was cyclosporin A and mycophenolate. Hematological toxicity and mucositis were mild, complete donor chimerism could be detected by STR-PCR on day + 13 in both patients. 12 (pt 1) and 10 (pt 2) months after allogeneic HSCT both patients are alive and well. Pt 1 requires CSA and steroid treatment due to chronic GVHD of the skin (extensive disease), whereas pt 2 lacks any symptoms of GVHD. While tumor response in pt 1 meets the criteria of stable disease, there is a nearly complete remission in pt 2. We conclude that there could be graft-versus-tumor effect in epithelial ovarian cancer. Further evaluation is warranted.

Significance of P-glycoprotein expression in childhood malignant tumors

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P-glycoprotein (PGP) is a protein involved in efflux of various compounds. Its overexpression in cancer cells decreases intracellular drug concentrations, and thus, confers a multidrug resistance phenotype. Quantitative and functional analysis of PGP is important in determining resistance and therapeutic response. The pretreatment expression of PGP is a prognostic sign of the failure of therapy in many different cancers. We present the results of PGP expression examination in 91 tumor tissue samples obtained from children treated for different malignant tumors. The correlation between the level of PGP expression and tumor histology, clinical outcome, use of therapy, relapse rate and metastatic disease was made. Patient ages ranged from 2 months to 22 years. Samples were analyzed by flow cytometry and Khi2 test was applied. The highest levels of PGP expression were frequently found in the group of soft tissue sarcomas, neuroblastomas, and Ewing family of tumors (p<0,05). Soft tissue sarcomas, neuroblastomas, and hepatoblastomas cells have shown significantly more frequent PGP expression (p<0,05). Brain tumors and nephroblastomas have shown significantly less frequent expression of PGP (p<0,05). In soft tissue sarcomas, we found a nonsignificant increased PGP expression in patients with relapse after or during chemotherapy (N.S., p<0,1). In patients with metastatic disease PGP overexpression was observed in soft tissue sarcomas and neuroblastomas (p<0,05). We found a significant PGP overexpression in soft tissue sarcomas, neuroblastomas, and hepatoblastomas. A significant increased PGP expression we proved in patients with soft tissue sarcomas and neuroblastomas with metastatic disease. In brain tumors and nephroblastomas we found significantly less frequent expression of PGP. PGP positive tumor cells were detected more frequently in disseminated disease. There was no significant difference between the tumors examined before the chemotherapy administration and the tumors influenced by the chemotherapy. Our results show that PGP positive phenotype is associated with a metastatic disease especially in soft tissue sarcomas and neuroblastomas. In those tumors PGP expression is frequently seen in recurrence and PGP presence in patients before chemotherapy may be a sign of low response to chemotherapy. We verify that flow cytometry is useful method for PGP detection not only in leukemias but also in solid tumors including brain and bone tumors.

17. Cytokines and Gene Therapy

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Mobilization of peripheral blood stem cells (PBSCs) with chemotherapy and granulocyte-colony stimulating factor (G-CSF): A randomized evaluation of different doses of G-CSF

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Objective: To date, there is no randomized study comparing different doses of G-CSF following mobilization chemotherapy (MC). Therefore, in this study the effect of different doses of G-CSF following MC on the yields of CD34+ PBSCs were evaluated in patients with hematologic malignancies and solid tumors. Methods: Fifty patients were randomized to receive G-CSF 8 (n = 25) versus 16 (n = 25) ug/kg/day following one of these mobilization regimens: cyclophosphamide (CY) and etoposide; CY and epirubicine; or CY and paclitaxel. Results: The median number of CD34+ cells collected after 8 ug/kg/day of G-CSF was 2.38x10^6/kg (range 0.21-7.80) as compared to 7.99 (range 2.76-14.89) after 16 ug/kg/day (P<0.001). Among patients randomized to 8 v 16 ug/kg, all (100%) achieved ≥ 4.0 x 10^6 CD34+ cells/kg and less aphereses were required to achieve ≥ 2.5x10^6 CD34+ cells/kg after the higher dose (P<0.001). Twenty of 25 (80%) patients in the low dose and 23 of 25 (92%) in the high dose G-CSF arm underwent high dose chemotherapy and autologous stem cell transplantation. Median days of WBC engraftment in patients mobilized with 8 ug/kg and 16 ug/kg of G-CSF were 12 and 9, respectively (P<0.001). But there was no difference between 2 groups regarding the other parameters of peritransplant morbidity such as days of platelet engraftment (P=0.10), RBC (P = 0.56) and platelet transfusions (P=0.22), days of TPN requirement (P=0.84), days of fever (P=0.93), and days of antibiotics (P=0.77). Conclusion: These data showed that higher doses of G-CSF following a MC were associated with a clear dose response effect based on the collected cell yields. Based on these results 8 ug/kg/day is as effective as 16 ug/kg/day except for a rapid neutrophil engraftment in the high dose arm. Therefore, in the routine clinical practice, despite some advantage in the use of higher doses of G-CSF, lower doses may be used for PBSC collections following chemotherapy based mobilization regimens in this cost-conscious era.

