations. The earliest event during DSB repair is phosphorylation of histone H2AX at S139 (so-called γH2AX) which is believed to serve as a focal point for the assembly of repair proteins at the DSB. In this work, we have demonstrated the formation of γH2AX foci in two human cell lines - K562 and HeLa - after suppression of topoisomerase II activity with etoposide. Furthermore, these foci remained visible at nuclear matrices and colocalized with the major components of non-homologous end joining system of DSBs repair. Thus, inhibition of topoisomerase II activity triggers assembly of NHEJ complexes at the nuclear matrix.

**SATELLITE DNA, MEIOTIC TELOMERES AND CHROMOSOME EVOLUTION**

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Exposure to ionizing radiation and genotoxic compounds leads to formation DNA double strand breaks (DSBs) that may trigger the formation of chromosome rearrangements, cancer or cell death. DSBs occur also in physiological context, e.g. during meiosis. Here DSBs are instrumental for generating crossing over, chromosome reduction, and genetic diversity in the offspring. During evolution, viable chromosome rearrangements propagated through the germ line have constantly reshaped karyotypes leading to the diverse karyomorphs encountered in extant species. Extreme karyotype variation and a particularly rapid karyotypic evolution characterize the genus *Muntiacus* comprising small Asian deer. Diploid chromosome numbers range from 2n=46 in the Chinese muntjac (*M. reevesi*) to as low as 2n=6/7 (female/male) in the Indian muntjac (*M. muntjak vag.*), with the latter being the lowest known chromosome number among extant mammals. The drastic chromosome number reduction in the Indian muntjac has involved numerous tandem and a few Robertsonian fusions. Molecular and FISH analysis suggests that repetitive DNA sequences found at centromeres and telomeres might have played a role in this fusion process. Genes involved in telomere metabolism appear to be functional in muntjac cells. Based on molecular and FISH analyses it is proposed that recombinogenic DSBs between telomere and muntjac satellite sequences during the bouquet stage of meiotic prophase, where chromosome ends cluster in a limited region of the nuclear periphery, may have sustained karyotypic orthoselection for tandem fusions that shaped the extant Indian muntjac karyotype.

## MOLECULAR MARKERS OF TUMOR PROGRESSION AND PROGNOSIS ESTABLISHED IN HEAD AND NECK CANCER

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Efficiency of head and neck cancer treatment remains low. Occurrence of multiple primary tumors (MPT), local metastasis and tumor relapse are the main causes of treatment failure. Identification of genetic markers of all the listed symptoms is a task for molecular epidemiology and experimental oncology studies.

The research efforts were focused on identification of a specific genotype predisposing MPT formation, differentiation between MPT and metastasis, discovering a marker of entering into micrometastasis, identification of genetic background of tumor tendency to relapse. The main results are as follows:

1. Cytogenetic (classical, FISH, CGH) findings indicate for the regions of oncogenes amplification and tumor suppressor genes deletion to be studied further for a loss of heterozygosity.
2. An amplification of 11q13 (*CCND1* locus) and translocations involving 11q13 region determined together with 3q gain is a reliable marker of poor prognosis. Interpretation of *CCND1* expression should take into account the cyclin D1 polymorphism.
3. Comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) appear to be a proper attempt to determine cancer cells clonality and to differentiate between metastasis and MPT.
4. LOH analysis in tumor and tumor free-areas of larynx confronted with laryngeal cancer progression requires a thorough selection of microsatellite markes to reach a predictive significance of tumor relapse.
5. Genotyping of the selected genes coding carcinogen metabolizing enzymes and DNA repair enzymes did not show a distinct genotype predisposing to MPT.

# Poster abstracts

**Only the posters with underlined titles enter the contest for best meeting communications.**

## 1. INFLUENCE OF TEMPERATURE ON RADIATION-INDUCED MICRONUCLEI IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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The level of cytogenetic damage induced by ionizing radiation under *in vitro* conditions in human peripheral blood lymphocytes is analyzed for the purpose of establishing calibration curves used in biological dosimetry and for assessing the intrinsic radiosensitivity of the blood donor. The irradiation of blood should be performed under strictly controlled physical conditions that allow a high reproducibility of the dose. A factor that is often not regarded is the control of blood temperature during exposure. Available data on the influence of blood temperature on the level of cytogenetic damage is scarce and somewhat contrdictory. We have, therefore, performed experiments to analyze the impact of blood temperature on the level of radiation-induced micronuclei. Blood was exposed to different doses of X-rays (200 kVp, 5 mA, 3 mm Cu filter) at 0, 20 and $37^0$C. Thereafter a standard micronucleus test was performed and micronuclei were analyzed optically on microscopic slides. The results of the experiments will be presented and discussed.

## 2. MODULATION OF THE ACTIVATION OF NFκB AND THE EXPRESSION OF NOS-2 AND COX-2 IN MOUSE EPIDERMIS BY TANNIC ACID – NATURALLY OCCURRING PLANT PHENOL

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Tannic acid (TA), the mixture of digallic acid esters of glucose, ubiquitous in edible plants, has been shown to possess antimutagenic and anti-carcinogenic activity in several experimental models, including mouse skin.

Mouse skin is one of the best animal models of chemical carcinogenesis which enables to study all stages of this process. Although most human skin cancers are not induced by chemicals, many events in this model could be extrapolated to humans. Moreover the biochemical changes observed in the mouse skin after application of tumor promoter, phorbol ester, 12-*O*-tertadecanoylphorbol 13-acetate (TPA) are the same as those in humans after UVB radiation. Our earlier studies [1,2] showed the inhibition of the formation of 7,12-dimethylbenz[a]anthracene-diol-epoxides (DMBADE)-dAdo adducts by tannic acid treatment in vitro and in vivo in mouse skin. The aim of the present study was to evaluate weather the inhibition of epidermal DNA adducts formation, which may affect the c-Ha-*ras* mutation by DMBA, may also influence the activation of NFκB and the expression of the enzymes controlled by this transcription factor.

We determined the effect of topical application of TA on TPA-induced NFκB subunits IkBα (p35) degradation, and p65 nuclear translocation and DNA binding by Western blot and ELISA assay. The activity and expression of inducible nitric oxide synthase (NOS-2) and cyclooxygenase 2 (COX-2) was measured using the same experimental protocol by specific enzyme activity assays and Western blot.

Pretreatment of mice with 16 μmoles of TA 15 minutes before TPA treatment, resulted in significant enhancement of IkBα retention in cytosol (2-fold, in comparison with TPA treated group). Nuclear translocation of p65 and p65-DNA binding caused by TPA was diminished by 60% and 50% respectively. TA significantly reduced the NOS-2 and COX-2 activities and their protein level. NOS-2 activity was reduced by 75% and its protein level by 40%, and COX-2 reduction was by 220% and by 140%, respectively.

These results indicate that polyphenol TA affect the NFκB activation which might be related to potential protection against c-Ha-*ras* mutation. Collectively the results of our present and earlier studies suggest that TA is capable of affecting more than one critical pathway of carcinogenesis and thus will have greater advantage over other single-target potential chemopreventive agents.

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## 3. SEVERE OXIDATIVE STRESS IN NEWBORN PIGLETS

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Reactive oxygen species (ROS) can damage different kinds of biomolecules, including proteins, lipids and DNA. Therefore, organisms developed a series of primary antioxidant defenses to protect against these lesions. However, in humans as well as in other mammals during early life the antioxidant systems are poorly developed. Moreover, partial pressure of oxygen in extrauterine environment is much higher than in a womb.
Although some studies suggest that increased oxidative stress in newborns may be responsible for many neonatal diseases the specific biochemical markers which can predict the onset of ROS induce damage are largely undefined.

The aim of the present study was to evaluate the oxidative status in healthy full-term newborns piglets in first month of their development. We assayed urinary excretion of 8-oxoGua, 8-oxodG and 5HMUra in newborn pigs using HPLC/GS/MS methodology. In addition concentrations of vitamins A, C and E were analyzed in blood serum of newborn piglets (HPLC with UV-absorbance and fluorescent detection).

Our experiments demonstrated that the level of all measured oxidatively modified bases/nucleotides excreted with urine increased sharply, shortly after the birth of piglets. Four days after the birth concentration of antioxidant vitamins increased significantly over the level observed in first day. From this moment on, the level of the vitamins dropped to the value characteristic for an adult animal. Simultaneously, excretion rates of the analyzed oxidatively modified bases/nucleosides decreased to the levels characteristic for adult pig. This findings combine indicate that antioxidant vitamins play a major role in the protection against oxidative DNA damage in newborns, scavenging ROS generated shortly after the birth.

## 4. GENE EXPRESSION SIGNATURE OF HEREDITARY BREAST CANCER

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**Introduction:** Hereditary breast cancer associated with mutations in BRCA1 gene is characterized by some pathological and clinical features that are distinct from sporadic breast cancer. These are e. g. earlier age of manifestation, high tumor grade, high proliferation index, characteristic pushing margins and lymphocyte infiltrate. Despite so bad prognostic indications, it is frequently observed that patients with BRCA1 mutations have overall survival similar to patients with sporadic breast cancer. The aim of this study was an attempt to elucidate molecular basis underlying described discrepancy by comparing gene expression profiles of BRCA1-associated hereditary breast cancer with sporadic breast cancer cases.

