

I/II trial to determine the safety and efficacy of a combination of arsenic trioxide, melphalan and ascorbic acid (AA) as preparative regimen in patients undergoing high-dose therapy (HDT) and autologous hematopoietic progenitor cell transplantation for multiple myeloma (MM). We also assessed the impact ATO levels on melphalan pharmacokinetics (PK), engraftment and toxicity.

Methods: Forty-eight patients with secretory myeloma (23 females, 25 males; median age: 54, range: 35-70) were treated between 4/04 and 8/05. All patient received melphalan 100 mg/m² IV on days -4 and -3 and AA 1000 mg/day IV on days -9 to -3. Patients were randomized to 3 arms; no ATO (arm 1), ATO 0.15 mg/kg IV on days -9 to -3 (arm 2) and ATO 0.25 mg/kg IV on days -9 to -3 (arm 3). Twelve patients had a prior autograft. Median CD34 cells dose infused was 4.5×10^6 /kg (range 2.3 -10.9).

Results: Patients in all 3 arms were evenly matched. With a median F/U of 17 months (range 6-29) post autograft, no dose-limiting toxicity or non-relapse mortality was seen. Toxicity was limited to grade I or II nausea, vomiting and diarrhea and was similar in all 3 arms. Melphalan PK was not altered by ATO pretreatment. Median time to neutrophil engraftment (ANC >500/ dl) was 9 days, with no engraftment failures or delays in the ATO arms. CR rate for the entire group was 23%, and overall response rate (ORR=CR + PR) was 75%. Progression-free survival (PFS) and overall survival (OS) after 17-month F/U were 68% and 82%, respectively. There was no significant difference in CR, ORR, PFS or OS between the 3 arms (p = 0.9, 0.9, 0.5 and 0.6, respectively). A prior autologous transplant (p = 0.02) and abnormal cytogenetics at transplant (p = 0.04) were associated with a significantly shorter remission.

Conclusions: ATO + melphalan + ascorbic acid is a safe, effective and well tolerated preparative regimen for patients with multiple myeloma undergoing an autotransplant. A prior autograft and abnormal cytogenetics are associated with worse outcome.

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AUTOLOGOUS STEM CELL TRANSPLANTATION FOR ELDERLY PATIENTS WITH MULTIPLE MYELOMA

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Background: Several trials have shown that autologous stem cell transplantation is superior to conventional therapy in terms of complete response (CR) rate, event-free survival (EFS) and overall survival (OS). This treatment, however, is generally limited to patients younger than 65 due to concerns about excessive toxicity and treatment-related mortality (TRM) in older patients. Previous reports have shown that age alone should not exclude patients from high-dose therapy, as long as they fulfill other eligibility criteria. In this report we analyzed the safety and efficacy of high-dose chemotherapy (HDT) and autologous transplant in patients with MM who were ≥ 70 years at the time of autotransplant.

Methods: Twenty-six patients (16 males, 10 females) with a median age of 72 (range 70-79) underwent HDT and an autograft between July 1999 and October 2005. The preparative regimen was melphalan 200 mg/m² in 19 patients (73%), melphalan 180 mg/m² in 6 and melphalan 140 mg/m² in 1 patient. Of the 26 patients, 12 were receiving first remission consolidation, 7 had primary refractory disease and 7 had relapsed disease. Clonal cytogenetic abnormalities were present in 5 patients (19%).

Results: Twenty-two of the 26 patients were alive after a median follow up of 25 months (range 8-74). Responses (complete + partial response) were seen in 20 patients (77%), five (19%) of which were complete responses. Median PFS was 24 months and median OS has not been reached yet. 100-day TRM was 0%. Median times to absolute neutrophil count of $\geq 0.5 \times 10^9$ /l and platelets $\geq 20 \times 10^9$ /l were 10 and 10 days, respectively. Three-year PFS and OS were 39% and 65%, respectively. A serum albumin <3.5 g/dl (p=0.02), abnormal cytogenetics at transplant (p=0.05) and >2 prior chemotherapy regimens (p=0.02) were associated with a shorter PFS. Patients transplanted with relapsed

disease had a shorter OS (p=0.0004). ISS stage, $\beta 2$ microglobulin level, lactic dehydrogenase (LDH) level, abnormal cytogenetics, CCI or HCT-CI at the time of transplant did not emerge as significant predictors of PFS or OS in this group of patients.