P714

Stem cell mobilization by G-CSF in solid and hematological malignancies - Single daily dose better than split dose in obese patients

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In the past, different results were reported for single daily and two divided daily G-CSF doses in stem cell collection where no study exists examining the effect of body mass index (BMI) on mobilization. Collected CD34+(+) cell numbers were compared in 86 patients with solid and hematological malignancies receiving either single daily 14 mcg/kg/day G-CSF (filgrastim) as group I(n=36) or divided daily two G-CSF doses 2X7 mcg/kg/day as group II (n=50). Both groups were subdivided into a and b groups according to their BMI as group a (BMI < 25 kg/m^2) and group b (BMI >25 kg/m^2). Two groups were similar in terms of age, gender and disease characteristics. All the patients have received G-CSF as a single or two divided doses subcutaneously and apheresis has been done on the 5th day. 4 hours after the last dose. No significant CD34(+) cell number difference between groups la and lb, groups lla and lib, groups la and lla were found. On the other hand, the median ratio and the number of CD34(+) cells in group...
treated patients (p=0.034 and p=0.040). No significant differences in the granulocyte count was observed in the G-CSF/EPO plus IL-2 series on day +20. These results demonstrate that low-dose IL-2 can be safely administered in combination with G-CSF/EPO early after PBSCT and that it exerts some positive effects on post-PBSCT myeloid reconstitution but not on immune recovery.

G-CSF and GM-CSF administration following PBPC transplantation in women with cancer - impact on transplantation outcomes in a randomized comparison

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PBPC transplantation (PBPC) combined with post-PBPC administration of myelopoietic growth factors is a valid therapeutic intervention to rapidly restore haematopoiesis after the delivery of intensive myeloablative anti-cancer chemotherapy. On the other hand, the best growth factor regimen to potentiate PBPC-mediated immuno-haematopoietic recovery has yet to be determined. We compared in a randomized evaluation the effects produced by post-PBPC G-CSF and GM-CSF on myeloid/lymphoid recovery and transplantation outcome in women with chemosensitive cancer. Thirty-seven ovarian cancer patients and thirty-four breast cancer patients ranging in age from 24 to 60 years were treated with carboplatin, etoposide and melphalan (CEM) high-dose chemotherapy and subsequently randomized to receive G-CSF (5 µg/kg subcutaneously) or GM-CSF (5 µg/kg subcutaneously) until day +13 post-PBPC. Patients were compared for their haematopoietic recovery, post-transplantation clinical management and immune recovery. Finally, clinical outcome was estimated as time to progression (TTP) and overall survival (OS). Haematopoietic recovery and post-transplantation clinical management were comparable in both the G-CSF and GM-CSF series. Conversely, significantly higher T lymphocyte counts were observed in G-CSF patients during the early and late post-transplantation follow-up. Patients who received G-CSF showed a significantly longer median TTP. A parallel analysis revealed an estimated time to progression (TTP) and overall survival (OS). Haematopoietic recovery and post-transplantation clinical management were comparable in both the G-CSF and GM-CSF series. Conversely, significantly higher T lymphocyte counts were observed in G-CSF patients during the early and late post-transplantation follow-up. Patients who received G-CSF showed a significantly longer median TTP. A parallel analysis revealed an estimated time to progression (TTP) and overall survival (OS). The enhancement in post-PBPC T cell recovery observed in G-CSF patients encourages the use of G-CSF to ameliorate immune recovery which seems to be significantly longer OS and TTP. The preliminary analysis of our data shows that the use of a low dose of G-CSF with a delayed beginning (from +5) is effective in reducing the need of parenteral nutrition (12 days in the G-CSF group vs 14 days in the control group). No statistical differences were noticed for other clinical parameters, such as platelet recovery, platelets transfusion requirement, duration of hospital stay and fever episodes.

