$10^6$ transplanted unilaterally) and single positive $RT1^n$ ($4.5 \times 10^6$ to $9.0 \times 10^6$) and $RT1^a$ ($6.0 \times 10^6$ to $12.0 \times 10^6$) cells transplanted bilaterally to naive Lewis rat. Donor-specific chimerism (for MHC class I antigens) and efficacy of MACS sorting was assessed by flow cytometry. Therapeutic effect of adoptive transfer of donor chimeric cells was evaluated following bilateral transplantation of skin flaps of donor origin (LBN and ACI). **Results:** In primary trimeras 13.1% of LBN donor positive cells ($RT1^n$) and 6.8% of ACI donor positive cells ($RT1^a$) was found. MACS-sorting revealed 87%-96% purity of double positive $RT1^n/RT1^a$ cells. At day 21 secondary trimera created via double positive cell transplantation ($RT1^n/RT1^a$) revealed 8.3% of $RT1^n$ and 11.3% of $RT1^a$ positive cells, whereas injection of single positive cells ($RT1^n$ and $RT1^a$) resulted in 6.0% of $RT1^n$ and 7.6% of $RT1^a$ chimerism. Prolonged skin flap survival was achieved over 84 days after double positive $RT1^n/RT1^a$ transplant and over 120 days in single positive chimeric cells recipients (still under observation). **Conclusions:** Intraosseous transplantation of bone marrow from two different MHC mismatched donors under a 7-day αβ-TCRmAb/CsA protocol resulted in creation of donor-specific trimera. Isolation and adoptive transfer of chimeric cells proved to be efficacious in extension of donors skin flap survival without prolonged immunosuppression.

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### BONE MARROW TRANSPLANTATION (BMT) FOR HEAVILY TRANSFUSED PATIENTS (pts) WITH SEVERE APLASTIC ANEMIA (SAA): 147 PTS TREATED AT THE SAME INSTITUTION WITH BUSULFAN (BU) + CYCLOPHOSPHAMIDE (CY)

*Bonfim, C., Bitencourt, M., Funke, V., Setubal, D., Ruiz, J., Doro, M., Medeiros, C.R., Zanis-Neto, J., Pasquini, R. Federal University of Parana, Curitiba, PR, Brazil.*

BMT from HLA identical siblings is currently the best treatment for young pts with SAA. However, heavily transfused pts have an inferior survival due to an increased risk of rejection and transplant related mortality (TRM). In developing countries not only the number but the quality of pretransplant transfusions should be considered because most of them are not filtered or irradiated. In 1993 we introduced BU to the preparatory regimen of pts with more than 15 pretransplant transfusions trying to reduce the elevated incidence of rejection observed in pts receiving only CY. In this study we retrospectively analyzed the results in 147 pts transplanted between 01/93-01/05 with BU + CY. Age: 2-46 y (M: 19). Previous blood transfusions: 15-675 (M: 34 UI). Disease duration: 1-114 mo (M: 4). All pts received bone marrow from HLA identical siblings. Preparatory regimen: BU 12 mg/kg+ CY 120 mg/kg. GVHD prophylaxis: cyclosporine + methotrexate. TNC infused: $1.17\text{-}6.55 \times 10^8$/kg (M: 3.01). Immediately prior to BMT, 39 pts (26%) had active bacterial or fungal infections. One hundred and four pts are alive with a median follow up of 2719 days (range: 189-4506). Eight pts died before day +28 and were not evaluable for engraftment. Primary graft failure (GF) occurred in 4/139 pts (2.8%) and all pts died. Late GF occurred in 19/135 pts (14%) between 207-1448 days after BMT (M: 584 days). 10/19 pts are alive (5 after a 2nd BMT and 5 after treatment with immune suppression). Mucositis grade III-IV occurred in 80 pts (54%). VOD: 2 pts. Hemorrhagic cystitis: 13 pts (8.8%). Acute GVHD grade II-IV: 20/135 pts (III-IV: 11 pts—8%). Chronic GVHD: 22/128 pts (extensive: 16 pts—12.5%). Forty-three pts died between 10-3072 days after BMT (M: 154 days). Death was mainly related to infections or bleeding. 17 pts died before day +100 and 76% of them had severe infections prior to transplant. 11/16 pts with extensive chronic GVHD died. Pts with more than 50 previous blood transfusions had an inferior survival when compared to those receiving between 15-50 UI (54% × 74%; $P < .005$). 28 pts are surviving more than 10 y after BMT with a satisfactory quality of life. We conclude that the use of BU + CY reduced the risk of rejection in this group of pts. Survival reached 70% in pts receiving between 15-50 transfusions. Alternative preparatory regimens could be offered to pts receiving >50 UI before transplant. Infections immediately prior to conditioning increased the risk of TRM.

