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BUSULFAN/MELPHALAN/ATG (BU/MEL/ATG) AS A PREPARATIVE REGIMEN FOR UNRELATED DONOR CORD BLOOD TRANSPLANTATION (UCBT): THE COBLT EXPERIENCE

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A non-TBI based preparative regimen, Bu/Mel/ATG was studied in the setting of UCBT. Children with infant ALL and older patients (pts) unable to tolerate TBI or with high risk/refractory leukemia were enrolled, n=38. Indications for transplant were 15 AML, 17 ALL, 1 undifferentiated leukemia, 2 JMML, and 3 MDS; most pts had high risk disease. Donor/recipient HLA matching was 5 or 6/6 (n=21) or 4/6 (n=17) by low resolution molecular typing for HLA A, B, and high resolution (HR) DRB1 typing. However by HR class I typing, 6 pts received 3/6 or less matched units. Bu was given on d −8 through d −5 either orally (1 mg/kg) or by IV (Busulfex 1 mg/kg if age ≤4 yrs or 0.8 mg/kg if age>4 yrs) q 6 h x 16 doses. Bu doses were adjusted to target Css 600 – 900 ng/mL. Mel (45 mg/m^2 x3 doses on d −4 through d −2). ATG (30 mg/kg/day) was given on d −3 through d −1. GVHD prophylaxis was cyclosporine and steroids. The median age was 2.0 yrs (range 0.5–17.3 yrs) and median wt was 10.9 kg (range 4.5–90.0 kg). The cord blood infusion had a median 9.7 x 10^{7}nucleated cells/kg (range 1.4 to 27.7) with a median 2.6 x 10^5 CD34+ cells/kg (range 0.2 to 9.5, n=31), precryopreservation. By d +42 post transplant 16/38 pts had not achieved ANC>500/mm^3. However, 3 patients engrafted on days +44, +49, +53; 3 had early relapse and 7 expired due to transplant related mortality prior to day +42. The cumulative incidence (CINC) of ANC>500/mm^3 was 0.66 (95% CI 0.53, 0.82). In pts achieving ANC>500, the median time to recovery was 27 d (range 11 to 53 d). The CINC of platelets > 20K was 0.52 (95% CI 0.34, 0.69). In pts achieving plts > 20K, the median time to recovery was 64 d (range 30 to 272 d). Gr III/IV aGVHD was seen in 11 (29%). CGVHD occurred in 4/20 (limited 3) pts who survived >100 d. VOD occurred in 3 pts(1 fatal). Failure after d +100 was due to relapse (3), nonengraftment (1), and cGVHD (1). The non-relapse mortality at 180 d was 0.35 (95% CI 0.19, 0.50). With a median f/u of 7.8 mos (2.5 – 27.2 mos), the 1 yr survival probability is 0.41 (SE=0.09). Conclusion: in this high risk patient cohort Bu/Mel/ATG may be sufficiently myeloablative and immunosuppressive to allow engraftment of UCBT in pts with acute leukemia. Heavily pretreated pts experienced a high incidence of early infections and regimen related toxicity.

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DONOR CD4$^+$CD25$^+$ REGULATORY T-CELLS, A CD4$^+$ POPULATION CAPABLE OF SUPPORTING HEMATOPOIETIC ENGRAFTMENT WITHOUT INDUCING GVHD

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Bone marrow transplantation (BMT) is a potentially curative treatment for many diseases, but the wider use of allogeneic BMT is limited by the frequent and severe outcome of graft vs. host disease (GVHD). Unfortunately, efforts to reduce GVHD by removing donor T-cells from the graft have resulted in poor engraftment and elevated disease recurrence. Alternative cell populations capable of supporting allogeneic hematopoietic stem/progenitor cell (HSPC) engraftment without inducing GVHD could increase the use of this treatment while broadening the pool of acceptable donors. Although unfractionated CD4$^+$ T-cells have not been shown to be an efficient facilitating population, we investigated the capacity of the CD4$^+$CD25$^+$ regulatory (T-reg) subset to support donor HSPC engraftment. In a murine allogeneic mismatched model of 1-2x10^6 C57BL/6 (B6) T-cell depleted bone marrow cells (BM-TCD) transplanted into 7.0 Gy sublethally irradiated BALB/c recipients, donor B6 T-regs (1x10^6) injected with BM-TCD supported significantly greater IL-3 responsive donor progenitor colonies one week after transplant and significantly increased donor chimerism in the blood, bone marrow, lymph nodes, and spleen one month after transplant. In contrast to BM-TCD supplemented with either CD25 depleted T-cells or highly enriched CD4$^+$CD25$^-$ T-cells, transplantation of allogeneic T-reg supplemented marrow did not result in GVHD. In immunological analyses 3-4 months post-BMT, chimeric recipients transplanted with T-regs did not generate killing against H-2^b (donor) or H-2^d (host) targets. However, recipients of only BM-TCD that rejected the donor graft did generate anti-H-2^b killing. Cells from T-reg recipients exhibited normal proliferation in response to LPS or anti-CD3 mAb. Furthermore, injection of T-reg recipients with allogeneic H-2^k cells resulted in a primed anti H-2^k CTL response. These results indicate that a CD4$^+$ T-cell population is capable of increasing donor engraftment without inducing GVHD. Importantly, T-reg recipients do not appear to be immunologically impaired, but may be specifically tolerant to donor and host cells. CD4$^+$CD25$^+$ T-cells may thus provide a useful alternative to unfractionated T-cells for supporting engraftment during non-myeloablative BMT.

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INTRAVENOUS BUSULFAN CONDITIONING PRIOR TO ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: MYELOABLATION WITH REDUCED TOXICITY

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Allogeneic transplantation is a potentially curative treatment for hematologic malignancies but is associated with a high rate of complications. Busulfan is a common component of pretransplant conditioning but has an erratic and unpredictable bioavailability when administered orally. Intravenous busulfan was recently introduced into clinical practice. Prior studies showed consistent and predictable drug delivery with tight control of busulfan plasma levels avoiding over and under-dosing. This study was designed to define the role of intravenous busulfan in different transplant and disease settings. It included 45 patients with various hematologic malignancies conditioned with high-dose intravenous busulfan containing regimens prior to allogeneic transplantation. The donors were HLA-matched siblings (n=24), matched unrelated (n=15) or 1-antigen mismatched related donors (n=6). Forty-four patients had initial engraftment. The toxicity profile was favorable. No patient developed VOD. Acute GVHD grade II-IV occurred in 18 patients (40%). Six patients died of treatment-related causes, five of complications related to acute GVHD and only one died of organ toxicity. Actuarial non-relapse-related mortality risk was 10% at day 100 and 17% at 2 years post-transplant. The actuarial 2-year OS and DFS rates are 62% and 44%, respectively. Disease status other than refractory relapse, myeloid disease, and no severe GVHD post-transplant predicted for longer DFS in a multivariant model. Intravenous busulfan containing regimens allows consistent engraftment of allografts from related and unrelated donors such that myeloablation is administered with a toxicity profile typical of non-myeloablative conditioning. Favorable outcome was seen in patients with myeloid leukemias and in early or intermediately advanced disease however this regimen may not be sufficiently cytoreductive in patients with very advanced or active leukemia and in ALL. Intravenous busulfan merits further studies to better define its role as a preferred substitute for oral busulfan in pretransplant conditioning.