**Material and methods:** We analyzed gene expression in 28 tumor samples obtained from 27 patients: 20 patients with hereditary breast cancer (11 with BRCA1 mutation, 1 with BRCA2 mutation and 8 without proven BRCA mutations) and 8 patients with sporadic breast carcinoma. We also analyzed 6 normal breast tissues. We used HG U133 Plus 2.0 (Affymetrix) oligonucleotyde microarrays. Target preparation, hybrydization and staining were done according to Affymetrix instruction manual. Preprocessing of the microarray data was performed by Robust Multiarray Analysis (RMA). Hierarchical clustering, statistical comparisons analyses and Principal Component Analysis (PCA) and were carried out in GeneSpring 7.2 software (Silicon Genetics). For gene selection, we used both non-parametric Mann-Whitney test and parametric Welch test, False Discovery Rate was estimated by Benjamini-Hochberg algorithm. Singular Value Decomposition was used to obtain orthogonal vectors called characteristic modes or supergenes which represent major independent variability patterns in analyzed data. Quantitative RT-PCR analysis was done using the ABI PRISM 7700 Sequence Detection System instrument and software (Applied Biosystems), and MasterAmp Real-Time RT-PCR Kit (Epicentre).

**Results:** We compared expression profile of over 47 000 transcripts in BRCA1 mutation-linked and BRCA1 mutation negative breast tumor tissues. We were able to select a set of 45 genes differentiating between both types of cancer; however we found that difference rather discrete and connected mostly to the tumor hormonal status. Interestingly, we found, that a difference in gene expression profile between BRCA1(+) and BRCAx tumors is more significant that between BRCA1(+) and sporadic tumors. We found that several genes connected with selenium metabolism or requiring selenium for their enzymatic activity, show differential expression between breast cancer and normal breast tissue, as well as between BRCA1(+) and BRCA1(-) tumors. These data were validated by Real-Time RT-PCR. These findings may be of potential practical value for prophylactics of breast cancer in mutation carriers by supplementing diet with selenium.

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## 5. POLYMORPHISM OF DNA REPAIR GENES, RELATION TO THE REPAIR PROCESS AND CANCER RISK

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The level of DNA damage after exposure to genotoxic factors, the rate of its repair, and the efficiency of repair vary considerably in cells of the same type from different individuals. The genetic background is likely to be one determinant of this diversity of responses, and in particular polymorphism in the coding or regulatory regions of genes which encode enzymes for DNA repair, a factor which also affects the risk of cancer.
The aims of the present study were:

- To estimate the changes in the risk of cancer that correlate with the presence of polymorphic variants of the genes NBS1 (Gln185Glu), APE1 (Asp145Glu), XRCC3 (Thr241Met), XPD (Asp312Asn and Lys751Gln), XRCC1 (Arg399Glu).
- To study the influence of these polymorphic forms on the process of DNA repair.

Polymorphisms were detected by PCR-RFLP using DNA isolated from frozen blood by standard SDS-proteinase K and RNase digestion and phenol-chloroform extraction. Functional tests were performed on lymphocytes isolated from fresh peripheral blood on gradients of Ficoll-Histopaque and exposed in vitro to ionizing radiation. Comet and micronucleus tests were used for assessement of DNA damage and repair. The experimental results for samples obtained from 46 head and neck, 33 lung, 30 cervix and 45 colon cancers patients and 84 healthy donors will be discussed.

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## 6. THE EFFECT OF I.P. ADMINISTRATION OF DIETHYLNITROSAMINE ON THE DNA DAMAGE IN BLOOD LEUKOCYTES IN RATS

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Oxidative stress related to carcinogen metabolism and activation is important pro-mutagenic and pro-carcinogenic factor as the reactive oxygen species (ROS) play role in all stages of tumorigenesis.

Diethylnitrosamine (DEN) is a food-derived carcinogen of gastro-intestinal tract in humans and it is commonly used for the induction of liver cancer in laboratory animals. After metabolic activation by cytochrome P450 (CYP 2E1) DEN becomes an indirect alkylating agent generating the DNA-ethyl adducts and induces oxidative stress due to the contribution to inflammatory reactions. The resulting generation of ROS, induction of NFκB and subsequently of iNOS and release of massive amounts of nitric oxide creates the pro-oxidative environment in which oxidative DNA damage occurs.

The aim of current study was the assessment of various i.p. doses of DEN (1, 15, 60 and 150 mg/kg body weight) on the DNA damage in peripheral blood leukocytes in male Wistar rats, measured in the single cell alkaline gel electrophoresis (Comet assay).

The rate of the DNA damage correlated to the increasing dose of DEN. At the doses of 1 and 15 mg/kg b.wt., no effects in comparison to control were observed (92 and 122% of control value, respectively). DEN administered in the doses of 60 and 150 mg/kg b.wt. caused significant DNA damage (129% and 164% increase in comparison to control, respectively) in the peripheral blood leukocytes.

The observed rate of the DNA damage may result from the oxidation of nucleic acid as well as from the formation of adducts. Since animals were sacrificed 24 hours after the DEN administration, a contribution from the repair enzymes should be taken into consideration. The lack of effects from lower doses might be then understood as no direct DNA oxidation and/or more efficient damage repair. Accordingly, in case of higher doses, the overall effect might be the result of increased oxidation and/or insufficient repair.

The obtained results indicate that the alkaline comet assay appeared to be a sensitive test for the DNA-damaging effects of carcinogen. The peripheral blood is easily available and the changes observed in leukocytes may reflect the changes appearing in the target organ(s).

## 7. COMPARISON OF EXPRESSION PROFILE IN BREAST AND OVARIAN CANCER

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**Background:** In majority, microarray studies exploit the differences between cancer and corresponding normal tissues or the molecular differences between tumor histotypes originating from one tissue. However, sound understanding of neoplastic transformation and progression will benefit from comparison of tumors originating from diverse tissues, especially if they share some biological or clinical properties. Such analysis may aid to seek novel therapeutic targets, which are rather tumor-specific than tissue-specific.

**The aim of our study** was to compare the expression profile in breast and ovarian cancer, two female adenocarcinomas with similar genetic background and comparable chemo- and radiosensitivity.

**Methods:** We compared expression profiles of 29 breast carcinomas (BC, analyzed within the project of gene expression profiling of hereditary and sporadic breast cancer, all grade 3 ductal or medullary tumors, 17 ER- ductal carcinomas, 7 ER- medullary ca samples and 5 ER positive tumors) and 16 serous ovarian carcinomas (OC, analyzed within the project of chemosensivity assessment by microarray analysis, all serous ovarian carcinomas, majority grade 3, three samples grade 2). We used GeneChip U133 2.0 Plus microarray and standard amplification procedure. We applied two methods of data pre-processing, GC-RMA and MAS5 algorithm, and compared the obtained results.

**Results:** It seemed clear that two different cancers shall exhibit different expression profiles. We measured the extent of difference between both groups and showed that the number of selected genes depends on the method of preprocessing (14790 genes by MAS5, 12843 by GC-RMA, FDR<5%). Moreover, by various preprocessing approaches different genes are missing. MAS5 algorithm is known to give large variance in low-expressing transcripts, and many low abundance genes were absent in MAS5-obtained data, as compared to GC-RMA. However, GC-RMA (which gives better precision than MAS5), due to quantile normalization which flattens the differences between two significantly distinct subclasses, was loosing many intermediate/high abundance transcripts.
In the next step, we performed the unsupervised analysis of BC and OC expression profiles. By Singular Value Decomposition we revealed that the samples were divided into three large clusters, which corresponded to two groups of breast carcinomas (BC1 and BC2) and a separate group of ovarian cancers (OC). These groups were properly separated by expression of two estrogen-related genes, *ESR1* and *GATA3*, which were low in BC1, showed variable and moderate expression in OC and very high expression in BC2. Both these genes were present in the third mode of SVD analysis.
We performed the supervised comparison between breast and ovarian tumors. We found out that one of the most upregulated GO classes in breast cancer compared to ovarian ca is that of the wound-response genes. We compared the expression of genes presented by Chang et al. (signature obtained by serum stimulation of fibroblasts), and we revealed that some of these genes differentiate between breast and ovarian cancer, including *AZGP1, PRLR* and *LTF*. To base the comparison of both classes on well-described transcripts, we also used the signature of neoplastic transformation, proposed by Rhodes et al. [2004] in a large meta-analysis of 40 cancer datasets. From 168 probesets that were corresponding to Rhodes genes, majority of genes showed no differences between both classes. 30 transcripts were differentially expressed between BC and OC (the strongest differences were within *KDELR2, PLK1, PPP2R5C, ACLY, G3BP, MMP9, TRA1, HSPD1*).

**Conclusions:** There are similarities in expression of neoplastic transformation signature genes between breast and ovarian carcinomas. However, such a comparison requires a careful selection of preprocessing method, which may strongly influence the obtained results. The important factor of similarity between the subgroups of breast and ovarian tumors is the estrogen receptor expression.

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## 8. FUNCTIONAL INTERACTIONS BETWEEN APOPTOTIC NUCLEASE DFF40/CAD AND HISTONE H1

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The major apoptotic nuclease, DNA fragmentation factor (DFF40/CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of programmed cell death. Previously we have demonstrated that several chromatin proteins, including HMGB1/2, histone H1 and topoisomerase II, greatly enhances naked DNA cleavage by this nuclease *in vitro* (histone H1 stimulates DFF40/CAD cleavage of DNA ~20-fold). Here we investigate the mechanism of stimulation of DNA cleavage by histone H1. Addition of histone H1 either during or after caspase-3 treatment of DFF causes the same stimulatory effect on DNA cleavage, indicating that histone H1 affects DFF40/CAD enzyme activity, but not caspase-3-dependent activation of the nuclease. We have found that each of the six somatic cell histone H1 isoforms, which differ in primary sequence, equally activate DFF40/CAD. Using a series of truncation mutants of recombinant mouse histone H1-0, we demonstrate that the H1-0 C-terminal domain (CTD) is responsible for activation of DFF40/CAD. We show further that the intact histone H1-0 CTD and certain synthetic CTD fragments bind to DFF40/CAD. These interactions enhance the ability of DFF40/CAD to bind to DNA. We have concluded that the interactions between the histone H1 CTD and DFF40/CAD target and activate linker DNA cleavage during the terminal stages of apoptosis.

