MAP4K3, CRKL and MAP2K4 were down-regulated, leading to cell cycle arrest and apoptosis.
Conclusions: Genistein induced growth inhibition and apoptosis in AML and APL cell lines in dose and time dependent manner. Our data suggests the potential clinical usage of genistein in anti-leukemia therapy. To our knowledge, this is the first description of genome-wide gene expression study for anti-leukemia effect of genistein in AML and APL cells. Our findings that genistein triggers different signaling pathways in AML and APL suggest that the impact of treatment in different hematologic malignancies can be prospectively monitored by measuring activities of distinct pathways.

Publication
Haematological malignancies

986 PUBLICATION

Clinical study of combining arsenic trioxide (As2O3), all-trans retinoic acid (ATRA) and idarubicin (IDA) for induction therapy on the patients with relapsed acute promyelocytic leukemia(APL)

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Purposes: To study the effects of treatment by combining 3 drugs including As_2O_3, ATRA, and IDA for long-term survival and relapse time on APL patients with relapse/relapses.
Methods: Between 1996–2003, long-term follow-up was carried out on the effects of treatment of combining 3 drugs including As_2O_3, ATRA, and IDA on 46 APL patients with relapse/relapses. All cases were diagnosed, according to the standard criteria of morphologic and cytogenetic examination. Among 46 cases, 36 cases had more than one relapse. The protocol of the 3 drugs administration included: As_2O_3 10mg iv and ATRA 30 mg p.o per body daily for continuous 32 days, combined with IDA 10mg per body per day on day 1, 3, 5, respectively.
Results: Among 46 cases, 37 cases achieved complete remission(CR), with CR rate of 80.4%. Five cases died related with treatment. Among 37 CR cases, 34 cases occurred infection during induction therapy, with infection rate of 90%, nevertheless, they all recovered after the administration of G-CSF and anti-infection agents. The 5-year disease free survival rate was 72%.
Discussion: The achievement of CR for APL patients resulted from inducing differentiation and apoptosis of the leukemia clone by the use of As_2O_3, ATRA, it also resulted from the cytotoxicity to directly destroy DNA of leukemic cells by the use IDA. In recent years, the relapsed APL patients were mainly treated by using combining drugs, with the effect of synergism. This study indicated that the combination of 3 drugs including As_2O_3, ATRA, and IDA could induce APL patients with relapse/relapses to achieve CR again, with 5-year disease free survival rate of more than 70%. Considering the relatively high incidences of infection and hemorrhage, it is advised to use this protocol in specialized hematology centers.

987 PUBLICATION

Rituximab maintenance therapy post Autologous Stem Cell Transplant (ASCT)

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Background: The anti-CD20 monoclonal antibody, Rituximab, is commonly administered after high-dose chemoradiotherapy and autologous stem cell transplant (ASCT) for B cell malignancies. Two series, one by Horwitz et al (2004) and another by Brugger et al (2004), using different schedules, administered Rituximab post-ASCT, importantly to Rituximab naive patients, and showed >80% two year event-free survival (EFS). The role of Rituximab post-ASCT in patients who have received Rituximab as part of front-line or salvage therapy has not been reported.
Materials and methods: We report on a series of 29 patients with either diffuse large B cell lymphoma (n = 19) or mantle cell lymphoma (n = 10) who received post transplant Rituximab maintenance therapy on one of three schedules: weekly ×4 weeks at day +42 and day +180 (n = 11); every 8 weeks for 6 treatments (n = 6); or other (n = 12). There were 19 males and 10 females. The mean age was 50 (range 22–69). All patients had received Rituximab as part of their initial chemotherapy regimens. All patients underwent PBPC mobilization after Rituximab and ICE (ifosfamide, carboplatin and etoposide). The transplant conditioning regimens were: BEAM (n = 17); CBV (n = 6); TBI/IFX/VP-16 (n = 1); TBI/IFX/CY (n = 4) and Mel/VP-16 (n = 1).
Results: Patients received a mean of 7.2 doses of Rituximab (range 1–16). The mean day of the start of Rituximab was day +65 post ASCT and concluded on day +293 post ASCT. Rituximab was administered in an outpatient setting.
The actuarial EFS at a median follow-up of 1.8 years is 83%. The mean absolute neutrophil count (ANC) nadir was 1.5 K/mcL. Ten patients (34%) experienced significant neutropenia (ANC <1.0 K/mcL) but all were afebrile and did not encounter any adverse clinical consequences of the neutropenia. Filgrastim or Pegfilgrastim was not used consistently in this cohort. None of the patients experienced clinically significant thrombocytopenia.
Conclusions: Rituximab is a well tolerated post-transplant maintenance regimen that is not schedule dependent and appears to improve EFS compared to historical controls. Neutropenia is common but with minimal consequences.

988 PUBLICATION

Aberrant cytoplasmic BCL10 expression reflects advanced disease in patients with mucosa-associated lymphoid tissue lymphoma of ocular adnexa

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Background: Some specific chromosomal aberrations are implicated in the development of mucosa-associated lymphoid tissue (MALT) lymphoma. These aberrations are also associated with BCL10 protein expression. However little is known about the relationship between the BCL10 expression and tumor progression.
Patients and methods: We reviewed clinical data and studied immunohistochemical analysis of BCL10 expression in 38 patients with MALT lymphoma of ocular adnexa treated with radical radiotherapy at our institution. Thirty-five patients had primary disease (33 stage IEA, 2 stage IIEA) and 3 patients had histories of lymphoma (2 stage IEA, 1 stage IIEA). The median follow-up duration was 48 months with a range of 21–159 months.
Results: According to the BCL10 expression pattern, patients were divided into three groups: aberrant nuclear expression (n = 10, 26%), cytoplasmic expression (n = 7, 18%), and normal staining (n = 21, 55%). Local control was achieved in all 38 patients. Extra orbital recurrence was observed in 6 patients (16%). Nuclear expression was detected in none (0%) of these 6 relapsing and in 10 (31%) of 32 non-relapsing patients, respectively. (P = 0.168). Cytoplasmic expression was detected in 3 (50%) of 6 relapsing and in 4 (13%) of 32 non-relapsing patients, respectively. (P = 0.063). Nine patients (24%) represented advanced disease with extra orbital lesions, including stage II, the history of lymphoma and recurrence. Nuclear expression was detected in none (0%) of these 9 advanced and in 10 (34%) of 29 non-advanced disease, respectively. (P = 0.079). Cytoplasmic expression was detected in 4 (44%) of 9 advanced and in 3 (10%) of 29 non-advanced disease, respectively. (P = 0.041).
Conclusions: In MALT lymphoma of ocular adnexa, aberrant cytoplasmic BCL10 expression is detected at a high frequency in advanced disease, while nuclear BCL10 expression tends to be detected in localized disease.

989 PUBLICATION

Result of acute lymphoblastic leukemia (MCP 841) protocol in a tertiary center from Eastern India

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Background: Acute Lymphatic Leukemia in children is a curable disease in the range of 80–90% in developed Countries by aggressive protocol like BFM, St. Judes'. In developing Countries like ours, patients can't tolerate those aggressive protocol because of Socio-economic and nutritional factors. The less aggressive Protocol like INCTR (International Network for Cancer Treatment & Research) are suitable in developing Countries like ours.
Materials and methods: We treated 331 Children (age range 1–25 years, median age of 7–8 yrs) with MCP 841 Protocol at Netaji Subhash Chandra Bose Cancer Research Institute, Kolkata, India a tertiary cancer centre of Eastern India during period from April'99 to Dec'04. There was female

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