Conclusions: The hepatic biopsy change the NHL stage in an important number of patients (5) even in those without clinical or analytic hepatic abnormalities. In these group of patients the value of hepatic biopsy was similar to bone marrow biopsy also with a greater morbidity.

Multiple myeloma

346 POSTER

Incidence and prognostic significance of deletions of tumor suppressor genes and aneuploidies in multiple myeloma: an interphase FISH study

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Recent studies indicate that chromosome aberrations are important prognostic factors in patients with multiple myeloma. In the present study, we performed interphase fluorescence in situ hybridization (FISH) in consecutively ascertained samples from 32 patients with multiple myeloma treated in our department from 1990 to 1999. In each case, a panel of 10 different FISH probes hybridizing to the chromosomal regions 8q24/CMYC 9p21/P16, 11q13/CyclinD1, 11q22, 13q14/D13S25, 17p13/P53 and to the centromeres of chromosomes 3, 7, 9, and 11 was applied. A simultaneous applied control probe served as an internal control for the hybridization efficiency. In addition to the patients' samples, 8 normal control samples were analyzed to define the cut-off levels for the probes under investigation. The FISH results were correlated with clinical data and the overall survival of the patients.

The majority of cases showed alterations of at least one locus analyzed (28 of 32 cases). The following abnormalities were detected: monosomies of 17p13/P53 in 11 of 31 cases (35%), monosomies of 13q14/D13S25 in 10 of 31 cases (32%), trisomies of 8q24/CMYC in 10 of 31 cases (32%), trisomies of 11q13/CyclinD1 in 10 of 32 cases (31%), trisomies of 11q22 in 7 of 31 cases (23%), trisomies of 9p21/P16 in 7 of 32 cases (22%), and trisomies of centromeres 3, 7, 9, and 11 in 11 of 32 (34%), 6 of 31 (19%), 4 of 29 (14%), and 10 of 32 cases (31%), respectively.

Deletions of D13S25 (18 vs. 30 months median survival, p 0,017) and P53 (20 vs. 32 months median survival, p 0,03), and overrepresentations of CMYC (16 vs. 30 months median survival, p 0,005) were associated with a significantly shorter overall survival as compared to patients without these aberrations.

The present study underlines the importance of chromosomal abnormalities in the assessment of the prognosis of patients with multiple myeloma.

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Spontaneous secretion of matrix metalloproteinases, urokinase plasminogen activator, its receptor and cytokines in primary long-term bone marrow cultures of multiple myeloma patients

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Purpose: Matrix metalloproteinases (MMP's), urokinase plasminogen activator (uPA), its receptor (uPAR) play a key role in dissemination of several cancers, and cooperate with cytokine network. There are only very limited report on their involvement in pathology of multiple myeloma (MM), a bone devastating neoplasia. We investigated spontaneous secretion of MMP's, uPA, uPAR, and cytokines by myeloma marrow microenvironment in primary long-term bone marrow cultures (LTBMC) in MM patients.

Methods: LTBMC's were established from bone marrows of 19 newly diagnosed myeloma patients, and 18 donors without any malignancy and any skeletal disease. Conditioned media from LTBMC's were analysed by gelatine zymography. Total MMP-1, MMP-2, MMP-3, MMP-9, uPA, uPAR, IL-6, IL-1beta, IL-10, IL-11 and TNF-alpha were measured by ELISA.

Results: The proteolytic activity corresponding to proMMP-2, proMMP-9 and their active forms was found by gelatine zymography in all cultures. Myeloma cultures secreted significantly more MMP-2, IL-6 and IL-10 than control cultures, but less MMP-3, MMP-1, IL-11 and TNF-alpha. MMP-1, MMP-9, uPAR, IL-6, IL-1beta levels were positively correlated with percentages of malignant plasma cells in bone marrows, however there were no differences in MMP-9, uPA, uPAR and IL-1beta levels between cultures. Further MMP-1 and MMP-9 levels in myeloma LTBMC's were positively correlated with uPAR and IL-1beta.