Delayed dose of glycosylated-G-CSF post autologous peripheral blood stem cell transplant: preliminary results of a randomized trial

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Although G-CSF utilization after PBSC transplantation determines a faster granulocytes recovery, not all studies have shown clear advantages associated with its usage and hence the necessity to conduct sufficiently extensive randomized trials in order to clarify this subject. Since a delayed start of G-CSF has been shown to have a comparable efficacy in respect to G-CSF started at day +1, we choose to study this schedule. We have initiated a prospective randomized clinical trial comparing low dose (4µg/Kg s.c./die) glycosylated G-CSF (Myelostim-Italfarmaco) starting from day +5 post transplant with a no treatment arm, in patients who underwent PBSC transplantation for LNH, HD, MM and solid tumors. Patients affected by acute leukaemia were excluded from the study. The treatment was stopped for neutrophils count > 500 for at least 2 days. Until now 31 patients have been enrolled in the study, 18 Lymphoma, 11 MM and 2 solid tumors; 15 patients were randomized to receive G-CSF from day +5 and 16 patients were randomized in no treatment arm. There were no significant differences in the two groups of patients regarding the characteristics of PBSC infused, age, state of diseases and type of the conditioning regimens used (HD-PAM, BEAM, TT-BUS-PAM). In the treatment group G-CSF was administered for an average of 7 days. Compared to control group, patients treated with G-CSF showed shorter neutropenia (N<500) (Logrank p=0.0004) and a reduction in the need of parenteral nutrition (12 days in the G-CSF group vs 14 days in the control group). No statistical differences were noticed for other clinical parameters, such as platelet recovery, platelets transfusion requirement, duration of hospital stay and fever episodes.

Low-dose interleukin-2 plus G-CSF/EPO administration early after autologous PBSCT transplantation - Results of a prospective study in women with breast and ovarian cancer

A. Perillo, L. Pierrelli, A. Battaglia, M.G. Salerno, E. Cortesi, A. Fattorossi, L. De Rosa, F. Ferraiu, M. Ludovisi, G. Leone, S. Mancuso, G. Scambia (Rome, Taormina, I)

This study evaluated the effects of low-dose interleukin-2 (IL-2) plus G-CSF/EPO on post-PBSC transplantation (PBSC) immune-haematopoietic reconstitution and NK activity in patients with breast (BrCa) and ovarian cancer (OvCa). To this end, two consecutive series of patients were prospectively assigned to distinct post-PBSC cytokine regimens. From day +1 to day +12, which consisted of G-CSF (5 µg/kg/d) plus EPO (150 UI/kg/every other day) in 17 patients (13 BrCa and 4 OvCa) or G-CSF/EPO plus IL-2 (2x10⁵ IU/m²/d) in 15 patients (10 BrCa and 5 OvCa). Haematopoietic recovery and post-transplantation clinical cares were manageable and comparable in G-CSF/EPO and in G-CSF/EPO plus IL-2 treated patients without significant side-effects attributable to IL-2 administration. In the early and late post-transplant period (day +20 and day +100) a significant higher granulocyte count was observed in the G-CSF/EPO plus IL-2 treated patients (p=0.034 and p=0.040). No significant differences were found between the two groups of patients in the kinetics of most lymphocyte subsets except naive CD45RA+ T cells which had a delayed recovery in G-CSF/EPO plus IL-2 patients (p=0.021). No significant differences was observed between NK activity in the two different groups, albeit a significantly higher NK count was observed in G-CSF/EPO plus IL-2 series on day +20 (p=0.020). These results demonstrate that low-dose IL-2 can be safely administered in combination with G-CSF/EPO early after PBSC and that it exerts some positive effects on post-PBSC myeloid reconstitution but not on immune recovery.

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High-dose chemotherapy followed by peripheral stem cell reinfusion in lymphoma patients: is post-reinfusion G-CSF administration useful?

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High-dose chemotherapy followed by autologous stem cell transplantation is widely used in lymphoma patients. Growth factor has been used after peripheral stem cell reinfusion with the aim to further reduce the duration of neutropenia. We retrospectively studied 63 consecutive lymphoma patients (NHL 37, HD 27) treated with high dose chemotherapy and peripheral stem cell transplantation, subdividing them in 3 groups on the basis of hematopoietic recovery. The first group received G-CSF starting 1 day after reinfusion independently from CD34+ number. In the second group, G-CSF was administered 5 days after reinfusion because CD34+ cells were less than 5 x 10^6/kg. Patients in the third group, did not received G-CSF because CD34+ cells were more than 5 x 10^6/kg. The number of CD34+ cells reinfused was significantly different between the study groups. However, we did not found relevant difference between group 1 and the third group in term of hematopoietic recovery. Only the number of days to obtain an ANC more than 1 x 10^9/L was significantly shorter in group 1 compared to group 3 (p = 0.05). On the other hand, the number of days with an ANC less than 0.5 x 10^9/L, and to obtain an ANC more than 0.5 x 10^9/L and 1 x 10^9/L were significantly shorter in group 2 compared to group 3 (p = 0.80, 0.0005, and 0.0001, respectively). Patients in group 3 experienced a shorter days with platelets less than 20 x 10^9/L (p = 0.05).

