Results: We identified 116 pts, which represents about 2% pts. 56% were female. Age was from 1.4 to 19.7, median 15y. There were 40 events: 1 toxic death, 17 no complete remission with the protocol and 22 relapses. 25 pts died. One major difficulty was the interpretation of a residual mediastinal mass. Prognostic factors were: LDH level >500 in the BFM series, and the association of size>10 cm+LDH > Nx2 in the FAB LMB96 series.
Conclusions: PMLBL is rare in children and national series are small, not allowing to draw clear conclusions. Data from the different databases will be extracted, merged and presented. The pooling of these data should enable a better description of these pts, and improve the analysis of events and prognostic factors, and will be the basis for a collaborative prospective study.

979 ORAL

Chemotherapy followed by low dose radiotherapy in childhood Hodgkin disease; retrospective analysis of results and prognostic factors

G.V. Arruda[1], P.E.R.S. Novaes[1], M.S. Castilho[1], R. Ferrigno[1], J.V. Salvajoli[1], M.A.C. Maia[1], R. Fogaroli[1], A.C.A. Pellizzon[1], C.G. Antoneli[3], W. Moura[3]. *[1]Hospital do Cancer A C Camargo – Brazil, Department of Radiation Oncology, Sao Paulo, Brazil; [2]Hospital do Cancer A C Camargo – Brazil, Department of Pediatrics, Sao Paulo, Brazil*

Purpose: To report on treatment results and prognostic factors of young patients with Hodgkin's disease treated with chemotherapy (CT) followed by low dose radiotherapy (RT).
Materials and Methods: This retrospective series analysed 166 patients under 18 years of age, treated from January 1985 to December 2003. Median age was 10 years (range 2–18). The male to female ratio was 2.3: 1. Adenomegalia was the most frequent complaint (68%), and the time of symptom duration was smaller than 6 months in 55% of the patients. In histological analysis Nodular Sclerosis was the most prevalent type (43%) followed by Mixed Celularity (41%). The disease was restricted to two nodal group (stage II) in 60% and to adjacent groups in 55% (stage III). The most frequent site of metastasis ware bone marrow (38%) and lungs (42%). Standard treatment consisted of chemotherapy (drug combination varied according to the current treatment protocol). Radiotherapy consisted of 21 Gy dose in 17 fractions in the majority of patients (90.2%), delivered to involved field or mantle field. 13.86% patients did not receive RT. Median follow-up was 101 months (mean 109, range 29–237).
Results: The Overall Survival (OS) and Event Free Survival (EFS) in 10 years were 89% and 82%. Survival according to clinical stage was 94%, 94%, 91% and 72% for stages I to IV ($p = 0.0215$). Ten years OS was 91% for patients who received RT and 76% for patients who did not ($p = 0.001$). Multivariate analysis showed presence of B symptoms and low platelet count to be associated with a worse prognosis.
Conclusions: This study shows that combining chemotherapy and low dose RT is effective in treating childhood HL, providing high cure rates (89% in 10 years), and disease control. So far it is not possible to exclude RT from treatment. And yet, attention to platelet count should be payed in order to improve survival. B symptom presenting children may be involved in more aggressive protocols so survival can be improved.
As the disease is highly curable, any data of long term follow-up should be presented in order to better direct therapy, improving outcome and lowering side effects.

980 ORAL

Suggestion of TNM staging system for the angiocentric T-cell and nasal type NK/T cell lymphoma

K. Kim[1], E. Chie[1], C. Kim[2], I. Kim[1,3,4], C. Park[1,3]. *[1]Seoul National University College of Medicine, Radiation Oncology, Seoul, Korea; [2]Seoul National University College of Medicine, Pathology, Seoul, Korea; [3]Institute of Radiation Medicine, Medical Research Center, Seoul National Univers, Seoul, Korea; [4]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea*

Background: To do comparative analysis of outcome of angiocentric T-cell and nasal type NK/T cell lymphoma after radiotherapy (RT) for appropriate staging of prognostic value.
Patients and methods: Between February 1989 and March 2001, 60 patients, with newly diagnosed angiocentric T-cell and nasal type NK/T cell lymphoma of Ann Arbor stage I and II involving the head and neck, underwent RT. There were 42 males and 18 females and the median age was 45 years. Twenty-five of them were treated with combined chemoradiotherapy (CRT), while 35 with RT alone. The tumors in the nasal cavity or paranasal sinuses were classified as the nasal cavity group (NC group; 35 cases), and those found in other regions as the non-nasal cavity group (NNC group; 25 cases). The median follow-up period was 74 months.
Results: The 5-year survival rate (5YSR) was 69%. The NC group was superior to the NNC group in 5YSR without significance (75% vs. 60%; $p = 0.40$). When the tumors were restaged by the AJCC TNM system of nasal cavity cancer in the NC group, patients with T1–2 tumors have not reached the time of median survival, whereas median survival time of T3 and T4 tumors was 50 and 10 months, respectively ($p = 0.013$). In the NNC group, however, Ann Arbor stage was relatively accurate in predicting the treatment outcome. The 5YSR of Ann Arbor stage I and II was 76% and 31%, respectively ($p = 0.060$).
Conclusions: Our results suggest that TNM stage of the nasal cavity cancer might be appropriate in predicting the treatment outcome in the NC group of the angiocentric T-cell and nasal type NK/T cell lymphoma rather than Ann Arbor system.

