Abstracts Speakers

S1 Lymphomas and Leukemias: Indian Experience

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Lymphomas and leukemias are heterogeneous groups of cancers. Marked geographic variation is noted in the incidence of these cancers and their subtypes. The incidence rates and patterns in India are significantly different from the rest of the world. Due to vast panoply of environments, lifestyles, and ethnic differences, within the country itself there are regional differences. Available information on the spectrum of leukemias and lymphomas as observed in India compared to that in the West is presented. In general the incidence of these malignancies is lower compared to that in the West; chronic myelogenous leukemia, however is higher. Within India, it appears that the incidence is highest in Delhi, followed by Mumbai, and lowest in Barshi (rural-based cancer registry).

A noteworthy feature common to these cancers is that these occur at a younger age in our country, almost a decade earlier compared to the West. Reasons are not entirely clear, however some postulations have been made. Higher frequency of mixed-cellularity Hodgkin's disease (70% vs. 30%), diffuse large-cell NHL (34% vs. 20%), T-cell lymphoblastic lymphoma (6% vs. <3%) and T- cell acute lymphoblastic leukemia (21-44% vs. 11-25%) is noted compared to that in the West. On the other hand, a lower frequency of nodular sclerosis Hodgkin's disease, follicular lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia is noted. Most patients present in advanced stages, with poor performance status and have poorer prognostic factors. Treatment results are comparable if stage wise distribution and poor prognostic factors are taken into account. The information presented in this paper confirms that various leukemias and lymhomas in India have a distinct clinico-pathological profile. v

S2 STAT Signaling In Leukemia

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Signal transducer and activator of transcription (STAT) proteins are a seven-member family of cytoplasmic transcription factors that contribute to signal transduction by cytokines, hormones and growth factors. STAT proteins control fundamental cellular processes including survival, proliferation and differentiation. Given the critical roles of STAT proteins, it was hypothesized that inappropriate or aberrant activation of STATs might contribute to cellular transformation and, in particular, leukemogenesis. Constitutive activation of mutated STAT3 has in fact been demonstrated to result in transformation. STAT activation has been extensively studied in leukemias, and mechanisms of STAT activation and the potential role of STAT signaling in leukemogenesis will be discussed. A better understanding of mechanisms of dysregulation of STAT signaling pathways may serve as a basis for designing novel therapeutic strategies that target these pathways in leukemia cells.

S3

Signaling Activities Of Human Gamma Herpes Viruses: Implications For Lymphomagenesis

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Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV-8) or Kaposi's sarcoma (KS) – associated herpesvirus are the two gammaherpesviruses that are known to infect humans. EBV has been implicated in a wide variety of both benign and malignant diseases of lymphoid and epithelial origin including infectious mononucleosis, Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma and T cell lymphomas. The newly discovered HHV-8 is the etiological agent of KS, and is also associated with primary effusion lymphoma and plasmablastic variant of multicentric Castelman's disease.

One well-known characteristic of all herpesviruses is that they can remain latent in the host cell they infect. During this quiescent stage of the virus cycle, the virus exists in the nucleus and displays a very restricted pattern of gene expression. The expression pattern and functions of EBV and HHV-8 latency genes point to mechanisms by which infection with these herpesviruses results in altered signaling leading to aberrant cell proliferation and development of lymphoproliferative and other disorders. An understanding of these signaling pathways and the cooperative effects of other viral and cellular genes will not only be relevant to the role of these viruses in neoplastic diseases, but will also help to elucidate the mechanisms regulating cell growth, survival and differentiation. These insights will offer new clues to therapeutic approaches that target key components of the pathways which play particularly important role in deregulating normal cellular functions.

S4

Ubiquitin-dependent degradation of p73, and its inhibition by promyeloid leukemia

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P73, similarly to its homologue p53, regulates apoptosis during DNA damage. The activity of p73 depends on its steady state protein levels, and a number of evidence suggest that post-transcriptional regulation rather than transcriptional control plays a major role in p73 response to DNA damage; both TAp73 (pro-apoptotic) and ÄNp73 (anti-apoptotic). However, the molecular mechanisms underlying the regulation of p73 protein stability remain largely unknown. p73 steady state protein levels increase in the presence of proteasome inhibitors, suggesting a role for this pathway in p73 degradation. Here we report that p73 stability is directly regulated by the ubiquitin/proteasome pathway and p73 is degraded through specific mechanisms, different from that of p53.

(1) we found that upon DNA damage (UV, doxorubicin, etoposide) ÄNp73 is rapidly degraded releasing the block exerted on p53 and TAp73 allowing cell cycle arrest and apoptosis to proceed.

(2) we found that p73 is degraded through a NEDD8-mediated mechanism and that the tumor suppressor protein PML modulates p73 half-life by inhibiting its degradation. PML-mediated stabilization of p73 is nuclear body (NB)-dependent, as PML mutants that do not localize to the NBs are drastically less effective in inducing p73 accumulation. We found that p300-mediated acetylation and consequent stabilization of p73 is impaired in PML-/- cells.

(3) we show that the protective effect of PML requires p73 phosphorylation by the p38 MAP kinase pathway. As a result, PML significantly increases the ability of p73 to transactivate

promoters of the bax and p21 genes and potentiates p73-dependent apoptosis and tumor suppressive activity. In turn, p73 pro-apoptotic function is markedly impaired in PML-/-cells. Thus, our findings demonstrate that PML plays a crucial role in modulating p73 function and provide further insights for the involvement of PML in tumor suppression.

(4) the PY motif-containing C-terminal region of p73á (not the isoform â, ã, ä) binds to Aip4/ Itch, an E3 Hect domain-containing (NEDD-4like) ubiquitin-protein ligase, resulting in ubiquitination and degradation of p73. The PY motif of p73 interacts with the WW domain of Itch. Interestingly Aip4/Itch is down-regulated upon DNA damage in a p73 dependent fashion allowing TAp73 levels to raise in response to this type of stress, and apoptosis to occur.

In conclusion, at least two distinct mechanisms of degradation exists for p73, NEDD8- and NEDD4-dependant. The former is finely regulated by PML in the nuclear body, resulting in a strict control of its function. The relative ratio of TAp73 (pro-apoptotic) and ÄNp73 (anti-apoptotic) finely regulates the sensitivity of cancer cell to chemotherapy. Therefore their relative degradation is crucial for the outcome of cancer cells. We showed that they are different regulated upon DNA damage, finely regulating apoptosis and chemosensitivity.

S5 High Throughput Genome Analysis Of B-Cell Lymphoma;Gene Discovery And Clinical Correlations

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The recent introduction of high throughput methods utilizing microarrays to globally scan tumor genomes for changes in gene copy number and gene expression have made possible new discoveries in the understanding genetic mechanisms and their clinical significance in cancers. The objective of this program is to determine copy number changes and expression patterns of known and newly discovered genes in B-cell lymphomas and investigate their role in lymphomagenesis and clinical outcome. Methods: Cohorts of at diagnosis diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) have been analyzed for changes in gene copy number and gene expression using BAC, cDNA, and oligonucleotide arrays. Results: Gene expression analysis of a panel of 46 at-diagnosis DLBCL with clinical follow-up using Affymetrix U95A oligonucleotide arrays identified the three expected subgroups, germinal center (GC), activated B-cell-like (ABC), and type 3. In contrast to some published results, no significant difference was seen in the clinical outcome of the three subgroups. Amplification of the REL gene did not correlate with its expression (RNA, protein) ruling it out as the functional target of the 2p12-16 amplicon, and indicating other functional target genes in the amplicon. Gene copy number changes in a panel of FL, that included matched pairs of initial and transformed biopsies assayed by a full genome cDNA array (~8000 ESTs) identified several novel targets that associated with transformation. Conclusions: Global genome scanning for gene copy number changes and gene expression patterns is a powerful method for discovery of novel genes and identifying genes that underlie clinical outcome of B-cell lymphoma.