Conclusions: HDT and autologous transplant is safe and feasible in selected patients ≥ 70 years of age. Patients transplanted with relapsed disease had a shorter OS.

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TOTAL MARROW IRRADIATION (TMI) USING HELICAL TOMOTHERAPY: DOSIMETRIC ANALYSIS DEMONSTRATES REDUCED ORGAN DOSES WHICH CORRELATE WITH REDUCTION IN ACUTE TOXICITIES AND PREDICT FOR ESCALATION OF DOSE TO TARGET MARROW BEYOND THAT ACHIEVABLE BY STANDARD TBI

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TBI dose escalation has been difficult due to associated organ toxicities. We recently demonstrated the feasibility of using Tomotherapy (an image guided IMRT delivery system) to deliver a more targeted dose of TBI to sites of greatest tumor burden (bone/marrow) with reduced dose to normal organs in a patient with multiple myeloma. This study provides a dosimetric analysis of target bone/marrow and normal organ doses from the first 13 patients treated, and details of set-up and delivery using this novel system.

Twelve patients with multiple myeloma (MM) were treated with Mel (200 mg/m²) followed 6 weeks later by TMI as part of a tandem autologous transplant Phase I/II trial. Total TMI doses were 10 Gy (3 patients), 12 Gy (4 patients), 14 Gy (3 patients) and 16 Gy (2 patients) delivered 2 Gy QD or BID over 5 days. One patient with AML was treated with TMI+TLI to 12 Gy (1.5 Gy BID) + concomitant Flu/Mel on a separate trial. Treatment time was 50 minutes, jaw size 2.5 cm, and pitch 0.45. Patients were treated supine with full body immobilization. Whole body CT imaging was performed by the Tomotherapy unit prior to each fraction to provide 3D alignment of patient anatomy to the intended target regions.

Median organ doses ranged from 15-65% that of the target bone/marrow dose. The degree of organ sparing was similar for all patients despite differences in thickness and habitus. Of the 84 TMI treatment sessions delivered, only one was temporarily interrupted due to nausea and vomiting. In the immediate post-TMI period, all MM patients experienced grade 1-2 nausea, with half experiencing no vomiting. Erythema, diarrhea, and mucositis were infrequent and grade 1-2. The AML patient experienced grade 2 nausea, grade 1 vomiting and grade 3 mucositis. This compares favorably to acute symptoms associated with large field hemibody RT or standard TBI.

This study confirms the feasibility of using Tomotherapy to deliver TMI. Dosimetric studies demonstrated reduced organ doses and predicted for reduced toxicities. Clinical results confirmed these predictions. The techniques used can be adapted by most centers with the technology. This study predicts for the ability to dose escalate to a level where normal organs receive comparable standard TBI doses with target regions receiving a significantly higher dose than achievable with TBI, offering the potential for improved outcomes in patients with hematologic malignancies.

GRAFT PROCESSING

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EX-VIVO EXPANSION (EvE) OF PREVIOUSLY CRYOPRESERVED CORD BLOOD (CB) INTO NATURAL KILLER (NK) CELLS WITH ENHANCED AML AND NEUROBLASTOMA CYTOTOXICITY: POTENTIAL ROLE OF CB NK CELLS IN ADOPTIVE CELLULAR IMMUNOTHERAPY (ACI)

