suspected. **Patients and Methods:** Six male and 2 female patients with hematologic malignancies, median age 45 (22-55) years, receiving unrelated stem cell transplants in our institution within the last 6 years, experienced primary (n = 5) or secondary (n = 3) graft failure. Match was 7/8 in one, 8/8 in three, 8/10 in three and 9/10 in one case. Primary conditioning was myeloablative in all patients and included rabbit ATG either Genzyme or Fresenius in all but one. Graft source was bone marrow in 7 and PBSC in one case, respectively. Mechanism of graft failure was evaluated by morphology, immunophenotyping, chimerism and kinetics and was judged rejection in three and probably immunologically mediated in 5 cases. At a median of 42 (35-71) days following the first transplant 7 patients received PBSC and one patient bone marrow as a second transplant, 4 from the same and 4 from a different unrelated donor. Matches of the new donors were 6/8, 7/8, 8/10 and 10/10. Two earlier patients were given fludarabin 100 and 120 mg/m^2 combined with ATG Genzyme 5 mg/kg BW, the later ones fludarabin 150 mg/m^2 together with ATG 7.5 mg/kg BW. **Results:** Seven out of 8 patients engrafted at a median of 14 days after second and 58 days after first transplant. One non-engraftment occurred in a polytransfused patient with refractory anemia and primary rejection, after reconditioning with fludarabin 100 mg/m^2 and ATG 5 mg/kg BW and grafting with the same donor. At a median observation time of 555 (266-2020) days 4 patients are alive and well. Four patients died at a median of 135 (75-337) days: two from relapse, one from aGvHD °IV and one from fungal sepsis. **Conclusion:** In primary and secondary graft failure after allogeneic stem cell transplantation, for reconditioning a highly immunosuppressive regimen with limited toxicity is warranted. Fludarabin 150 mg/m^2 and ATG Genzyme 7.5 mg/kg BW is a very efficient and well tolerated combination enabling engraftment even across a two HLA antigen barrier.

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COSTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Purpose: To determine the total costs after allogeneic hematopoietic stem cell transplantation (ASCT) and the pre- and post-transplant conditions or factors associated with increases or decreases in costs. **Patients and Methods:** We collected all in- and outpatient costs during five years in 93 patients who had undergone ASCT in 1998 and 1999 at Huddinge University Hospital in Stockholm, Sweden. The inpatient costs included all those related to a patient from the first day of admission until discharge and then all costs of readmission to any hospital in the Stockholm area. **Results:** The total median cost of five posttransplant years was 163675.61 (61160.87-405790.22) USD. The costs were highest during the first year—i.e., median inpatient and outpatient costs 118165.68 USD and 15339.82 USD, respectively. The total costs during the first year were higher in patients with acute graft-versus-host disease grades III-IV (relative hazards [RH] 1.35, P = .003), bacteremia (RH 1.33, P = .005), veno-occlusive disease of the liver (RH 1.32, P = .005), prophylaxis with granulocyte colony-stimulating factor (G-CSF; RH 1.31, P = .01), acute leukemia (RH 1.32, P = .008) and treatment in hospital instead of at home (RH 1.20, P < .07). During the early transplant period, a second transplantation (RH 1.35, P = .004) and hemorrhagic cystitis (HC) (RH 1.24, P = .03) were also associated with higher costs. The total five-year cost declined with longer survival rates (r = 0.4028, P < .001) and reduced intensity conditioning (RH 0.79, P = .024). **Conclusion:** Higher costs of ASCT were associated with retransplantation, acute leukemia, G-CSF prophylaxis, hospital care, myeloablative conditioning and major transplant-related complications.

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HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) USING FLUDARABINE, BUSULFAN AND THYMOGLOBULIN: A MATCHED COMPARISON TO HCT WITH BUSULFAN AND CYCLOPHOSPHAMIDE

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Allogeneic HCT after standard myeloablative conditioning is associated with significant risks of regimen related mortality and graft versus host disease (GVHD). Over the past 5 years, a novel fludarabine-based conditioning regimen using a low dose of rabbit ATG (Thymoglobulin) with fludarabine and intravenous busulfan has been used at The Alberta Blood and Marrow Transplant Program in Calgary. Initial data suggest a lower incidence of acute GvHD with this approach in a diverse population of patients with acute leukemia (n = 48), chronic myelogenous leukemia (n = 21), chronic lymphocytic leukemia (n = 9), myelodysplasia (n = 22), lymphoma (n = 23) and multiple myeloma (n = 11). To assess further this single center experience, we performed a retrospective analysis comparing their outcomes with matched controls receiving HCT after conditioning with standard busulfan and cyclophosphamide during the same time period. We attempted to select two controls for each case from 574 eligible patients reported to the CIBMTR. All patients were 18 to 65 years of age, received HLA-identical sibling peripheral blood or bone marrow transplants for the diseases noted above from 1999-2003 and were given cyclosporine and methotrexate for GVHD prophylaxis. Controls were selected to match on disease and disease status at transplant and to minimize age differences. Two matches were found for 95 cases, 1 for 26 and none for 13. The latter 13 were excluded from further study leaving 121 cases and 216 matched controls available for comparison. Median follow-up was 30 (range, 12-61) months for cases and 35 (range, 2-72) months for controls (P = .47). Compared to the 216 controls, the 121 cases had lower Karnofsky scores before transplant, and were more likely to receive a peripheral blood transplant. Outcome comparisons used multivariate Cox regression, stratified on the matched pair, to adjust for these differences. The risk of grades II-IV acute GvHD (relative risk [RR] 0.34, 95% confidence interval [CI], 0.20-0.59, P = .0001) and overall mortality (RR 0.48, CI, 0.29-0.77, P = .003) were significantly lower in cases versus controls. The risk of chronic GvHD was similar in the cases and controls. These results suggest that the novel regimen fludarabine, busulfan and Thymoglobulin decreases the risk of acute GvHD and improves survival after HLA-identical sibling HCT and support the development of a prospective multicenter randomized clinical trial to confirm these findings.