S6 Defective Epigenomic Pathway In Leukemic Cells

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Leukemia is an epigenetic disease at the same level that it can be considered a genetic disease. Advances in our knowledge have recognized double-stranded RNAs (dsRNAs) as the primary signals required for converting non-specific sequence information into distinct chromatin states, thereby regulating the plasticity of the "epigenome". The source of these dsRNAs is bi-directional transcription of repetitive genetic elements, transposable elements and DNA satellite sequences. In order to understand the dsRNA-mediated epigenomic pathway in leukemic cells, we attempted to explore the small interfering RNA (SiRNA)-mediated C-myc gene regulation using Burkitt's

lymphoma (BL) –derived EB-3 cell line as an archetype cellular model. Such a study revealed that EB-3 cells possess 4-fold higher expression of Dicer gene coupled with 2-fold higher activity of RNA polymerase-III than observed in normal human lymphocytes. SiRNA derived from EB-3 cells had the inherent capacity to suppress C-myc expression in normal cells but not in native cells. Further, EB-3 were unable to express genes coding for Receptor-Ck and PPAR-g that in turn regulate genes involved in cell growth, differentiation, apoptosis and inflammation. Based on these findings we have proposed a novel SiRNA-mediated C-myc gene regulatory pathway that may be responsible for BL.

S7 A Variety Of Tumor Associated Antigens Can Elicit A CD8 T Cell Response In Vivo In Patients With CML.

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As a consequence of the t(9,22) translocation associated with chronic myeloid leukemia (CML), a novel bcr-abl fusion protein is produced. Several peptides from the junction or extra-junction region of the chimeric molecule bind with high affinity to HLA class I molecules and elicit cytotoxic T lymphocyte (CTL) responses in vitro. To determine whether such anti-tumoral CTLs are present and functional in CML patients, we used a panel of HLA-A2 tetramers. In addition to the bcr-abl neoantigen, three other tumoral antigens were studied: - proteinase 3, a myeloid tissue-restricted serine protease overexpressed in leukemia cells,

The Wilm's tumor gene-encoded transcription factor (WT1) normally expressed in immature CD34+ progenitor cells and overexpressed in CML cells, and - telomerase reverse transcriptase (h-Tert), a universal tumor antigen. Peptide sequences used to generate HLA tetramers were chosen either from in vitro binding or stability experiments with purified HLA class I molecules, or from prediction algorythms for proteasomal cleavages and HLA class I binding motifs. None of the bcr-abl junction peptides tested allowed in vitro refolding of HLA-A2, suggesting that these peptides cannot bind efficiently to HLA-A2 molecules and therefore should not be exposed at the cell surface in vivo. Longitudinal FACS analysis was performed in 33 HLA-A2 patients studied at time of diagnosis, in partial or complete remission during treatment with IFNa or with the selective tyrosine kinase inhibitor imatinib, or in complete remission following allogeneic bone marrow transplantation. Our results indicate that a specific CD8 T cell response directed to at least one tumoral peptide is observed in most patients in remission (0.1 to 12% of circulating CD8 T cells), but is never observed in healthy individuals. Combined perforin and intra-cellular cytokine staining experiments suggested that these CD8 T cells may be functional in vivo. These results provide evidence that leukemia-specific CTL may contribute to CML eradication, provided anergy mechanisms do not preclude the effector potential of these specific T cells. Strategies to boost immunity against leukemia antigens might thus be beneficial for the treatment of CML.

S8 Immunological Trade-Off During Therapy For APML: Molecular Mechanisms Of Arsenic-Induced Apoptosis In T Cells

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Arsenic trioxide (As_2O_3) has been used successfully in the treatment of acute promyelocytic leukemia. However, effects of As_2O_3 in normal peripheral blood T cells have not been studied in detail. The purpose of this study was to investigate if As_2O_3 would induce apoptosis in normal T cells and therefore may have immunosuppressive side effects.

Apoptosis was measured by TUNEL assay, caspase activation by flow cytometry and colorimetric assays, and mitochondrial transmembrane potential ($\Delta\Psi_m$), oxygen species (ROS) and intracellular reduced glutathione (GSH) by flow cytometry using FACSCalibur. The release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria was measured by confocal microscopy and the expression of molecules regulating apoptosis (Bcl-2, Bcl-xL, Bax, XIAP, VDAC) was analyzed by Western blotting. Overexpression of Bcl-2 by transfection was confirmed by Real time RT-PCR.

As_2O_3 at clinically achievable therapeutic concentrations, induced apoptosis in peripheral blood CD4+ and CD8+ T cells. As_2O_3 induced apoptosis was associated with reduced $\Delta\Psi_m$, enhanced generation of intracellular ROS, decreased intracellular levels of GSH, release of both cytochrome c and AIF from the mitochondria, activation of caspase-9 and caspase-3, downregulation of VDAC (voltage-dependent anion chanel), Bcl-2 and Bcl-x_L and upregulation of Bax expression. In addition, exogenous GSH protected lymphocytes from As_2O_3-induced apoptosis. Furthermore, overexpression of Bcl-2 inhibited As_2O_3-induced apoptosis and blocked depolarization of $\Delta\Psi_m$, generation of ROS, and release of both cytochrome c and AIF.

Conclusions: These data indicate that As_2O_3 induces apoptosis in T cells by enhancing oxidative stress and Bcl-2 appears to play a major role in As_2O_3-induced apoptosis. Therefore, As_2O_3 may have side effects of immune suppression during therapy of APML.

S9 The Mitochondrial Pathway Of Apoptosis And P53

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Apoptosis induced by the activation of p53 is a major mechanism of tumor suppression. While it is abundantly clear that p53 acts as a transcription factor to regulate the expression of genes involved in apoptosis and cell cycle arrest, there is accumulating evidence that p53 also acts via a transcription independent process to promote cell death. Using cells that stably express a chimeric $p53ER^{tam}$ protein we investigated this process. The activation of p53 in these cells by addition of 4-OHT induces expression of p21 in these cells and triggers apoptosis. Addition of cycloheximide (CHX) blocked induction of protein expression, but did not prevent p53-induced apoptosis in several cell lines. This effect was not seen in a line expressing $p53ER^{tam}$ lacking the proline rich domain, which has been implicated in apoptotic signaling. Similarly, cytoplasts from cells expressing $p53ER^{tam}$ underwent apoptotic changes upon addition of 4-OHT. These transcription-independent effects were dependent on Bax. Native p53 from UV-irradiated cells, recombinant p53, or purified $p53ER^{tam}$ were all capable of inducing cytochrome c release from isolated mitochondria, provided that Bax was present. Using synthetic lipid vesicles, p53 was shown to directly activate Bax to permeabilize membranes in the absence of other proteins. Further, p53 was found to bind to Bcl-xL, and in doing so Bid or Bax bound to the latter were then released. These interactions of p53 with Bcl-2 family proteins resemble those of BH3-only members of this family.

S10 Mechanisms involved in antibody based immunotherapies

W. Knapp

Antibody based immunotherapies particularly in the last couple of years became an absolute success story. even to experts in the field this came in its extent to many as a surprise.

