grade IV aGvHD and these values were significantly higher as compared to grade I aGvHD cases (15,2%±2,7 vs 8,4%±1,2 for grade IV and I of aGvHD, respectively, p=0,05). *Summary.* It appears that higher proportions of CD4+CD25+ in blood lymphocytes measured soon after HSCT tended to be associated with mixed chimerism but importantly associated with early manifestation of aGvHD and heralded a severe course of this complication.

0362

SHORTENING OF NEUTROPENIA IN LYMPHOMA PATIENTS AFTER TRANSPLANTATION OF LIN ENRICHED CELLS EXPANDED *EX VIVO*

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Background. Hematopoietic stem cells are able to regenerate hematopoiesis in all lineages. They are clinically used in transplantation of bone marrow or peripheral blood stem cells (PBSC) after myeloablative regimens of chemotherapy in the patients with diagnosis of leukemia or lymphoma. *Aims.* The methods of enrichment, isolation, cultivation and expansion of hematopoietic stem cells open the way for specific cellular therapy. In this study, the influence of *ex vivo* expanded Lin' enriched stem cells on the speed of engraftment was evaluated. *Methods.* Authors analyzed expansion of hematopoietic stem cells (HSC) selected by immunomagnetic separation of Lin' cells in the culture of serum-free medium *in vitro* with combination of 5 cytokines (SCF, Flt-3-L, IL-3, IL-6, G-CSF). Cell counts, morphology, immunophenotyping, S-phase, electron microscopy, and biological tests of LTC-IC, CFU-GM and CFU-Meg were analyzed. Clinical protocol was designed based upon these *Results.* Hematopoietic stem cells were enriched from apheresis products collected from patients undergoing mobilization chemotherapy by Lin' separation and expanded *in vitro*. Clinical transplantation protocol based on these results was developed. 10 patients with diagnosis of Hodgkin's or non-Hodgkin's lymphoma indicated for high-dose chemotherapy and autologous PBSC transplantation were enrolled to the protocol. All patients underwent standard PBSC collection, BEAM chemotherapy regimen from day -7 and autologous transplantation at day 0. Besides that, an extra PBSC graft was collected, hematopoietic stem cells were enriched by Lin' procedure and cells were frozen. At day '14, enriched cells were thawed and cultured in the presence of 5 cytokines in serum-free medium. Expanded cells were infused at day 0 to the patients at the escalating dose from 5.10^7 to 3.10^9 cells. Patients were closely monitored, side effects and time to engraftment in leucocytes and platelets was observed. The results were compared to historical controls of 143 patients with diagnosis of lymphoma transplanted with identical BEAM regimen and PBSC grafts. *Results.* Isolated Lin' cells in culture differentiate, the relative proportion of CD34+ cells decreases below 5% at day +14. Growing number of granulocytic progenitor cells correlates with number of CFU-GM colonies. The highest number of CFU-GM colonies and total cell expansion was observed at day +14 in cytokine combination SCF+IL-3+FLT-3-L and IL-6, which was used in the clinical protocol. The procedure of Lin' cells transplantation was free of side effects in all patients. Engraftment in leucocytes occured from day +6 to day +9 in the study group. Compared to historical controls, there was a significant shortening of neutropenia to 5.6 days in average and to 5.0 days in patients who received doses over 1.10^9 cells. There was no significant change in the engraftment in platelets (day +10 versus day +11). *Conclusions.* Hematopoietic stem cells can be enriched from PBSC grafts, cultured and expanded *ex vivo*, and safely used in the cellular therapy protocols. At higher doses of infused cells, the procedure resulted in shortening of critical period of pancytopenia.