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CD56⁺ NK subsets exhibit differential NK receptors (NKR) such as cytotoxicity profiles including killer-Ig-like receptors (KIR), C-lectin (NKG2) and natural cytotoxicity receptors (NCR)

involved with tumor target recognition (Farag et al *Blood*, 2002). In particular, AML and Neuroblastoma are NK sensitive pediatric tumors. CB is limited by the absence of available donor effector cells (NK, CTL, LAK and NKT cells) for infusion after UCBT (Cairo et al *Transfusion*, 2005). We demonstrated the ability to EvE CB in short-term culture with IL-2, IL-7, IL-12 and anti-CD3 with increased CD3⁺/16⁺/56^{dim} and bright subsets expressing KIR3DL1, KIR2DL2, KIR2DL1/S1 and CD94/NKG2a with increased NK and LAK cytotoxicity (Ayello/Cairo et al *BBMT*, 2006). In this study, we compared short-term culture (48 hrs) with prolonged cultures (4-10 days) on expansion of NK cells expressing NCR, KIR, NKG2, lytic ability and mechanisms of tumor lysis. Rethawed CB cells were cultured 2-10 days with anti-CD3 (50 ng/ml), IL-2 (5 ng/ml), IL-7 (10 ng/ml) and IL-12 (10 ng/ml) [ABCY]. NKR expression (KIR2DS4, NKG2D, CD94, NKp46), intracellular granzyme B and LAMP-1 receptor (CD107a) expression were determined by flow cytometry. Cytotoxicity of EvE effector CB cells was measured by europium release assay at 20:1 E:T ratio with tumor targets K562 (NK), Daudi (LAK), Kasumi-1 (AML) and SYSY5Y (neuroblastoma). KIR2DS4 was significantly increased at day 10 vs 2 in ABCY in both CD3⁺/16⁺/56^{dim} and dim subsets (16.9±0.4 vs 2.1±0.2% and 22.3±0.3 vs 0.9±0.2%, p<0.001, respectively). C-lectin receptor CD94/NKG2D expression was increased at day 7 vs 2 (41.4±0.43 vs 23.7±2.0%, p<0.001). NCR expression in CD3⁺/16⁺/56^{dim} NKp46 subset was increased at day 7 vs 2 (10.1±0.06 vs 2.62±0.8, p<0.001). Granzyme B expression was increased from day 2 to 10 (25.8±1.7 vs 45.1±1.7%, p<0.001). CD107a expression was significantly increased at day 7 vs 2 (12.9 ±1.4 vs 69.3±2.2%, p<0.001). Additionally, increased cytotoxicity was demonstrated at day 7 vs 2 with tumor targets K562 (71.5±0.81 vs 53.8±3.9%, p<0.001), Daudi (63.9±0.73 vs 38±1.1%, p<0.001), Kasumi-1 (56.6±0.4 vs 31.8±1.8, p<0.001) and SYSY5Y (59.5±5.35 vs 32.6±4.9%, p<0.01). In summary, CB MNC may be thawed at time of transplantation, cryopreserved, rethawed at a later date, expanded and activated up to 10 days to yield viable NK subsets which appear to be cytolytic against AML and Neuroblastoma and could be potentially used as ACI post UCBT.

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EARLY HEMATOPOIETIC CELLS, INCLUDING MEGAKARYOCYTE PROGENITORS, ARE RECOVERED IN ALDH BRIGHT CELL POPULATIONS ISOLATED BY CELL SORTING FROM PREVIOUSLY FROZEN UMBILICAL CORD BLOOD

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ALDH bright [ALDH^{br}] cell populations sorted from fresh umbilical cord blood [UCB] on the basis of their high aldehyde dehydrogenase [ALDH] activity are known to include hematopoietic progenitor cells [HPC]. However, neither the hematopoietic potential of ALDH^{br} cells recovered from previously frozen UCB, nor the ability of any ALDH^{br} cells to generate platelets has been reported. We have measured hematopoietic [CFC-H] and megakaryocytic [CFC-M] colony forming cells in ALDH^{br} and ALDH^{dim} [depleted of ALDH^{br} cells] populations from thawed UCB. Cells were washed, immunomagnetically depleted of cells expressing glycophorin A and CD14, reacted with ALDEFUOR[®] for flow cytometric detection of ALDH, and sorted to yield ALDH^{br} and ALDH^{dim} populations. ALDH^{br} cells comprised 0.38 ± 0.15% [mean ± SD] of thawed UCB cells. CD34⁺ and CD133⁺ cells comprised 90.9 ± 3.1% and 57.7 ± 9.4 % of ALDH^{br} cells, respectively. CD41⁺ and CD110⁺ cells were 23.4 ± 11.7% and 1.7 ± 3.4 % of the ALDH^{br} population, respectively. CFC-H activity was enriched 1116-fold in the ALDH^{br} population compared to the ALDH^{dim} population [256 ± 104 colonies/1000 cells vs. 0.23 ± 0.33 colonies/1000 cells], and 65% of the CHC-H were recovered in the ALDH^{br} population. The ratio of erythroid to myeloid colonies generated from ALDH^{br} population was 3.7. Others have shown that most ALDH^{dim} HPCs in fresh UCB are short term progenitors, whereas earlier long-term culture initiating cells and cells that establish long-term grafts in NOD-SCID

