79%, and 96% respectively. Similar results were obtained for the validation sample, including 215 patients. In the total population, 90% of patients with a monoclonal gammopathy could be classified correctly as having MM or a non-myeloma condition.

Conclusion: The Myeloma Risk Score can identify patients with a paraproteinaemia at risk for MM. These patients are candidates for bone marrow and X-ray examination.

401 POSTER

Treatment of peripheral blood progenitor (PBPC) harvests by two-stage immunomagnetic selection: Yields comparable to positive selection alone

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Purpose: To determine the feasibility of sequential positive and negative selection to enrich haemopoietic progenitors and deplete tumour cells from PBPC's for rescue following high-dose chemotherapy.

Methods: Aliquots of 9 PBPC harvests (0.59–2.3 × 1010 cells) were processed by immunomagnetic selection using antibodies (Ab's) to CD34 for enrichment of haemopoietic precursors, followed by depletion of tumour cells using a cocktail of Ab's to either 5 lymphoid or 3 epithelial antigens for lymphoma (5 pts) or breast cancer (4 pts) respectively. Numbers of CD34+ cells were measured by flow cytometry and CFU-GM enumerated in the apheresis product and at each stage of the procedure.

Results: Initial mean concentration of CD34+ cells was 1.73% (±0.81), increased to 90.8% (±8.25) following enrichment and 92.6% (±7.05) after both stages. CFU-GM were enriched a mean 241-fold (±132) in the final product. For the enrichment step the mean yield of CD34+ cells was 34.5% (±11.8), for depletion 92.1% (±6.7). Overall mean yield of CD34+ cells was 33.6% (±9.15). By projecting numbers of CD34+ cells from the total harvest on 2 days apheresis, all 5 patients with lymphoma and 1 patient with breast cancer would have had sufficient numbers (>2 × 106/kg) for rescue after both stages. The proportion of CD34+ cells in the apheresis product was a good predictor of adequate numbers of cells remaining after processing, with a cut-off at 1%.

Conclusion: Two-stage selection of PBPC gives yields of early progenitors very similar to single stage enrichment, suggesting that this is a feasible method for in vitro treatment to remove tumour cells.

402 POSTER

Treatment of multiple myeloma with short-term infusion of liposomal daunorubicin in combination with vincristine and dexamethason

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The continuous infusion of vincristine and doxorubicin over 96 h in combination with oral dexamethason (VAD) is one of the most effective treatment options for patients with relapsed or primary refractory multiple myeloma. On the other hand, a retrospective analysis of quality of life in patients treated with VAD in our clinic showed that this regimen which requires a central venous catheter and hospitalization in most cases is associated with considerable inconvenience. We have initiated a phase I/II trial with a modification of this protocol by replacing doxorubicin with short-term infusion of liposomal encapsulated daunorubicin, which provides sustained intracellular anthracycline levels. Furthermore, the rate of alopecia and cardiotoxicity of liposomal daunorubicin seems to be substantially lower. Patients receive a bolus injection of vincristine 1 mg/m^2 on day 1, and 40 mg dexamethason on d1–4, 9–12 p.o. The starting dose of liposomal daunorubicin is 40 mg/m^2 and will be escalated interindividually in 10 mg/m^2 steps. The treatment courses are repeated every three weeks.

Quality of life is assessed with special emphasis on bone pain and preliminary results indicate an advantage over VAD. Multiple clinical and laboratory parameters of disease activity, number and immuno-phenotype of the myeloma cells in the bone marrow, P-glycoprotein expression, functional assays of the multidrug resistance are measured before and during the chemotherapy course to identify subgroups of patients with different probabilities of remission and survival within this protocol.