Patients in group 2 showed a higher incidence of infections (FUO + documented infections) even if without consequence on number of days with antibiotics, antimycotics or time to hospital discharge. In conclusion, the routine administration of G-CSF after peripheral stem cell is not recommended if an adequate (more than 8 x 10^6/kg) CD34+ cells is reinfused. When this target is not obtained, G-CSF starting 5 days after reinfusion can reduce the aplasia period with an economical advantage compared to early administration (day +1).

Effects of epoietin beta on RBC transfusion in inflammatory breast cancer patients receiving sequential high dose chemotherapy: Pegase 05, a randomized FNCLCC trial

T. Palangie for the Working Party Sessions

Purpose: To assess in a randomised trial the effects of Epoietin Beta on transfusion requirements in patients with inflammatory breast cancer treated with sequential high dose Doxorubicine (D) + Cyclophosphamide (C) and Taxotere (TXT). Patients: 54 entered into the trial. Treatment consisted in 7 courses of D 75mg/m² + C 6g/m² in cycle1 (C1) and C2 every 3 weeks; TXT 100mg/m² in C3, C4 and C5 every 2 weeks; D 75mg/m² + CPM 3g/m² in C6 and C7. PBSC were collected after C2 + G-CSF, and infused after C6 and C7. G-CSF was administered on day 5 post transplant or following chemotherapy. Only 40 patients with Hb level < 14 g/dl at baseline were randomised to Epoietin Beta 150 UI/ kg 3 times per week, versus control. The primary end point was the reduction of RBC units transfused in treatment group (n = 21) / control (n = 19).

Results: 15 out of 21 patients in the treatment arm and 13 / 19 in the control, completed the study successfully; reasons for withdrawal were infectious toxicity and patient's decision. Epoietin Beta therapy resulted in significant decrease in the rate of transfusion per chemotherapy cycle as reported in the table:

| N transfusion / cycle | control | epo | p   |
|-----------------------|---------|-----|-----|
| 0                     | 57.4%   | 72.2%| 0.02|
| 1-2                   | 32.2%   | 26.2%|     |
| >= 3                  | 10.4%   | 1.6% |     |

Peripheral-blood progenitor-cell collection was increased in patients treated with G-CSF and EPO as compared to patients with G-CSF alone, but the difference was not significant. Conclusion: The result from this randomised trial suggest that Epoietin Beta decrease transfusion requirements in patients treated with sequential high dose chemotherapy.

Persistence of anemia due to inadequate erythropoietin production after allogeneic stem cell transplantation

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Background: Anemia due to inadequate erythropoietin (EPO) production is well known in the first weeks after allogeneic stem cell transplantation (SCT). Persistence of posttransplant anemia and delayed red cell reconstitution are associated with infections, graft-versus-host-disease (GVHD), renal insufficiency or hemolysis. The role of EPO in these conditions is unclear. We report 3 patients with persisting anemia and inadequate low serum EPO responsive to therapy with recombinant human (rhu) EPO.

Patients: Patient 1: 38 year old female with Multiple Myeloma IgG kappa had a low haemoglobin (Hb) of 87 g/l 8 months after SCT. EPO was low with 12.7 IU/l (normal 12-23). RhuEPO at a dose of 3 x 10'000 IU was started and later tapered to 4000 IU weekly. Hb remained stable around 135g/l on continuous rhuEPO therapy for one year.

Patient 2: 36 year old male with AML M4 eo (inv 16) in 1st relapse developed anemia with Hb 90g/l 10 weeks after transplant. EPO was low with 12.0 IU/l. RhuEPO at a dose of 2 x 10'000 IU weekly raised the Hb up to 150g/l. RhuEPO was tapered and finally discontinued. His Hb remained stable at 120g/l 3 months after stopping rhuEPO.

Patient 3: 25 year old female with CML in chronic phase had stable anemia with Hb 93g/l 10 months after SCT. EPO was inadequately low with 25.6 IU. Therapy with rhuEPO at a dose of 2 x 10'000 weekly raised Hb to 110 g/l after 4 weeks of therapy. All patients received a peripheral blood precursor SCT either from an HLA identical sibling (patients 1 and 2) or an HLA identical donor (patient 3). They are in continuous complete remission for 7, 12 and 20 months. All patients developed steroid responsive acute GVHD grade II, which was inactive at the time of anemia and rhuEPO therapy. No patient had active infection or hemolysis. Renal function was slightly impaired in all patients (Creatinine clearance 50, 64 and 80 ml/min).