## 9. HALOGENATED ANESTHETICS: GENOTOXICITY AND INFLUENCE ON HUMAN GENOME

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Inhalation anesthetics exert some side effects. Health effects for short-term (patients) and long-term occupational exposure differ. An increased hepatotoxicity, nephrotoxicity, migraine and reproduction disorders in operating room personnel were reported. Papers concerning genotoxic and cytotoxic effects of halogenated anesthetics are discordant.

The studies aimed for testing genotoxicity of volatile anesthetics (halothane, desflurane, isoflurane and sevoflurane) in human lymphocytes in vitro using comet assay. Because of a high volatility of drugs and to minimize DNA repair, all processes were carried out at 4ºC. The negative control was water and 1 % DMSO (used as a solvent), when halothane (already proven as genotoxic) served as positive control. Mann-Whitney U-test was employed to estimate statistical significance.

An induction of DNA fragmentation by desflurane was as effective at that of halothane. Genotoxicity of isoflurane / sevoflurane did not differ significantly from controls. Genotoxic activity of desflurane was dose-dependent (0.1mM - 10mM). However, when pharmacokinetics is taken into account desflurane appears to be less harmful than halothane for the exposed staff.

In studies on inhalation anesthetics concentration ($N_2O$, halothane, sevoflurane, isoflurane) in operating halls of two Poland regions, supported by KBN project No. 6PO5C00521 we found a considerable exceed of highest admissible concentrations.

Using comet assay two groups were examined (100 persons each) for DNA damage. The exposed group consisted of surgeons, anesthesiologists and nurses, the control group were the persons not exposed on inhalation anesthetics. The studies did not reveal statistically significant ($p<0.05$) differences between exposed and group control. Further division in view of work position showed onto anesthesiological nurses as a group of an increased risk.

With the aid of genotyping were not affirmed essential differences in distribution of genotypes of DNA repair genes (XRCC1, XRCC3, XPDex6, XPDex23) in the exposed and control group.

## 10. THE ROLE OF BIOREDUCTIVE ACTIVATION OF 4'-O-TETRAHYDROPYRANYL-DOXORUBICIN IN THE CYTOTOXIC ACTIVITY AGAINST LEUKAEMIA HL60 SENSITIVE CELL LINE AND ITS MULTIDRUG RESISTANT SUBLINES.*IN VITRO* AND SPECTROFLUOROMETRIC STUDIES

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The clinical usefulness of anthracycline antitumour drugs (e.g. doxorubicin, DOX; daunorubicin, DR) is severely limited by the occurrence of multidrug resistance (MDR) associated with the presence of the membrane transporters (e.g. P-glycoprotein, MRP1), responsible for the active export of drugs out of resistant cells. DOX is a well-known bioreductive drug. In our recent study (*Kostrzewa-Nowak et al., Br. J. Cancer, 93, 89-97, 2005*) we have evidenced the important role of bioreductive activation of DOX by exogenously added NADPH-cytochrome P450 reductase (CPR) and NADPH in cytotoxic activity against human promyelocytic leukaemia HL60 cell line as well as its MDR sublines exhibiting two different phenotypes of multidrug resistance related to the overexpression of P-glycoprotein (HL60/VINC) or MRP1 (HL60/DOX).

The aim of this study was to examine the reductive activation of 4'-O-tetrahydropyranyl derivative of DOX (THP-DOX, pirarubicin) by human liver CPR and its impact on increasing the cytotoxic activity against sensitive as well as resistant HL60 cell lines.

It was found that the presence of tetrahydropyranyl group at the sugar moiety of DOX did not disturb the ability of this derivative to undergo reductive activation by CPR with the formation of reactive metabolites. It was evidenced, similarly to results obtained previously for the parent drug (DOX) that, upon CPR catalysis, THP-DOX underwent only the redox cycling (at low NADPH concentration) or multi-stage chemical transformation (at high NADPH concentration). We have also found, using superoxide dismutase (SOD), that the first stage undergoing according to the mechanism of the redox cycling had the key importance for the metabolic conversion of both compounds examined. Results of *in vitro* studies confirmed the same behaviour of reductively activated parent drug (DOX) and its tetrahydropyranyl derivative. Our assays showed that the presence of CPR catalysing only the redox cycling of both compounds had no effect in increasing their cytotoxicity against sensitive and MDR tumour cells. In contrast, an important increase in cytotoxic activity of DOX as well as THP-DOX, after their metabolic conversion by CPR, was observed against sensitive HL60 as well as multidrug resistant HL60/VINC and HL60/DOX cells. The interactions of THP-DOX alone (non-activated), THP-DOX acting in the redox cycling as well as reactive metabolites of the drug obtained upon CPR catalysis with naked DNA and intact cells were examined using spectrofluorometric method (*Tarasiuk et al. Biochim. Biophys. Acta 1013, 109-117, 1989*). Similarly to results obtained for THP-DOX alone, an important quenching of the fluorescence signal for THP-DOX operating in the redox cycling was observed after the addition of naked DNA or during the incubation with sensitive HL60 as well as resistant cells (HL60/VINC and HL60/DOX) due to the intercalation of the drug between base pairs of DNA. In contrast, under the same experimental conditions only a slight decrease in the fluorescence signal was observed for THP-DOX undergoing reductive conversion that indicates another type of interaction of the drug with naked DNA and nuclear DNA of intact cells. The formation of covalent adducts of reactive metabolites of THP-DOX with DNA could be assumed.

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## 11. BIOLOGICAL ACTIVITY OF PREPARATIONS FROM *UNCARIA TOMENTOSA* (WILLD.) DC

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*Uncaria tomentosa* (Willdenow ex Roemer & Schultes) De Candolle also called una de gato, cat's claw and Katzenkralle is a woody wine of South and Central America. Aqueous decoctions of this plant have been used in local folk medicine for at least two thousand years as a remedy on digestive tract, arthritis and rheumatism, immunological and genital diseases. Potential imunostimulating, antinflamatory and anticancer activities have been widely studied. Several phytochemical analyses showed that such activity is associated with pentacyclic and tetracycylic oxindole alkaloids, quinine glycosydes, ursolic acid and a number of phenolic compounds.

In this study antiproliferative activity of different extracts obtained form leaves and bark of *Uncaria tomentosa* originated from Peru and supplied by Andean Medicine Centre were evaluated. The preparations were standardized by HPLC for obtaining their total oxindole alkaloids contents which were from 430 to 50401 mg/100g. Qualitative analyses were also performed indicating different oxidondole alkaloids profiles in the fractionated preparations. The biological assays were performed on HL-60 acute promyelocytic human cell lines, ranging exposed on different extracts for 72 hours. The proliferation rates and cytotoxicity of each preparation were evaluated by applying trypan blue staining and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay (MTT). The highest antiproliferative activity was showed for enriched alkaloid fraction Bsrt and generally for ethanolic preparations. Total activities of all preparations were determined and compared with particular alkaloid determinations. By calculating Pearson's correlation factors, pteropodine/isomitraphilline and isopteropodine were found to possess the strongest antiproliferative activity (r=0.91 and r=0.74, respectively).

## 12. MODIFICATION OF THE RATE OF OXIDATIVE DNA DAMAGE REPAIR BY DIETARY FACTORS AND INFLAMMATION IN NEWBORN PIGS

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The diet exerts an important effect on the development and functioning of the digestive tract. We were investigating the effect of supplementation of the diet of sows with grains rich in polyunsaturated fatty acids (PUFA: flax-seed, rape-seed 4 kg/100 kg of feed each), L-carnitine (15 g/100 kg), taurine (100 g/100 kg) and vitamin E (15 g/100 kg) on the rate of oxidative DNA damage excision in colons of their offspring. Diet supplementation was performed from the 80$^{th}$ day of pregnancy till 28$^{th}$ day of lactation. Repair activities in colons of the offspring from one sow were similar in the first, fourth and seventh day after delivery, however they differed between piglets from different mothers. Diet supplementation of mothers had no effect on the repair activity (measured by the nicking assay) of ethenocytosine (εC) in the colons of newborn pigs. However the repair activity for 8-oxoG and εA increased about twice in pigs, whose mothers were fed with supplemented diet. Thus dietary factors can modulate repair capacity for 8-oxoG, and εA, but have no effect on excision rate of εC from pig intestines.

## 13. PHOTODYNAMIC EFFECT, CELL DEATH PATHWAYS AND SUBCELLULAR LOCALISATION OF TWO LIPOSOME-INCORPORATED SYNTHETIC PORPHYRIN DERIVATIVES

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Photodynamic therapy (PDT) of cancer diseases relies on the combined use of photosensitisers (virtually non-cytotoxic by themselves) and harmless visible light. Target cells are photodamaged as a result of singlet oxygen and other reactive oxygen species formation. Photofrin® was the first photosensitiser approved for human use. Search for novel drugs with improved efficiency and fewer side effects has been continuing, however, for the past decade.
In the present study, two synthetic porphyrin derivatives: 5-(4-hydroxyphenyl)-10,15,20-tritolylporphyrin (**C16-TTP**) and 5-(4-hexadecyloxyphenyl)-10,15,20-tri-pyridylporphyrin (**TPYR-PP**) were compared with respect to photodynamic efficiency, cell localization as well as mode of induced cell death.