Antibody therapy experienced many ups and downs in history. The start of it was fulminant and led to the first Nobel Prize in medicine awarded to Emil von behring in 1901 for his work on the generation of protective antitoxins together with S.Kitasato.highlight in the ontongeny of todays antibody therapy clearly was the introduction of antibody producing hybridoma technology by Koehler and Milstein. Still, the necessary steps frm bench to bed and clinical application took a

long whle to take, as Cesar Milstein and H.Waldmann recently pointed out in a very personal editorial commentary. The real breakthrough in terms of therapeutic applications when humanized or human antibody preparation became available and when it was realized that epitope and functional specificity are decisive parameters which control therapeutic efficiency. A particularly interesting example in this respect is the CD20 antibody C2B8 (Rituximab) which directly affects survival of malignant and normal B-cells and as we could show, promotes cross-priming of cytotoxic T cells by bystander DC. Our results in this respect with chronic lymphocytic leukemia will be presented.

S11 Application Of RNA Interference Technology In NF-κB Signaling Pathway

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RNA interference technology has several potential applications not only in functional genomics analyses but also in therapeutics. We have developed a panel of siRNAs in viral and non-viral plasmid vectors to knockdown expression of a variety of genes in the NF-κB signaling pathway. Nuclear factor-κB (NF-κB) activation by most agents involves the activation of IκBα kinase leading to the serine phosphorylation of IκBα , followed by ubiquitination-dependent degradation of I κB α, thus releasing the p65 subunit of NF-κB for translocation to the nucleus. The suppression of individual genes involved in this pathway, such as, IκBα , IKKα , IKKβ , IKKϒ, and p65 were tested by transfecting HeLa cells with plasmid vectors expressing appropriate shRNA target sequences. The gene suppression was tested by western blot analysis, DNA gel shift assay, and inhibition of downstream signaling. Suppression of IκBα phosphorylation was observed in cells transfected with IKKα and IKKβ shRNA plasmids. Inhibition of NF-κB translocation into the nucleus in IKKα, IKKβ and IκBα gene suppressed cells were tested by an ELISA system (p65 ActivELISA TM, Imgenex). Another mechanism of NF-κB activation is through tyrosine phosphorylation of Iκ Bα . We found that treatment of human myeloid KBM-5 cells with H_2O_2 activated NF-κB in a dose- and time-dependent manner much as TNF does, but unlike TNF, H_2O_2 had no effect on Iκ Bα degradation. We found that H_2O_2 induced the tyrosine phosphorylation of IκBα , which is needed for NF-κB activation. The data presented here suggest that the Syk protein tyrosine kinase is involved in H_2O_2-induced NF-κB activation. The reduction of Syk transcription using small interfering RNA inhibited H_2O_2-induced NF-κB activation. The silencing of individual proteins in the NF-κB pathway and their role in cellular signaling will be discussed.

S12 The IL-2 and IL-15 Receptor Systems: Targets for the Immunotherapy of Leukemia and Lymphoma

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After decades of disappointment, monoclonal antibodies have come of age for the therapy of leukemia and lymphoma. To date, the U.S. Food and Drug Administration (FDA) has approved 11 such antibodies and at least 400 other monoclonal antibodies are in clinical trials. This success has reflected the use of humanized antibodies, the arming of monoclonal antibodies with toxins or radionuclides as well as the definition of more effective antigenic targets including growth factor and cytokine receptors. We have recognized that the IL-2 receptor represents an extraordinary useful therapeutic target, since resting cells do not express IL-2R alpha whereas this receptor subunit is abundantly expressed by a variety of leukemia and lymphoma cells including those in HTLV-I associated adult T-cell leukemia. The FDA has approved our humanized antibody, anti-Tac (daclizumab) that is directed toward IL-2R alpha (CD25) for use in humans to

prevent acute kidney-transplant rejection. It has been proven to be of value in the treatment of adult T-cell leukemia. More recently we have armed this antibody with toxins and radionuclides to increase its effector action. In a clinical trial involving ^{90}Y-anti-Tac (anti-IL-2R alpha) therapy for patients with HTLV-I associated ATL we have observed a partial or complete remission in over 50% of patients. However, the long serum survival of intact monoclonal antibodies following the administration of antibody conjugated radionuclides prolongs the exposure of normal tissues, particularly the bone marrow, to irradiation. One way that we are utilizing to increase the dose of radionuclide delivered to the tumor cell involves the separate administration of the antibody and radionuclide, with the aim of improving tumor to normal organ ratios. We have used a multi-step process. In the first step Streptavidin is targeted to a specific antigen on the cell surface of the tumor using an antibody single-chain variable region targeted to IL-2R alpha fused to Streptavidin. The final step is the administration of radiolabeled DOTA—Biotin which is used to target a radionuclide to the tumor localized Streptavidin. Utilizing this approach we were able to cure IL-2R alpha expressing leukemias and lymphomas in murine xenograft models

As part of our study of HTLV-I associated adult T-cell leukemia we co-discovered the cytokine IL-15 that stimulates T-cell proliferation and is essential to NK-cell development. Abnormalities of IL-15 expression have been defined in patients with autoimmune disorders as well as diseases including adult T-cell leukemia associated with the retrovirus HTLV-I. In efforts to inhibit the actions of IL-15 for the therapy of autoimmune disease and leukemia, we have demonstrated that a monoclonal antibody humanized MiK-Beta-1 directed toward IL-2/IL-15R beta prevents the presentation of IL-15 thereby inhibiting its actions. We are translating these observations concerning IL-15 blockade from animal models to clinical trials for the therapy of patients with leukemia and lymphoma. Thus, the use of monoclonal antibodies that target IL-2, IL-15 and their receptors is providing a new perspective for the treatment of select lymphocytic leukemias and lymphomas.

S13 Noval Insidghts into the Biologyand Therapy of Chronic Lymphoaytic Leaukemia

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Abstract Not Available

S14 Acute Lymphoblastic Leukemia In Children : AIIMS Experience

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Advances in the treatment of acute lymphoblastic leukemia (ALL) in the form of combination chemotherapy, effective central nervous system (CNS) prophylaxis, risk adapted therapy based on the various prognostic factors determined by several multicentric trials has resulted in dramatic improvement in treatment outcome and over 70% children with ALL are now cured. The development of effective CNS prophylaxis resulting in the decrease of CNS relapse to about 5% has been regarded as one of the important milestone in the success story of cure of ALL. But in spite of the best treatment about 25-30% of ALL patients still relapse. Male sex, age less than one or more than ten years, high white blood cell (WBC) count >50,000/mm3, CNS leukemia, 'T' cell immunophenotype, nucler hyperdiploidy ((chromosome number <40) of the blasts, cytogenetic features like t9;22, t4;11 translocations in the blasts have been traditionally described as poor prognostic factors for occurrence of relapse. In the modern era of intensive therapy, treatment has been regarded as the most important prognostic factor as it has been shown that most of the prognostic factors except age and WBC count loose their

predictive value with good therapy. Unfortunately most of these therapeutic trials have been conducted in developed countries and hence reflect the status of leukemia in these populations. There is a need for such large multicentric trials to be conducted in our country to understand the biology of leukemia in our patients, identify prognostic factors and evolve therapeutic regime tailor made for our patients to achieve/ obtain optimal results with minimal side effects. This study was conducted in Pediatric Oncology division of the Department of Pediatrics at All India Institute of Medical Sciences, New Delhi to study the relapse pattern of ALL patients in our study group and also identify high risk factors for relapse in these patients.

255 children (<15 years age) with ALL were treated on MCP 841 protocol from June 1992 to June 2002. Male: female ratio was 3.8:1. 27.5% patients presented with WBC >50,000/mm^3 and 77.7% patients had platelet less than 100,000/cumm. Lymphadenopathy was seen in 88.6% patients and 96.9% presented with hepatosplenomegaly. CNS disease was seen in 6.3% patients.

