preponderance in the study group. Fever (58.91%), Lymphadenopathy (44.71%) and Haepatosplenomegaly (31.72%) were the major clinical presentation. 29 (8.76%) patients were present with hyper Leukocytosis. C-ALL phenotype were the largest group though the incidence of the T-ALL were quite high (29.90%).
Results: Remission induction were seen in 93.65% of the patient. In a follow-up period of 1–56 months (with an average of 35 months) the Disease Free Survival (DFS) was 67.97% with an overall survival of 73.41%. The isolated Bone Marrow relapse was seen in majority of the cases and the major Relapse was in maintenance and first 6 months of completion of therapy. The major cause of morbidity was infection (66.76%) followed Metabolic Complications (17.82%), Hemorrhage (10.87%), Neurology(2.11%), Hepatitis (1.2%) and Pancreatitis(0.9%). The major cause of the mortality was infection (75.52%) followed progressive disease (7.25%) and Hemorrhage (5.74%).
Conclusion: The initial data from Eastern Part of India is encouraging.

990 PUBLICATION

Mutant N-ras activation in primary human hemaopoietic progenitor cells: biologic, phenotypic and genetic sequelae

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Constitutive activation of Ras signalling through mutation is one of the most frequently detected genetic abnormalities in myeloid disorders including acute myeloid leukemia (AML). This work sought to analyse the mechanism(s) of oncogenic Ras activation and leukemogenesis, and to identify potential new therapeutic targets for gene therapy in leukemia.
A retroviral vector expressing mutant N-ras (*N-ras^m*) was used to efficiently transduce primary hematopoietic progenitor cells (HPCs) (cord blood CD34+ cells) with both *in vitro* and *in vivo* NOD/SCID mouse readout (Shen et al, *Experimental Hematology* 2004;32:852–860). Retrovirally transduced human HPCs efficiently engrafted and repopulated bone marrow of sub-lethally irradiated host mice, and reconstituted both the lymphoid and myeloid lineages. *In vitro* analysis revealed that *N-ras^m* differentially affects lineage/maturation specific hematopoietic cells. Introduction of *N-ras^m* into HPCs resulted in an increase of myelomonocytic lineage cells, both in liquid culture and in clonogenic assay, at the expense of erythroid and lymphoid lineage cells. Growth suppression following *N-ras^m* transduction was observed in the CD34+/N-ras+ cell population, but not in the CD34-/N-ras+ cell population. cDNA microarray was used to identify the transcriptome induced by *N-ras^m*, and showed (subsequently confirmed by real-time RT-PCR) a significant increase in expression of cyclin-dependent kinase inhibitors *p16^{INK4a}* and *p21$^{CIP1/WAF1}$* in CD34+/N-ras+ cells, but not in CD34-/N-ras+ cells.
When transplanted into NOD/SCID mice, *N-ras^m* HPCs displayed not only higher engraftment of the cells themselves, but also promoted engraftment of co-transplanted HPCs not expressing *N-ras^m*, indicating that expression of *N-ras^m* in HPCs induces the release of soluble factor(s) that promotes survival and/or homing of HSCs to the bone marrow and engraftment.
This hypothesis is supported by the transcriptome analysis in which a large array of soluble growth factors were shown to be significantly increased in *N-ras^m* HPC.
Taken together, these results indicate that 1) *N-ras^m* promotes myelomonocytic differentiation and suppresses proliferation of primitive HPCs; 2) *N-ras^m* alone is not sufficient to initiate leukemogenesis; and 3) *N-ras^m*-associated leukemogensis requires collaborative secondary event(s) of inactivation of tumor suppressive pathways.