Singlet oxygen yields and lifetimes were determined by directly measuring phosphorescence at 1270 nm. In vitro studies were conducted on three cancer cell lines: human malignant melanoma (Me45), murine melanoma (B16(F10)) and human colon adenocarcinoma (Hct-116). Cationic liposome-plasmid DNA complexes (lipoplexes) were used as vehicles transporting these hydrophobic porphyrins. Cell viability following PDT treatment was monitored by MTS-tetrazolium reduction assay and by a test assessing clonogenic potential of cells. The mode of cell death was investigated with fluorescence microscopy using acridine orange/ethidium bromide (AO/EB) double-staining method. The sensitisers' localisation was analysed using a confocal microscope

Both compounds are characterised by high quantum yield of singlet oxygen generation and they displayed high photostability under the conditions used for PDT tests. They revealed negligible dark cytotoxicity but high phototoxicity. The results were both light dose- and cell line-dependent. To reach $LD_{50}$, only 5 J/cm$^2$ was required. TPYR-PP was slightly more phototoxic than C16-TTP. Analysis of data gathered suggests that the manner of cell death following use of either one of the examined compounds depends on light irradiation dose. In either case, when high dose (15 J/cm$^2$) was used, the dominating effect was necrosis. Almost total inhibition of cellular proliferation was then observed. Confocal laser scanning microscopy showed that both compounds localized in the cytoplasm but not in the nucleus of cells, which explains no DNA damage detection under dark conditions and when using photodynamic doses.

Both compounds are photodynamically active, effectively inducing cell death when light activated, presumably due to efficient generation of singlet oxygen. We conclude that porphyrin derivatives of this type seem to be promising agents for PDT treatment of neoplasms.

## 14. ANALYSIS OF SUCCYNYL DEHYDROGENASE (SDH) SUBUNITS GENE MUTATIONS IN PATIENTS WITH PARAGANGLIOMAS

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Tumors derived from chromaffine tissue include pheochromocytomas (tumors located in adrenal medulla) and paragangliomas (extraadrenal tumors). These tumors are in 20-25% inherited. Paragangliomas are even rarer and are presented either as familial disease or pheochromocytoma-paraganglioma syndrome (PPS). The mutations in SDH genes (*SDHB*, *SDHD*) are suspected for causing the syndrome.

The aim of present study is to look for germline mutations in *SDHB* and *SDHD* genes in patients with pheochromocytomas and/or paragangliomas.

DNA was isolated from peripheral blood leukocytes obtained from patients with pheochromocytomas and paragangliomas. The polymerase chain reaction (PCR) was performed for *SDHB* (exons 2, 3, 4, 6, 7) and *SDHD* (exons 1, 2, 3). The PCR product was then analyzed with the use of MSSCP (Multiplex Single-Strand Conformation Polymorphism). When the change in the conformation of DNA strand was found, it was then identified by sequencing.

We have so far analyzed DNA from 12 patients with diagnosed paragangliomas; we have also examined 72 patients with pheochromocytomas only in order to seek for pheochromocytoma-paraganglioma syndrome. We have found two types of mutations of *SDHD* in 6/12 (50%) of paraganglioma cases. 33 TGC-TGA substitution was associated with benign tumors, whereas N 721 G-A occurred in the only patient with malignant paraganglioma.

Conclusions: 1) Mutations in the *SDHD* gene appear to be frequent in patients with paragangliomas. 2) Among inherited cases of pheochromocytomas no correlations of the type of *SDH* mutation with malignancy have been found so far.

## 15. THE ROLE OF NUCLEOSIDE ANALOGUES AND VITAMINS, ATRA AND $D_3$, IN EPIGENETIC CHANGES (METHYLATION) OF *PTEN* AND *APC* GENE PROMOTERS IN MCF-7 BREAST CANCER CELLS

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Hypermethylation of promoters of tumour suppressor genes is epigenetic modification of DNA and takes part in combined regulation of gene transcription. We previously documented that the actions of the adenosine analogues, 2-chloro-2'-deoxyadenosine (2CdA, cladribine) and 9-β-D-arabinosyl-2-fluoro-adenine (F-ara-A, fludarabine) lead to a decrease of genomic DNA methylation. Additionally, results of several studies indicated that natural compounds such as vitamins, which are ligands of nuclear receptors, can be synergists with anticancer drugs.

The experiments were aimed at estimation of methylation changes of promoter regions of selected tumour suppressor genes: *ERα*, *BRCA1* (with a specific sequence for CREB), *E-cadherin*, *PTEN* and *APC* in MCF-7 cells (ERα (+) and non-invasive cells), which grew in the presence of the mentioned above adenosine analogues and natural compounds: all-*trans* retinoic acid (ATRA) and vitamin $D_3$.

MCF-7 cells were cultured (72 hr) in the presence of 2CdA, F-ara-A, 5-aza-deoxycytidine (5-aza-dCyt, a potent inhibitor of DNA methyltransferase), ATRA, and vitamin $D_3$ at their $IC_{50}$ concentration: 0.17 μM, 15.0 μM and 0.6 μM, 0.34 μM, and 1.5 μM, respectively. The methylation status of gene promoters was estimated using methylation-sensitive restriction analysis (MSRA).

We noted that among selected genes only promoters of *PTEN*, *APC*, and *BRCA1* genes were methylated in MCF-7 cells cultured without drugs. In MCF-7 cells treated with nucleoside analogues or natural compounds for 72 hr we observed that: (i) methylation of *PTEN* promoter (fragment between −281 and +5 bp) was partially reduced by 2CdA, ATRA, and vitamin $D_3$ and completely eliminated by F-ara-A and 5-aza-dCyt, (ii) methylation of *APC* promoter (fragment between −113 and + 205 bp) was completely eliminated by 2CdA, F-ara-A, 5-aza-dCyt, and ATRA, and partly reduced by vitamin $D_3$, (iii) no effect of any tested drugs and natural compounds on methylation status of *BRCA1* promoter (region included sequence for CREB, fragment between −317 and −24 bp) was observed.

The findings indicated that promoter methylation of *PTEN* and *APC* genes, encoding proteins implicated in regulation of intracellular oncogenic signal transduction (PI3K/Akt and MAP kinase dependent pathways – in the case of *PTEN*, and Wnt/APC/β-catenin pathway – in the case of *APC*) can be modulated by nucleoside analogues and vitamins as well. This fact has dual significance for anticancer strategies. Firstly, it indicates that actions of 2CdA and F-ara-A, antimetabolites of natural nucleosides, are implicated not only in inhibition of DNA synthesis but also in indirect regulation of cell development due to influence on activity of proteins (i.e. PTEN and APC), crucial for cellular signaling pathways. Secondly, the possibility of hypermethylation decrease of tumour suppressor genes (i.e. *PTEN* and *APC*) by natural vitamins (i.e. ATRA and $D_3$) seems to be important for carcinogenesis prevention as well as synergistic effects with drug used in chemotherapy.

## 16. THE REPAIR OF GAMMA-RADIATION-INDUCED DNA DAMAGE IS INHIBITED BY MICROCYSTIN-LR, THE PP1 AND PP2A PHOSPHATASE INHIBITOR

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We studied the effect of microcystin-LR, the PP1 and PP2A phosphatase inhibitor, on the repair capacity of radiation-induced DNA damage in human lymphocytes and human glioblastoma cell lines MO59J and MO59K.

Human lymphocytes in $G_0$-phase of the cell cycle were pre-treated with MC-LR for 3 hours and irradiated with 2 Gy of gamma radiation. The kinetics of DNA repair was assessed by the comet assay. In addition γ-H2AX foci and the frequencies of chromosomal aberrations were analyzed. The pre-treatment with MC-LR inhibited the repair of radiation-induced damage, resulted in reduced numbers of γ-H2AX foci and lead to enhanced frequencies of chromosomal aberrations.

In order to elucidate the impact of MC-LR on DNA-PK we examined the kinetics of DNA repair in human glioblastoma cells MO59J cells that are deprived of the catalytic subunit of DNA-PK (DNA-$PK_{cs}$) and in the MO59K cells that have a normal level of DNA-$PK_{cs}$. Both cell lines were exposed to 10 Gy of X-rays and DNA repair was analyzed by the comet assay. While a strong inhibitory effect was observed in the MO59K, MC-LR also moderately inhibited the repair of damage in the MO59J cells. These results indicate that apart from DNA-PK, other enzymes involved in DNA repair could also be inhibited by MC-LR.

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## 17. CHROMOSOMAL RADIOSENSITIVITY IN PERIPHERAL BLOOD LYMPHOCYTES OF LARYNX CANCER PATIENTS

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Patients treated with identical radiotherapy schedules show a substantial variation in the degree of early and late normal tissue reactions. The identification of radiosensitive patients before therapy would allow its optimization. The aim of our study was to investigate whether the *in vitro* radiosensitivity of lymphocytes derived from a blood sample predicts the effect of radiotherapy in larynx cancer patients. In addition, there is data suggesting that the sensitivity to ionising radiation of peripheral blood lymphocytes of cancer patients is higher than that of healthy donors. This effect is especially prominent when chromosomal aberrations induced in $G_2$ phase of the cell cycle are analysed. The second aim of our study was to investigate if the $G_2$- aberration frequencies in lymphocytes of patients with larynx cancer are higher than in the case of healthy individuals.