225/255 (88.2%) achieved complete remission after induction therapy. Induction death occurred in 28 patients (11%) and remission deaths in 35 patients (15.5%). Total number of relapses were 40/225 (17.7%). 33/40 (87.5%) relapsed patients were between 1-10 years of age and their male: female ratio was 9:1. The sites of relapse were bone marrow (BM)- 23 (57.5%), central nervous system (CNS) -7 (17.5%), testes -2 (5%), BM+testes- 5 (12.5%), BM+CNS, CNS+testes and isolated bone relapse 1 (2.5%) each. 4 of 17 (13%) patients with CNS leukemia at diagnosis relapsed but none of them had a CNS recurrence. Overall Survival was 67.5 ± 3.5 and event free survival was 51.6 ± 3.8.

Majority of our patients belonged to high risk category. Many of the patients relapsed on-therapy. Bone marrow relapses were the commonest and was evenly distributed in all phases of therapy. None of the traditionally described poor prognostic factors like age<1 or >10 years, male sex, WBC>50,000/cumm or Tcell phenotype were significant for occurrence of relapse. This study emphasizes the need of recognition of this high-risk group of patients and intensification of chemotherapy in these patients.

S15

Arsenic Trioxide In The Treatment of Acute Promyelocytic Leukemia

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Acute promyelocytic leukemia(APL) is a disease characterized by bleeding diathesis, abnormal promyelocytes in the peripheral blood and marrow (faggot cells) and characteristic cytogenetic abnormalities. The identification of t(15;17) revolutionized the treatment of APL since differentiation therapy using All trans retinoic acid (ATRA) could be administered to patients with excellent results. ATRA combined with chemotherapy achieved initial remission of 80-90% with long term remission rates ranging from 70-75%. The treatment however is quite expensive with the average cost of treatment varying between Rs 6 – 8 lakhs and hence alternative treatment modalities were investigated.

Arsenic trioxide was initially used in the 19th century as Fowlers solution for the treatment of a number of ailments including asthma, pemphigus, chorea, Hodgkin's disease and pernicious anemia. In the early 20th century, Ehrlich used an organic arsenical (salvarsan) for the treatment of trypanosomiasis. The initial use of arsenic in relapsed APL was derived from Chinese medical tradition. Studies from China (Niu et al) and USA (Soignet et al) showed that 80-90% of patients with relapsed APL achieved remission with 50-60% achieving long term remission.

Arsenic trioxide is believed to act mainly on the PML portion of the RARá-PML complex. Arsenic facilitates profound cellular alterations including induction of apoptosis, inhibition of proliferation, stimulation of differentiation and inhibition of angiogenesis. In vitro studies have shown that arsenic trioxide exerts a dose dependant effect on NB4 APL cell lines and fresh APL cells. At high doses (0.5 to 2 umol/L), arsenic trioxide triggers apoptosis of APL cells. At lower concentrations (0.1 to 0.5umol/L), arsenic causes morphological differentiation along with expression of CD11b and decreased expression of CD33 on the cell surface. This

differentiation is however not terminal as with ATRA as most of the cells are blocked at the myelocyte/metamyelocyte stage of differentiation.

At our center, we have treated 63 patients with acute promyelocytic leukemia using arsenic trioxide as single therapy. This included 49 newly diagnosed patients and 14 relapsed patients with APL. As_2O_3 was administered daily over 4 hours at a dose of 0.15mg/kg/day. Therapy consisted of induction therapy where As_2O_3 was administered till hematological remission was achieved (or a maximum of 60 days) followed by consolidation therapy where As_2O_3 was administered for 28 days, one month after completing induction therapy and then maintenance therapy which consisted of cycles each lasting for 10 days monthly for a period of 6 months. Patients were followed up for molecular remission using RT-PCR from peripheral blood.

There were 13 children and 50 adults in the whole group. The mean WBC count at diagnosis was 10.9 x 10^9/L (range: 0.5 to 96.5). All patients had the hypergranular type of APL. 54/63 patients (85.7%) achieved hematological and molecular remission. The median time to achieve hematological remission was 44.5 days. This included 40 newly diagnosed patients (81.6%) and 14 relapsed patients (100%). All 9 patients who did not achieve hematological remission expired early during therapy. Death occurred mainly due to intracranial bleeding (7 patients) and infection (2 patients). No major toxicity was seen during treatment except for hyper leukocytosis in 35 patients (64%) which was controlled with hydroxyurea, prolonged neutropenia in 1 patient with fibrosis in the bone marrow and ECG abnormalities in 1 patient. No toxicity required discontinuation of arsenic except for the patient with marrow fibrosis. At a median follow up of 30 months (range: 1 – 70), 49/63 (77.7%) remain in hematological and molecular remission. Among the newly diagnosed patients, 38/49(77.5%) are in remission while among relapsed patients 11 patients (78.5%) remain in remission.

Arsenic trioxide achieves hematological and molecular remission in >80% of patients with APL.

A number of questions still need to be answered -

1. What are the long term remission rates with arsenic?
2. What is the long term toxicity associated with the use of arsenic especially with regard to children?
3. Can the duration of administration of arsenic trioxide be reduced without reducing the efficacy?
4. What are the other risk factors that are associated with increased risk of relapse?
5. What is the concentration of arsenic in various tissues including the CNS – to look at both efficacy and toxicity ?
6. Is a combination of arsenic and chemotherapy/ATRA better than arsenic alone?
7. What are the differences in the various isoforms with response to arsenic?

We hope that an Indian multicenter trial using indigenous arsenic trioxide will be able to answer atleast some of these questions.

S16

Recent Advances In Chronic Lymphocytic Leukemia (CLL)

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Among the major advances in CLL in the past 5 years, the improved ability of clinicians to use an accurate and clinically accessible method of risk-adapted approach to make therapeutic decisions for individual patients with this disease ranks the highest. Until recently, clinical staging, assessment of disease activity using such criteria as lymphocyte doubling time,serum beta-2 microglobulin, serum LDH,etc were the only available methods, but they all have been known tc have limitations in clinical practice. The advances in genomic and cytogenetic assessment ir

CLL using FISH technology, first reported by the German investigators in a large series of patients (Dohner et al, NEJM, 2000) clearly demonstrated that del 13q was the most frequently seen abnormality , followed in decreasing incidence, by normal karyotype, trisomy 12, del 11q, and del 17p (p53 aberration). The median life expectancy of patients with these abnormalities also was in decreasing order starting with the longest among those patients who had del 13q as the sole abnormality. The second advance in helping us with improved method in prognostication was reported by two groups of investigatorrs independently of each other but simultaneously in the same issue of the journal Blood of 15 Sept 1999. One of these 2 papers (Damle et al) was from the lab of Dr Nicholas Chiorazzi, one of the leading faculties in this Conference, and the other (Hamblin etal) was from the lab of Dr Freda Stevenson, in the UK.These papers demonstrated that CLL patients with their leukemic lymphocytes showing hypermutations in the Ig heavy-chain gene in the variable region had excellent prognosis, significantly better than without mutations. Damle etal also showed that leukemic lymphocytes in CLL patients without co-expression of CD 38 had better prognosis than those those patients with leukemic lymphocytes coexpressing CD38. The progress made with the recent discovery that the expression of zeta-associated protein-70 (ZAP-70) by the leukemic lymphocytes in CLL patients had significantly worse prognosis than patients without ZAP-70 expression, was reported by Crespo et al in the May 1,2003 issue of NEJM (with an Editorial by Rai and Chiorazzi), and this observation also was made in an issue of Blood in 2002 (just a few months prior to the NEJM paper by Crespo et al) by another important faculty member of this Conference,Dr Tom Kipps. As mutation status testing is not a clinically available tool and is not likely to be for some time in the future, ZAP-70 has become an attractive test to be developed as a practical tool in the Flow Cytometry Labs.

