353 POSTER

Eosinophilia in bone marrow after hematopoetic stem cell transplantation: does it indicate the onset of acute graft-versus-host disease ?

M. Koldehoff, B. Schmetzer, E. Roemer, M.G. Kiehl, A.A. Fauser, N. Basara. *BMT-Center, Idar-Oberstein, Germany*

Objective: Acute and chronic graft-versus-host disease (GvHD) are frequent complications after allogeneic hematopoetic stem cell transplantation (HSCT) mediated by alloreactive lymphocytes and proinflammatory cytokines. Acute GvHD has been recognized as TH-1 associated process. Eosinophilia has been linked with a variety of diseases such as allergies and parasitic infections and is associated with a TH-2 response triggered by IL-5. Few studies have explored bone marrow morphologic findings in patients (pts) after HSCT. Eosinophilia in HSCT following BU/CY or TBI/CY preparartive regimens has been regarded by some authors as a valuable indicator of evolution to chronic GvHD. However, the significance of eosinophilia in acute GvHD is presently unknown. Significant CD25 antigen expression on the eosinophilia has been recently demonstrated in pts with acute GvHD.

Patients and Methods: We perform prospectively both bone marrow smear and histologic evaluation at day +30 and day +100 in 237 pts transplanted with allogeneic HSC from July 1987 to September 2000 in our department. Ninety-four of them were diagnosed with AML or MDS, 68 with CML or MPS, 46 with ALL, 16 with NHL and Hodgkin lymphoma and 13 with non malignant hematological diseases. 129 pts (54%) received unrelated HSCT and 106 pts (48%) received related HSCT. 20 pts died before the first bone marrow evaluation at day +30.

Results: A significant eosinophilia of more than 7%, determined at 400 bone marrow cells, was found in 32 pts (15% of pts). Twenty-eight (87,5%) of those pts developed acute GvHD grade II-IV. Four pts died due to severe GvHD, 24 pts developed chronic limited GvHD. Conclusions: In conclusion, bone marrow eosinophilia after HSCT, probably mediated by endogenous IL-2 predicts acute GvHD. The use of allogeneic G-CSF mobilized stem cell grafts in pts, which contain a high number of TH-2 polarized lymphocytes, may contribute to the development of eosinophilia in association with GvHD. However, the functional significance is not known and should be determined.

354 POSTER

Total body irradiation (TBI) with stem cell transplantation (SCT) in multiple myeloma (MM)

G. Perry[1], R. Donker[1], S. Dahrouge[1], P. Genest[1], I. Bence-Bruckler[2], H. Atkins[2], C. Bredeson[3], L. Huebsch[2]. [1] *Ottawa Regional Cancer Centre, Radiation Oncology, Ottawa, Canada;* [2] *The Ottawa Hospital, Clinical Hematology, Ottawa, Canada;* [3] *Medical College of Wisconsin, International Bone Marrow Transplant Registry/Autologous Blood & Marrow Transpla, Milwaukee, USA*

Background: Until recently, TBI has been part of the conditioning regimen prior to SCT. Most centers use fractionated TBI. At the ORCC single fraction has been the standard. This study reports on the toxicity associated with this regimen.

Methods: A retrospective analysis of 52 consecutive patients (pts) having received SCT following melphalan 140 mg/m2 + etoposide 60 mg/kg and 500 cGy TBI.

Results: Allogenic/autologous cells were used in 4/48 (8%/92%) pts. Median age was 53 years (27-66). Prior therapy consisted of chemotherapy (8%); radiotherapy (XRT) and both (17%). Induction chemotherapy of VAD was given to 48 (92%) pts. Median/mean dose rate of XRT was 90/87 cGy/min (79-94). Objective response was seen in 42 (81%) pts (25% CR). Median EFS and OS were 30 and 36 months. 4 pts (8%) had deaths potentially related to regimen toxicity: 1 esophageal bleeding and aspiration secondary to thrombocytopenia 1.5 weeks after TBI (wat); 1 pneumonia 5 wat; 1 multi-organ failure 1.5 wat in a pt with prior amyloidosis and nephrotic syndrome; and 1 renal failure 73 wat. 2 pts had grade (gr) 3, 3 gr 4 transient dyspnea < 2 wat. 1 pt died of respiratory failure secondary to graft vs host disease 41 wat. Infection/febrile neutropenia was documented in 40 (77%) pts. Interstitial pneumonitis was observed in 3 pts and resolved within 3 months. FEV1 and/or DLCO were compromised in 7 of 21 evaluable pts 11-50 wat, which in 2 pts returned to normal within 13 and 22 weeks. 26 (50%) pts had diarrhea/colitis (3 were gr 3); 23 (44%) had nausea/vomiting (6 gr 3); 36 pts (69%) had mucositis/dysphagia (16 gr 3 and 1 gr 4). GI symptoms usually occurred <1 wat and always resolved. 8 (15%) pts had cardiovascular manifestations, all of which resolved but one (arrythmia) 0.5-5 wat. 5 (10%) pts developed endocrine abnormalities: hypogonadism, hypothyrodism (HT) (x2), HT+SIADH and transient diabetes; 35-100 wat. 1 pt had mild elevation in creatinine 72 wat and recovered after 1 year; 4 other pts had transient renal insufficiencies <3 wat. 1 pt has blurred vision wat, 1 optic neuropathy 55 wat, 2 cataracts 46-52 wat.

