ceived anti-thymocyte globulin 30 mg/M2 days -1, 1, 3, and 6. Engraftment was assessed by whole blood RFLP analysis or XY FISH (sex mismatched transplants). 30/32 patients, including all 11 haplo-stem cell recipients and all 12 unrelated donor recipients, engrafted stably. Only transient, incomplete engraftment was seen in the cord blood recipient and one 5/6 matched recipient. Engraftment was >90% donor in 19/32 (59%) by day 30 and in 20 of 25 (80%) evaluable patients by day 100. 3/5 patients with suboptimal engraftment at day 100 had residual circulating CLL cells. Significant renal dysfunction (creatinine >2.5mg/dl) occurred in 15/32 by Day 100 with 2 patients needing dialysis. Renal failure resolved or abated greatly when tacrolimus was reduced or discontinued. Day 100 transplant related mortality was 3/11 (36%) in haplo-transplant patients and 1/20 (5%) of URD or 5/6 (low risk) transplant patients. Acute GVHD (<D100) was seen in 21 of 32 patients (66%), but was in general easily managed with steroids, particularly after low risk transplants. Day 100 transplant related mortality was 3 of 11 haplo recipients and 1 of 19 low risk patients. In total 20/32 patients survive at a median 209 days post-transplant. 14 of these 20 patients have been free of progression of malignancy since transplant and one more has been disease free for 16 months since chemotherapy and donor lymphocyte infusion (ie. 47% currently progression free). Sirolimus added to tacrolimus and methotrexate appears to facilitate engraftment, minimize graft versus host disease, and potentially contribute to control of malignant disease.

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PERFORIN, FASL, TNFR1, TWEAK, TRAIL, AND DR3-TL1A ARE NOT REQUIRED FOR EFFICIENT T CELL MEDIATED RESISTANCE AGAINST ALLOGENEIC BONE MARROW GRAFTS

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Immunologic resistance to allogeneic BMT is a major concern in clinical transplantation, however the molecular pathway(s) mediating this resistance remains unclear. In antigen sensitized CD8+ T cell mediated resistance models, mice deficient in perforin and FasL (B6-cdd), demonstrate efficient rejection of MHC mismatched or matched allogeneic BM. Surprisingly, resistance remains intact in the absence of TNFR1 or R2 on donor cells, as well as in the presence of blocking mAb to TRAIL. This study further investigates the *in vivo* effector mechanism(s) in the absence of these molecules. TWEAK and TL1a are expressed on T cells and can induce apoptosis when bound to their receptors Fn14 and DR3 respectively. To sensitively examine their role in resistance, blocking mAb's against these ligands were applied with the simultaneous disruption of perforin, FAS-L, TNF, and TRAIL function. B6-cdd mice (H-2^b) were primed 3 weeks prior to 9.0Gy TBI and transplant with 10^7 BALB/c (H-2^d) TNFR1$^{-/-}$ or C3H.SW (H-2^b) BM-TCD to assess both MHC mismatched and matched allogeneic resistance models. On day 0 and +1 post-BMT, recipients received 250 ug of α-TRAIL (N2B2) and α-TWEAK (MTW-1) mAb. In the MiHA disparate model, mice additionally received α-TL1a mAb (L466) or a control non-blocking mAb to TL1a(L3A10). Early presence (Day +5) of both multi-potential CFU-HPP and lineage committed CFU-IL3 progenitor populations was assessed in recipient spleens. Syngeneic control recipients exhibited significant CFU numbers. In contrast, low or absent numbers of both CFU progenitor populations were detected in allogeneic transplants lacking perforin, FasL, and TNFR1. Notably, mice additionally receiving α-TWEAK, TRAIL, and TL1a also exhibited effective resistance. Therefore, resistance remained intact despite the simultaneous disruption of these six candidate effector pathways. These findings highlight the question, what precisely, is the role of apoptosis in allogeneic BMT rejection and also raise the possibility for the involvement of other molecular players distinct from death inducing ligands. In the MHC disparate model, marrow from allogeneic B6 bim$^{-/-}$ donors (kind gift of Dr. A. Strasser) was effectively rejected in BALB/c recipients. These observations indicate that if the intrinsic apoptotic pathway is required for primed T cell resistance against allogeneic progenitors, it is not engaged by Bim. Further studies are in progress to delineate the contribution of these pathways.

Table. *Total Donor Splenic CFU% of Controls*

Recipient (n ≥ 3)	Donor (10^7 Bone Marrow TCD)	mAb Administered (D0, D + 1)	CFU-IL3% Control	CFU-HPP % Control
B6-cdd	B6 gfp	none	100%	100%
B6-cdd	BALB/c TNFR1$^{-/-}$	none	<1%	<1%
B6-cdd	TNFR1$^{-/-}$	αTRAIL + αTWEAK	<1%	<1%
B6-cdd	C3H.SW	none	<3%	<5%
B6-cdd	C3H.SW	αTRAIL + αTWEAK	<1%	<5%
B6-cdd	C3H.SW	αTRAIL + αTWEAK + αTL1a(control)	<8%	<3%
B6-cdd	C3H.SW	αTRAIL + αTWEAK + αTL1a	<7%	<1%

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ACTIVATED ALLOGENEIC NK CELLS AS SUPPRESSORS OF HOST ALLOREACTIVE RESPONSES

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Nonmyeloablative conditioning regimens offer promise in lowering toxicity and extending the applicability of bone marrow transplantation (BMT). However, despite many means of host immunosuppression, marrow rejection by host effector cells remains a significant concern. As NK cells have been shown to be potent immunoregulatory cells both *in vitro* and *in vivo*, we studied the ability of activated donor NK cells to specifically suppress or delete host reactive immune cells, thereby facilitating engraftment of donor marrow. We initially tested this hypothesis *in vitro*. Activated H2^d ALAK (adherent lymphokine activated killer, IL-2 activated T cell depleted bone marrow and spleen cells, >98% NK cells) cells from BALB/c mice significantly suppressed the proliferation of H2^b splenocytes from C57BL/6 (B6) mice in mixed lymphocyte responses (MLR) stimulated with irradiated H2^d splenocytes from BALB/c mice ($p < 0.01$). The ability for H2^b splenocytes to kill H2^d tumor targets was also significantly inhibited by activated H2^d ALAK cells ($p < 0.01$). The same number of H2^b ALAK cells or H2^d splenocytes did not show the same suppressive effect. Interestingly, in a secondary MLR using an allogeneic B6 (H2^b) spleen T cell line (stimulated by irradiated BALB/c splenocytes), activated H2^d ALAK cells also greatly suppressed the activity of the B6 T cells killing H2^d tumor targets. These results suggest that activated H2^d ALAK cells can specifically suppress the anti-H2^d activity of the H2^b splenocytes in both primary and secondary responses. Anti-TGFβ antibody blockade did not diminish this suppressive effect of ALAK cells, suggesting that this activity was not dependent on TGFβ secretion. We are currently testing these findings *in vivo* using a nonmyeloablative BMT model. These *in vitro* studies suggest that activated donor NK cells provide a promising way to promote donor engraftment without involving systemic and nonspecific suppression of the immune system.

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HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THE TREATMENT OF FANCONI ANEMIA USING A FLUDARABINE-BASED CYTOREDUCTIVE REGIMEN AND T-CELL DEPLETED GRAFTS FROM ALTERNATIVE DONORS

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