($P < 0.001$). Direct DNA sequencing of *C. psittaci* was performed in 10 OAL cases with *C. psittaci* infection, and 6 different sequences of *C. psittaci* were identified. However, infection rates of *C. trachomatis* and *C. pneumoniae* were very low in both OAL and NNOAD: *C. trachomatis* was not observed in any cases, and *C. pneumoniae* was found in 9% of OAL cases and in 4.7% of NNOAD cases ($P = 0.492$).
Conclusion: In this study, we observed high infection rate of *C. psittaci* in OAL cases. The results may suggest *C. psittaci* may play a role as a causative antigen to stimuli the development of OAL.

Poster presentations (Wed, 2 Nov)
Haematological malignancies

983 POSTER

Prevalence, incidence, risk factors and other anemia patterns in multiple myeloma patients: results from European Cancer Anaemia Survey (ECAS)

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Background: Although anemia is a common complication of multiple myeloma (MM) patients (pts), information on the evolution of anemia during follow up, relation with age and performance status, risk factors for its development and treatment practices was not available.
Methods: ECAS is a large, prospective, epidemiologic survey which enrolled 720 pts with MM of 15,370 pts with cancer at any stage of their disease. Survey data were collected for up to 6 data points or 6 months of scheduled visits. [1] Logistic regression modeling was applied to identify risk factors for anemia in ECAS lymphoma (L) and MM pts who were not anemic at enrollment ($n = 469$) and started on and receiving at least 2 chemotherapy (CT) cycles. [2]
Results: 28% of the 720 pts with MM were <60 years (yrs), 32% were 60 to 69 yrs and 40% were 70+ yrs old. Demographics included: 52% male, mean age of 65.7 yrs, and a mean Hemoglobin (Hb) level of 11.0 g/dL. Half of MM pts were on CT and 44% had a WHO score of 2–4. Data analysis showed 69% of MM pts were anemic (Hb <12 g/dL) at enrollment, with 30% Hb < 10 g/dL and 39% Hb of 10 to 12 g/dL. 85% were anemic at some time during the survey; 78% of those <60 yrs, 85% of those 60–69 yrs and 90% of those 70+ were ever anemic. Adverse WHO score correlated with low hemoglobin ($r = -0.346$). Despite the 59% of those who became anemic having a nadir Hb < 10 g/dL, 53% received no anemia treatment, 3% received iron, 21% transfusion and 24% received epoetin. 75% of CT pts became anemic during ECAS, 60% of those <60 yrs, 88% of those 60–69 yrs and 100% of those 70+ yrs. Logistic regression analysis of L/MM pts revealed 4 variables significantly predicting anemia development. They were assigned score values based on the respective adjusted odds ratios: Initial Hb (adjusted odds ratio (AOR) 4.2), persistent/recurrent disease (AOR 2.8), female gender (AOR 1.5), and treatment with platinum-based chemotherapy (AOR 5.5) were found to independently predict anemia ($P < 0.001$), with an area under the receiver operating characteristic (ROC) curve of 0.821 (95%-CI; 0.763–0.878), indicting acceptable predictive accuracy of the model. To help better identify the L/MM patients most likely to develop anemia, three levels of risk (low [24%], moderate [51%], and high [72%]) were calculated from the model scores ($\chi^2_{(2)} = 112.6$; $P < 0.001$). [2]
Conclusions: Prevalence of anemia was high (69.2%), increased with age, correlated with poor WHO score; anemia was found in 85.3% of pts at least once during the 6 months survey. The identification of predictors of anemia allows early intervention with appropriate anemia treatment in order to optimize overall patient care.

References

[1] Ludwig H, *et al.* The European Cancer Anaemia Survey (ECAS). *EJC* 2004; 40 (15): 2293–2307.
[2] Ludwig H, Van Belle S and Gascon P. Development, prediction and treatment of anemia in patients with lymphoma/multiple myeloma: finding of two European surveys (ECAS and BEPOS). *Blood* 2004; 104: 856a (abstract 3133).

