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

$IR_{50}$and IR_{50A} groups received 50 Gy doses. The amifostine groups, C_A, $IR_{25A}$and IR_{50A}, were also incubated with 3 mg/mL amifostine for 15 minutes before irradiation. C group cells delivered neither radiation nor amifostine.
All groups were incubated in 37ºC and 5% CO_2 pressure for 12 hours. Prior to and 12 hours following the irradiation all samples were assessed in terms of total viable cell counts, colony numbers detected in mixed colony cultures and percentage of apoptosis by flow cytometry. The group means were compared for statistical analysis and significance level was set at $p < 0.05$.
Results: There was no statistical difference found for all assessments between control groups of C and C_A. Meanwhile, viable cell counts were detected higher in amifostine groups than those irradiated only ($0.9x10^9$/L, IR_{25} vs. 3.9 $x10^9$/L, IR_{25A}, $p<0.01$; 0.2 x 10^9/L, IR_{50} vs. 2.5×10^9/L, IR_{50A}, $p < 0.05$). In addition, the colony numbers were significantly higher in both dose levels (80, IR_{25A} vs. 20, IR_{25}, $p < 0.01$; 41, IR_{50A} vs. 12, IR_{50}, $p < 0.05$). The percentage of apoptosis was less in amifostine group but only for 25 Gy (46.0%, IR_{25A} vs. 29.7%, IR_{25}, $p < 0.05$).
Conclusions: Purging the malignant cells from stem cell grafts is done with several pharmacological agents and there are some data on amifostine preventing the normal bone marrow cells in procedure. Our study showed radiation may safely be administered in 25 Gy for the same purpose but with an amifostine like protector and further in vivo studies are required to test the feasibility.

Head and Neck Cancer

Oral presentations (Tue, 1 Nov, 13.45–15.45)
Head and neck cancer

994 ORAL

Paclitaxel and gemcitabine vs. paclitaxel and pegylated liposomal doxorubicin in advanced non–nasopharyngeal head and neck cancer. A phase III study conducted by the Hellenic Cooperative Oncology Group (HeCOG)

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Background: Advanced head and neck cancer (HNC) is an incurable disease and bears grave prognosis. Median survival with platinum-based chemotherapy does not exceed 6 months. In a series of phase II studies, we have shown that the combinations of paclitaxel (P) with gemcitabine (GEM) or with pegylated liposomal doxorubicin (PLD) have promising activity in patients with HNC. Median survival with these combinations, were 9 and 7.9 months respectively. The aim of the present study was to compare the efficacy of these two novel regimens with proven activity in phase II studies in patients with non-nasopharyngeal advanced HNC.
Patients and methods: From 15/9/1999 until 9/11/2004, 159 eligible patients entered the study. 5 patients presented with locally advanced and 154 with recurrent /metastatic disease. Patients randomized to Group A (n = 83) were treated with 6 cycles of P 175 mg/m^2 by 3-hour infusion on day 1 and GEM 1000 mg/m^2 on days 1, 8 of each cycle every 3 weeks. Patients randomized to Group B (n = 76) received 6 cycles of P, as in Group A, and PLD (Caelyx®) 40 mg/m^{2}every 4 weeks.
Results: There were 139 men and 20 women with a median age of 64 years and median PS of 1. Primary tumor site was larynx (48 vs. 46), hypopharynx (7 vs. 4), oropharynx (2 vs. 1), oral cavity (24 vs. 21), and other (2 vs. 4). 37 (45%) patients in Group A and 34 (45%) in Group B completed 6 cycles of chemotherapy. In 158 evaluable for response patients, the overall response rate was 20.5% vs. 29% (p = 0.215). After a median follow-up of 33.1 months, median time to progression was 4.4+ vs. 6.3+ months (p = 0.0568) and median overall survival (OS) 8.6 vs. 11.5+ months (p = 0.2784) in Group A and B, respectively.
Major (grade 3–4) toxicities included leukopenia (7.2% vs. 6.8%), anemia (0% vs. 3%), allergic reactions (1% vs. 7%, p = 0.02), peripheral neuropathy (1% vs. 0%), diarrhea (1% vs. 0%), infection (2% vs. 0%), fatigue (1% vs. 0%), skin (0% vs. 5.5%, p = 0.045), fever (2% vs. 1.4%), and hand and foot syndrome (0% vs. 3%). The incidence of neutropenia (12%), thrombocytopenia (1%) and pain (1%) was similar in the two groups. Alopecia was universal.
Conclusions: The present study has clearly demonstrated that there was no significant difference in OS between the two groups. Further, both regimens are accompanied with confirmed promising efficacy in advanced HNC and should be compared with the reference regimen of cisplatin and 5-day continuous infusion of fluorouracil.