At therapeutic level, the major advances in CLL, are the introduction of monoclonal antibodies, Campath-1H and rituximab. These antibodies target all lymphocytes with anti CD52, humanised Campath-1H (CD52 is expressed by all lymphocytes, normal or malignant, B or T-cells), or B-cells expressing CD20 with anti CD20 chimeric antibody, rituximab. The use of these antibodies in combination with chemotherapy drugs such as fludarabine, cyclophosphamide, etc, has resulted in increasing percentage of complete remissions (CRs) in CLL (Keating etal Blood May 15 2002,Rai et al Blood [ASH abst]2002 and 2003,Byrd et al Blood Jan 1, 2003, and Keating et al Proc ASCO [abst] 2003). These advances and the promise of newer drugs such as anti-bcl2 oligonuleotide Genasense in combination with other chemotherapy agents and proteasome-inhibitor Velcade (PS341) also in combination with other drugs and monoclonal antibodies including anti CD23 (IDEC 152) indicate that in the next few years this otherwise incurable disease will become amenable to be brought in long-term control.

S17

Fratricidal Retrovirus: A New Twist In Gene Therapy

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To test the concept that a replication-competent retrovirus carrying a suicide gene could have potential utility in the control of the natural virus infection in mammalian species, we constructed derivatives of a feline leukemia virus (FeLV) that is commonly associated with leukemia-lymphomas in this species. Besides FeLV vectors with suicide transgenes, such as, HSV thymidine kinase and yeast cytosine deaminase, a similarly constructed FeLV with the GFP marker gene was included to serve as a control in the study. It is found that the expression of the transduced gene can be maintained in cultures of infected cells for months, and cytotoxicity could be induced following treatment with the appropriate prodrug enzyme substrates. A strong by stander effect on uninfected cells, co-cultured with the infected cells, is also demonstrated. Feline cells infected with different subtypes of FeLV could be readily superinfected by the FeLV suicide vector to target them for killing. Most interestingly, superinfection appears to be also effective within the same subtypes, only if initiated early enough before the full blockade of the respective cell surface receptors from the original virus infection. To obtain a proof-of-the-principle for the utility of engineered FeLV to deliver the therapeutic genes in vivo, we initially evaluated the status and nature of persistence of the GFP gene introduced in cats by

FeLV-GFP DNA construct. Despite the detection of predominant viral species with various deletions in the viral genome, stable GFP expression could be visualized in tissues even after 3 months following infection. It is estimated that about 1-3% of the total cell population was GFP positive in the lymphoid tissues, e.g., thymus, spleen and lymph nodes. Co-localization of immunofluorescent cells in the lymphoid organs appears to indicate that cell types, such as CD3-positive T cells, dendritic cells and macrophages are the major targets for the expression of the GFP protein. Motivated by these results we have now initiated experiments with suicide gene FeLV vectors in cats. Early results are again encouraging for further development of this new concept to combat leukemia virus infection, a strategy which could complement other therapeutic protocols in controlling the virus load or spread. This type of vectors could also potentially act as cytotoxic agents for retroviral induced malignancies.

The title of this Abstract is adopted from the commentary by A.B. Rabson on our paper which appeared in Cancer Biol. & Therap. 2:92-99, 2003

S18 Use of Liposomal Technology to Deliver Therapy of Leukenia and Lymphonia.

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Abstract Not Available

S19 Chronic Myeloid Leukemia : STI 1571 Versus Allo – BMT

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The treatment of Chronic Myeloid Leukemia continues to evolve rapidly. The exciting success of Imatinib has dramatically revised the way clinicians think about treating Chronic Myeloid Leukemia. Although Bone Marrow transplant is at present only curative approach in Chronic Myeloid Leukemia, the prolonged cytogenetic remissions seen with Imatinib therapy gives us a hope of complete eradiation of disease, becoming a reality without any major treatment associated mortality an morbidity. Imatinib is an inhibitor of Bcr-abl tyrosine kinase that is central to pathogenesis of Chronic Myeloid Leukemia. It competes with ATP for its kinsase-binding site, and prevents the kinase from transferring phosphate from ATP to tyrosine residues of the substrates. This action inhibits downstream signaling from the kinase, which switches the balance towards apoptosis. The treatment with Imatinib mesylate results in reduction in the tumoral metabolism and growth. In the treatment of chronic phase CML, Imatinib produces much better hematological and cytogenetic responses than interferon -a (IFN-α) with most patients sustaining these responses. In newly diagnosed CML the major cytogenetic response on Imatinib therapy is 83% with 68% complete responses (compared with 20% and 7%, respectively, with interferon + Ara-C). However, there are as yet no data to conclusively demonstrate that Imatinib improves long-term survival for CML patients when compared with an interferon-containing regimen.

We have treated 39 patients with Chronic Myeloid Leukemia with Imatinib. 29 were males and 10 patients were females. 100% of patients showed complete hematological remission with Imatinib. Cytogenetic response could be evaluated in 18 patients, of which 6 patients revealed complete cytogenetic response and other 10 patients showed partial response. None of our patients had any serious side effects

Allogeneic Bone Marrow transplant is time-tested treatment for CML. Nearly 50% of patients undergoing transplant with us are CML – Total number of patients who received myeloablative regimen is 55, with median age 30 years. All of them received preparative regimen with Busulfan and Cyclophosphamide. Mean duration of ANC recovery after transplant was 18 days. At the end of five years of follow up 50% (27) patients are alive and 25 patients are disease free.

S20 Improving outcome in adult ALL in India

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Abstract Not Available

S21 Clinical, Haematological and Histomorphological profile of Adult, Myelodysplastic Syndrome, Study of Ninety Six Case in a Single Institute

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Myelodysplastic syndromes are clonal haematopoietic stem cell disorders characterized by ineffective and dyspoietic haematopoiesis. The natural history of these disorders is variable and ranges from a chronic to a rapid course towards leukemic progression. Certain shortcomings have been encountered in the FAB classification over the years, therefore, a need was felt for an alternative method of classification. In 1999 the WHO published a revised classification of MDS.

In the present study we have analysed the clinical, haematological and histomorphological features in 96 cases of primary MDS seen in the department of haematology at AIIMS in last six years (1996-2001). Both FAB and WHO classification have been incorporated and Bournemouth scoring system applied in each case at presentation with a view to prognosticate the cases. In summary, classification of MDS based on WHO revised criteria seems better than the classification based on FAB criteria because it offers proper characterization and consistency on long term follow up. The Bournemouth scoring system, in the absence of cytogenetic study offers a good prognostication and long term survival estimate.

S22 Laboratory Evaluation of Acute Leukemia

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Acute leukemia, a malignancy of leukocytes, results forms uncontrolled growth of blasts/ promyelocytes. Its laboratory evaluation assists in its management by providing accurate diagnosis, determination of prognosis at presentation and evaluation of response to therapy. Diagnosis of leukemia is based on the number of blasts being >30% (FAB classification) in the bone marrow. The morphological examination of peripheral smear and bone marrow helps in further subtyping the leukemia in approximately 50% of cases. In the rest, cytochemistry and immunophenotyping need to performed for accurate subtyping. Despite this, in 5-10% cases, subtyping may be difficult. The advent of molecular/cytogenetics has helped bridging this gap. Thus demonstration of specific cytogenetic abnormalities helps in accurate diagnosis of some leukemias, like acute promyelocyte leukemia, AML (M2) etc.