Conclusion: Spontaneous secretion of MMP's by bone marrow microenvironment is significantly disturbed in MM and could reflect angiogenic potential of malignant plasma cells. The study gives reason for introduction of synthetic selective matrix metalloproteinase inhibitors into therapy of multiple myeloma.

348 POSTER

The Antwerp experience with thalidomide in relapsed/refractory multiple myeloma

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Background: Barlogie and coworkers showed the marked anti-tumor effect from thalidomide in high risk refractory multiple myeloma (MM) in 1998.

Aim of the study: To evaluate in a retrospective way the effect of thalidomide in patients with relapsed/refractory MM.

Patients: Since 12.07.1999 51 patients with relapsed/refractory MM have been treated; 50 are evaluable. The mean age is 69 years with the youngest being 45, the oldest 87; 26 are males, 24 are females. Before the start of thalidomide, patients were treated with an average of 4 different schemes of chemotherapy and/or irradiation (1-18). Fifteen patients underwent an autologous transplant; one patient was transplanted twice. The distribution of the different subtypes is as follows: IgG k (24), IgG l (11),IgA k (6), IgA l (4), light chain k (2), light chain l (1) and unknown (2).

Treatment: Ten patients were treated for 14 days or less at the moment of evaluation and are not included for further evaluation. The mean duration of treatment in the other patients is 176 days, with a minimum of 15 and a maximum of 448 days. The average oral dosage was 200 mg daily (100-400).

Results: Of the 40 evaluable patients, 15 reached a partial response (PR) (37.5%), 3 a good PR (GPR) (7.5%), 13 had stable disease (32.5%) and 9 developed progressive disease (PD) (22.5%).

Discussion: The effect of thalidomide can be quite dramatic in a positive sense. Moreover, the majority of patients only complained of mild side-effects.

Bone marrow transplantation/cytokines

349 POSTER

Dose intensified chemotherapy with growth factors, in patients with advanced cancer

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Background: Inadequate drug dose is a major factor responsible for the failure of chemotherapy (CT). Carboplatin (CBDCA), Cyclophosphamide (CTX) and Etoposide (VP-16) are active drugs in many solid tumors. In a previous phase I study (Clin Ter 148:201-207,1997) we found the maximum tolerated dose of the three drugs given in combination with growth factors. In another study we ascertained the value of the sequential administration of G-CSF and GM-CSF (Am J Clin Oncol 20:209-214,1997) after dose intensified (HD) CT. Aim of this phase II study was to find the activity and toxicity of HDCT in a group of patients with advanced tumors.

Patients and Methods: A group of 48 patients, were entered in this phase II study of HDCT from 1-93 to 11-99. Patient's characteristics: median age was 52 years (range 18-75). Patients had been treated previously as follows: surgery-37, radiotherapy-7, all had received CT (total 330 courses, median-6 courses/patient, range 4-13). Diagnosis: 26-breast cancer, 13-lung cancer, and 9 miscellaneous tumors. 21 patients had metastatic disease at the diagnosis, 16 patients had a median disease free interval of 18.5 months before developing metastases, while 11 patients had inflammatory breast cancer. Patients received two consecutive courses of the same CT, over 3 days, with CTX and VP-16 (1500 mg/m2 and 400 mg/m2 respectively). CBDCA was administered at the AUC of 8. After CT patients received a sequential combination of growth factors (G-CSF and GM-CSF, both at the

dose of 5 mcgr/kg/day, for 7 days each. The 48 patients received a total of 96 cycles of HDCT.

Results: Toxicity: grade IV leukopenia and thrombocytopenia were observed in all patients. Twenty episodes of febrile neutropenia were observed. Transfusions: platelet to 13 patients, red blood cells to 14 patients. No treatment related death occurred. Complete response in 17 patients (35.4%), partial response in 13 patients (27.1%), for an overall response rate of 62.5% (95% confidence interval 47.3%-76.01%). Stable disease was observed in 6 patients (12.5%) and progressive disease in 12 patients (25%). Median TTP was 9 months (range 1-91). Median survival was 15.1 months (range 3-91). 1 and 3-year survival rate were 58% and 30%, respectively, after a median follow-up of 35 months.