403 POSTER

Correlation between the number of CD34+ cells reinfused and complications and mortality of high-dose chemotherapy with stem cell support

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Reinfusion of peripheral blood autologous stem cells (PBSCs) allows hematologic recovery after high-dose chemotherapy (HD-CHT). There is an inverse correlation between the number of CD34+ cells and the duration of aplasia. There is little information as to the clinical correlates of this observation, that is, are the severity of complications (comp) and the mortality affected by the number of CD34+ cells infused?. The severity of comp in 45 consecutive patients (pts) treated with HD-CHT+PBSC at our institution (1995–6) was evaluated by an investigator unaware of the CD34+ counts, and subsequently correlated with the number of CD34+ cells reinfused. Pts with mild comp were those with fever lasting <48 hours, grade 0–2 mucositis and diarrhoea and no need of total parenteral nutrition. Pts with severe comp were those with grade 4 diarrhoea plus either peritonitis or sepsis of intraabdominal origin, and those who required admission in the Intensive Care Unit. All others were labeled intermediate comp. Eighteen pts had mild comp, 19 had intermediate comp and 8 had severe comp. The median numbers of CD34+ cells × 10^{-6}/Kg infused were 4.3 (range 2.3–18.5), 3.7 (2.2–16.5) and 2.9 (2–5.3) respectively ($p < 0.05$ mild vs severe). Since the evaluation of comp is subjective, we then compared CD34+ cell numbers in pts with toxic death (5 pts) vs those who survived (40 pts). Medians were 2.6 (2–5.3) and 3.7 (2.2–18.5) ($p < 0.05$). None of 12 pts who received >5.5 × 10^6 CD34+ cells had severe comp vs 24% of those who received <5.5. Infusion of high numbers of CD34+ PBSCs not only results in shorter aplasia but also in milder extrahematologic comp and lower mortality

404 POSTER

High-dose therapy with peripheral blood progenitor cell (PBPC) autografting in multiple myeloma (MM)

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Purpose: Dose-escalated therapy combined with autografting improves the response rates and survival in patients (pts.) with MM (Attal et al. 1996).

Methods: In a prospective study we treated MM-pts. with high-dose cyclophosphamide or ifosfamide/mitoxantrone followed by filgrastim (R-metHuG-CSF, 300 μg/day) and myeloablative therapy consisted of total body irradiation (TBI) + Melphalan (MEL) or MEL alone.

Results: 131 pts. have been transplanted. Autografts contained a median of 3.3 × 10^6 CD34$^+$ cells/kg BW (range 2.0–29.0). A neutrophil count of 0.5 × 10^9/l and an unsubstituted platelet count of >20 × 10^9/l was reached after a median of 14 days (range 9–22) and 11 days (range 5–157), respectively. Two pts. died of transplantation-related complications. As a result of HD-therapy, the remission status (EBMT criteria) in 60 pts. was improved. The median event free survival period was 23 months. The median overall survival (OS) has not yet been reached. We found no difference in EFS and OS between the two high-dose treatment regimens (TBI + MEL vs. MEL).

Conclusion: >2.0 × 10^6 CD34$^+$ cells/kg BW predicts a rapid hematopoietic reconstitution in MM patients. The functional capacity of CD34$^+$ cells is not influenced by treatment before PBPC mobilization. To improve the results of HD-therapy, we have started a multicenter protocol using CD34$^+$ selected PBPC for tandem autografting followed by α-interferon maintainance therapy.

405 POSTER

Apoptosis induction by fludara and anti-Fas monoclonal antibodies on B-chronic lymphocytic leukemia cells

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Purpose: Apoptosis occurs in responce to many different stimuli. We examined the induction of apoptosis by Fludara (Fludarabine phosphate) in vitro against freshly isolated B-chronic lymphocytic leukemia (B-CLL) cells

and in vivo in B-CLL patients. We also studied the expression and function of CD95 (Fas/APO-1) antigen in B-CLL cells with the help of anti-Fas monoclonal antibodies (Mabs).

Methods: Apoptosis was investigated using flow cytometric method of measurement of hypodiploid DNA, labelled with propidium iodide. Antigen expression was studied by indirect immunofluorescence assay using flow cytometry (FACScan, Becton Dickinson).

Results: Fludara activated in vitro apoptosis on freshly isolated B-CLL cells after 24 hours and in vivo on 3–6 days after the start of treatment. CD95 antigen was expressed on minority of B-CLL cells and this expressi modified during treatment Fludara. Anti-Fas Mabs induced apoptosis in Fas(+) cells.