Determination of laboratory findings like TLC at presentation (>50,000 in children with ALL: bad prognosis, >30,000 in adults with ALL: bad prognosis, etc), age at presentation and CD10 positivity

prognosis, >30,000 in adults with ALL: bad prognosis, etc), age at presentation and CD10 positivity serve to prognosticate the disease. Presence of cytogenetic/ molecular transcripts for TEL-AML (t12:21), t15:17, t8:21 serve as good prognostic makers wereas presence of t 9:22, t 4:11 serve as poor prognostic markers. In case bad prognostic markers as present, more aggressive treatment regimens may be implemented. In cases with APML or AML-M2 with t 8:21, residual disease may be assessed using RT-PCR and quantitated using real time PCR. These studies help in evaluating the response of therapy. It is thus concluded that laboratory evaluation plays an important role in management of leukemia.

S23 Primary CNS Lymphoma : Indian Experience

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During the last two decades, the frequency of primary CNS lymphoma (PCNSL) has been reported to be increasing in the West. This study was undertaken to establish the clinicopathological spectrum as well as hospital based trend of PCNSL in India.

A total of 8865 intracranial tumors were diagnosed in AIIMS during a 20-year period of which 80 were PCNSL. Similarly, in NIMHANS, 51 out of 5319 intracranial tumors were PCNSL. The histopathological features of these cases were reviewed and immunohistochemical staining done for Leucocyte Common Antigen, B cell and T cell markers. Clinical features were noted and HIV testing done in all patients diagnosed after 1990. Chi-square test for trend was applied to find out the hospital based prevalence. Since the number of brain lymphoma cases recorded in each year interval was small, prevalence rates for periods of at least 3 years was calculated to provide more stable estimates.

PCNSL constituted 0.9% of all intracranial tumors in this series. No actual increase in hospital-based prevalence of PCNSL was noted. Neither was there any alteration in the ratio of PCNSL to glial tumors. Only 2 cases were immunocompromised – 1 renal transplant in the AIIMS series and 1 HIV positive in the NIMHANS series. The mean age was a decade younger than reported in the West. There was male preponderance and the frontal lobe was the commonest site of involvement, followed by the temporal and parietal lobes. Majority were diffuse large cell lymphomas, high grade, B cell type. Only 1 case was T cell lymphoma. Present series compares well with data obtained from other centres in India (PGIMER, Chandigarh).

Thus, despite the increase of AIDS in India, there appears to be no changing trend of PCNSL and majority of our PCNSL patients are young and immunocompetent. This is further substantiated by the low frequency of PCNSL reported in large HIV positive / AIDS series from India. This is in contrast to the Western trend and forms an interesting topic for further investigation. It is possible that in the Indian context, AIDS patients die early of infection and do not survive long enough to develop in primary CNS lymphoma.

S24 Current Use Of Autologous Stem Cell Transplantation

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In year 2003-2004 we have to rethink the appropriate diseases and suitability of various types of hematopoietic Stem Cell Transplants (HSCT). With the rapid proliferation of HSCT use, we have learned alot and we have made significant alterations to the procedure. Availability of newer drugs and instrumentation has to be amalgamated with the lessons learned from the clinical trials done from 1976 until now. The issues to be discussed include: 1. Sources of stem cells; 2. Autologous stem cell preservation and processing guidelines; 3. Timing of stem cell collection; 4. Biology of each cancer; 5. Appropriateness of transplant for different types of cancer; and 6. When to

secondary cancer, heart, lung, etc.

Hodgkins and non-Hodgkins lymphoma, leukemias, multiple myeloma, pediatric cancers and chemo responsive breast cancer are the main targets of properly designed studies. Improvements and optimum use of the above technologies will be discussed.

S25 Tailoring Therapy in Stem Cell Transplantation

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Classical allogeneic stem cell transplantation (SCT) requires a HLA matched (A, B, DR) donor, usually a sibling, to provide the graft that has to be used to replace the hematopoietic stem cells in the recipient with hematological disease (malignancy, marrow failure or hereditary genetic disorders). A standard dose of chemo / radiotherapy is used to prepare the recipient ('conditioning') followed by infusion of the stem cells, again in empiric doses, originally obtained from the bone marrow but more recently from peripheral blood and other sources, as well. Up to 50% of patients undergoing SCT, develop complications related to the conditioning (veno-occlusive disease of the liver, hemorrhagic cystitis, interstitial pneumonia, mucositis, hemorrhage and infections) or engraftment (graft versus host disease) or the lack of it (graft rejection). Empiric doses of drugs are used for conditioning and as prophylaxis against the immunological complications of SCT. Many of these complications can be potentially attributed to lack of individualization of therapy and poor understanding of the donor – recipient interactions. The optimal dose and the way to individualize administration of these drugs in high doses remains to be defined. What also continues to be unclear is the basis of the immunological interactions between the recipient and host cells and the role played by the many other immunologically active cells in the graft (various lymphocyte subsets, natural killer cells and dendritic cells).

In recent years, considerable work has gone into trying to understand the basis of many of these complications and graft – host interactions. Over the last 10 years, much data has accumulated regarding the pharmacokinetics of busulfan and more recently, cyclophosphamide, and their correlations with outcome. The pharmacogenetics of these drugs is also being worked out emphasizing the role played by the glutathione S transferase and the cytochrome P450 enzyme systems. Such data may help predict pharmacokinetics of these drugs in different individuals. Towards understanding the immunological interactions between the host and donor cells, one of the approaches has been to study the role of polymorphisms in genes associated with various host defense and inflammatory responses. Such data has shown that engraftment, incidence of GVHD and infections and survival can be correlated with these genetic markers. Great interest also exists towards evaluating the role of natural killer (NK) cells in allogeneic SCT and particularly the role of their killer immunoglobulin-like receptors (KIR). Very recent work has shown that the interaction of NK cells with host tissues may be crucial in determining the incidence of GVHD / rejection even in matched related transplants and could possibly be of immense value in selecting suitable donors in partially matched donors for SCT. Unlike the HLA system, where the aim has been to find the perfect match for good outcome of SCT, here the paradigm seems to be to find the perfect mismatch to achieve the best outcomes.

This presentation will discuss some of these issues in SCT and present data of the work done at the Chistian Medical College, Vellore.

S26 Some unusual infections in BMT -their successful management

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Abstract Not Available

S27 Chronic Myeloid Leukemia : Newer Advances In Treatment And Prognostic Scoring

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Chronic myelogenous leukemia (CML) is the commonest leukemia among adults in North India. In a review of 437 patients, We have observed certain differences in clinical presentation compared to Western population ; CML occurs relatively at a younger age in India (median age, 35 years vs 47-57 years) with higher M : F ratio (1.6:1 vs 1.4;1) and higher frequency of advanced disease at diagnosis (12% vs 6%). Presence of systemic symptoms (fever – (55% vs 18%), hepatomegaly (>5cm below costal margin, 55% vs 8%) and splenomegaly (>10 cm, 33% vs 2%) is seen in more no of cases in our patients population. .

More recently, a new drug STI-571 (Imatinib) has been introduced; this oral drug has a novel mechanism of action and is associated with higher hematological and cytogenetic remission rates. 118 patients have been entered into the study at IRCH till June 2003. ; chronic phase (CP) n=79 (IFN-a resistant n=44, IFN-α intolerant n=7, IFN-α naïve n=28) and accelerated phase (AP) n=23; blast-crisis (BC) n=16]. The median duration of therapy was 5 months (range: 1-29 months). The complete hematological remission (CHR) was seen in 95% CP and 35% AP/BC patients at a median duration of 19 days (range: 7-87 days. 53 of 79 CML-CP patients, were evaluable for cytogenetic response (CGR); 25% had complete CGR. Common toxicities were - edema (48%), weight gain (54%), myalgia (27.8%), muscle cramps (16.7%), nausea (68%), fatigue (11%), and headache (19%). Grade 3-4 hematological toxicities were - anaemia (6%), thrombocytopenia (15%) and neutropenia (17%). These results are superior to those obtained with hydroxyurea and interferon alfa. We have also developed a simple isocratic HPLC run method to determine imatinib levels in the patient's plasma. This method is capable of detecting Imatinib at the concentration of 30 ng/ml plasma. The interday and intraday variations were less than 5% (RSD).