Conclusion: Inadequate EPO production can persist for months after allogeneic SCT. It might result from clinically inapparent GVHD or even minimal renal insufficiency. Patients with low EPO may seem to profit from therapy with rhuEPO.
was studied in 14 patients with leukemia/lymphoma relapsing following NST. IFN-a (Roferon-A) 3 MU IU/day s.c was given to patients with early relapse following transplantation from mismatched or matched unrelated donors (MUD) whenever donor lymphocyte infusion (DLI) was ineffective in 6 patients. In patients with overt relapse, IFN-A was combined with DLI. Patients were off treatment if grade 3 toxicity and or grade 2 GVHD occurred. Fourteen patients (9 females; 5 males), median age 27 years (range 7-57), 5 AML, 5 ALL, 2 CML, 1 Richter’s syndrome and 1 NHL, were included. Following NST (Judarabine 30mg/m2x6; busulfan 4mg/Kgx2; 8 with ATG 10mg/Kgx4; 3 without ATG: 1 with additional TBI; 1 with additional Campath-1H in vivo; 11 received peripheral blood stem cells (PBSC) from MHC identical siblings, 2 from MUD and 1 from haploidentical sibling (with additional Cytokaxan and CD34+/CD4- CD8- PBSC). Treatment was started in median time of 3 mo. post NST (range 1-30). Out of 14 patients, 6 patients developed AGVHD (5 patients grade II-III and 1 patient grade IV). Two out of 6 patients with acute GVHD received DLI in addition to IFN-a. Out of the 6 patients who developed GVHD, 4 had cutaneous skin lesions of disease that disappeared within 7 (range 7-30) days. Three of them maintain complete response for 16-24 mo. (median of 23). Overall, 6 patients, 3 treated with IFN-a alone and 3 with DLI plus IFN-a, are alive without evidence of disease, for 6-42 mo. (median 22). Two patients are alive with disease, 2 patients died due to sepsis and 5 due to disease progression. We conclude that IFN-a therapy alone and mostly with DLI can induce GVL effects and long-term remission following NST, but further investigations are needed.

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Flow cytometric determination of intracellular cytokines in monocytes and association with the development of acute graft-versus-host disease (aGVHD) and hepatic veno-occlusive disease (VOD) in allogeneic stem cell transplant (allo-SCT) recipients

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Inflammatory cytokines (TNF-a, IL-1, IL-6) play a crucial role in the development of aGVHD, and have also been implicated in the pathogenesis of VOD. In a prospective study, we employed flow cytometric (FC) detection of intracellular cytokines (ICs) to assess the production of cytokines by monocytes and investigate its association with the development of aGVHD and VOD in allo-SCT recipients. Flow cytometric determinations of TNF-a and IL-1b in peripheral blood monocytes were performed on days -8, -5, -3, -1, 0, +13, +16, and +20 in 16 patients, who underwent allo-SCT for hematological malignancies from an HLA-identical sibling (n=14) or an alternative (n=2) donor. The conditioning regimen was combination chemotherapy in all cases. The source of stem cells was bone marrow in 11 and peripheral blood in 5 patients. For detection of ICs in monocytes, whole blood was incubated with brefeldin A, with or without the addition of lipopolysaccharide (LPS), for 4 hours. Surface staining of monocytes was performed with CD14-FITC monoclonal antibody (mAb), followed by intracellular staining with TNF-a or IL-1b-specific PE-conjugated mAbs. The percentage of TNF-a or IL-1b-positive monocytes was calculated by two-color FC. The patients were classified into 3 different groups according to the occurrence of aGVHD or VOD: group 0 (no aGVHD or VOD), n=6; group 1 (aGVHD only), n=6; and group 2 (aGVHD and VOD), n=4. The values of ICs in unstimulated (u-TNF-a/IL-1b) and LPS-treated (s-TNF-a/IL-1b) monocytes were compared among the 3 groups. The mean values of ICs at all time points (with the exception of u-TNF-a on day -8) were higher in groups 1 and 2 compared to group 0. This trend reached statistical significance only for u-IL-1b on day +13. Among groups 1 and 2, the proportion of patients with increased values of u-IL-1b (above the highest value in group 0) was 40% and 50% respectively on day -8 (p=0.07), 20% and 50% on day -5 (p=0.06), and 50% and 100% on day +13 (p=0.014). We conclude that intracellular production of cytokines by monocytes may correlate with the development of aGVHD or VOD after allo-SCT.