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LOW TREATMENT RELATED MORTALITY AND IMPROVED SURVIVAL IN ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANT: TARGETED INTRAVENOUS BUSULFAN COMBINED WITH FLUDARABINE (tBuFlu) AS CONDITIONING REGIMEN

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Myeloablative doses of intravenous busulfan in combination with fludarabine have been employed as conditioning before hematopoietic cell allografts with reduced treatment-related toxicity and mortality. In this report, we describe the early results of a targeted busulfan pharmacokinetic dosing strategy (tBuFlu) used in combination with fludarabine before either related or unrelated grafts We treated 61 pts with tBuFlu prior to allogeneic peripheral blood stem cell transplantation. The median patient age was 48 (range 22-68) years. Patient diagnoses included AML (13 de novo, 7 with prior MDS, and 7 treatment related), MDS (7 pts), MF (5), NHL (6 pts), ALL (6 pts), CML (5 pts), CLL (2), MM (2) and PNH (1). Five patients had received a prior autologous HCT. Donors were

HLA-A, B, C, DRB1, DQB1 matched siblings (29), matched unrelated donors (22), or unrelated donors mismatched for one HLA antigen (6), homozygous mismatch (1), one HLA allele (2), or two HLA alleles (1). Fludarabine 40 mg/m^2 was given intravenously daily for four days, with each infusion followed immediately by intravenous busulfan. The dose of busulfan for days 1 and 2 was 130 mg/m^2. Pharmacokinetic analysis was performed after the first infusion of busulfan; in 59 pts, the goal was to adjust busulfan doses for days 3 and 4 to achieve an average targeted Css level of 800-1000 ng/ml. Levels were drawn incorrectly in 4 of these pts and doses were not changed. Thirty-five (59%) pts had their doses adjusted, increased in 27 and decreased in 8, while 20 pts had Css within the desired range without adjustment. Patients received tacrolimus and standard doses of methotrexate for GVHD prophylaxis, with the exception of five patients. Engraftment occurred in 58 (95%) pts. Thirty (64%) of 47 pts followed for at least 100 days experienced acute GVHD requiring treatment. Six pts have died of transplant-related complications and 7 pts have failed to achieve remission or have relapsed. Median follow-up is 174 days (range 26-448 days). The 100-day K-M estimate of survival for the whole cohort is 92%, and event-free survival 88%. The 100-day mortality in this study compares well with the 100-day mortality reported to the IBMTR for patients with AML, ALL, MDS, and CML transplanted from either HLA-matched siblings or unrelated donors. These preliminary results indicate that tBuFlu is a promising myeloablative regimen that can be utilized in older patients with low early treatment-related mortality.

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LACK OF IMMUNE BARRIER TO ALLOGENEIC HEMATOPOIETIC STEM CELL ENGRAFTMENT IN T, B, AND NK CELL DEFICIENT (RAG2ΓC$^{-/-}$B6) MICE