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0363

CONTINUOUS INFUSION IDARUBICIN AND ORAL BUSULPHAN (IBU) AS CONDITIONING FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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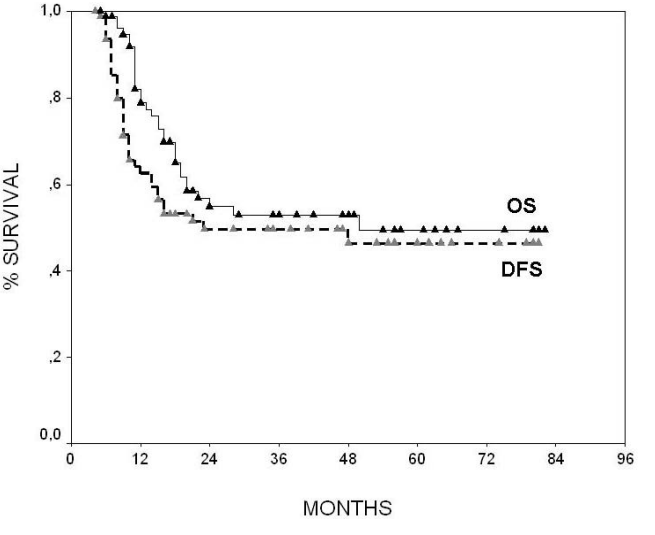
Background. One way for reducing the relapse rate after autologous stem cell transplantation (ASCT) in acute myeloid leukemia (AML) in first complete remission (CR) is the adoption of new conditioning regimens. We developed an original conditioning program, named IBu, consisting of the combination of high dose idarubicin (IDA), given at 20 mg/sqm as 3 days continuous infusion from day -13 to -11 and busulphan (Bu) at 4 mg/kg from day -5 to -2, whose feasibility was previously demonstrated in a phase II study on 14 patients (Ferrara *et al.,* THJ 2001). *Aims.* To report results from a series of 80 AML patients autografted in first CR conditioned with IBu regimen. *Patients and Methods.* There were 50 males and 30 females with a median age of 53 years (16-77). All patients had non M3-AML autografted in first CR. Karyotype was evaluable in 75 cases, with favourable, intermediate and unfavourable cytogenetics being found in 4, 60 and 11 cases, respectively. All patients received peripheral blood stem cells (PBSC) collected after consolidation plus G-CSF. The median interval between CR achievement and ASCT was 3 months (3-10). The median number of CD34+ cells infused was $6,5\times10^6$/kg (2,1-29). In patients aged more than 60 years (n=24), IDA and Bu were reduced to two and three days, respectively. *Results.* One case of transplant related death (1.2%) occurred in a patient aged 55 years, due to septyc shock. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 10 (7-21) and 12 (6-168), respectively. The median number of platelet and blood units transfused was 3 (0-8) and 2 (0-12), respectively. Extra-hematological toxicity mainly consisted of grade WHO III-IV stomatitis (62/88 or 77%) requiring in all cases total parenteral nutrition, while 2 patients had grade III hepatic toxicity and one experienced transient hallucinations. Furthermore, most patients had FUO, while 3 experienced documented infection. Median days of intravenous antibiotics, required in 75 cases, were 11 (4-28). LVEF examination post-ASCT did not reveal any cardiac toxicity. Finally, median time of hospitalization was 28 days (22-49). At the time of writing, 43 patients (54%) are in continuous CR, while 36 have relapsed at a median time from ASCT of 5 months (1-44), with only three patients relapsing after more than one year from ASCT. One patient died in CR from gastric cancer. After a median follow-up for surviving patients of 29 months from ASCT, median overall and disease free survival are 52 months and 48 months, respectively, as shown in the figure. Patients aged more than 60 years did not experience more complications than younger patients. *Conclusions.* Our data demonstrate the efficacy of the IBu regimen in patients with AML, due to a substantial reduction of relapse rate. The most relevant toxicity of the regimen was severe mucositis requiring TPN.



0364

HIGHER DOSE OF CD4+ T CELLS IN THE ALLOGRAFT AND THE OCCURRENCE OF ACUTE GVHD ARE ASSOCIATED WITH IMPAIRED KIRS EARLY RECONSTITUTION AFTER UNMANIPULATED HLA-MISMATCHED/HAPLOIDENTICAL BLOOD AND MARROW TRANSPLANTATION

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Backgrounds. The beneficial effect of killer immunoglobulin-like receptors (KIRs) driven alloreactivity of NK cells had been proved in the T-cell-deplete hematopoitic stem cell transplantation (HSCT), but with the inconsistent effects in the T-cell-replete HSCT. These differences seemed to result from the differences in transplant protocols that utilize different extents of T cells depletion *in vitro* or *in vivo* with the existence of antithymocyte globulin (ATG). *Aims.* The goal of this study was there-