We have employed a mouse model for neuroblastoma, AGN2a cells injected subcutaneously into strain A/J mice, to demonstrate that transfection of AGN2a with CD80, CD86, CD54, and CD137L transforms this lethal cell line into an effective cell-based vaccine (AGN2a-4P). When tumor vaccines are administered in the context of Treg blockade (with anti-CD25 antibody), the anti-tumor effect is heightened. Our previous work also demonstrated that the inclusion of CD137L in the vaccine preparation is uniquely responsible for a strong anti-tumor T cell response. However, when we switched from tumor-challenge to tumor-bearing regimens the vaccine was ineffective. Even the inclusion of gene expression vectors encoding GM-CSF, IL-15, lymphotactin, or SLC did not inhibit tumor progression. In order to determine if any impact could be made in tumor-bearing animals, we initiated a tumor-bearing model system featuring HSCT. Analysis of lymphoid reconstitution post-HSCT revealed that up to day 21, mice remained severely lymphopenic. However, it was during this time period that vaccination with AGN2a-4P proved most effective in a tumor-challenge experiment. Moreover, vaccination early post-HSCT was markedly improved when T cells were adoptively transferred 3 days after HSCT, just prior to the initiation of AGN2a-4P vaccination. ELISPOT data with purified CD4 and CD8 cells indicated that adoptively transferred lymphocytes early post-conditioning generated an IFN-γ-producing tumor-specific effector population. Furthermore, splenic reconstitution studies at day 21 clearly indicated that animals given lymphocyte adoptive transfer had greater percentages of donor (as opposed to residual host) T cells in their spleens. This indicates that combining adoptive T cell transfer with HSCT alters the lymphocyte populations present early post-HSCT, and that these populations are crucial to the generation of anti-tumor effector cells. We then tested our post-HSCT vaccine strategy in a tumor-bearing model. 1 x 10^6 live tumor cells were given on day -8, TBI on day -1, HSCT consisting of bone marrow plus 6 x 10^6 T cells on day 0, and irradiated AGN2a-4P vaccine on days 2 and 7. Effective neuroblastoma therapy required HSCT, T cell transfer and AGN2a-4P vaccination. Our results support the current practice of autologous HSCT for neuroblastoma therapy, and suggest that the addition of vaccination and adoptive immunotherapy (T cell transfer) to these regimens may improve outcomes.
1 secondary RA; 1 RAEB in CR1), and JMML (1 PR3). Patients received busulfan 8.5mg/kg and cyclophosphamide 110–1150mg/m², fludarabine 30mg/m²/day, and thymoglobin, 2.5 mg/m²/day x 4 days for RD and x 4 days for UD and UCB. Two pts received their grafts after a failure of transplant, 1 after a first failure of transplant and 1 a second failure of transplant. Two pts died prior to day 100 due to toxicity (6%) and the overall NRM is 16%. Acute GVHD (grade I-II) occurred in 3/25 (12%, no grade III-IV) and chronic GVHD occurred in 21/25 (84%, 5% chronic extensive, 3% chronic severe). Median follow-up is 12 months (range 1-35 m). Of 10 pts >30 days out from transplant, 10 pts relapsed. Two year EFS and OS are 49% (SE 9.8) and 49% (SE 11%), respectively. Two relapsed pts are alive at 5 and 1 months after a second transplant and DLI, respectively. One patient who received a second RIC, engrafted, and is alive with cGVHD. Of the 8 pts surviving more than one year from transplant, 4 had AML/MDS (2 in CR2; 1 secondary AML; 1 secondary MDS), 3 had ALL (1 in CR2; 2 in CR1), and 1 had HD in CR3. In conclusion, RIC using bu/flu/ATG in a large cooperative group setting leads to engraftment in >90% of very high risk pediatric pts using a variety of stem cell sources. In spite of significant prior therapy and comorbidities in this cohort, rates of NRM and GVHD and TRM are low. Prolonged relapse free survival has occurred not only in pts with myeloid disease as expected, but also in pts with ALL and lymphoma. Flu/bu/ATG is a promising therapeutic approach for pediatric pts otherwise ineligible for myeloablative transplant.

SIROLIMUS (SRL)-BASED GVHD PROPHYLAXIS TO DECREASE RELAPSE IN PEDIATRIC RELATED AND UNRELATED TRANSPLANT RECIPIENTS WITH VERY-HIGH-RISK ALL: PRELIMINARY RESULTS OF A MULTI-INSTITUTIONAL PILOT STUDY

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Relapse and NRM after allogeneic SCT remain significant barriers to success in treating very high-risk ALL. Preclinical models have shown activity of SRL against human ALL, causing apoptosis and cell death at serum levels used for immune suppression. We hypothesized that SRL would decrease relapse after HSCT for ALL, with acceptable rates of GVHD, thus improving EFS. This multi-institutional pilot trial assessed the feasibility/toxicity of a SRL-based GVHD prophylaxis in RD, UD, and UCB HSCT. After a preparative regimen of TBI, thiotepa and cyclophosphamide, pts received continuous IV tacrolimus (start d-2, target 5-10ng/mL) for 21 days, CSA and MMF. Stem cell source was PBSC in 5/14 patients, including 60% predicted, c) Bilirubin >50 mM or ALT >4 x normal or d) TPN dependent enteropathy. The conditioning regimen consisted of Alemtuzumab 0.2mg/kg x 3 (MUD) or 0.1mg/kg x 3 (MSD) on D-8 to 6, YTH 24.5/34.12 0.4mg/kg on D-5 to D-2, with Fludarabine 150mg/m² and Cyclophosphamide 1200mg/m². GVHD prophylaxis was with CSA and MMF. Stem cell source was PBSC in 5/14 patients, BM in 8/14 and cord blood in 1/14. The Mabs were well tolerated. Median time to neutrophil recovery > 0.5 x 10⁹/L was 9 days (range 0-15). The patient who received a MUD cord blood graft rejected 12/13 evaluable patients who received BM/PBSC engrafted. 7/13 patients achieved 100% donor chimerism, 4/13 had 1-99% donor chimerism (MC in both myeloid and lymphoid lineages and 1/13 low level MC). Six patients developed significant aGVHD (3 grade 2, 3 grade 3). Since BM has been used as the stem cell source, only 1/8 has had GVHD ≥ grade 3. 4/10 evaluable patients have cGVHD (limited or extensive). At a median follow up of 13 months, all patients are alive. 12/13 are stably engrafted and those with > 6 months follow-up have good immune reconstitution. In summary, Mab-based conditioning is well tolerated and achieves engraftment even in patients with severe organ toxicity or DNA repair defects, with a short period of neutropenia. The protocol enables transplantation in patients who previously would not be candidates for such a procedure and may additionally reduce late effects.

REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWED BY TARGETED CONSOLIDATION IMMUNOTHERAPY WITH GEMTUZUMAB OZOGAMICIN IN CHILDREN AND ADOLESCENTS WITH CD33+ ACUTE MYELOID LEUKEMIA

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