Conclusions: These data indicate that dose intensified CT may be delivered safely without bone marrow or peripheral stem cell support.

350 POSTER

IL2 effect on T lymphocyte phenotype of PBPC from healthy donors and patients

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Interleukin 2 (IL2) can increase the anti-tumoral activity of peripheral blood progenitor cells (PBPC) used in autologous haematopoetic transplantation. The aim of this work was to evaluate the in vitro activation of T lymphocytes (lymps) from PBPC of healthy donors (HD) (n=6) and patients (n=4) following IL2 incubation. The patient diagnosis were Non Hodgkin Lymphoma (2), Acute Myeloid Leukemia (1) and Hodgkin's Disease (1). PBPC were incubated for 24hrs with 1000 U/ml IL2 and the cells immunophenotypes, pre and post incubation, were evaluated by flow cytometry, using 4-colour staining.

In this study, PBPC from HD were used as controls and when gated on CD3+ lymps the % of positive cells (median values) were: CD2bright 51; CD4 51; CD7 bright 48; CD8 39; CD16 0.6; CD25 17; CD28 84; CD56 6; CD57 13; CD69 5; CD94 5; HLA-DR 16; Granzyme B 15; NKb1 1 and the CD4/CD8 ratio was 1.4. When gated on CD8+ lymps the % of CD25+ and CD122+ cells were 5 and 7 respectively. Following the IL-2 incubation we observed a significant increase ($p<0.05$) in the % of cells expressing the following antigens (pre-post IL2 incubation): CD69+ 5-33; CD69+ CD56-CD16- 3-32; CD8+ 39-44 and CD8+CD28+ 29-36. We also detected a reduction in the % of cells which were: CD4+ 51-45; CD8+CD122+CD25-6-0.4. There was also a decrease in the CD4/CD8 ratio 1.4-1. So far, no significant changes in the % of cells expressing other antigens were observed.

In comparison with HD, patients T lymps showed increased % of cells expressing CD57, HLA-DR, Granzyme B and CD8+ cells with an inverted CD4/CD8 ratio. After IL2 stimulation patients T lymps showed an increase in the % of CD69+ cells, in particular in the same population as the HD (CD3+ CD56- CD16-). For the other antigens studied we did not observe any other variation trend.

In summary, our preliminary results show that IL2 incubation leads to some changes in T lymphocyte phenotype in particular a significant increase in %CD69+ cells both in HD and patients. Our results are in agreement with previous reports showing that CD69 is expressed shortly after T cell activation. Since, CD69 has been associated with an increase in T cell cytotoxicity, it seems that the IL2 incubation of PBPC will rise the cytotoxic potential of the graft.

Bone marrow and stem cell supported therapies

351 POSTER

A phase I study of high-dose paclitaxel for stem cell mobilization in patients with metastatic breast cancer

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Background: Infusion of large numbers of peripheral blood stem cells to support high-dose chemotherapy has been correlated with milder toxicity. Patients with prior exposure to multiple courses of chemotherapy have difficulties to mobilize stem cells. There is a need for better stem cell mobilization strategies that reliably yield sufficient numbers of stem cells even in heavily chemotherapy-pretreated patients. Paclitaxel (PTX) at a dose of 200 mg/m2 in combination with cyclophosphamide is an effective mobilization inducer. However, there is a lack of data on the mobilizing effect of different doses of PTX alone, and the optimal mobilizingdose remains to be established.