991 PUBLICATION

Quantitative analysis of WT1 gene for detection of minimal residual disease in acute leukemia by Real-time RT-PCR

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Introduction: WT1 gene encodes a transcription factor which is involved in differentiation and proliferation of Hemtopoeitic precursor cells as well as some other tissues like kidney, ovary, heart etc. It is also expressed in 80% of Acute Leukemia cases (AML, ALL) as determined by various qualitative and quantitative RT-PCR methods. It is proposed to be a useful marker in minimal residual disease (MRD) detection and leukemia management.
Methods: To assess the relevance of this gene, sequential peripheral blood samples from 72 leukemic patients (62 AML and 10 ALL) were analyzed for the expression level of WT1 mRNA, using Real-Time Quantitative RT-PCR. Samples from patients obtained at the time of diagnosis, and during treatment (follow-up), in remission, relapse and after relapse.
Results: Samples of diagnosis and relapse showed significantly higher WT1 expression levels (90%), compared to samples from patients in complete remission (CR) or healthy volunteers. No significant difference in expression levels was found between various AML subtypes. ALL patients showed lower levels of WT1 expression compared to AML ones. Our study revealed that rising of WT1 expression predicts a forthcoming relapse 1–6 months before overt hematologic or clinical relapse. A linear correlation between quantities of WT1 and PML-RARa fusion transcripts could be seen in APL patients treated with arsenic trioxide.
Conclusion: There was a strong correlation between WT1 and specific fusion gene expression in leukemic patients, showing the significant potential of WT1 as a non-specific leukemia marker (NSLM) for monitoring of MRD and treatment approaches in leukemia.

992 PUBLICATION

Fluorescence in situ hybridization in conjunction with karyotyping in detection of cytogenetic abnormalities in B-cell chronic lymphocytic leukemia and its prognostic value

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Background: B-cell lymphocytic lymphoma (B-CLL) is a relatively common condition accounting for 0.8% of all cancers. Routine cytogenetic analysis frequently fails to identify an abnormal clone due to poor response to mitogen stimulation. Fluorescence in situ hybridization (FISH) suggest that chromosomal abnormalities occur more frequently, most commonly trisomy 12, retinoblastoma gene deletion (Rb1 gene) and P53 gene deletion.
Purpose: In the present study thirty three B-CLL patients were studied to assess the possible incidence of trisomy 12, Rb1 gene deletion and P53 gene deletion by karyotyping and FISH technique and to correlate these with clinical features and survival.
Patients and methods: 33 patients with B-CLL were enrolled in the trial from 2 centers in Cairo, Egypt during the period May 2000 to January 2001, 3 patients were excluded because of non compliance. Karyotyping and FISH assessment for possible chromosomal abnormalities (trisomy 12, Rb1 gene and P53 gene) were done at initial diagnosis; patients were treated according to center protocols. Results of cytogenetic abnormalities were correlated with clinical picture and survival.
Results: The median age was 57.4 years (range 40–75), clinical staging of B-CLL patients showed 20% of them were Binet stage A, 43% were stage B and 37% were stage C. Karyotyping technique showed that no metaphase could be detected in 30%, 63% showed metaphase with normal karyotyping, cytogenetic abnormalities were detected in 2 cases (1 trisomy 12 and 1 deletion in chromosome 13). FISH examination of interphase and metaphase nuclei revealed cytogenetic abnormalities in 15 cases (50%), trisomy 12 in 9 cases (30%), Rb1 gene deletion in 5 cases (17%) and P53 gene deletion in 1 case. At diagnosis, patients with trisomy 12 were significantly associated with advanced stage and absolute lymphocyte of $\geqslant$30,000/mm3. 4 years overall survival for the whole group was 55.8%. Univariate analysis showed that absolute lymphocyte count $\geqslant$30,000/mm^3 ($p = 0.017$) and trisomy 12 ($p = 0.0433$) were associated with poor survival.
Conclusion: Interphase and metaphase FISH studies improve the cytogenetic diagnosis of chromosomal abnormalities when performed in conjunction with karyotypingin B-CLLwhich showed significant worse prognostic value.

993 PUBLICATION

The protective effect of amifostine on irradiated haemapoetic cells: ex vivo study

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Background: To evaluate the protective effect of amifostine on ex vivo irradiated human bone marrow cells in purging procedure.
Materials and methods: Human bone marrow cell samples of healthy volunteers were divided into six groups as control (C and C_A), 25 Gy (IR_{25}), 50 Gy (IR_{50}), 25 Gy+amifostine (IR_{25A}) and 50 Gy+amifostine (IR_{50A}), respectively. Samples of $IR_{25,}$and IR_{25A} were irradiated with 25 Gy whereas