Peripheral blood of 40 patients was collected before the onset of radiotherapy, cultured and irradiated with Co-60 after 67 hours of culture time. Irradiation was performed in the Swietokrzyskie Oncolgy Center which is located elsewhere in Kielce. Therefore, blood cultures were transported to and from the Center and irradiated on ice. Chromosome specimens were prepared from cells fixed at 72 hours of culture time. Colcemid was added for 2 hours before harvest. Lymphocytes of 40 healthy donors were cultured and irradiated in the same way like in the case of larynx cancer patients.

No statistically significant correlations were observed between aberration frequencies in lymphocytes and degrees of both early and late normal tissue reactions. The aberration frequencies in lymphocytes of patients were on average higher than in the case of healthy donors. This result suggests that the radiation sensitivity of lymphocytes of patients with larynx cancer might be a marker of cancer predisposition.

## 18. SEARCH FOR THE MOLECULAR SIGNATURE RESPONSIBLE FOR DRUG RESISTANCE IN OVARIAN CARCINOMA

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Ovarian cancer is usually diagnosed at advanced stage. In the majority of cases treatment of the disease encompasses maximal possible cytoreduction and adjuvant chemotherapy. Standard chemotherapy regimen was based on administration of cisplatin and cyclophosphamid. During last few years cisplatin with taxanes became a favored regimen, however this combination is much more expensive. While in general more effective, this regimen not always results in good response of the tumor. About 20 - 30% of patients are not responding to this type of chemotherapy. There are also some evidences, that patients with germline mutation in BRCA1 and 2 genes respond better to standard, cisplatin based chemotherapy. Thus, there is a need for more precise criteria for classification of patients for appropriate chemotherapy.

Our aim was to find a set of genes the expression of which is changed among ovarian cancer cases, those that are sensitive to chemotherapy and those that are resistant. Among those genes we expect to find potential molecular markers suitable for prediction of individual drug response.

We used DNA microarray technology (HG U133 Plus 2.0 oligonucleotide microarrays, Affymetrix) to analyze gene expression profile in ovarian cancer tissues. We analyzed 32 cancer samples from patients that were treated with cisplatin based chemotherapy. We also analyzed 20 samples from patients treated with taxane based chemotherapy.

Microarrays from both experiments were normalized together by RMA algorithm, while data analysis was performed for both data sets separately. For genes selection we used Welch test with Benjamini-Hohberg correction for multiple comparison and limma algorithm based on linear models with empirical Bayesian approach. For data analysis patients were divided into several groups according to the level of chemosensitivity/chemoresistance.

The poster will show our preliminary results of data analysis. We found, that despite the set of samples from cisplatin treated patients was larger, the difference in gene expression pattern between responders and non-responders in this group was smaller than in the taxane treated cohort. This may be related to the fact that each chemotherapeutic agent has a different mode of action. Probably, in cisplatin treated tumors, p53 status (presence and type of mutation, protein accumulation) is crucial for prediction of response to chemotherapy.

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## 19. THE EFFECT OF 1-METHYL PYRIDINIUM SALTS ON THE CYTOTOXIC ACTIVITY OF VINCRISTINE TOWARDS RESISTANT SUBLINES OF HUMAN PROMYELOCYTIC LEUKAEMIA HL60 CELLS, HL60/VINC AND HL60/DOX

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Vincristine is among the most effective drugs available for the treatment of leukaemia diseases. However, its antitumour activity is drastically reduced towards tumour cells developing the multidrug resistance (MDR) against a wide range of drugs, structurally dissimilar and having different intracellular targets. MDR phenomenon is associated with the presence of the membrane transporters (e.g. P-glycoprotein, MRP1), responsible for the ATP-dependent export of drugs out of resistant cells.

Vincristine exhibits a high cytotoxic activity against human promyelocytic sensitive leukaemia HL60 cell line (IC50 = 1.6 nM). However, an important decrease in its cytotoxic activity is observed against MDR sublines exhibiting two different phenotypes of multidrug resistance related to the overexpression of P-glycoprotein (HL60/VINC, resistance factor IR = 978) or MRP1 (HL60/DOX, IR = 15.8). The aim of this study was to examine the effect of selected 1-methyl pyridinium salts: $MNP^+$, (1-methyl-3-nitropyridinium salt) and $MDION^+$ (3,3,6,6,10-pentamethyl-3,4,6,7-tetrahydro-[1,8(2H,5H)-dion]acridine salt) on the cytotoxic activity of vincristine against HL60/VINC and HL60/DOX cells. In our previous study (Wieczorkowska et al., Free Radic. Res. 37, 1157-62, 2003) it was evidenced that these agents are able to effectively shift an equilibrium between NADH and $NAD^+$ playing an important role in energy metabolism of cells. $MNP^+$ and $MDION^+$salts were much less cytotoxic themselves against HL60 cells (at micromolar concentration) and conserved an important cytotoxic activity towards resistant HL60/VINC and HL60/DOX cells (IR = 2÷4). The high synergistic effect of $MNP^+$ and $MDION^+$ salts at 1-2 µM on the cytotoxic activity of vincristine towards both MDR resistant cell lines was observed in the wide range of vincristine concentration (up to 1 µM and 100 nM in the case of HL60/VINC and HL60/DOX, respectively).

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## 20. THE ROLE OF LYSOSOMAL IRON IN •NO SIGNALING

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Recently, it has been proved, that a considerable amount of cellular iron remains in the form of complexes with low molecular mass ligands, labile enough to enter the Fenton reaction. This pool has been called the labile iron pool (LIP) [1]. It plays a role, among others, in cellular iron transport, expression of iron regulatory genes, control of the activity of iron containing proteins, and catalysis of the Fenton reactions. It was proposed that a part of the cellular pool of labile iron is confined within the acidic vacuolar compartment (lysosomes). In the present reports the contribution of lysosomal iron to the total amount of cellular labile iron pool is vividly discussed [2-3].

Our previous results show that LIP plays a role in the regulation of nitric oxide-dependent biochemical pathways, forming dinitrosyl iron complexes (DNIC), a group of physiologically important transducers of nitric oxide. Formation of nitrosyl iron complexes, known as 2.03 complexes due to the g value of their characteristic EPR spectra, has been found by several groups of researchers in many kinds of bacteria, plants and animals. It is postulated that DNIC are important factors in nitric oxide-dependent regulation pathways in the cell [4-6]. It has been shown that low molecular-weight DNIC possesses endothelium derived relaxing factor (EDRF) activity [7-8]. In addition, the low molecular-weight DNIC have been shown to modulate redox properties of the cellular interior through the inhibition of glutathione-dependent enzymes, such as reductase, transferase, and peroxidase of glutathione [9-12].

The sources of iron forming DNIC *in vivo* are still not precisely defined, one of the putative sources being the labile iron pool and another – iron proteins. Neither are defined the cellular compartments, in which DNIC are formed.

In the report presented here we show that depletion of lysosomal labile iron pool by either chelation with deferoxamine or lysis inhibition leads to a considerable decrease (down to 50%, depending on the incubation time) of DNIC forming in the cells under the influence of nitric oxide. This would indicate a vital role of lysosomal labile iron in DNIC formation. Taken together, our present and previous results confirm the thesis on the considerable contribution of lysosomal iron to the total labile iron pool in the cell.

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## 21. GENE EXPRESSION PROFILE OF B16(F10) MURINE MELANOMA CELLS UNDER HYPOXIC CONDITIONS *IN VITRO*

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Rapidly growing tumors are exposed to hypoxic conditions due to inadequate blood supply. Hypoxia is not only an obstacle for effective radiotherapy and chemotherapy but also strongly contributes to tumor progression. Therefore, better understanding of this phenomenon might improve cancer diagnosis and treatment.

We investigated gene expression profile in murine melanoma B16(F10) cultured cells under: (i) hypoxia and (ii) hypoxia-mimicking conditions. Cells were exposed to hypoxic (nominal 1% $O_2$) or hypoxia-mimicking conditions (100μM or 200μM $CoCl_2$), for 24h. Total RNA was isolated and cRNA was hybridized to microarrays (Affymetrix).

Data analysis revealed huge changes in gene expression profile when comparing experimental samples and controls: for 1% oxygen experiment 2448 transcripts (app. 19% of total probset number, FDR 5%) and for cobalt chloride 364 transcripts (3.5% of total probset number, FDR 5%) showed differential expression. Genes significantly modulated by cobalt chloride, in comparison to those modulated by hypoxia are related to cytokinesis, mitosis and melanin biosynthesis. Upregulated mRNAs in hypoxia vs. control samples included known hypoxia-induced genes (*vegf, p4ha2, hig1*), genes previously related to cancer (i.e. *ctgf, anxa2, adm, lgals3, nppb, mitf*) and genes, to our knowledge, so far not associated with hypoxia (i.e. *rras, rnf19*).

This experiment indicates novel potential hypoxia and/or prognostic markers as well as therapeutic targets for melanoma treatment. It also demonstrates that use of cobalt chloride to induce hypoxia mimicry results in a somewhat different gene expression profile of B16(F10) cells compared to that induced by low oxygen tension.