mouse models are ALDH^{br}. Consistent with this, we found that GEMM colonies are about ten times more frequent in ALDH^{br} CHC-H [6.6 ± 4.5 GEMM/1000 cells] than in ALDH^{dim} [0.6 ± 1.2 GEMM/1000 cells] cells from thawed UCB. Furthermore, CFC-M activity was 2015-fold higher in the ALDH^{br} population than the ALDH^{dim} population [60 ± 35 colonies/1000 cells vs. 0.03 ± 0.02 colonies/1000 cells]. Cells giving rise to large megakaryocyte colonies, usually considered to be CFC-M with the most self-renewal potential, were particularly enriched in the ALDH^{br} populations; all the large colonies we detected [38.0 ± 17.4 large CFC-M/1000 cells] were derived from ALDH^{br} populations. The rare CFC-M colonies from ALDH^{dim} cells were small or non-megakaryocytic. These results suggest that ALDH^{br} cells recovered from thawed, banked UCB could be used to reconstitute erythroid and myeloid, including megakaryocytic, blood elements after transplantation.

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PD-1 IS REQUIRED TO INDUCE PERIPHERAL CD8 T CELL TOLERANCE IN RECIPIENTS OF ALLOGENEIC BONE MARROW TRANSPLANTATION WITH ANTI-CD154

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We examined the mechanisms involved in peripheral CD8 T cell tolerance induced by mixed chimerism achieved with non-myeloablative conditioning with low-dose (3 Gy) total body irradiation (TBI) and 2mg of anti-CD154 antibody. CD8 T cell tolerance is CD4 dependent and is characterized by a specific anergic state toward donor antigens prior to specific deletion of donor-reactive cells. We tested the role of the PD-1 pathway in a model in which only CD4 peripheral tolerance is required (i.e. TBI 3 Gy and depleting anti-CD8 mAb Day -1, 2mg of anti-CD154 Day 0 followed by allogeneic BMT) and in a model in which both CD4 and CD8 peripheral tolerance is required (i.e. TBI 3 Gy Day -1 and 2mg of anti-CD154 followed by allogeneic BMT) for the development of mixed chimerism. PD1^{-/-} (C57BL/6 background) or C57BL/6 wild-type mice received fully allogeneic bone marrow cells (B10.A). While WT control mice showed successful mixed chimerism induction with both regimens, the PD1^{-/-} recipients failed to develop mixed chimerism unless they were CD8 depleted. These results indicate that PD1 is required for the tolerance of peripheral donor-reactive CD8 cells but not for that of CD4 T cells. We confirmed these results using blocking anti-PD1 and anti-PD-L1 mAb in WT B6 recipient mice. While control groups again showed successful engraftment, recipient mice treated with blocking anti-PD-1 and anti-PDL-1 mAb failed to develop mixed chimerism unless they were depleted of CD8 cells. Chimeric mice that were CD8 depleted and received PD-1 blockade accepted donor skin grafts while rejecting third party grafts. Thus, a functional PD1-PDL1 pathway is critical to achieve donor-specific CD8 T cell tolerance in our model.

GVH/GVL

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BLOOD EOSINOPHILIA AS A MARKER OF FAVORABLE OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Eosinophilia is observed in a variety of systemic disorders including acute and chronic graft-versus-host disease (GVHD) after allogeneic stem cell transplantation (allo-SCT). The clinical records of 237 adult patients who underwent allo-SCT were retrospectively reviewed. Peripheral complete blood cell counts (CBCs) had been performed at least two or three times a week until day 100 in all cases, and white blood cell differentiation was