Methods: Twelve patients (pts) with metastatic breast cancer and a median number of 9 prior courses of chemotherapy (6 adjuvant + 3 for metastases) were enrolled in an ongoing phase I trial of tandem high-dose chemotherapy consisting of: A) 1 course of high-dose PTX (400-650 mg/m2) delivered by 24-hour infusion (day 1) with G-CSF: 10 mcg/kg/day s.c. on days 3-12 (and ciprofloxacin 750 mg BID po on days 3-12), with stem cell apheresis beginning on day 11; followed by B) 1 course of high-dose chemotherapy (CTCb) with stem cell support. Patients were discharged as soon as the PTX infusion was completed.

Results: Median numbers of cell harvested for pts treated with each of the four dose levels of PTX tested were as follows:

PTX dose mg/m2	Total MNC/kg	Total CD34+/kg	Days of apheresis 1st apheresis	MNC/kg in 1st apheresis	CD34+/kg in
400: 3pts	11.4	10.1	2	6.4	6.2
500: 3pts	7.2	14.6	1	7.2	14.6
600: 3pts	6.6	17	1	6.6	17
650: 3pts	7	3.8	3	3	1.4

The study is still ongoing since maximun tolerated dose of PTX has not been reached yet. Only 1 pt treated with 650 mg/m2 had to be admited for neutropenic fever.

Conclusion: the optimal dose of paclitaxel for mobilization of maximum numbers of peripheral blood stem cells in pts with metastatic breast cancer is 500-600mg/m2.

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Increased renal toxic effect of total body irradiation in combination with radioimmunotherapy

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Purpose: Renal toxicity of total body irradiation (TBI) alone and with radioimmunotherapy (RIT) in conditioning regimes for bone marrow transplantation (BMT) was examined retrospectively. Since 3/98 we have performed additional RIT with Re-188-labeled anti-CD66 monoclonal antibody for high-risk leukemia patients. Dosimetry showed an uptake of labeled 188Re-Mab not only in the bone marrow but also in the kidneys.

Methods: From 10/97-11/99 58 adults underwent TBI for BMT and survived at least 9 months posttransplant. We performed TBI alone in 35 patients (15 female, 20 male, median age 40.5 y (17 - 60 y)). 23 patients with high risk-leukemia (8 female, 15 male, median age 47 y (16 - 58 y)) received additional RIT. All patients had normal serum creatinine values (female <96 micromol/l, male <110 micromol/l) before conditioning. We evaluated serum creatinine levels 6, 9 and 12 months after transplantation and their association with the application of nephrotoxins and the delivered dose to the kidney.

Results: The mean kidney dose due to the radiolabeled antibody was 8.3 Gy (2.3 - 11.6 Gy). The additive dose of TBI was reduced using renal shielding to 6 Gy. Patients who underwent TBI alone received 12 Gy to the kidney (2 x 2 Gy daily). 4/35 patients with TBI and 16/23 patients with TBI and RIT subsequentely had increased serum creatinine levels. Before transplantation and after 6, 9 and 12 months the median creatinine was 77.14 mmol/l (11.01), 85.66 mmol/l (20.58), 85.00 mmol/l (17.95), 89.39 mmol/l (20.18) for TBI alone and 78.22 mmol/l (12.63), 119.33 mmol/l (35.72), 126.95 mmol/l (32.78), 149.47 mmol/l (69.30) for TBI and RIT. Foscavir, Gancyclovir or Cidofovir were given to 12/35 patients with TBI over a median period of 2.83 months and to 12/24 patients with TBI and RIT over a median period of 4.08 months. 8 patients with TBI and 3 patients with TBI and RIT received amphotericin. Median delivered dose to the kidney by RIT in patients with pathologic serum creatinine was 7.25 Gy (2.3 - 11.5 Gy) and in patients with normal serum creatinine 9.4 Gy (5.2 - 11.6 Gy).

Conclusion: The incidence of severe bone marrow transplantation nephropathy is low, when TBI is performed with a dose of 12 Gy, fractionated 2 x 2 Gy daily. Intensifying the conditioning regime by RIT for high-risk patients, exposed to several nephrotoxins increases the risk of renal damage.