984 POSTER

Novel anti-cancer compounds – jasmonates, kill leukemic cells from chronic lymphocytic leukemia patients: selectivity and mechanism of action

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Background: Jasmonates have recently been shown by us to be a novel class of anti-cancer agents in vitro and in vivo. We found that jasmonates killed various types of cancer cells while sparing normal lymphocytes. Many chemotherapeutic drugs induce mitochondrial membrane permeability transition, membrane depolarization, osmotic swelling, and release of cytochrome C, involving the opening of the mitochondrial permeability transition pore complex (PTPC), and resulting in cell death. Since jasmonates exert their cytotoxic effects independent of transcription, translation and p53 expression, we hypothesized that these compounds act directly on mitochondria, and that this may be the basis for their selective activity against cancer cells.
Methods: Blood cells were purified by density gradient centrifugation. Three-color FACS analysis determined the percentage of leukemic cells in blood samples from chronic lymphocytic leukemia (CLL) patients. Mitochondrial membrane depolarization was determined by flow cytometry, and cytochrome C release by Western blotting analysis. Mitochondria were isolated by mechanical lysis and differential centrifugation. Cytotoxicity was measured by a tetrazolium-based assay, and mitochondrial swelling by spectrophotometry.
Results: A correlation was found between the ex-vivo cytotoxicity of methyl jasmonate (MJ), and the percentage of leukemic cells in the blood sample of the respective CLL patient. Moreover, exposure of blood cells from CLL patients to MJ caused the preferential death of the leukemic cells (CD5+/CD19+). MJ and additional jasmonates induced membrane depolarization in CLL cells. In addition, jasmonates induced swelling and release of cytochrome C in mitochondria isolated from CLL cells, but not in mitochondria isolated from 3T3 non-transformed cells or from normal lymphocytes, in a manner dependent on PTPC opening.
Conclusions: Jasmonates act directly on mitochondria derived from CLL cells in a PTPC-mediated manner, and could therefore bypass pre-mitochondrial apoptotic blocks. Also, jasmonates are endowed with the unique capability to selectively damage mitochondria from transformed cells (reflecting probably specific characteristics of mitochondria in cancer cells), resulting in preferential killing of cancer cells. Thus, we predict that jasmonates might be devoid of side-effects; and propose that they are promising candidates for the treatment of CLL and other types of cancer.

985 POSTER

Naturally occurring tyrosine kinase inhibitor, genistein, exerts distinct anti-leukemia mechanisms in AML and APL cells

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Background: Acute myeloid leukemia (AML) in general, has a poor long term outcome even after intensive debilitating chemotherapy. Novel and less toxic therapy are urgently needed. Genistein, an isoflavone which is a naturally occurring tyrosine kinase inhibitor present in soybeans has been shown to be an active agent in APL (NB4) harboring PML-RAR fusion gene in our previous study (Khan et al. Blood 104: 692a, 04). We extend our study to test genistein in other AML cell lines with different doses and time points with the aim to elucidate biological pathways affected by genistein in APL and AML cells using DNA microarrays.
Material and methods: Leukemia cells were cultured in RPMI 1640+10% FBS. Cell growth and apoptosis were measured and compared with untreated group. Gene expression analysis was carried out with Affymetrix human genome HU133Av2 chip. Data analysis was done using R and GeneSpring softwares.
Results: Genistein inhibited NB4, HL-60, K562, KG-1 and NOMO-1 growth (IC50 20–30 μM) equally well in dose and time dependent fashion from 20 to 50 μM in 24, 48 and 72 hours. Flow cytometry showed treated cells were blocked at G2/M followed by apoptosis. Two cell lines, HL60 and NB4, representing AML and APL were chosen for DNA microarray studies. Interestingly, gene expression profiles in HL60 varied greatly from NB4 cells. 684 and 364 genes were differentially regulated by more than 2-fold in HL60 and NB4, respectively. However, only 26 genes of these are in common. Although MAPK signaling and apoptosis pathways are among the most affected pathways by genistein treatment, the patterns differ significantly between HL60 and NB4 cells. In HL60, FGFR1 and Ras were activated, leading to the activation of transcription factors Jun and FOS, resulting in predominant signaling for differentiation. In NB4, TGFβ,

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