## 22. INDIVIDUAL RADIOSENSITIVITY OF PATIENTS WITH BREAST CANCER AND HEALTHY DONORS: ANALYSIS OF DNA DAMAGE AND REPAIR IN PERIPHERAL BLOOD LYMPHOCYTES

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Individual radiosensitivity of the human population is heterogeneous. In the case of patients undergoing radiotherapy an enhanced curability could be achieved by adjusting the treatment scheme to the individual sensitivity of the patient. There is data suggesting that the radiosensitivity of peripheral blood lymphocytes (PBL) may serve as a model cell system for determining the individual sensitivity to ionising radiation.
The analysis of DNA damage and repair in PBL of breast cancer patients undergoing radiotherapy may be important for estmating the susceptibility of the patients to side effects of radiotherapy and their risk of developing seconadary cancer.
An interesting question is if the kinetics of DNA repair in PBL of cancer patients is different from that of healthy donors and wheather can be correlated with chromosomal aberrations and resistance/sensitivity of the patient to therapy.
The aim of our study was to compare the kinetics of DNA repair in PBL of 30 healthy donors and 20 breast cancer patients estimated by the comet assay and to compare the frequency of chromosomal aberrations induced by radiation.
Peripheral blood lymphocytes were collected from cancer patients (before radiotherapy) and from healthy donors and irradiated with 2 Gy $^{60}$Co. Chromosome slides were prepared from cells fixed at 50 hours after irradiation. The frequency of chromosome aberrations was scored in cells in the first mitotic division. The level of DNA damage was estimated by the alkaline comet assay after 0, 15, 30, 60 and 120 minutes post exposure.
The results suggest that the frequency of chromosomal aberrations in cancer patients was higer than in healthy donors and no difference exists between the kinetics of DNA repair and the frequency of chromosomal aberrations.

## 23. BRAF INITIATING MUTATIONS IN PAPILLARY THYROID CARCINOMA

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**Introduction:** The major molecular pathway leading to papillary thyroid cancer (PTC) includes the activation and alterations in MAPK pathway. Currently, three major points of the initiation have been identified: RET tyrosine kinase (or NTRK) rearrangements cause very early initiation of the whole pathway, while point mutations occurring in the later steps of signaling, i.e. in RAS genes or in BRAF kinase gene constitute the alternative initiating mechanisms. Somatic *BRAF* mutations have been found in melanomas - in 98% this was a transversion from thymine to adenine at position 1796 (V600E). This mutation activates consitutively the enzymatic activity of the molecule by preventing the hydrophobic interactions between the residues of activation centre and ATP binding residues which are responsible for inactive conformation. $BRAF^{T1796A}$ mutation has been described in 36-39% PTC cases, also in poorly differentiated and anaplastic thyroid ca.

**Aim:** We aimed to study the frequency of somatic *BRAF* mutations and to relate it to the incidence of *RET* rearrangements and to the differences in gene expression pattern in papillary thyroid carcinoma.

**Material and methods:** The analysis was carried out in the collection of 45 PTC tumors in which expression profile and *RET/PTC* was previously analyzed. Total RNA was extracted from postoperative tumor tissue; cDNA synthesis was carried out with gene-specific primers. Exon 15 of *BRAF* gene was amplified by PCR and analyzed by automated sequencing. .

**Results:** The $BRAF^{T1796A}$ mutation was found in 24 cases of papillary thyroid carcinoma. In one case of PTC we identified a nucleotide substitution, A1799T, coexisting with $BRAF^{T1796A}$ mutation. Similarly only in one patient with papillary thyroid carcinoma we found a deletion of 14 nucleotides. *RET/PTC* rearrangement were identified in 11/42 cases of PTC, in 20 cases we found no RET rearrangement; in 6 patients we interpret the result without ambiguity. We found *BRAF* $^{T1796A}$ mutation in two patients with previously detected *RET/PTC* rearrangement. Now we are verifying this results by repeated sequencing and we carry out the comparison of gene expression profile of papillary thyroid cancer with RET rearrangement vs. BRAF mutation, to reveal whether the difference in initiating event confers the changes in expression profile of these tumors. We also perform a comparison with a similar study by Giordano et al., from which raw microarray data were available.

**Conclusions:** Two molecular events leading to PTC, BRAF mutation and *RET/PTC* rearrangements differ in frequency of occurrence in PTC: in our group BRAF mutation prevailed over RET rearrangement and was more than two times more frequent. Microarray analysis allows to delineate changes in gene expression profile associated with these alterations of MAPK pathway.

## 24. ASSOCIATION BETWEEN PATIENT- AND TUMOR-RELATED FACTORS AND THE GENE EXPRESSION PROFILE OF PAPILLARY THYROID CANCER

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We performed the analysis of correlation between gene expression profile of the papillary thyroid cancer PTC tumors and known clinical factors influencing the outcome.
Gene expression profile was assessed on Human Genome U133A array (Affymetrix). We examined tumors samples obtained from 49 patients diagnosed with PTC. For further evaluation we included following factors: sex, age, tumor size, capsule invasion, multifocality, vascular invasion, lymph node and distant metastases.

We used corrected Welch t-test estimation to analyze how many genes are associated with each factor. We found out that poor differentiation/early recurrence (2 tumors) is connected with a prominent difference in gene expression profile (812 genes changed). For the further analysis we excluded these 2 samples (because of the large scale of difference between two poorly differentiated tumors and the rest of PTCs) and performed the analysis on the remaining 47 well-differentiated PTCs.
The strongest factor in this group was sex with 10 sex-related. Large difference appeared for distant metastases: 1486 transcripts showing moderate level of statistical significance. To rank the remaining clinical factors, with low significance, we performed the analysis of uncorrected Welch t-test p-values. In such classification distant metastases are the strongest factor. We compared gene expression profile of 8 patients with mets to 39 samples who did not present metastases within the short follow-up time. We selected 150 genes differentiating both groups of patients. By Support Vector Machine approach this dataset correctly predicted the occurrence of metastases in 45/49 patients.

**Our conclusions:** 1) Some clinically relevant features in PTC (poor differentiation, sex) are strongly and significantly associated with gene expression profile. 2) We revealed that presence of distant metastases is related to gene expression and that it is possible to specify the metastatic signature in PTC.

## 25. INTRACELLULAR LOCALISATION AND PHOTOTOXIC ACTIVITY OF LIPOSOME ENTRAPPED AMINO ACID PORPHYRIN DERIVATIVES; STUDIES ON HUMAN MALIGNANT MELANOMA

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Numerous clinical trials have demonstrated that PDT is an effective and safe treatment for cancer with comparatively mild side effect and low general toxicity. Much experience has been gained with PDT for malignant disorders of the skin, since skin is readily accessible to treatment by light. PDT plays a substantial part in treatment of non-melanoma skin cancer however poor therapeutic results were reported for pigmented human melanoma and experimental melanoma cell lines. This might be explained by a high content of melanin, which can act as a screen for white light and also as free radical scavenger although the extend of such effect is limited. Melanoma responds poorly also to chemical and radiation therapy and the most effective treatment is surgical excision before the tumour is well advanced.

In this contribution we describe the results of experiments with the new potential photosensitisers for PDT, different amino acid porphyrin derivatives. We chose human malignant melanoma (Me45) cell line derived from a lymph node metastasis of skin melanoma in 35-year-old male. It is known that the mode of transfer strongly influences subsequent localization of photosensitiser in cells and consequently modulates cell death pathway therefore cellular distribution and mode of cell death was also explored. Our study using confocal microscopy indicated that use of cationic liposomes as a carrier of porphyrins enabled their efficient transfer into cells. Some from a panel of amino acid porphyrin derivatives (glycine-porphyrin) appeared very efficient photosensitiser for melanoma cells with low dark toxicity. The mode of cell death was dependent on applied energy of light and time of post treatment incubation, shifting the apoptosis towards necrotic death at higher doses and longer incubation.

**26. <u>PML ISOFORMS I-VI FORM BODIES PARTIALLY CO-LOCALIZING WITH NUCLEAR STRUCTURES BUILT BY WRN PROTEIN AND DIFFERENTIALLY REGULATE THE ACTIVITY OF GENE PROMOTERS</u>**

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PML protein may suppress tumor formation by regulating the induction of apoptosis, cellular senescence and by participation in maintenance of genomic stability. These PML functions require its interaction with p53 tumor suppressor protein, and with proteins involved in DNA repair or transcription. The significance of these interactions is only beginning to emerge. Moreover, PML is strongly induced by interferon and is targeted by many viral proteins, indicating that it is a crucial element of cellular, antiviral machinery. To better understand the functioning of PML protein, we began to study the regulation of cellular localization of PML and WRN - the pleiotropic protein involved in recombinational DNA repair, gene transcription and maintenance of correct telomere structure. We created the expression vectors coding for the PML isoforms I to VI fused with the green fluorescent protein (EGFP) and for the WRN fused with the red fluorescent protein (mRFP). We found that mRFP-WRN fusion protein is localized in nucleoli or in numerous, small, nucleoplasmic foci. However, in cancer cell lines of different origin, mRFP-WRN forms distinct, nuclear, toroidal bodies. The *WRN* point mutation (1184:Leu>Arg), disrupting the native structure of the protein, prevents both: nucleolar localization of WRN and formation of “donut-shaped” bodies, what indicates that the bodies form when structural integrity of WRN is preserved. In spite of the fact that these “donut-shaped” structures morphologically resemble PML bodies, they do not extensively colocalize with any of the studied EGFP-PML isoforms. However, we noticed, that in a subset of cells expressing both fluorescent proteins, the surface of WRN bodies is covered by EGFP-PML protein, what was clearly seen for isoforms I, III, IV. Thus, the isoforms show differences in their ability to interact with WRN bodies. Moreover, we noticed that the co-expression of mRFP-WRN and PML isoforms V or VI significantly increases the frequency of cells with large number of WRN “donuts” and increases the amount of mRFP-WRN, but not the endogenous WRN protein. Using the luciferase reporter assay, we found that the PML V and VI significantly increase the activity of viral promoter (CMV) driving the transcription of the mRFP-WRN sequence from the expression vector. The influence of the PML isoforms on the human gene promoters is conspicuously different. The activity of examined promoters (*BRCA1, BRCA2, p16, XPA, WRN*) was significantly increased only by isoform IV or by isoforms IV and VI. We conclude that differences in biological activities (e.g. induction of cellular senescence) exerted by PML isoforms, may partially result from differences in sets of genes activated or repressed by these isoforms. Moreover, our data indicate that some PML isoforms may participate in formation of nuclear “compartments” regulating the stability of nuclear proteins.