995 ORAL

A phase II trial of BAY 43–9006 in patients with recurrent and/or metastatic head and neck squamous cell carcinoma (HNSCC). A Southwest Oncology Group (SWOG) trial

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Background: BAY 43–9006 is a potent raf kinase inhibitor of kinases of Raf-1 and B-Raf of the RAS/RAF/MEK/ERK pathway. The compound also inhibits protein tyrosine kinases associated with VEGFR-2 and 3 as well as PDGFR-B. We conducted a phase II trial to evaluate the efficacy of BAY 43–9006 in chemotherapy naive patients with metastatic or recurrent HNSCC.
Methods: Chemotherapy naïve patients with histologically proven squamous cell carcinoma of the head and neck either metastatic, persisted or recurred following definitive surgery and/or radiation therapy, and not amenable to salvage surgical resection with measurable disease were eligible. Patients must not have received any previous chemotherapy for the recurrent or metastatic disease. Patients who have received induction or adjuvant chemotherapy are eligible, provided that at least six months have elapsed since the last course of chemotherapy was administered. Patients may have received only one induction or adjuvant regimen. Patients must have adequate cardiac, hematologic, renal and hepatic function and a Zubrod Performance Status of 0 or 1. We obtained specimens from either archival or fresh pre-treatment biopsies and planned to obtain a second tissue specimen at the time of progression of disease for biologic correlative studies. BAY 43–9006 was administered orally at 400 mg BID on a continuous basis, in 28-day cycles. Respnses were evaluated every 8 weeks according to RESIST criteria.
Results: Twenty-two patients (17 males, 5 females, median age 65 years) have been enrolled to date. Fourteen patients are evaluable for toxicity. The drug was generally well tolerated. The grade 3 toxicities included one patient with hand/foot syndrome and another with stomatitis. The most common grade 2 toxicity was fatigue (3 pts.), and anorexia (3 pts.), nausea (1 pt), weight loss (1 pt), lymphopenia(1 pt), AST/ALT elevation(1 pt), and stomatitis (1 pt). The trial was temporarily closed on April 1, 2005 after reaching its first stage accrual goal and will reopen if any responses are documented.
Conclusion: BAY 43–9006 is well tolerated. Updated toxicity data will be reported. Response, time to progression and survival data will be presented, if the trial has met its final accrual goal and has permanently closed. If any response is noted the trial will re-open to accrue an additional 20 patients.

996 ORAL

Randomized phase III study in squamous cell carcinoma of the head & neck (SCCHN) using Lipoplatin: First safety results of a multicenter trial

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Background: Based on a metanalysis of >10.000 patients, cisplatin emerges as an essential chemotherapy drug for the treatment of advanced SCCHN. However, its clinical use is impeded by its severe adverse reactions, especially renal toxicity, peripheral neuropathy, and ototoxicity. In a randomized, multicenter phase III trial, we replaced conventional cisplatin by a liposomal formulation of cisplatin (lipoplatin), and compared the safety profiles of patients in the two treatment arms.
Material and methods: Main inclusion criteria selected patients with histologically confirmed SCCHN (primary metastatic or patients with relapsed/progressive disease) between 18–75 years of age with sufficient renal function defined as creatinine clearance >50 ml/min. After stratification (criteria: primary metastatic disease, recurrent or progressive SCCHN, prior chemotherapy, no prior chemotherapy, prior cisplatin-based chemotherapy, prior non-cisplatin based chemotherapy and center), patients were randomized between the following arms: Arm A: 100 mg/m^2/d lipoplatin (d 1, 8, 15) plus 1000 mg/m^2/d 5-FU (d 1–5) q3w for 6 cycles; arm B: 100 mg/m^2/d cisplatin (d 1) plus 1000 mg/m^2/d 5-FU (day 1–5) q3w for 6 cycles. Main endpoints for this interims analysis were hemato-, neuro- and nephrotoxicity. We tested for non-inferiority.