(n = 10), NHL (n = 7), Hodgkin's lymphoma (n = 6), advanced CML (n = 4), and advanced CLL (n = 4). 9 pts had previously undergone autografting. 80% received HLA-identical grafts. All pts were conditioned with fludarabine (30 mg/m^2/day, days -7 to -3), busulfan (0.8 mg/kg/dose IV × 8 doses) and rabbit ATG (2.5 mg/kg/day, days -4 to -2) followed by micro-dose methotrexate and tacrolimus. Stem cell source included peripheral blood (n = 26) or bone marrow (n = 4). All pts engrafted neutrophils and platelets promptly (median 15 and 16 days, respectively). There were no primary graft failures. Rates of grade II-IV and III-IV aGVHD were 43% (n = 13) and 23% (n = 7) respectively. 9 pts (30%) developed cGVHD but extensive cGVHD was seen in only 10% (n = 3). Day 100 TRM was 10% (n = 3). CMV and EBV reactivation occurred in 30% (n = 9) and 20% (n = 6) respectively. 2 pts developed PTLD requiring rituximab. 3 pts had BK-virus associated hemorrhagic cystitis. Chimerism analysis showed 100% donor CD33+ at all time points (days 30, 60, 100) and median donor CD3+ chimerism of 94% at day +30 and 100% at day +100. One pt had secondary graft failure. 23 pts (76%) were in CR after SCT. Kaplan-Meier estimates of overall survival (OS) and progression free survival (PFS) at 1 year are 62% and 43% respectively. OS (P = 0.95) and PFS (P = 0.65) was not statistically significant between recipients of matched and mismatched grafts. In conclusion, FBA and tacrolimus based GVHD prophylaxis achieved rapid donor chimerism and a favorably low incidence of TRM and cGVHD despite being tested in poor risk pts. However the rates of EBV reactivation and disease relapse warrant further exploration of this approach using lower doses of ATG (e.g. 5–6 mg/kg total dose).

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ALLOGENIC STEM CELL TRANSPLANTATION IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA AND FANCONNI ANEMIA: A SINGLE CENTER EXPERIENCE OVER 11 YEARS

Rosales, C., Abello, V., Esguerra, H.J., Pedraza, E., Linares, A., Rosales, M.L. Clínica de Marly, Bogotá, Colombia.

We performed a retrospective analysis, of allogenic stem cell transplantation (allo-SCT) results, in 40 patients with diagnosis of acquired aplastic anemia or Fanconni Anemia, in a transplant center in Bogotá, Colombia, between 1996 and 2007.

During that period, 42 transplants were performed in 40 patients. 28 (70%) acquired aplastic anemia and 12 (30%) Fanconni's Anemia. 17 (42.5%) female/25 (62.5%) male. Mean age was 22 years (4–57).

Stem cells were obtained from peripheral blood in 36 (90%) and from bone marrow in 6 (15%). The majority of patients were of high risk; the mean time from diagnosis to transplant was 27.8 months (2–141) and 57.5% of them had received more than 20 transfusions before transplantation. Patients were conditioned with Cy-ATG in 24 (57.1%), high dose Cyclophosphamide (Cy) in 11 (26.2%), fludarabine-Cy-ATG in 3 (7.14%) and Alemtuzumab-Cy in 4 (9.52%).

Mean CD34+ cell dose was 3.3 (1.08–6.66), TNC: 9.7 (1.2–59.2).

Neutrophil engraftment was achieved at day +16 (3–54) post-transplantation.

At a mean follow up of 19.5 months (7–128), overall survival is 65%.

Comparison of results of different conditioning regimens shows that overall survival for patients conditioned with Cy alone is disappointing, 8/11 patients died, 6 due to GVHD and 2 due to infection. After adding ATG to Cy, mortality due to GVHD was significantly reduced. Only 7/24 patients died, GVHD was the cause of death only in 2. Infection was the cause of death in other 4. 2 patients had secondary graft failure, one died. The other received a second transplant and is alive with mixed chimerism, but free of transfusion support.

Of 4 patients treated with alemtuzumab containing regimens, 3 had secondary graft failure, 2 died, and one received a second transplant successfully.

On this group of patients Cy-ATG was the best conditioning regimen in terms of overall survival and GVHD incidence. The majority of patients in this cohort were remitted to transplant late in their disease, early treatment will probably improve the outcomes, as it has been confirmed in other papers.