## 27. EFFECT OF HEME OXYGENASE-1 ON PROLIFERATION, VIABILITY AND ANGIOGENIC POTENTIAL OF MELANOMA CELLS IN MICE

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**Background:** Heme oxygenase-1 (HO-1), an enzyme degrading heme to biliverdin, carbon monoxide and iron ions, may augment angiogenesis. Additionally, HO-1 can regulate cell cycle and apoptosis. Here we investigated the effect of HO-1 overexpression on the proliferation, viability and angiogenic potential of B16(F10) murine melanoma cell line *in vitro* and *in vivo*.

**Methods and results:** Establishing the HO-1 overexpressing cell line (B16(F10)-HO-1) was confirmed by PCR, RT-PCR and western blotting. Overexpression of HO-1 led to a significant increase in melanoma cell proliferation, as measured by BrdU incorporation. Moreover, B16(F10)-HO-1 cells were more resistant to oxidative stress. Four-hour exposure to 200 µM $H_2O_2$ resulted in massive death of wild-type melanomas, whereas almost 70% of B16(F10)-HO-1 cells were still viable, as assessed by trypan-blue exclusion. Accordingly, low concentration of $H_2O_2$ (0.78-3.12 µM, 18 h) induced apoptosis in B16(F10), but not in B16(F10)-HO-1, as measured by TUNEL assay. Finally, conditioned media harvested from B16(F10)-HO-1 more potently induced endothelial cell proliferation and formation of capillaries by endothelial spheroids than those collected from B16(F10). This effect was not modified by pre-treatment of media with antibodies blocking the vascular endothelial growth factor (VEGF). In accordance, HO-1 overexpression did not influence the generation of VEGF as checked at the promoter, mRNA and protein levels in melanoma.

To check the effect of HO-1 overexpression on tumor development 0.2 x $10^6$ B16(F10) or B16(F10)-HO-1 cells were injected intracutaneously into the back of syngenic C57BL/6 mice. Overexpression of HO-1 in melanoma cells shortened survival time of B16(F10)-bearing mice. Surprisingly, there were no differences in tumor size and in tumor necrotic areas between mice bearing B16(F10) and B16(F10)-HO-1 cells. Preliminary analyses suggest, however, that HO-1 overexpression was associated with reduced inflammatory edemas in the tumors and with increased density of melanoma cells. In accordance, the levels of tumor necrosis factor (TNF), in serum and tumor lysates from mice injected with B16(F10)-HO-1 cells were lower and the levels of tumor necrosis factor receptor I (sTNFRI) were higher than from those injected with the wild-type melanoma. Moreover, immunohistochemical staining using anti-CD31 antibodies reveals also the stronger vascularization of HO-1 overexpressing tumors. Interestingly, the levels of vascular endothelial growth factor (VEGF) was significantly increased in tumor lysates from mice injected with B16(F10)-HO-1 cells than from those injected with the wild-type melanoma.

**Conclusion:** HO-1 increases viability, proliferation and angiogenic potential of murine melanoma cell line. Accordingly, overexpression of HO-1 in tumor cells leads to decrease in survival time of melanoma-bearing mice, inhibition of inflammatory reaction, and increase in tumor vascularization. It suggests that reduction of HO-1 activity might be beneficial in therapy of melanoma.

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**28. <u>NOVEL GENES IDENTIFIED IN HEPG2 CELLS ACTIVATED BY IL-1 AND IL6</u>**

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Interleukin-1 and interleukin-6 are principal cytokines involved in regulation of expression of acute-phase proteins. Both cytokines are released from activated macrophages, fibrobalsts, keratinocytes and endothelial cells and stimulate liver and other tissues to synthesis of acute phase proteins. To evaluate the changes in the transcript level of hepatoma-derived cell line after stimulation with both cytokines a differential display analysis was carried out. HepG2 cells were treated with IL-1 or IL-6 alone or with both cytokines for different time periods.

Based on the homology data all identified transcripts were classified into 13 groups according to the function of proteins encoded by these transcripts. These proteins are engaged in cellular metabolism, protein synthesis, trafficking, transport, signal transduction, DNA interaction, transcription, posttranscription modification, posttranslation modification, cell proliferation, stress protection, proteases and proteins functionally not yet classified. Forty transcripts (10 - under IL-1 stimulation, 21 – under IL-6 stimulation and 9 – under IL-1/IL-6 stimulation) had no homology with known and functionally classified genes deposited in GenBank entries. Transcript sequences matched BAC and PAC or hypothetical cDNA sequence clones with the identity reachable in more than 85%. Two of them are being now studied in details. Both genes were cloned to analyze their regulation under proinflammatory cytokines treatment and their importance in cell viability. Using Northern and Western blot analysis we studied modulated expression of both transcripts and occurrence of protein products on cellular and tissue level.

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## 29. MICRODELETIONS AND CHROMOSOMAL BREAKPOINTS OF RGS AND RGS-LIKE CANCER SUSCEPTIBILITY GENES IN BREAST CANCER

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**Background**: The RGS (regulators of G protein signalling) genes have a very high cancer potency because they encode for proteins which are involved in cellular proliferation, differentiation, response to neurotransmitters, membrane trafficking and embryonic development. These genes are located within a 370 kb region at the chromosome 1q25.3 to which the hereditary prostate cancer (HPC1) locus is tightly linked. The loss of heterozygosity/allelic imbalance results provided information about high level of chromosomal instability reflected in the breakpoints localized between the analyzed microsatellite loci. This study aimed at a detailed mapping of the position of these potential breakpoints in patients with breast cancer.

**Methods**: The breakpoint screening PCR-based analyses were performed in order to determine the size of the deleted parts of the examined RGS genes at the chromosome 1q25.3. The fragile sites flanked by microsatellite markers were divided into small fragments and PCR amplified. The sizes of these fragments varied from 500-1200 bp. A set of 60 primer pairs spaced across chromosome 1q25.3 containing the affected RGS genes were used.

**Results**: PCR analysis revealed intragenic chromosomal breakpoints in the RGSL2, RGS16 and RGS8 in breast tumours. The major common chromosomal breakpoint region was found inside RGS8 and involved intron 3, exon 4 and intron 4. The size of a typical microdeletion localized within the amplified segment (27 kb) ranged from 1 kb (26%), 2 kb (22%), 2.8 kb (11%), 4 kb (4%) to 5 kb (4%). The analyzed region was completely deleted in two breast tumours. Frequent deletions were also found in the potential promoter region of RGS16. Ten of the thirty one patients (32%) shared a common microdeletion of 1.4 kb localized in the close vicinity of exon 1 in RGS16. The third, significant fragile site within the 370 kb region I have identified was located between the RGSL2 in2 and RGSL2 ex4 microsatellite loci. This chromosomal breakpoint involves exon 3 and 4.

**Conclusion**: These results suggest a possible relationship between microdeletions in the RGS genes and the protein expression. Frequent 1q25.3 chromosomal breakpoints are likely to play a role in breast tumorigenesis as they affect potential tumour suppressor genes. Furthermore, a more extended screening for 1q25.3 chromosomal breakpoints and their implication in breast cancer is required.

***Key terms****: breast cancer, loss of heterozygosity/allelic imbalance, microsatellite markers, RGS genes, microdeletions, chromosomal breakpoint.*

## 30. DNA INTERSTRAND CROSSLINKS ARE INDUCED IN CELLS PRELABELLED WITH 5-BROMO-2′-DEOXYURIDINE AND EXPOSED TO UVC RADIATION

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It has been observed previously that 5-bromo-2′-deoxyuridine (BrdU) potentates the effect of UVC radiation on the level of sister chromatid exchanges. It is not known which type of DNA damage is responsible for this enhancing effect and we have proposed this to be the DNA interstrand crosslink (ICL) which, theoretically, may arise in cells that are labeled with BrdU for one round of replication and exposed to UVC radiation. The aim of the present investigation was to verify if ICLs are indeed formed in this irradiation scenario. CHO-K1 cells were prelabelled with BrdU and exposed to UVC. ICLs were detected by the modified version of the comet assay that relies on the reduction of induced DNA migration in the agarose gel. Carboplatin was used as a positive control.

We found that BrdU+UVC treatment indeed results in a reduction of the damage induced by -radiation. Furthermore, we observed that CL-V4B cells exposed to BrdU+UVC, but not to UVC alone, showed a very high level of chromosomal damage. These cells have a deficient Rad51C paralog that renders them extremely sensitive towards ICLs.

Taken together these results clearly show that ICLs are formed in DNA that is prelabeled with BrdU and exposed to UVC radiation.