Conclusions: Results suggest this regimen of single fraction, high dose rate TBI does not appear to result in higher toxicity levels. However the use of TBI containing conditioning regimens in general does not appear to provide superior control rates over chemotherapy conditioning regimens alone. For that reason, this approach was discontinued.

355 POSTER

Allogeneic peripheral blood stem cell transplantation following reduced-intensity chemotherapy for refractory sarcoma

P. Pedrazzoli[1], G. Giorgiani[2], G. Landonio[1], D. Montagna[2], M. Renga[1], R. Schiavo[1], F. Locatelli[2], S. Siena[1]. [1] *Ospedale Niguarda Ca' Granda, Divisione di Oncologia Falck, Milano, Italy;* [2] *Università di Pavia e IRCCS Policlinico S. Matteo, Dipartimento di Pediatria, Pavia, Italy*

An immune-mediated graft-versus-tumour (GVT) effect has been recently reported in patients given an allogeneic transplant of hematopoietic stem cells (HSCT) for renal cancer and occasionally in patients with other solid tumors. To evaluate whether refractory sarcomas can benefit from a GVT effect, we are conducting a pilot trial of allogeneic HSCT in patients not amenable to further conventional treatments. The 3 evaluable patients enrolled so far had stage IV, progressive disease: Ewing sarcoma (ES; age 18, lung and bone mets), rhabdomyosarcoma (RMS; age 10, soft tissue and limphnode mets), gastric sarcoma (GS; age 45, liver metas). Preparative regimen consisted of reduced intensity chemotherapy (CT) with fludarabine 30 mg/m2 days from day -5 to day -2 and cytoxan 30 mg/kg days -5 and -4. Patients were transplanted with peripheral blood stem cells from HLA identical siblings (CD34+ cells: 2.4, 4.9 and 6.5x10-6/kg). GVHD prophylaxis consisted of CyA and short-term MTX. All patients experienced mild treatment related complications limited to short-lasting neutropenia and thrombocytopenia </= grade 3 and no organ toxicity. At day +90, 100% donor chimerism was documented by minisatellite PCR in the 2 cases previously treated with intensive CT including autologous HSCT), and mixed donor chimerism was observed in the patient who had previously received only one line of CT (GS). Acute grade 2-3 GVHD occurred in 2 patients. One patient (ES) had initial radiographic evidence of tumor progression (PD) and subsequent 5 months disease stabilisation (SD) after CyA withdrawal and development of GVHD; he died of PD on day 390. GS patient had SD for 6 months post transplant and subsequently had a low progressing disease; he is alive at day +280. Following allogeneic HSCT, the patient with RMS achieved complete remission lasting 5 months, but he subsequently relapsed and died on day 231. We conclude that: 1) allogeneic HSCT following CY/FLU reduced conditioning is feasible in patients with refractory sarcoma; 2) full donor chimerism may be more easily achieved in patients who had received previous intensive CT; 3) tumor regressions or disease stabilisation occurred in these patients thus indicating that a GVT effect can be generated in vivo against sarcoma cells. Two additional patients (1 ES and 1 liposarcoma) received an allogeneic HSCT more recently. The procedure was uneventful and no adverse events were recorded at 1 month post-transplant.

356 POSTER

Allogeneic hematopoietic stem cell transplantation in patients with beta-thalassemia

J. Zakrzewski[1], M. Koldehoff[2], V. Tran[2], S. Günzelmann[2], A. Dürr[2], G. Lentini[2], W. Nürnberger[1], N. Basara[2], M. Kiehl[2], A.A. Fauser[2]. [1] *Bone Marrow Transplantation Center, Pediatric Hematology/Oncology/BMT, Idar-Oberstein, Germany;* [2] *Bone Marrow Transplantation Center, Idar-Oberstein, Germany*

Introduction: Beta-thalassemia major is a hereditary disease characterized by a defective synthesis of the beta-chains of the hemoglobin molecule. The course of the disease in childhood depends almost entirely on whether the child is maintained on an adequate transfusion program. Until now the only curative is an allogeneic hematopoietic stem cell transplantation (HSCT). We herein report on nine patients (six children, three adults, median age: nine years) with beta-thalassemia undergoing allogeneic bone marrow-(BMT) or peripheral blood stem cell transplantation (PBSCT).