To predict the prognosis of an individual case at diagnosis, we have developed a scoring system using factors – peripheral blood blast %, phase of disease, platelet counts at diagnosis and sex. Following equation was derived.

R= 0.54*blast (0 when blast <10% otherwise 1)+1.81*diagnosis

(0 when Chronic Phase otherwise 1)-0.42*platelet (0 when

plateletl count<280*10**9/L otherwise 1)+0.54*sex (Male 1, Female 2)

Value of £ 2.57 = Low risk; 2.57 - 3 = Intermediate risk, >3 = High risk

According to this score, median survival is 75 months ± 9.63(SE) for low risk, 38 months ± 8.23(SE) for intermediate risk and 9 months ± 2.66(SE) for high risk patients. The results of this score has been validated in our patients population and compared with other 2 international prognostic scoring systems.

S28 Impact Of Busulfan And Cyclophosphamide On The Glutathione S-Transferases-Its Relevance To Stem Cell Transplantation

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Busulfan (Bu) in combination with cyclophosphamide (Cy) is the most commonly used conditioning regimen for BMT for both malignant and non-malignant conditions. Wide inter individual variation in the pharmacokinetics and pharmacodynamics of these drugs has been reported in the BMT

setting. We observed 2-12 fold variation in Bu kinetic parameters in patients with beta thalassemia major undergoing BMT. This variation was attributed to the activity of hepatic glutathione S-transferase (GST) in these patients. GSTs are phase II drug-metabolizing enzymes involved in the detoxification of various drugs & environmental toxins. Both Bu & the metabolites of Cy are metabolized by the GSTs in the liver. The pharmacokinetics of these drugs has been well studied & first dose monitoring and target dose adjustment are being done in many centers. Others and we have reported previously that the Cl/F of Bu at steady state is faster than the first dose Cl/F (Bone Marrow Transplant 1999) suggesting that Bu induces glutathione S-transferase (GST), an enzyme responsible for the conjugation of Bu in the body. In Cy metabolism, it has been shown that Cy metabolites are substrates for GSTA1, an isoform of GST. Hence this study was planned in the rat model to evaluate the effect of Bu and Cy on the hepatic and enterocyte GST A1 expression.

Male Wistar rats were treated with Bu & Cy either alone or in combination: Group I; Untreated rats, Groups II & Group III; received Bu (30mg/kg orally by gavage), but sacrificed after 24 & 72 hours of Bu respectively. Groups IV & V received Cy (100mg/kg i.p) and sacrificed after 24 hours & 72 hours of Cy respectively. Group VI & VII received a combination of Bu & Cy at 24 hour interval and sacrificed after 24 & 48 hrs of Cy treatment respectively. Liver & enterocyte homogenates are subjected to ultracentrifugation to collect the cytosolic fraction, which is used for measuring GST activity spectrophotometrically and by ELISA. Total RNA was isolated from liver & enterocyte homogenates, reverse transcribed with Superscript system and subjected to real time quantitative PCR analysis of GST alpha (with 18S as the control gene) using ABI 7700.

GST alpha levels & expression in rat liver:

GST α levels & expression in rat liver (A) and enterocytes (B)

A	G I	G II	G III	G IV	G V	G VI	G VII
GST/18S	3.48	317	4.6	27.8	7.5	27	11.4
GST α ELISA	945	1563	1541	1316	1098	1273	925
GST activity	1.03	1.63	2.12	1.62	1.83	1.92	2.09
B	G I	G II	G III	G IV	G V	G VI	G VII
GST/18S	0.216	0.42	0.24	0.64	0.31	0.77	0.09
GST α ELISA	466	1344	1066	681	1263	636	904
GST activity	0.14	0.25	0.3	0.21	0.27	0.38	0.35

(GSTα by ELISA was expressed as ng/mg protein; GST activity was expressed as units/mg protein)

Bu showed peak induction in GST alpha expression (GSTα/18S) at 24hrs in the liver about 100fold compared to the untreated group & the effect was almost totally lost at 72 hrs reaching base line values. Cy has a small independent effect on hepatic GST alpha expression at 24 hours and falling by 72 hrs to near base line. In combination, in the BuCy48h group, the effect of Bu is almost lost at 48h, reflecting only the effect of Cy after 24 hrs. In the BuCy72h group, the expression is almost similar to the Cy72h group. At the protein level, (both by ELISA & by spectrophotometry), the induction was similar, but the levels did not come down like the GST alpha RNA expression, probably due to longer half life of the protein than RNA. In the enterocytes, there was a similar, definite but less pronounced induction of GST alpha at all time points like that of the liver.

This study shows that Bu and to a lesser extent Cy, induces GST alpha in the liver & enterocytes. This could explain the faster Bu Cl/F at steady state as compared to the first dose that we observed in our previous study. In support of this finding, the plasma GST alpha levels in most of the thalassemic patients showed 1.5 to 30-fold increase post Bu treatment, as compared to pre Bu levels. A few patients however showed decreased levels after Bu treatment. This effect may be influenced by GST A1 genotype of the patient. Coles et al have shown that the hepatic expression of GST A1 and A2 depends on the GST A1 genotype. The need now is to do further studies in GST genotyped models to assess whether this response and its magnitude can be

correlated with particular genotypes. The ultimate aim is to define all the parameters that can alter Bu PK, so that doses can be individualized more predictably to improve efficacy and reduce toxicity in BMT.

S29

Post-Hematopoietic Stem Cell Transplant Monitoring Using Molecular Tools

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The ultimate success of an allogeneic haematopoietic stem cell transplant (HSCT) is affected by underlying disease relapse and the relative proportion of persisting donor cells. Successful transplantation occurs when the donor stem cells are durably engrafted in the recipient, a state referred to as "chimerism" (Latin: *chimera* means *monster*). Several genetic markers including sex specific markers are being used to monitor post BMT chimerism. The most useful loci are those consisting of tandem repeats of very short (3-5 bp) or short (15-60bp) specific repetitive base sequences, referred to as short tandem repeats (STR) or variable number of tandem repeats (VNTR) respectively. These markers can be easily evaluated with GENESCAN using an automated DNA sequencer. Complete chimerism is in general associated with myeloablative treatment while mixed with nonmyeloablative. Complete chimerism is often accompanied with GvHD, less rate of relapse and longer disease free survival. But mixed chimerism is associated with less severe GvHD and GvL, higher chance of relapse and shorter disease free survival. Early assessment of engraftment in the immediate post-transplant period and subsequent monitoring by chimerism may be useful for prevention and management of rejection, and for detection of persistent disease and imminent disease relapse. Although originally used primarily to document engraftment of the bone marrow, studies of chimerism also have the potential to explain mechanisms of Graft vs Host Disease (GvHD), graft rejection, a comparative evaluation of different conditioning regimens and pathological situations. Classical cytogenetics has been the mainstay of diagnosis and monitoring leukemias. However, these traditional methods are being complemented by flourescent in-situ hybridisation, to detect the BCR-ABL translocation. RT-PCR is used for both diagnostic purposes and its quantitative follow-up, particularly after allogeneic stem cell transplantation fro detection of minimal residual disease (MRD). Improved molecular methods of quantification have enabled reliable monitoring of leukemic tumor load and allowed for therapeutic interventions before the occurrence of hematological relapse. An accurate and long-term follow-up of monitoring MRD is essential for optimum clinical management of hematological malignancies. The maximum sensitivity is achieved by mRNA based molecular studies (Real-time Taqman based PCR assays). Since the introduction of the novel therapy Imatinib mesylate (tyrosine kinase inhibitor), a number of patients have developed resistance to Imatinib. There are number of possible mechanisms for this resistance that are being investigated, including point mutations within the Abl kinase domain of the Abl moiety of the Bcr-Abl kinase. Unification of precise molecular analysis and establishment of prospective follow-up studies should allow the application of chimerism / MRD based interventional therapy in various transplant settings that would be beneficial for optimum management of leukemia/ lymphoma patients.