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DOG LEUKOCYTE ANTIGEN (DLA)-IDENTICAL SIBLING CORD BLOOD TRANSPLANTATION (CBT) FOLLOWING MYELOABLATIVE TOTAL BODY IRRADIATION (TBI)

Lesnikov, V.[1], Baran, S.[1], Abrams, K.[1], Zellmer, S.[1], Yang, Y.-J.[1], Heimfeld, S.[1], Lesnikova, M.[1], Nash, R.A.[1,2], Georges, G.E.[1,2]. [1]Fred Hutchinson Cancer Research Center, Seattle, WA; [2]University of Washington, Seattle, WA.

Cord blood (CB) is increasingly used for hematopoietic cell transplantation due to its rapid availability and less stringent HLA matching requirements. However, low cell dose of CB units and delayed engraftment remain significant obstacles for increased use of CBT in adults. We aim to develop a large animal model of CBT by using outbred dogs to improve the understanding of engraftment across histocompatibility barriers and cell dose limitations of CBT. We harvested and cryopreserved individual units of canine CB obtained from litters following Caesarian section at day 54 to 60 of gestation. We asked if single or multiple units of DLA-identical sibling CB could engraft in DLA-identical recipient dogs. Eight adult dogs received 920 cGy TBI followed by intravenous infusion of thawed CB, either a single CB unit (n = 3) or multiple [2 to 4] CB units (n = 5) with a combined total nucleated cell (TNC) dose range 0.3–2.6 × 10^7/kg. Transplanted total CD34$^+$ cell dose was 0.2–2.5 × 10^5/kg. Postgrafting immunosuppression was cyclosporine + mycophenolate mofetil for 35 and 28 days, respectively. G-CSF was given until recovery of neutrophil counts. Three dogs died on days 13–17 due to neutropenic sepsis. Five dogs engrafted and survived; and are currently 265–621 days after CBT. Sustained neutrophil recovery >1000/μL occurred 29–35 days after CBT, and platelet recovery >20,000/μL was 38–84 days after CBT. Monthly chimerism analysis was assessed by PCR using informative microsatellite markers. Among each of the 4 surviving recipients of multiple unit CBT, all transplanted donor CB units contributed to hematopoiesis with sustained multi-donor chimerism. However, in all 4 dogs, 1 of the CB units eventually dominated hematopoiesis with sustained 75–95% donor chimerism. In all cases the dominant CB unit had the highest TNC dose. There was no acute or chronic GVHD. From 3–8 months after CBT, immune reconstitution studies normalized including T cell proliferation allo-stimulation index, 1^o and 2^o immune response to sheep red blood cells and recovery of the absolute number of CD4 and CD8 T-cell subsets. In summary, cryopreserved DLA-identical CB successfully engrafted and provided durable hematopoietic recovery. There was stable multi-donor chimerism and the CB unit with the greatest TNC dose predicted the dominant donor graft. The approximate minimum cell dose threshold for successful engraftment of a single CB unit with this conditioning regimen model was 0.8 × 10^7 TNC/kg.

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TACROLIMUS DOSING IN ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION RECIPIENTS RECEIVING VORICONAZOLE

Trifilio, S.M., Pi, J., Singhal, S., Frankfurt, O., Evens, A., Gordon, L., Tallman, M., Winter, J., Williams, S., Mehta, J. Northwestern University, Chicago.

Tacrolimus (TAC) is primarily metabolized by the CYP450 3A4 isoenzyme. Voriconazole, often used to prevent fungal infections after allogeneic HSCT, is metabolized by the CYP450 3A4, 2C9 and 2C19 isosenzymes. Clinical trials in healthy volunteers have shown significant drug interactions between the two requiring TAC dose reduction. Ordinarily, TAC is started at the dose of 0.03 mg/kg IV daily on day -1. After starting it at this dose and having to reduce the dose substantially within 2–3 days in all patients receiving concomitant voriconazole 200 mg twice daily orally from day 0, we implemented a simple, preemptive TAC dose reduction strategy to maintain steady-state levels between 5 and 15 ng/mL. As a first step, IV TAC was initiated at the reduced dose of 0.022 mg/kg/day. As a second step, dose was reduced by 30–40% if the steady-state level 48 h after initiation of TAC (day +1) was between 7 and 10 ng/mL, and by 40–50% if the level was between 10 and 15 ng/mL. No change was made if the level was <7 ng/mL. Subsequently, levels were monitored 2–3 times a week and the dose