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### THERAPEUTIC ANTI-TUMOR IMMUNITY MEDIATED BY TRANSIENTLY ENGRAFTING ALLOGENEIC LYMPHOCYTES: THE "ALLOGENEIC EFFECT" REVISITED

*Symons, H.J., Levy, M.Y., Wang, J., Zhou, X., Fuchs, E.J. Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD.*

The "allogeneic effect" refers to the induction of host B cell antibody synthesis or host T cell cytotoxicity, including tumoricidal activity, by an infusion of allogeneic lymphocytes. We have previously shown that treatment of mice with cyclophosphamide (Cy) followed by infusion of CD8+ T cell-depleted allogeneic spleen cells (Cy + CD8− DLI) induces anti-tumor activity in a model of minimal residual leukemia, even though the donor cells are eventually rejected by the host immune system. The purpose of the current investigation was to test the activity of Cy + CD8− DLI in the treatment of well-established cancer, and to characterize the mechanisms of the anti-tumor effect. BALB/c mice were inoculated intravenously (IV) with the syngeneic A20 lymphoma/leukemia or the RENCA renal cell carcinoma on day 0 and were then treated with nothing, Cy alone on day 14, or Cy + $CD8^-$ DLI from MHC-mismatched C57BL/6 donors on day 15. In both tumor models, the combination of Cy + $CD8^-$ DLI significantly prolonged survival compared to mice treated with nothing or with Cy alone. While depletion of CD4+ T cells from the DLI significantly diminished the beneficial effect of CD8− DLI, purified CD4+ T cells alone were inactive, demonstrating that donor CD4+ T cells and another population of cells were required for optimal anti-tumor activity. Several observations pointed to an active role for the host immune system in the anti-tumor activity of Cy + CD8− DLI. First, host T cells participated in the anti-tumor effect of treatment with Cy alone, since the drug's activity was diminished in tumor-bearing scid mice or in normal BALB/c mice depleted of T cells. Second, while Cy + CD8− DLI caused no GVHD in tumor-bearing but immunocompetent BALB/c recipients, it caused fatal acute GVHD in either tumor-bearing scid or T-cell depleted BALB/c mice. Finally, the anti-tumor effect of Cy and Cy + CD8− DLI was also significantly inhibited in BALB/c mice depleted of CD8+ T cells. These results demonstrate that transiently engrafting T cells administered after Cy can induce significant anti-tumor effects against solid and liquid tumors. We propose that upon recognition of alloantigen on host antigen-presenting cells (APCs), allogeneic donor $CD4^+$ T cells deliver activating ligands to the APCs, thereby generating effective "help" to break tolerance in tumor-specific host CD8+ T cells. This mechanism may correspond to the "allogeneic effect" in the anti-tumor response described over three decades ago.

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### TREATMENT OF MYELODYSPLASTIC SYNDROMES WITH ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)—IMPACT OF NON-RELAPSE MORTALITY ON OUTCOMES

*Ativitavas, T.[1], Saliba, R.[2], Sergio, G.[2], Ghosh, S.[2], Champlin, R.[2], de Lima, M.[2] [1]Mahido University, Bangkok, Thailand; [2]Department of Blood and Marrow Transplantation, Houston, TX.*

**Introduction:** Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders potentially curable with allogeneic HSCT. Non-relapse mortality (NRM) is a major cause of treatment failure in this context. Preparative regimens may impact survival (S) differently given dissimilar toxicity rates. We investigated this hypothesis in a dataset of patients with MDS (as defined by WHO criteria) treated in our institution from January 1993-December 2004. **Methods:** We reviewed 88 patients (65% males); 30 patients (34%) received induction chemotherapy prior to HSCT (4 RA, 4 RARS, and 22 RAEB) and 58 patients (66%) did not (30 RA, 3 RARS, 21 RAEB, and 4 refractory cytopenia). Median age was 50 years (20-69). IPSS was high risk in 27%, intermediate 2 in 40%, intermediate 1 in 28%, and low in 5%. Cytogenetics were poor in 52%, intermediate in 17% and good in 31% of patients. 53 patients (60%) were treated with myeloablative regimens including 12 Gy TBI-based (11/53), Bu/Cy (17/53) and Bu/Flu (25/53). 35 (40%) patients were treated with reduced intensity regimens (Flu/Mel, FAI or low-dose busulfan/Flu). Donor was matched-related in