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The way that allogeneic hematopoietic stem cells (HSC) resist engraftment is not completely understood. Natural killer cells (NK) and lymphocytes are thought to mediate the allograft barrier in mice that are mismatched at the major histocompatibility complex (MHC). The clearing of niche space is also thought to be required for donor cell engraftment. Here we attempt to dissect the relative contribution of these host elements to hematopoietic resistance using genetically defective Rag2$^{-/-}$ (*H2^b*) mice lacking T and B cells, or Rag2γc$^{-/-}$ (*H2^b*) mice lacking in T, B, and NK cells as recipients. We have previously shown that HSCs encounter greater resistance to engraftment when compared to unfractionated bone marrow (BM), and the resistance can be quantitated by titrating numbers of HSCs needed to rescue lethally irradiated recipients. Rescue of syngeneic or CD45 congenic recipients requires only 200 HSCs, whereas higher HSC doses are required as the genetic disparity increases. In this study, radioresistant MHC-mismatched AKR/J (*H2^k*) HSCs were transplanted into lethally irradiated (950 cGy) B6.WT (*H2^b*). All B6.WT mice died of hematopoietic failure despite attempted rescue with 1000 AKR/J HSC. No significant improvement in engraftment was observed in Rag2$^{-/-}$ mice when compared to B6.WT mice. However, an impressive difference was noted in the Rag2γc$^{-/-}$ mice, in which the immune barrier completely disappeared. A dose of 300 HSC rescued all irradiated Rag2γc$^{-/-}$ mice and even 200 AKR HSC, an amount equivalent to a congenic dose rescued 100% of recipients. We then sought to determine if engraftment could be achieved using non-myeloablative conditioning, or no radiation at all. Rag2γc$^{-/-}$ recipients of 6000 AKR/J HSCs treated with 500 cGy-300 cGy resulted in 100% donor engraftment. Additionally, unconditioned Rag2γc$^{-/-}$ also engrafted since 10-20% of donor AKR/J granulocytes were detected. In contrast, unconditioned Rag2$^{-/-}$ mice showed no evidence of donor cell engraftment. We also studied the trafficking of allogeneic FVB (H-2^q) HSC in irradiated versus unirradiated Rag2γc$^{-/-}$ (H-2^d) recipients by in vivo bioluminescence imaging. HSC were observed to enter the marrow space of irradiated mice within minutes following infusion, whereas unirradiated mice demonstrated no luciferase signal until day +5 post-infusion. We conclude that Rag2γc$^{-/-}$ mice have a profound reduction in the immune barrier to allogeneic HSC engraftment and, that in irradiated mice, HSC rapidly enter the marrow.

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POST-TRANSPLANTATION CYCLOPHOSPHAMIDE (Cy) AS A SINGLE AGENT FOR GVHD PROPHYLAXIS AFTER HLA MATCHED RELATED AND UNRELATED BONE MARROW TRANSPLANTATION

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In animal models of BMT, a properly timed high dose of Cy post-BMT selectively eliminates host-versus-graft and graft-versus-host reactive T cells, thereby preventing graft rejection and reducing GVHD. We hypothesized that high dose posttransplant Cy (50 mg/kg IV) administered on days +3 and +4 after BuCy conditioning may be effective in preventing GVHD and can limit, or entirely eliminate the need for, standard postgrafting immunosuppression. This should lessen immunosuppression and allow early institution of additional posttransplant immunotherapy such as DLI. 28 patients with advanced hematologic malignancies were conditioned with busulfan (PO or IV) on days −7 to 3 and Cy on days −2 and −1, transplanted with non–T cell–depleted marrow, and treated with Cy on days +3 and +4 as only postgrafting immunosuppression. 15 patients (median age 41 years) were allografted with bone marrow from HLA-identical siblings. Time to neutrophil (>500/μl) and platelet (>20000/μl, untransfused) engraftment was 22 and 31 days, respectively. One patient experienced secondary graft failure and was successfully rescued. Acute GVHD occurred in 7/15 patients at a median of 43 days after transplantation (range 20-68 days) and was exclusively grades I (2 patients) and II (5 patients). All 7 patients with GVHD responded completely to standard therapy (steroids only or steroids + FK-506) and all of them were successfully rapidly weaned from all immunosuppressive agents. With a median follow-up of 290 days (range 50-380), 10/15 patients are alive (all 5 patients died of relapsed disease) of which 7 are in remission. 13 patients (median age 41 years) received bone marrow from HLA-matched unrelated donors. Primary graft failure occurred in 2 recipients of unrelated marrow, and was fatal in one. One patient died from VOD. Time to neutrophil and platelet engraftment was 25 and 71 days, respectively. Of the 11 patients that engrafted, 1 developed grade I, 4 developed grade II and 1 developed grade III acute GVHD. All of them rapidly responded to standard therapy. From an overall survival perspective, 10/13 patients are alive of which 6 are in remission, with a median follow-up of 290 days (range 75-430). This preliminary analysis suggests that high dose post-transplantation Cy is effective as a single agent in the prophylaxis of severe GVHD after myeloablative conditioning and HLA-matched related BMT and should be studied in patients with standard risk hematologic malignancies.

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OUTCOME OF ALTERNATIVE DONOR TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA CAN BE COMPARABLE TO OUTCOME WITH MATCHED RELATED DONORS

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Matched related donor (MRD) bone marrow transplantation is the treatment of choice for pediatric patients with severe aplastic anemia (SAA); however, only 25% of patients will have an HLA-identical sibling. Alternative donor transplants may be an option for these patients, but such therapies have been associated with greater incidences of graft failures and graft-versus-host disease (GVHD). We retrospectively analyzed 32 pediatric patients who have undergone 34 hematopoietic stem cell transplants for severe aplastic anemia at our institution from April 1997 to April 2005. One patient had a MRD transplant followed by a matched unrelated donor (MUD) transplant, while another had an HLA-mis-