adjusted as needed. Here, we evaluate the pharmacokinetic effects of concomitant voriconazole administration on blood TAC levels in 27 consecutive allogeneic HSCT recipients (28–64 y; median 55) in whom pre-emptive dose-modification was used during the first 2 weeks. A total of 170 levels (3–12 per patient; median 5) were checked between day +1 and day +16. None of the levels was subtherapeutic (<5 ng/mL), and 34 (20%) were >15 ng/mL. 24 of 27 patients required dose-reduction from day 0 to day +1 based on levels. Each patient required dose-reduction at least twice. An increase in the dose was needed in only 2 patients after initial dose-reduction. TAC doses (median, range) on days 0, 7 and 14 were 1.6 (1–2), 0.6 (0.13–1.4), and 0.4 (0.13–1.1) respectively indicating that the median absolute TAC dose, the median TAC mg/kg dose, and the median per cent TAC dose (100% being the baseline) declined substantially. However, the median TAC level over the first 2 weeks remained between 10 and 14.5. It is clear that lack of pre-emptive dose-reduction would have resulted in TAC levels climbing steadily. Based on this, we recommend starting TAC at 0.02–0.022 mg/kg rather than at 0.03 mg/kg if patients are on concomitant voriconazole, checking levels regularly, and reducing the dose by 30–40% if the 48-h level is 7–10 and by 40–50% if it is 10–15.

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LOW RELAPSE RATE AFTER ALLOGENEIC-SCT FOLLOWING A MYELOABLATIVE PREPARATIVE REGIMEN WITH FLAMSA CHEMOTHERAPY AND TOXICITY-REDUCED CONDITIONING IN PATIENTS WITH HIGH RISK MYELOID MALIGNANCIES AGED 60 YEARS OR OLDER

Schleuning, M.[1], Judith, D.[1], Heshmat, M.[1], Burlakova, I.[1], Taube, R.[1], Jedlickova, Z.[1], Baurmann, H.[1], Kolb, H.-J.[2], Schwerdtfeger, R.[1]. [1] German Diagnostic Clinic Foundation, Wiesbaden, Germany; [2] University of Munich and GSF Research Center for Environment and Health, Munich, Germany.

With the introduction of toxicity-reduced conditioning (TRC) allogeneic stem cell transplantation (allo-SCT) may be considered also for elderly patients (pts). However, non-myeloablative regimens are associated with a high relapse risk. Our strategy combined intensive chemotherapy to induce a status of minimal residual disease, TRC, GVHD prophylaxis with in vivo T-cell depletion and adjuvant donor lymphocyte transfusions (aDLT). This report summarizes our cumulative experience in a cohort of 45 consecutive elderly pts (60–70 y, median 63 y) with myeloid malignancies (18 with high risk cytogenetics) treated with the FLAMSA-RIC protocol, consisting of a 4-day course of chemotherapy (AraC 2 g/m^2, Fludarabine 30 mg/m^2 and Amsacrine 100 mg/2) followed by 3 days rest and TRC with 4Gy TBI, cyclophosphamide and antithymocyte globuline prior to allo-SCT. The underlying diseases were AML (de novo n = 20; sAML n = 16) or progressive MDS (n = 8) with only 5 pts being in CR at the time of transplant. One pt suffered from CML in refractory myeloid blast crisis. To further reduce toxicity TBI was replaced by intravenous Busulfan (8 × 3.2 mg/kg) in the last 16 pts. All but one pt received mobilized peripheral blood stem cells as graft and 9 pts had a sibling and 36 an unrelated donor. GVHD prophylaxis consisted of Cyclosporine A and MMF. The procedure as a whole was clearly myeloablative as evidenced by full donor chimerism at d + 30 in all pts. Engraftment occurred after 10–48 d (median 18 d). Of 25 eligible pts (being alive and free of leukaemia at d + 120) 8 pts actually received aDLT and none of these relapsed. Despite the high relapse risk we observed only 6 deaths because of recurrent leukaemia. Severe acute GVHD occurred in 20% and the non relapse mortality was 31% with 3 pts dying from GVHD, 10 from infection and one from cardiac failure. With a median follow-up of 10 months for surviving pts the Kaplan-Meyer procedure estimates a 43% probability of survival at 2 y after transplantation. Although, the follow-up of pts having received i.v. Busulfan is rather short there seems to be a strong tendency of further reduced toxicity without increased relapse incidence in this cohort. Our data support the notion that toxicity-reduced but still myeloablative conditioning followed by allo-SCT from related or unrelated donors can safely be applied in elderly pts with high risk myeloid malignancies and provides an excellent platform for adoptive immunotherapy after induction of tolerance.