Methods: The preparative regimens consist of busulfan 3,5 mg/kg orally on four consecutive days, cyclophosphamide 50 mg/kg intravenously on four consecutive days followed by HSCT. Graft versus host disease (GvHD) prophylaxis included cyclosporine A (CSA) starting on day 2 with 5 mg/kg

and prednisolone starting at day +7 with 0,5 mg/kg, increased to 1 mg/kg on day +14.

Results: Seven patients (3 BMT from a matched related donor (MRD), 1 PBSCT from MRD, 3 BMT from a matched unrelated donor (MUD)) demonstrated a sustained engraftment, chimerism analysis revealed 89% to 100% donor cell origin in peripheral blood samples.

Two patients (PBSCT from MUD) suffered from graft failure after initial engraftment: one patient had full thalassemia recurrence with 0% donor cells, the other patient lost the graft with autologous recovery (early mixed chimerism) followed by re-occurrence of aplastic marrow.

Organ toxicity: most patients demonstrated mucositis grade I-III of the oral cavity and the intestinal tract, reversible elevation of liver enzymes and kidney function tests; CSA associated complications such as seizures, hypertensive crisis and visual hallucinations occurred in two patients.

Graft versus host disease (GvHD): no severe acute GvHD occurred, two patients developed chronic limited GvHD of the skin and liver.

Survival: Seven out of nine patients are well and alive (days +1400 to +60). One patient died due to graft failure, the second patient died two years post-transplant due to intestinal bleeding.

Conclusion: HSCT from MRD and MUD is a well-established treatment in patients with beta-thalassemia. The clinical course and outcome of MRD-BMT seems considerably better than that of MUD-PBSCT. Our results suggest that allogeneic HSCT can even be performed in adult thalassemics without increase in toxicity and infectious complications.

357 POSTER

Idarubicin containing regimen in mm: preliminary results of pilot study of a modified "tandem" transplant program

. *G. Martinelli[1], A. Agazzi[1], P. Greco[2], K. De Luzio[1], R. Pastano[1], A. Alietti[1], P. Mancuso[1], A. Vanazzi[1]*

[1] European Institute of Oncology, Hematoncology Division, Milan, Italy; [2] Pharmacia & Upjhon Pharmaceuticals, Milan, Italy

Background: The definitive role of double HDCT with APBSC has been recently established. However, the optimal myeloablative regimen to be used before HSC transplantation in MM remains questionable. Preliminary results arising from EBMT registry suggest a possible benefit in terms of EFS for those patients who received combination CT as a part of tandem transplant. Idarubicin, antracycline analogue, has demonstrated its activity in some hematologic malignancies. However few data are up to now available on its use in high CT setting.

Materials and methods: From January 1997 to April 2001 we treated in our Institution 15 MM consecutive pts (median age 62 years, range 48-69, ratio male/female 3:1, 10 IgG, 5 IgA, 2 stage II, 11 stage III Durie-Salmon at diagnosis) after previous VAD regimen (median 3 cycles, range 2-6). MGUS preceded MM in 9 (60%) pts, elevated B2 microglobulin was present in 6/14 (42%) and high erytrocyte sedimentation value in 11/15 (73%). Skeletal lesions were demonstrated in 11 (73%): pamidronate was given to 6/15 (40%) and RT in 4/15 (26%) previous HDCT. Five pts received tandem traditional transplant with Melphalan alone and 10 pts, after one cycle of high dose Melphalan, an additional cycle with Melphalan and IDA combination (180 mg/sqm and 45 mg/sqm c.i. respectively) according to PS and age. Each cycle was supported by APBSC reinfusion (at least 2.0 x 10 6 CD34+/kg).

Results: After the VAD chemotherapy, 2 CR (13%), 12 PR (80%) and 1 SD (7%) were observed. After HDCT (12 pts evaluated), the ORR reached to 6 CR (50%) and 6 PR (50%). 4/6 CRs were observed in pts receiving HD IDA containing regimen. With a median follow-up of 22.3 months (range 8-80), 3 pts are still in CR after 27, 3 and 2 months respectively; two of these pts received IDA containing regimen transplant. One pt died of disease. Hematological toxicity observed was more severe for pts receiving antracycline containing regimen; time to WBC recovery was 12 days vs 6 days for the double Alkeran schedule. 57% of pts receiving IDA experienced G3 mucositis and febrile neutropenia. No toxic death was recorded.

Conclusions: Our preliminary results seem to confirm that double HDCT is feasible also in older MM pts. The high rate of CR observed after HDCT confirm a dose-response relationship. The addition of HD IDA in c.i. at least in the second HDCT procedure seems to increase the complete remission rate despite higher hematological and non-hematological toxicity.