P723

Assessment of cytokine gene polymorphism in patients developing hepatic veno-occlusive disease following stem cell transplantation

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Hepatic veno-occlusive disease (HVOD) is one of the major and potentially life threatening complications of stem cell transplantation (SCT). The pathogenesis of HVOD is not known and high serum levels of proinflammatory cytokines were found to be associated in some studies. Cytokine gene polymorphism genotype was found to be associated with GVHD, another major SCT complication. The objective of this study was to analyze and compare the cytokine profile in patients undergoing allogeneic SCT and in their donors in respect to the development of HVOD. Five cytokines: TNF-alpha, TGF-beta, IL-10, IL-6 and INF-gamma were studied in 15 patients/recipients pairs with HVOD and in 13 patients/recipients pairs without evidence of HVOD. DNA polymorphism, based on single nucleotide polymorphism, was assessed by a POR-SSP approach (One Lambda Inc). Both groups of patients were comparable with regard to allelic profile of TNF- alpha, TGF-beta, IL-10 and IL-6. The majority of patients typed as homozygous low producers of TNF- alpha, (100% in both groups), high producers of TGF-beta both homozygous and heterozygous (57% vs. 50% in patients with VOD and without VOD, respectively), heterozygous intermediate producers of IL-10 (71% vs. 60%, respectively) and high producers of IL-6 both homozygous and heterozygous (92% vs. 87%, respectively). Similar results were found in the donors. Smaller proportion of patients with HVOD were low producers of INF-gamma as compared to patients without HVOD (15% vs. 30%) p=0.025, OR-8. In conclusion: It is an intriguing possibility that low production of INF-gamma has a protective role against development of HVOD, whereas the profile of the other cytokine production in our series did not seem to be associated with HVOD. Larger number of patients should be evaluated to confirm these results.

P724

Polychronic expansion of primary human T-lymphocytes for retroviroly mediated gene transfer can lead to functional impairment and skewed distribution of memory and effector T-cell subsets

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Retrovirally mediated gene transfer into primary human T-lymphocytes usually involves strong polychronic stimulation before transduction. Although little is known about the functional profile of these cells after expansion, this is a critical aspect for all applications involving adoptive transfer of transduced T-cells into patients. Actually, several clinical trials with transduced T-cells have reported defective function in vivo. To explore the impact of polychronic stimulation in T-cell immunocompetence, PBLs from donors were expanded in parallel with various doses of either PHA or immobilized anti-CD3 and anti-CD28 (3/28). The proportion of 3/28-expanded cells to third party stimulators were consistently higher than those of their PHA-counterparts, though this advantage was smaller at higher doses of stimuli. In addition, greater than 80% of the 3/28-CD8+ T-cells expressed intracellular perforin compared to less than 30% of the PHA-CD8+ T-cells. For transduction experiments, expanded T-cells were incubated on days 4 and 5 with VSV-G pseudotyped particles of pCC1-tNGFR. Both PHA and 3/28-stimulated cells were transduced at comparable levels (11-25% vs 15-40%, respectively). Interestingly, functional profiles were similar in tNGFR+ and tNGFR- fractions. T-cells can currently be classified into naïve
(CCR7+/CD45RA+), central memory (CCR7+/CD45RA-), effector memory (CCR7-/CD45RA-) and effectors (CCR7-/CD45RA+). CCR7- subsets secrete IFNγ and express intracellular perforin. PHA-expansion leads to a rapidly skewed distribution of these T-cell subsets, with an average 6-fold increase of the central memory cells and a marked decrease of all the others. Conversely, cells from the same donors expanded with comparable levels of 3/28-stimulus retained their physiological profile to a larger extent. When stimulated with autologous dendritic cells pulsed with CMV or EBV-derived HLA-A2 peptides, PHA and 3/28-cells from HLA-A2 donors gave rise to comparable percentages of HLA-A-tetramer positive cells. However, tetramer positive PHA-cells expressed lower levels of perforin, half the amount of IFNγ upon peptide-specific stimulation, and a lower percentage of effector cells when compared with their 3/28 counterparts. In conclusion, polyclonal expansion of T-cells with PHA skews their functional profile and impairs their immunocompetence in vitro compared to 3/28. This might contribute to a defective function of the transduced T-cells after their adoptive transfer in vivo.