981 ORAL

N-CoR is a target of a serine protease specifically activated in acute promyelocytic leukaemia (APL)

M. Khan. *National University of Sinagpore, Oncology Research Institute/Medicine, Singapore, Singapore*

Acute promyelocytic leukaemia (APL), which is caused by fusion protein PML-RAR, is characterized by accumulation of immature myeloid cells arrested at the stage of promyelocytic development. APL tumour cell exhibits disintegration of nuclear domains known as PODs (PML oncogenic domains) while treatment of APL patients with retinoic acid (RA) results in clinical remission associated with reorganization of PODs. We recently identified that PML-RAR promotes accumulation of mis-folded nuclear hormone receptor co-repressor (N-CoR) in Endoplasmic Reticulum (ER). Here, we report that N-CoR is proteolytically processed in APL tumor cell, while N-CoR in non APL cells showed no sign of processing. Cellular lysate of APL tumor cell NB4, as well as that of human APL primary cell, contain an activity that cleaved the N-CoR protein.
Expression analysis using RNA prepared from APL and non APL cells revealed selective expression of the activity in APL tumor cell. It is likely that mis-folded N-CoR in the ER becomes a target of cellular protease that is activated in response to accumulation of mis-folded protein. Biochemical purification of the activity from the NB4 cell and its spectrometric analysis revealed that the N-CoR cleaving activity is a serine protease. Through small scale screening of known protease inhibitors, we identified a specific agent capable of inhibiting the activity and the processing of N-CoR in NB4 cell. Treatment of NB4 cells with the protease inhibitor, as well as with retinoic acid (RA) stabilizes the N-CoR protein, suggesting a role of N-CoR in the differentiation of promyelocytic cells. Indeed, down regulation of N-CoR through RNAi abrogated RA induced differentiation of NB4 cells. Targeting the N-CoR cleavage activity with its inhibitor promotes apoptosis and differentiation of NB4 cells, suggesting a crucial role of the protease in malignant transformation of APL tumor cell.
We have identified a previously uncharacterized protease that appears to be crucial for transformation of promyelocytic cells. Moreover, we have identified an agent capable of inducing differentiation and apoptosis of APL tumor cells through targeting the cleavage of N-CoR protein. These finding will improve our understanding about the pathogenesis of APL and will lead to the designing and development of newer diagnostic and therapeutic measure.

982 ORAL

Ocular adnexal lymphoma is highly associated with Chlamydia psittaci

C. You[1], M. Ryu[2], J. Huh[3], J. Park[2], H. Ahn[4], Y. Lee[4], T. Kim[2], H. Chang[2], J. Lee[2], Y. Kang[2]. *[1]Asan Medical Center, Seoul, Korea; [2]Asan Medical Center, Internal Medicine, Seoul, Korea; [3]Asan Medical Center, Pathology, Seoul, Korea; [4]Asan Medical Center, Ophthalmology, Seoul, Korea*

Background: Ocular adnexal lymphomas (OAL) are mostly of low-grade MALT type. Recently, an association between *C. psittaci* and OAL was suggested (Ferreri AJM et al. J Natl Cancer Inst 2004;96:586). We conducted this study to confirm the relationship between *C. psittaci* and OAL.
Methods: Between 1993 and 2004, a total of 33 OAL cases were identified in Asan Medical Center, Seoul, Korea. DNA was extracted from formalin-fixed, paraffin-embedded OAL tissues, and then touchdown enzyme time release-PCR was performed to identify three Chlamydia species (*C. psittaci, C. tracomatis,* and *C. pneumoniae*). DNA extraction and PCR for Chlamydia species were also performed in 21 cases with non-neoplastic ocular adnexal disease (NNOAD).
Results: In all OAL cases, histologic type was low-grade MALT lymphoma. The median age was 42 yrs (range, 22 to 73 yrs). Male to female ratio was 1.1. *C. psittaci* was highly associated with OAL: *C. psittaci* was found in 78% of OAL cases, while it was observed only in 23% of NNOAD cases

($P < 0.001$). 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)

H. Ludwig[1]. *For the ECAS investigators (Hospital Clinics, Cancer Centers, Europe).* [1]*Wilhelminenspital, Center for Oncology and Haematology, Vienna, Austria*

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

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984 POSTER

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

O. Fingrut[1], D. Blickstein[2], M. Shaklai[2], A. Harel[1], E. Flescher[3].
[1]*Sepal Pharma, Ness-Zionna, Israel;* [2]*Rabin Medical Center Beilinson Campus, Felsenstein Medical Research Center, Petach Tikva, Israel;* [3]*Sackler Faculty of Medicine, Tel Aviv University, Department of Human Microbiology, Tel Aviv, Israel*

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

C. Chen, S. Wong, B. Tay, J. Shen, M. Khan, J.-H. Han. [1]*National University of Singapore, Singapore, Singapore*

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,