Conclusion: Thus, Fludara may induce apoptosis in vitro and in vivo in B-CLL cells and activation of cell death is an indicator of susceptibility of B-CLL cells to chemotherapy.

406 POSTER

Low incidence of myelodysplasia (MDS) or acute leukemia (AML) after autologous blood or marrow transplant without total body radiation (TBI) for lymphoma

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Purpose: To evaluate the incidence of MDS/AML in long term survivors following high dose chemotherapy and ABMT for relapsed or refractory Hodgkin's disease (HD) or non Hodgkin's lymphoma (NHL), using a regimen that does not employ TBI.

Methods: From 12/86 to 11/96, 335 pts (160 HD, 175 NHL) received etoposide 60 mg/kg + melphalan 160–180 mg/m^2 supported by autologous BM or PBPCs; 54 NHL pts also received TBI 2 Gy bid x 6 fractions. 150/305 pts are continuously disease free (DF) > 1 year post ABMT (median F/U 45 months, range 12–116).

Results: Seven pts have developed MDS (5) or AML (2) post ABMT; one pt developed MDS following XRT for local relapse and the other 6 remain DF. None of the MDS/AML pts received TBI. The crude rate of MDS/AML is 2.3% (95% CI 1.1–4.6%) 5 yr post ABMT. Actuarial probability of clonal bone marrow disorders in pts who remain DF is 7% (±3%SE) at 4 yrs. Of 4 pts with MDS/AML who had bone marrow cytogenetics performed, 2 pts had -7 and one had t(10;11)(p12;q23).

Conclusion: Compared to previous reports, MDS/AML is less common after ABMT with etoposide + melphalan in pts with lymphoma, but clearly can arise in pts who do not receive TBI.

407 POSTER

Number of CD34+cells infused and duration of aplasia after high-dose chemotherapy

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It has been previously shown that the number of CD34+ peripheral blood mononuclear cells (PBMCs) is inversely correlated to the duration of aplasia after high-dose chemotherapy (HD-CHT). However, the minimum number of CD34+ needed for early and sustained engraftment and the number above which no further acceleration in hematopoietic recovery is obtained remain controversial. CD34+ cell number has been correlated with the duration of aplasia post-HD-CHT in 49 courses of HD-CHT administered (Oct 1994–May 1996) to patients (pts) with breast cancer (26), non-Hodgkin's lymphoma (9), myeloma (3), acute leukemia (3) or other solid tumors (3). PBMCs were collected with a Fenwall CS3000 Plus continuous-flow cell separator and harvested at −80°C after movilization with G-CSF (26 pts) or CHT+ G-CSF (23 pts). Mean cell numbers/Kg harvested: 5.2 x 10^8 PBMCs (0.8–12.4), 4.8 x 10^6 CD34+ (0.9–37) and 7.6 x 10^4 CFU-GM (1.2–29.3) (in 22 pts). Mean days to recovery: 9.6 (7–16) to ANC > 0.5 x 10^9/L, 13.1 (13–34) to platelets (PLT) > 20 x 10^9/L, 16.8 (10–41) to PLT > 50 x 10^9/L. According to CD34+ cell number:

CD34+ cell number	Days to ANC > 0.5	Days to PLT > 20	Days to PLT > 50
<2 x 10^6/Kg	15 (14–16)	26 (19–34)	30 (22–41)
>2 x 10^6/Kg	9 (8–14)	12 (7–24)	16 (10–37)

One pt (receiving <2 x 10^6/Kg) died with graft failure after initial engraftment. The number of CD34+ cells infused (but not PBMCs or CFU-GM number) was inversely correlated with the duration of neutropenia and thrombopenia.

Conclusions: The threshold for rapid and sustained engraftment is 2 x 10^6/Kg CD34+ cells. Even though differences above that threshold are smaller, engraftment is faster with higher numbers of CD34+ cells.

