

0316**IMMUNO HAEMATOLOGICAL RECONSTITUTION AFTER T-CELL-DEPLETED HLA-HAPLOIDENTICAL STEM CELL TRANSPLANTATION FOR THALASSEMIA**

A. Isgrò,¹ B. Erer,¹ P. Sodani,¹ P. Polchi,¹ M. Marziali,² W. Leti,² C. Gramiccioni,² W. De Santis,² M. Campagna,² G. Del Giudice,² M.C. Sirianni,² F. Aiuti,² G. Lucarelli¹

¹Mediterranean Institute of Hematology, ROME, Italy; ²Department of Allergy and Clinical Immunology, ROME, Italy

Background. We evaluated haematological and immunological characteristics of four thalassemia patients after T-cell-depleted HLA-haploidentical stem cell transplantation. **Methods.** We evaluated the clonogenic capability by the colony forming cell assay (CFC) and the long term culture-initiating cell (LTC-IC) assay at baseline and 20 days after transplant. Stromal cells were obtained from long term culture of bone marrow mononuclear cells (BMMCs) and analysed by immunohistochemistry. Lymphocyte subsets were studied by flow cytometry; and stromal IL-7 production by BMMCs was analysed by ELISA. **Results.** At baseline, no significant differences were observed in haematological and in immunological parameters in thalassemia patients when compared with a group of normal subjects. Day + 20 after transplant, a reduced clonogenic capability was observed (4±2 vs. 41±40 CFU-E, 17±9 vs. 109±22 BFU-E, 3±1 vs. 9±6 CFU-GEMM and 16±10 vs. 66±23 CFU-GM). The number of primitive bone marrow (BM) progenitor cells was also decreased (1.8±1.4 vs. 15.4±3.6 LTC-CFC/106 BMMCs). In addition, stromal cells secreted lower IL-7 levels (0.3 + 0.1 pg/mL vs. 0.8 + 0.1 pg/mL, in controls) and displayed by immunohistochemistry an altered phenotype. Upon light microscopy examination, the majority (75%) of these cells appeared as moderately large cells, frequently rounded, with abundant cytoplasm, whereas in control subjects about 90% of the stromal cells exhibited a different morphology characterized by irregular or spindle shape and branching cytoplasmic processes (fibroblast-like). Compared with normal subjects, thalassemia patients showed: reduction of naïve CD4+ T-cells (2±0.5% vs 50±10%), reduction of thymic naïve CD4+ T-cells (1±0.2% vs 40±12%), and a significant increase of CD4+ cells activation markers (CD95, HLA-DR and CCR5). IL-7 receptor (CD127) expression was also significantly decreased on CD4+ T-cells and on naïve CD4+ T-cells (CD4+/CD45RA+CD62L+/CD127+). NK cells were among the first lymphocytes to repopulate the peripheral blood, and up to 70% of these cells were CD56 bright whereas CD16+ NK cells were decreased. **Conclusions.** Twenty days post transplant, an impaired growth and differentiation capacity of stem/progenitor cells were observed in thalassemia patients, in parallel with an altered homeostasis of T-cells and a reduction of T-cell naïve compartment. We hypothesize that the damage of T cell compartment may be at least partially due to an altered production of new T cells starting from the haematopoietic stem/progenitor cells. CD56+ NK cells develop more rapidly than other lymphocytes, but CD16+ NK cells (with cytotoxic potential) require more prolonged exposure to maturation factors (IL-2) in the bone marrow. An IL7/IL7R pathway dysregulation has been also observed, possibly involving bone marrow stromal cells. *in vitro* studies are ongoing about the use of cytokines (IL-2, IL-7, IL-2 plus IL-7) supporting T cell development.

0317**HIGH EFFICACY OF PULSE CYCLOPHOSPHAMIDE IN CORTICOSTEROID-REFRACTORY LIVER GRAFT-VERSUS-HOST DISEASE**

M. Krejci, J. Mayer, M. Doubek, Y. Brychtova, J. Kamelander, J. Vorlicek

Faculty Hospital Brno, BRNO, Czech Republic

Background. Corticosteroid-refractory GvHD is difficult to manage, and is associated with high morbidity and mortality. Cyclophosphamide (Cy) is an established immunosuppressive and cytotoxic drug widely used as a part of conditioning regimens. Pulse Cy in the GvHD treatment is based on the Cy efficiency for the treatment of many autoimmune disorders and the autoimmune nature of GvHD. In our previous work, we showed that intestinal GvHD responded poorly to pulse Cy, whilst liver, skin and oral GvHD responded well. The liver GvHD is more frequent than other GvHD forms. **Aims.** We used pulse Cy in the treatment of corticosteroid-refractory liver GvHD with aims to evaluate efficacy, toxicity and influence of Cy to some clinically significant parameters. We analyzed our new data concerning liver GvHD. **Methods.** This is a retrospective study of 20 patients (pts) with hematological malignancies after allogeneic peripheral blood stem cell transplantation: 12 pts had acute GvHD (2 pts grade I, 3 pts grade II, 7 pts grade III), 4 pts had chronic extensive GvHD and 4 pts developed liver GvHD upon DLI. Three pts had only liver GvHD, 17 pts had GvHD with involvement of liver and/or oral mucosa, skin, gut. Nine patients had hepatic variant of liver GvHD (serum aminotransferase ALT or AST elevation above 10 times the upper normal limit). All patients were treated by cyclosporine A and steroids in dose 2 mg/kg before pulse Cy, six patients had another previous therapy (mycophenolate mofetil, tacrolimus, ATG, alemtuzumab). Steroid-refractory GvHD was defined as lack or response to steroids administered for at least 5 consecutive days. Twenty pts with corticosteroid-refractory liver GvHD were treated by Cy at median dose of 1g/m² (range 460 mg/m²-1500 mg/m²). Sixteen patients received one pulse Cy, 4 patients two pulses of Cy. **Results.** There were 55% CR (11/20), 10% PR (2/20) and 35% NR (7/20). However, in 3 pts with NR their clinical status stabilized and they responded to another treatment. Eight pts (89%) from nine pts with hepatic variant of liver GvHD reached CR. Five pts died, 3 from intractable liver and intestinal GvHD, 1 from intestinal GvHD with liver GvHD in PR, and 1 from relaps of leukemia. No influence of pulse Cy to chimerism and disease status was observed. Leukopenia and/or thrombocytopenia WHO grade 4 developed in 5 patients. When myelosuppression appeared, it was usually short-lived (1-4 days). Twelve infectious complications occurred in 8 of 20 pts (pneumonia 2x, febrile neutropenia 1x, CMV positivity 6x, BKV positivity 3x), all of them resolved after antimicrobial therapy. No other significance toxicity after Cy pulse was observed. Overall survival is 75%, with median and maximum follow-up of 12 and 58 months, respectively. **Conclusions.** Pulse Cy has a good toxicity profile and the cost of the drug is negligible. According to our results, pulse Cy is very effective therapy of steroid-refractory liver GvHD.