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SIMILAR SURVIVAL AFTER SIBLING VS UNRELATED DONOR ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING

Stein, S., Goldstein, S.C., Smith, J., Luger, S., Loren, A., Stadtmauer, E., Schuster, S., Nasta, S., Tsai, D., Perl, A., Andreadis, B., Frey, N., Kasner, M., Cole, S., Hinkle, J., Porter, D.L. Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA.

Although recent studies have shown comparable survival outcomes between unrelated donor (URD) and sibling donor stem cell transplantation in the myeloablative transplant setting, little comparative data based on donor source is available in the setting of non-myeloablative/reduced intensity conditioning (RIC), where it is presumed that GvL effects must play a key role in long term survival. In this retrospective analysis, we compare the outcome of 111 patients receiving RIC followed by either matched sibling (n = 65) or unrelated donor (n = 46) peripheral blood stem cell (PBSC) transplantation for hematologic malignancies. All patients were deemed ineligible for myeloablative conditioning based on institutional standards for age, comorbid disease, and/or prior therapy. All sibling and 38 of 46 unrelated recipients received A, B, DR matched grafts. The median recipient age in both cohorts was identical; sibling 52 y (range 12–75 y) and unrelated 52 y (range 29–69 y). Conditioning regimens were primarily fludarabine/cytoxan-based in both cohorts, with URD recipients skewed toward the addition of Alemtuzumab pre-transplant (69% URD vs 25% sib), and the use of TBI 200 for 21/23 myeloma pts. The distribution of diagnoses was similar between both cohorts in patients with lymphoid malignancies (NHL, HD, CLL n = 66) 34 sib vs 32 URD, and leukemia/MDS (n = 22), 10 sib vs 12 URD; the diagnosis of myeloma (n = 23) was skewed toward sibling donors (21 vs 2 URD). Kaplan-Meier estimate of overall survival (OS) for all patients at 2 years was 31%. At a median f/u of 43 weeks in both cohorts, overall survival was nearly identical (57% sibs; 55% URD). Of note, there was no statistically significant difference in 2 year OS between sibling and URD recipients (p = 0.25), nor was there a difference in K-M estimates of OS between sibling and URD recipients when patients with lymphoid and myeloid disease were analyzed separately. Among expired patients, there was no difference in the incidence of disease-related (26/42 sibs vs 18/32 URD) or treatment-related [organ failure, infection and GvHD] (16/42 sibs vs 14/32 URD) causes of death. Furthermore, a statistically significant higher proportion of GvHD-related death among URD patients was not seen. These data support the pursuit of unrelated donors for RIC transplantation as an alternative to sibling donors without compromising overall survival.

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DEVELOPMENT AND ANTI-BALB.B REACTIVITY OF T CELLS EMERGING FROM BONE MARROW CELLS TRANSDUCED WITH A CLONAL TCRVβ14-Jβ2.4 SEQUENCE

Appel, M.Y.[1], Friedman, T.M.[2], Korngold, R.[2]. [1] Thomas Jefferson University, Philadelphia, PA; [2] Hackensack University Medical Center, Hackensack, NJ.

In previous experiments utilizing spectratype-sequencing, we reported a dominant T cell receptor (TCR) Vβ14-Jβ2.4 sequence prevalent in the thoracic duct lymphocyte (TDL) population of irradiated BALB. B mice transplanted with C57BL/6 (B6) mouse bone marrow and CD8$^+$ T cells. CD4-dependent CD8$^+$ Vβ14$^+$ T cells mediate lethal graft versus host disease (GVHD) in a B6→BALB. B transplantation model. Therefore, we wanted to investigate the alloreactive potential of T cells expressing the particular isolated TCRβ sequence. We harvested bone marrow from 5-fluorouracil (5-FU) treated TCRβ$^{-/-}$ mice. The bone marrow cells were retrovirally transduced with a construct containing the cDNAs for Green Fluorescent Protein (GFP) and the specific Vβ14-Jβ2.4 TCR sequence. The marrow was then used to reconstitute irradiated B6 mice. 20–40% of the peripheral blood mononuclear cells (PBMC) in these mice were GFP$^+$Vβ14$^+$, of which greater than 95% were CD4$^+$ and 1–4% were CD8$^+$. When the marrow was used to reconstitute MHC II$^{-/-}$ mice, bred to a B6 background,