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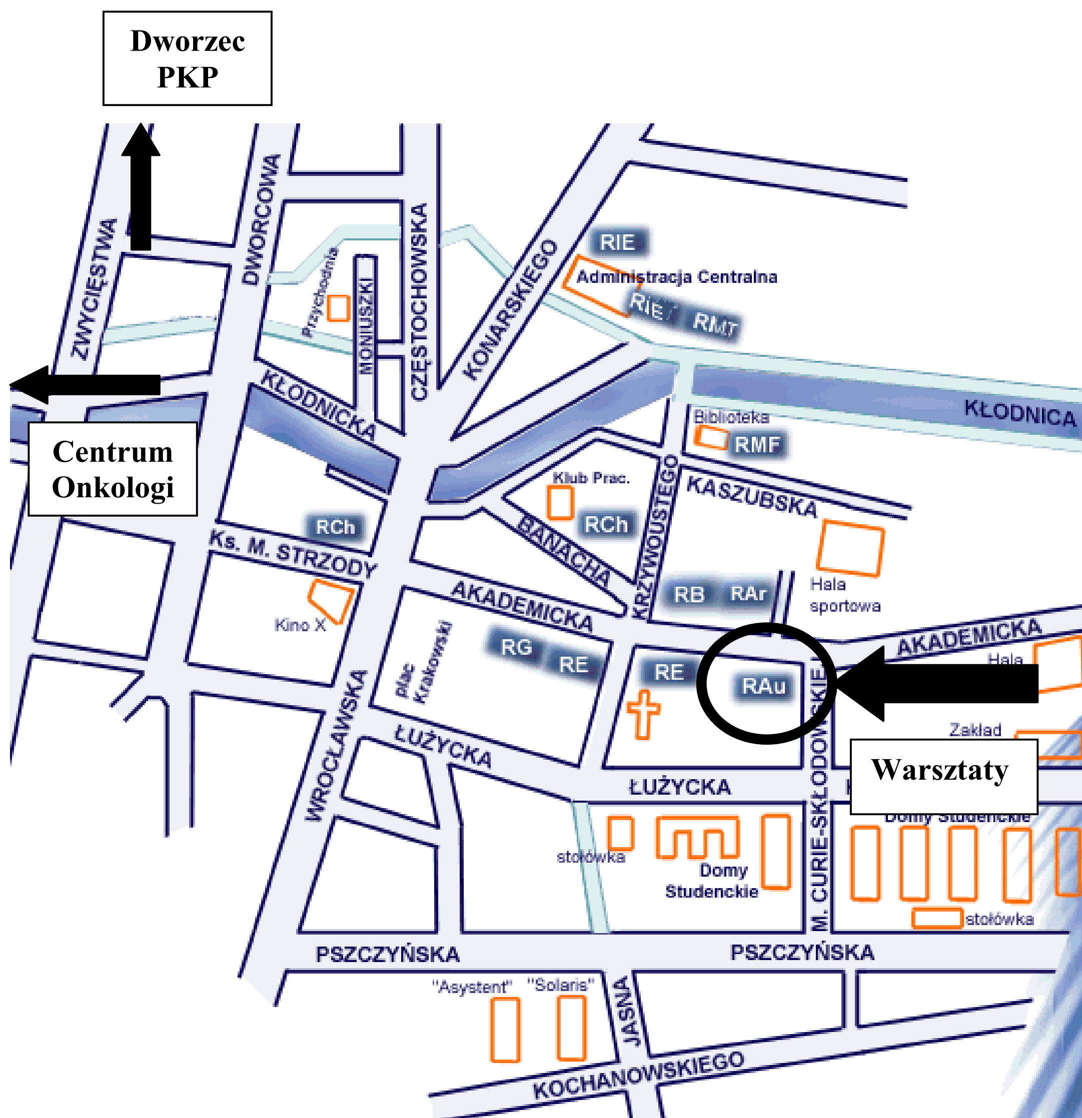

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**Autobus nr 71**

Przystanek: Gliwice Plac Piastów

Kierunek: Łabędy Huta

**Dni robocze:** $5^{15}$ $6^{15\ 45}$ $7^{15}$ $8^{00\ 40}$ $9^{15\ 55}$ $11^{05\ 35}$ $12^{20}$ $13^{00\ 35}$ $14^{15\ 50}$ $15^{30}$ $16^{10\ 45}$ $17^{25}$ $18^{35}$ $20^{25}$

**Soboty:** $5^{30}$ $6^{50}$ $8^{00}$ $9^{15}$ $11^{05}$ $12^{20}$ $13^{35}$ $14^{55}$ $16^{10}$ $17^{25}$ $18^{35}$

**Niedziele i święta:** $13^{35}$ $14^{55}$ $16^{10}$ $17^{25}$ $18^{35}$

**Autobus nr 710**

Przystanek: Gliwice Plac Piastów

Kierunek: Osiedle Kopernika Kąpielisko

**Dni robocze:** $4^{29\ 47\ 59}$ $5^{29\ 47}$ $6^{19\ 28}$ $7^{25\ 54}$ $8^{25\ 59}$ $9^{30}$ $10^{00\ 30}$ $11^{00\ 30}$ $12^{00\ 31}$ $13^{01\ 25\ 56}$ $14^{25}$ $15^{01\ 35}$ $16^{05}$ $17^{00\ 29\ 59}$ $18^{39}$ $19^{09\ 50}$ $20^{29}$ $21^{10\ 49}$

**Soboty, niedziele i święta:** $5^{52}$ $7^{52}$ $9^{52}$ $12^{27}$ $14^{52}$ $17^{22}$ $19^{49}$

**Autobus nr 932**

Przystanek: Gliwice Plac Piastów

Kierunek: Osiedle Kopernika Kąpielisko

**Dni robocze:** $5^{10\ 30\ 50}$ $6^{10\ 30\ 50}$ $7^{10\ 30\ 50}$ $8^{10}$ $13^{10\ 30\ 50}$ $14^{10\ 30\ 50}$ $15^{10\ 30\ 50}$ $16^{10}$

Nie kursuje w okresach przerw w zajęciach szkolnych

**Autobus nr 692**

Przystanek: Gliwice Plac Piastów

Kierunek: Osiedle Kopernika Kąpielisko

**Dni robocze:** $4^{38\ 57}$ $5^{33\ 57}$ $6^{27\ 57}$ $7^{27\ 57}$ $8^{27}$ $9^{07\ 32}$ $10^{27}$ $11^{07\ 52}$ $12^{47}$ $13^{27\ 57}$ $14^{27\ 57}$ $15^{27\ 57}$ $16^{57}$ $17^{27}$ $18^{22}$ $19^{07}$

**Soboty, niedziele i święta:** $4^{31}$ $5^{30}$ $6^{11}$ $7^{11}$ $8^{11}$ $9^{11}$ $10^{11}$ $11^{11}$ $12^{11}$ $13^{11}$ $14^{11}$ $15^{11}$ $16^{11}$ $17^{11}$ $18^{11}$ $19^{11}$

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Przystanek: Osiedle Kopernika Kąpielisko

Kierunek: Gliwice Plac Piastów

**Dni robocze:** $6^{26}$ $7^{01\ 31}$ $8^{21\ 51}$ $9^{36}$ $10^{36}$ $11^{06}$ $12^{01\ 31}$ $13^{16\ 51}$ $14^{31}$ $15^{06\ 36}$ $16^{26}$ $17^{06\ 41}$ $18^{16}$ $19^{51}$ $21^{31}$

**Soboty:** $6^{26}$ $7^{36}$ $8^{51}$ $10^{36}$ $12^{01}$ $13^{16}$ $14^{31}$ $15^{51}$ $17^{06}$ $18^{16}$ $19^{31}$

**Niedziele i święta:** $14^{31}$ $15^{51}$ $17^{06}$ $18^{16}$ $19^{31}$

**<u>Autobus nr 710</u>**

Przystanek: Osiedle Kopernika Kąpielisko

Kierunek Szczygłowice Kopalnia, (trzeba wysiąść na przystanku Gliwice dworzec PKP)

**Dni robocze:** $4^{42}$ $5^{15\ 40}$W $6^{10\ 40}$W $7^{10\ 45}$W $8^{15}$W $^{45}$ $9^{15\ 45}$ $10^{15\ 45}$ $11^{15\ 40}$W $12^{10}$P $^{40}$W $13^{15}$W $^{45}$ $14^{15\ 45}$ $15^{15\ 40}$ $16^{15\ 45}$ $17^{20}$ $18^{05\ 48}$W $19^{03}$G $^{27}$ $20^{07}$W $^{47}$ $21^{22}$G $22^{02}$

**Soboty, niedziele i święta** $6^{\underline{10}}$ $8^{\underline{15}}$ $10^{\underline{45}}$ $13^{\underline{00}}$ $15^{\underline{40}}$ $18^{\underline{05}}$ $20^{\underline{07}}$

**<u>Autobus nr 932</u>**

Przystanek: Osiedle Kopernika Kąpielisko

Kierunek: Zabrze Goethego

**Dni robocze:** $5^{37\ 57}$ $6^{17\ 37\ 57}$ $7^{17\ 37\ 57}$ $8^{17}$P $^{37}$P $13^{37\ 57}$ $14^{17\ 37\ 57}$ $15^{17\ 37\ 57}$ $16^{17\ 37}$P

**<u>Autobus nr 692</u>**

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Kierunek: Osiedle Waryńskiego Pętla

**Dni robocze** $4^{50}$A $5^{10\ 45}$ $6^{15}$A $^{45}$A $7^{15\ 45}$ $8^{15\ 45}$ $9^{20\ 55}$ $10^{45}$ $11^{45}$ $12^{50}$A $13^{20\ 50}$A $14^{20\ 50}$ $15^{20}$A $^{50}$A $16^{20}$ $17^{10\ 50}$ $18^{35}$P $19^{20}$P

**Soboty, niedziele i święta:** $4^{41}$ $5^{41}$A $6^{41}$ $7^{41}$ $8^{41}$ $9^{41}$ $10^{41}$ $11^{41}$ $12^{41}$ $13^{41}$ $14^{41}$ $15^{41\ 49}$ $16^{41}$ $17^{41}$ $18^{41}$ $19^{41}$