S30 Current Status Of Allogeneic Hematopoietic Stem Cell Transplant For Leukemia

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In the last three decades treatment of acute and chronic leukemia both in adults and pediatrics has advanced significantly, improving event free survival in these patients. Hematopoietic stem cell transplant is one of the major treatment strategies, which has changed the road map of treatment of leukemia.

Myeloablative therapy with total body irradiation and/or chemotherapy till recently has been the mainstay of conditioning regimen for allogeneic hematopoietic stem cell transplant. Rationale for such intensive therapy is reliance on cytotoxic effect of these therapies to eradicate the disease. However, the notion of eradication of leukemia by allogeneic stem cells does not depend entirely on myeloablative therapy given during the conditioning for stem cell transplantation. The concept of graft versus leukemia has brought forth the role of donor leukocyte infusion (DLI) after transplant to sustain or reverse the remission.

Recently reduced intensity or non-myeloablative regimens for transplantation have gained lots of attention. Clinical studies indicate that eradication of immune hematopoietic cells, can be achieved by adoptive allogeneic cell therapy with DLI following induction of host versus graft tolerance, mediated by engraftment of donor stem cells in the course of transplantation

Availability of alternative sources of hematopoietic stem cells and recruitment of a larger population in the donor registry has expanded our capability of identifying a suitable donor for a patient who does not have match donor in the family. Selection process of alternative donor with high resolution DNA technology has significantly improved the outcome of these transplants

S31 Unrelated Donor Marrow Registries : Ethnic influence

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Over the past 25 years, allogeneic hematopoietic stem cell transplantation has become a standard therapy for several otherwise fatal hematologic diseases. Initially allogeneic transplantation was limited to the use of HLA genotypically identical marrow donors. Given the average family size of less than three children, and the 25% probability that a given sibling pair would be HLA compatible by inheritance, 30-35% of all patients will have an HLA-matched sibling. This led to the need for alternative to family donors. New developments in immunosuppression and supportive care have allowed the use of donors other than HLA-matched siblings. With advancements in histocompatibility testing and the possibility of precisely distinguishing matched vs mismatched HLA alleles at the DNA level, it is now feasible to consider the use of volunteer unrelated marrow donors. These developments have led to the establishment of large, international, HLA-typed marrow donor registries, which now list approximately 8 million potential donors.

HLA studies in the North Indian population have confirmed the historical documentation of racial admixture. Although population studies have indicated that the major HLA gene pool in the Indian population is much similar to that of Western Caucasoids, allele level studies have revealed appreciable heterogeneity with the presence of both Caucasoid and Oriental HLA genes, as well as new recombinants among Indians. In addition, a significant number of 'novel alleles' and 'unique haplotypes' were observed both in the MHC class I (HLA-A2,-A19, -B27 etc) as well as class II region (HLA-DR2,-DR4 etc). Our studies indicate that the Indian population constitutes a transition zone between Caucasians and Negroids on one hand and Australoids and Mongoloids on the other. The allelic diversity and uniqueness among North Indians is contributed by new allele sequences and immense heterozygosity in haplotypic combinations. The novel alleles and

haplotypes may confer some selective survival advantage to the population as a whole. Immense racial admixture, influence of natural selection and potential genetic conversions over centuries in this subcontinent may be responsible for the observed allelic repertoire and generation of ubiquitous and unique alleles.

Knowledge on the occurrence of the novel alleles and unique HLA haplotypes is of critical importance for donor selection during organ and bone marrow transplantation. Therefore developing an Asian Indian registry of volunteer donors would be of immense help to patients of Indian descent who may otherwise fail to find an unrelated HLA-matched donor from other registries. Unlike other parts of the world, unrelated marrow donation is not a common practice in India so far. We at the All India Institute of Medical Sciences (AIIMS), have taken the initiative in 1994 for establishing a registry of volunteer Indian donors and this has been functioning under the title, 'Asian Indian Donor Marrow Registry' (AIDMR). With the main objective of recruiting large donor pool, this modest effort has resulted in the enrolment of nearly 3000 donors, majority of which belong to the states from North India. These are already tested for HLA class I alleles by serological methods and approximately 40% of them are also tested for HLA class II alleles using molecular technologies. The AIDMR is the only registry of voluntary donors in India and has developed collaborative arrangements with similar registries elsewhere e.g. NMDP (USA), SAMAR (USA), BMDW (Holland). It is our endeavor to expand the registry appreciably in the near future for the benefit of those unfortunate patients who may not have an HLA identical sibling in the family.

Liver Based Stem Cell

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Abstract Not Available

Cord Blood Transplantation: Indian Scenario

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Cord blood transplantation and cord blood banks appear to be potentially useful options in India, with its high birth rate and large family size. There is little data about the cord blood characteristics from India, and what percent of these would be useful for cord blood transplantation. To determine this, a study was carried out at Army Hospital R&R, New Delhi from 1997 to Aug 2002 and CD 34 estimation was done at Dr Lal's Lab. Twenty consecutive cord blood collections were done after normal vaginal delivery. In addition, ten related cord blood collections were done over a 3 year period for possible cord blood transplantation. All mothers were nutritionally healthy. These 30 collections were analysed for collection volume, total nucleated cell counts and CD 34 counts. The nucleated cell dose of each collection was calculated for a potential transplant in a 10 kg and 20 kg recipient taking a value of 3.7×10^7/kg as an optimal dose with > 85% chance of engraftment [N Engl J Med 1997; 337 ; 373-81]. Only 50 % of cord blood samples had volumes > 60 ml. The nucleated cell dose > 3.7×10^7/kg for a 10 kg recipient was present in 80% (24/30) collections and for a 20 kg recipient in 20% (6/30) collections. Details are given in Table 1.

Table. 1

	Volume (ml)	Nucleated cells	Total CD 34 x 10^6	Nucleated cell dose x 10^7/kg. for 10 kg Pt	Nucleated cell dose x 10^7/ kg for 20 kg Pt
Median	59.6	9.75	1.1	5.9	2.945
Range	25-160	5-27.6	0.05-6.4	2.21-22.99	1.1-13.1

HLA identical sibling cord blood transplantation was done in 3 cases, and in a fourth case combined bone marrow and cord blood transplantation was done. The cord blood was cryopreserved in a mechanical freezer at -71°C.
Details are given in the Table 2.

Table 2.Transplant Characteristics of 4 cord blood transplants.

Diagnosis	Nucleated cell does infused x 10^7/kg	Days of cryopreservation at - 71°C	Days to Neutrophil engraftment	Days to platelet engraftment	Complications
AML(refractory) in1st CR	4.5	105 days, (95%)	15	20	Fever
AML in 2ndCR	1.7	124 days, (90%)	Died on Day +21	-	VOD liver, Sepsis
Thalassemia Major	15.0	80 days, (84%)	13	18	Fever, seizures
Thalassemia major	3.53 (+ bone marrow 2.26 X 108/kg)	18 months, (86%)	13	13	Fever