408 POSTER

Dose escalation of paclitaxel in combination with cyclophosphamide, thiotepa and carboplatin with stem cell rescue

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Paclitaxel (P) and alkylators are synergistic in preclinical studies when P is administered first. Synergistic myelotoxicity may preclude exploitation of this sequence in the absence of stem cell (PBSC) rescue. The triple alkylator combination of high-dose cyclophosphamide, thiotepa and carboplatin (CTCb) is active against breast cancer and is devoid of severe extrahematologic toxicities such as neuropathy or mucositis that might be overlapping with those of P. For these reasons, a phase I study of P in combination with CTCb was performed.

Purpose: was to determine the DLT, MTD and recommended dose of P + CTCb.

Methods: Escalating doses of P (500–800 mg/m^2) were infused infused in 24 hours on day -7 and followed on days -6 to -2 by a simultaneous 96-hour infusion of C (6 g/m^2), T (500 mg/m^2) and Cb (800 mg/m^2) (Antman et al, J Clin Oncol 1992, 10, 102). PBSCs (>2.5 x 10^6/Kg CD34+) were infused on day 0.

Results: Eighteen patients (pts) were treated (16-breast, 1-ovarian, 1-unkown primary). The DLT at 700 mg/m^2 was grade 4 mucositis (3/6 pts) with sepsis and lethal adult respiratory distress syndrome (ARDS) in 2/6 pts. SWOG grade 3 sensory neuropathy appeared in 1/3 pts receiving 500 mg/m2 of P, 0/3 at 600, 1/3 at 650, 3/6 at 700 and 2/3 at 800. The neuropathy reverted to grade 0–1 and nerve conduction studies improved in all pts within 3 months. The recommended dose for Phase II studies is P 650 mg/m^2 plus CTCb. **Activity:** Objective responses were seen in all 13 pts with evaluable metastatic breast cancer treated. This activity is to be confirmed during an ongoing Phase II study in pts with evaluable metastatic breast cancer. We conclude that significant escalation of P combined with full-dose CTCb is possible.

409 POSTER

Comparison of two systems for stem cell movilization (G-CSF ± chemotherapy) in breast cancer patients

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Sufficient numbers of peripheral blood stem cells (PBSCs) to support high-dose chemotherapy (CHT) can be movilized with either G-CSF or CHT+G-CSF. Potential advantages of G-CSF alone include predictability of the day (d) to start PBSC harvest and avoidance of the adverse effects of CHT. Potential advantages of CHT+G-CSF include no need to stop CHT to harvest PBSCs and fewer apheresis, with potential cost savings. Twenty-six patients (pts) with metastatic or high-risk breast cancer (median age 45 years (30–64)) were randomly assigned to movilization with G-CSF (group A) (10 μg/Kg/day sc from d0, start PBSC harvest on d + 4) (15 pts) or with CHT+G-CSF (group B) (5-FU 600 mg/m^2, adriamycin 50 mg/m^2 and cyclophosphamide 600 mg/m^2, iv on d0, followed by G-CSF 5 μg/Kg/day sc from d + 2; start CD34+ cell counts on d + 9 and apheresis upon CD34+ peak)(11 pts). Mean numbers of apheresis and mononuclear cells (CMN x 10^8/Kg), CD34+ cells (x 10^6/Kg) and CFU-GM (x 10^4/Kg) harvested:

Group	No. apheresis	CMN	CD34+	CFU-GM
A (G-CSF)	2.4 (1–5)	5.5 (1–11.6)	3.6 (0.9–5.9)	8.4 (1.2–29.3)
B (CHT+G)	1.8 (1–3)	4 (0.8–9.6)	6.7 (2–23.7)	7.4 (4.5–12.9)

The difference in CD34+ cell yield is significant ($p < 0.05$). No significant differences in hematologic recovery after high-dose-CHT+PBSC were seen between groups A and B. Mean times were 9.3 vs 9.7 d respectively to ANC > 0.5 x 10^9/L, 12 vs 13.4 d to platelets >20 x 10^9/L and 16 vs 18 d to platelets >50 x 10^9/L. Either CHT+G-CSF or G-CSF alone are valid methods for PBSC movilization. Even though significantly higher CD34+ cell numbers were harvested with CHT+G-CSF, no differences in hematologic recovery were seen.