358 POSTER

Tbi using compensators: 16 years of experience in patients with b cell malignancies

R. Schneider[1], M. Jensen[1], J. Schultze[1], N. Schmitz[2], B. Kimmig[1].
[1] University Kiel, Clinic for Radiotherapy, Kiel, Germany; [2] University Kiel, 2nd Medical Dept., Kiel, Germany

Purpose: Total body irradiation (TBI) in preparation for BMT and ABSCT is a routine treatment of B Cell Lymphoproliferative Disorders. The aim of this study is to report 16 years of experience with special focus on side effects.

Project: Since 1984 TBI prior to BMT or ABSCT is performed as a preconditioning regimen in B Cell Lymphoproliferative Disorders. The total dose of 12 Gy and a reduced lung dose of 11 Gy is delivered within 6 fractions on 3 subsequent days using a bilateral compensator technique. For calculation of the individual compensators, one for each hemibody, a series of up to 80 CT-scans of the entire body is used to take into account the patient's contour and density distribution of tissue. This controlled optimized dose distribution should minimize the side effects in general. Up to now we treated a total of 218 patients with B Cell malignancies. We treated 33 patients with ALL, 35 with Follicular Lymphomas grade I,II, one pat. with grade III, 14 with Mantle Cell Lymphomas 12 with Lymphoplasmocytic Lymphomas, 9 with Plasmacytoma and 14 with B-CLL. For determination of 3-year-survival-data and incidence of effects associated with TBI we evaluated data of all those 118 pat., treated during the time intervall 12/84 to 12/97. 75 pat. underwent an ABSCT and 43 an Allogenic Stem Cell Transpl. or BMT.

Results and conclusions: Without discrimination of age, sex, disease, chemotherapy etc. we found an overall 3-year-survival-rate of 65%. The overall survival rate up to now is 59%. The follow up time of the survived pat. is 56 months (12 to 17months).Interstial pneumonitis occurred in 7 of 118 pat., 5 of them died. 12 pat. developed other pulmonary complications, 4 of them died. Nausea and vomiting occurred 57% and 32% during the acute phase with radiation, chemotherapy and tranplantation. 22 pat. developed an acute GvHD, 4 of them died.

Long term side effects were seen in 5 cases of cataract complications, in 6 cases of reduced pulmonary function and in 5 cases of reduced kidney and liver function. 13 pat. developed a cGVHD grade I-II. 10 pat. complained a lower capacity or a fatigue. Other complications were observed only in individual cases. 40 pat. had a relapse. Up to now 32 of those died. 60% of the survived pat. did not get any long term side effects.

The TBI using compensators seems to be as effective as other regimen. The rate of acute and long term side effects is obviously lower than using conventional treatment schemes.

359 POSTER

Apoptosis detection on CD34+ cells by flow cytometry on fresh and cryopreserved/thawed leucapheresis products

M.E. de Sousa[1], M.I. Godinho[1], S. Roncon[1], A. Avila[1], P. Torres[1], P. Pimentel[2], A. Carvalhais[1], I.L. Barbosa[1]. *[1] Instituto Português de Oncologia, Serviço de Imuno-Hemoterapia, Porto, Portugal; [2] Instituto Português de Oncologia, UTM, Porto, Portugal*

The apoptotic program is characterised by certain morphological features, including loss of plasma membrane asymmetry. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, exposing PS to the external cellular environment. Annexin V is a phospholipid-binding protein with a high affinity for PS and binds to the cells that exposed it. After the loss of membrane integrity, DNA fragmentation occurs. So, using Annexin V conjugated with phycoeritrin (PE) and use a vital dye like 7-amino-actinomycin D (7-AAD), we can identify the different stages of apoptosis and dead cells. The aim of our work is to look at the effect of cryopreservation on the apoptosis of the CD34+ cells, in G-CSF mobilised PBPC collections, obtained by apheresis. Leucapheresis products (LP) were frozen on a Planner, cryopreserved at -190°C in liquid nitrogen and thawed at +37°C, in a water bath. B So far, we have analysed 7 LP from 4 patients on fresh and thawed samples using the Annexin V-PE Apoptosis Kit I (PharMingen - ENZIfarma, Portugal), CD34 FITC and CD45 APC MoAb (BD - ENZIfarma, Portugal). Cells were labelled according to the manufacturer instructions and analysed by flow cytometry in a FACSCalibur (BD - ENZIfarma, Portugal). After gating on CD34+ cells (based on CD34+ FITC/SSC), the different apoptotic subpopulations were defined by Annexin V and 7-AAD according to the following criteria: Annexin V-/7-AAD- (live cells), Annexin V+/7-AAD- (early apoptotic cells) and Annexin V+/7-AAD+ (late apoptotic or dead cells). In the majority of fresh and thawed samples > 80% CD34+ cells are viable; only in one case, due to freezing problems, all thawed cells were dead. Our