P725
Bcl-2 antisense in the treatment of large cell anaplastic lymphoma with relapse after autologous hematopoietic cell transplantation – a case report
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Mitochondrial protein bcl-2 is an important inhibitor of apoptosis. Blocking expression of bcl-2 gene by antisense oligonucleotides (AS-ODN) administration may result with lower production of bcl-2 protein what makes neoplastic cells more sensitive to chemotherapy.
We used bcl-2 AS-ODN in the treatment of 11-years old boy with Anaplastic Large Cell Lymphoma, which underwent two autologous PBSCST. The diagnosis was made in March 1999. The patient was initially treated with CALM BFM 93 protocol and due to partial response autologous PBSCST was performed (BEAM conditioning). The patient was in complete remission for 18 months and relapsed in May 2001. First relapse was treated according NHL BFM 95 for relapsed LCAL and was followed by the second auto-PBSCST in September 2000 with conditioning consisted of Busulfan, Vepesid, Cyclophosphamid. No remission was achieved. Palliative therapy was introduced in October 2000, (Vinblastin) and resulted with transient and short remissions. In the June and September 2001 bcl-2 antisense infusion was given and was combined with chemotherapy based on Topotecan and Vinblastin with good result – stable remission is observed for 20 weeks. No toxicity of bcl-2 AS-ODN was observed. This observation shows that bcl-2 AS-ODN may be useful in the treatment of relapsed lymphomas after conventional chemotherapy and BMT.

P726
Efficient and durable gene marking of cord blood stem cells: comparison of two cytokine cocktails
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Gene marking of cord blood (CB) hematopoietic stem cells (HSCs) is crucial to investigate the role of HSCs in hematopoietic reconstitution after transplantation. We evaluated the efficiency of the infection of CB CD34+ cells by Gibbon Ape Leukemia Virus pseudotyped retroviral supernatant, containing the low affinity nerve growth factor receptor (LNGFR) marker gene, under the control of Moloney murine leukemia virus long terminal repeat (study 1; n=7). Moreover, we determined the maintenance of the gene marker in long term culture initiating cells (LTC-IC) present in the suspension of the infected cells (study 2); isolated CD34+ cells from CB were prestimulated for 24 hours in a serum-free medium containing cocktail A (used in our previous studies to expand HSCs compartment): IL-6 (10 ng/ml), IL-11 (10 ng/ml), FL (50 ng/ml) and TPO (10 ng/ml) versus cocktail B (used by Thrasher et al, 1998): SCF (100 ng/ml), FL (100 ng/ml), IL-6 (20 ng/ml) and IL-3 (20 ng/ml). The cells were then infected on a retroenone coated plate previously loaded with retroviral supernatant. The infection efficiency was carried out by exposing cells to retroviral supernatant containing the above cocktails for 3 times every 12 hours. Results of study 1: the median and range fold expansions of nucleated cells (NC) were 2.8 (2.1-8.8) and 6.8 (3.9-17.7) with cocktails A and B respectively. The median fold expansions of CD34+ cells were 2.6 (1.6-6.7) and 5.1 (3.5-12.4) with cocktails A and B respectively. The percentages of LNGFR positive NC were 59% (21-79) (A) and 77% (31-84.5) (B). The percentages of LNGFR positive CD34+ cells were 67% (23-86) (A) and 86% (34-97) (B). Differences were statistically significant by the Wilcoxon signed-rank test (p<0.05). Results of study 2 were as follows. By using a flow cytometry gate on live cells recovered after 4 weeks of the LTC-IC culture, we found 3.7% (0.7-14) CD34+ cells, of which 35.5% (25-95) were LNGFR+ with cocktail A; and 4.8% (1.1-11) CD34+ cells, of which 65.5% (37-98) were LNGFR+ with cocktail B. Differences did not reach statistical significance. Our data suggest that, probably due to IL-3, cocktail B shows about 10% higher efficiency in transducing HSCs.

Additional abstracts to this topic
Influence of post-transplant granulocyte-colony stimulating factor (G-CSF) administration on peritransplant morbidity in patients undergoing autologous stem cell transplantation (Asct)
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Objective: To evaluate the effect of post-transplant G-CSF on the parameters of peritransplant morbidity. Methods: Three sequential and consecutive cohorts of 20 patients each with hematological malignancies and solid tumors received either post-transplant G-CSF at a dose of 5 μg/kg/day IV in the morning started on day 0, day 5, or no G-CSF. G-CSF was given until the absolute neutrophil count was greater than 5 x 10^9/L. Engraftment kinetics and other parameters of peritransplant morbidity such as transfusion and TPN requirements, fever, antibiotic administration as well as hospital stay were evaluated with univariate and multivariate analysis. Results: Patients receiving post-transplant G-CSF starting on day 0 and 5 recovered granulocytes more rapidly than those not receiving post-transplant G-CSF (P=0.00 for ANC>0.5 and 1x10^9/L). Post-transplant G-CSF was not significantly associated with more rapid platelet engraftment in the univariate and multivariate analysis. Post-transplant G-CSF starting on day 0 and 5 were significantly associated with a decreased duration of fever (P=0.002 and 0.001, respectively) and antibiotic administration (P=0.000 and 0.006,respectively) compared to reference group of without G-CSF. Post-transplant G-CSF were also significantly associated with a short hospital stay compared to reference group of without G-CSF (P=0.000 and 0.001,respectively). There was no difference among three arms regarding TPN and transfusion requirements. There was also no difference between day 0 and day 5 arms regarding the parameters of peritransplant morbidity. Conclusion: Post-transplant G-CSF is associated with a faster granulocyte recovery and shortened duration of hospitalization, fever as well as nonprophylactic antibiotic administration in patients who receive Asct. This study also showed that post-transplant G-CSF on day 5 may be as effective as day 0 administration on the clinical outcome. Day 5 administration instead of day 0 may be an economical approach in the routine clinical practice in this cost-conscious era.
18. Graft Engineering

