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.

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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.

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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.

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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.

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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.