P727
Relation between CFU-GM and CD34+ doses and engraftment
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Autologous Peripheral Stem Cells Transplantation (AP SCT) is standard therapeutic approach in the treatment of many haematological malignancies. Its success depends on many factors, one of the most important is quality of autologous graft. The quality of graft is most frequently evaluated by CD34+ cells count determination and by CFU-GM progenitor cells cultivation in ap-heresis product. Engraftment time, haematologic reconstitution and frequency of infective complications depends on CD34+ and CFU-GM reinfused cells count. Minimal recommended doses in autograft are 2.0 - 2.5 x 10^6/kg body weight of CD34+ cells and 2 - 4.1 x 10^4/kg of CFU-GM colonies.

We have studied 30 patients with different haematological malignancies, who received autologous peripheral stem cell transplantation since November 1999 till December 2001: 8 AL (CR1), 12 MM (CR1, PR1), 8 NHL (CR1,PR1,2HD (CR2,PR2). Ratio M/F was 13/17, patient age ranged from 19-65 years (median 42). Transplant procedures were performed at mean time of 12 months after achieving CR or PR. PBSCs were obtained after chemotherapy with HD-CY (22) or HAM (8) followed by G-CSF. Patient was conditioned with BuCy2 (7), BuMel(1), HD-Melphanal (12) or BEAM (10) according to diagnosis. Median CFU-GM dose was 114,1 x 10^4/kg (range 6,90-275,40) and 3,8 x 10^6/kg CD 34 positive cells (range 1,22-10,5).

No significant correlation was found between doses of progenitor cells and hematopoietic recovery time or infectious complications in our patients. Engraftment depended on diagnosis and conditioning regimen.

P728
CD 34+ enriched - CD 19 depleted autologous peripheral blood stem cell transplantation for chronic lymphoproliferative disorders: High purging efficiency but increased risk of severe infections
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Introduction: Purging procedures have been developed to decrease the incidence of relapse after autologous stem cell transplantation. We used a 'positive' (CD34) and 'negative' (CD19) double selection procedure to improve the efficacy of 'single' purging of hematopoietic harvests in poor prognosis lymphoproliferative disorders.

Material and methods: The inclusion criteria were patients under 65 years old with a diagnosis of B-CLL in stage C, or B with poor prognosis factors; follicular lymphoma after a non-localized relapse (or in first response with a 4-5 IPI score) and mantle cell lymphoma, diffuse or blastic variants, in first or subsequent response. Patients with chemoresistant disease, or in partial remission with a bone marrow neoplastic infiltration >50% were excluded. Mobilization regimens were Flotamidine-VP16 or CY 3g/m2, both adding G-CSF. Patients were conditioned with CY- TBI or BEAC. All patients were treated in the study with autologous purging procedure in which CD34+ and CFU-GM reinfused cells count. Minimal residual disease (MRD) was studied by flow cytometry and PCR techniques during the purging procedure and after transplantation.

Results: Twenty-six patients fulfilled entry criteria. Median age of patients was 50 years (range: 33-66), 17 were male and 9 female. Thirteen (50%) of the patients mobilized an adequate number of CD34+ cells (≥3 x 10^6/kg) to proceed with the double selection protocol. Twelve of the 13 harvests became PCR negative after purging. Ten patients were grafted with the selected products and all but one engrafted without delay. After a median follow-up of 30 months, 2 of 10 patients suffered a molecular relapse at 7 and 19 months, respectively. The earlier relapse was observed in the patient who received a MRD+ product. Only one patient has experienced a clinical relapse. Three patients died due to infections bronchiolitis, pneumococcal sepsis and septic shock of unknown origin, respectively, and three others presented life-threatening infections.

Conclusions: Therefore, CD34+/CD19 positive/negative selection is an effective purging approach in patients with chronic lymphoproliferative disorders. This favorable effect is, however, counterbalanced by the high frequency of life-threatening infections.