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Classic strategies for peripheral blood progenitor cell (PBPC) mobilization include daily administration of a growth factor, such as filgrastim, alone or with marrow suppressive chemotherapy. A single injection of pegfilgrastim has been shown to be comparable to daily injections of filgrastim in the treatment of chemotherapy-induced neutropenia. One of the objectives of this phase 1–2 study was to provide dose-finding information regarding the efficacy and kinetics of cytokine-alone PBPC mobilization with pegfilgrastim. **Methods:** Chemotherapy-naive subjects with a variety of solid tumors were randomized to receive a single administration of 6, 12, or 18 mg of pegfilgrastim on day 1 or daily administration of 10 μg/kg filgrastim from day 1 until day 7. Daily blood samples for peripheral CD34+ analysis were collected on days 1–7 and then on days 9 and 12. No chemotherapy was administered during this cycle. For each subject, peak CD34+ cell count was defined as the maximum cell count observed between days 3 to 7, inclusive. **Results:** Of 61 subjects randomized into the study, all received study drug (15 subjects each in the pegfilgrastim 12 mg and 18 mg and filgrastim groups and 16 subjects in the pegfilgrastim 6 mg group). The treatment groups were balanced in terms of demographics and baseline characteristics. The most common tumor types were non-small cell lung cancer (n = 23 [38%]) and ovarian (n = 19 [31%]). The mean peak CD34+ cell count was similar in the filgrastim and pegfilgrastim 6mg groups (4.51 and 4.24 × 10^4/ml, respectively), whereas the pegfilgrastim 12 mg and 18 mg groups had higher mean peaks (8.18 and 9.96 × 10^4/ml, respectively). Pegfilgrastim 12 mg and 18 mg mobilized significantly more peripheral CD34+ cells than filgrastim (P = .034 and .006, respectively). The subject incidence of SAEs was low and comparable between groups. **Conclusion:** In chemotherapy-naive subjects with solid tumor, single-day administration of pegfilgrastim is at least as efficacious as repeated daily injections of filgrastim (10 μg/kg) in its ability to successfully mobilize peripheral CD34+ cells. A dose-response relationship of CD34+ mobilization is observed. Pegfilgrastim 12 mg and 18 mg mobilize higher numbers of CD34+ cells than does daily administration of filgrastim. Pegfilgrastim at 6 mg, 12 mg, and 18 mg is generally safe and well tolerated in this setting. The trial is ongoing, and updated results on safety and efficacy will be presented.

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INTERIM ANALYSIS OF A PHASE II STUDY OF RISK-ADAPTED INTRAVENOUS MELPHALAN FOLLOWED BY ADJUVANT DEXAMETHASONE (D) AND THALIDOMIDE (T) FOR NEWLY DIAGNOSED PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS (AL)

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High-dose melphalan (M) with autologous stem cell transplant (ASCT) is effective therapy for AL, but treatment-related mortality (TRM) remains high, and hematologic complete responses (CR) occur in a minority of patients. In this study, we investigated whether a risk-adapted approach to intravenous (IV) M dosing could decrease TRM, and whether adjuvant D ± T could improve hematologic and amyloid-involved organ response rates. Low-risk patients (1 or 2 major organs involved, no advanced cardiac disease) receive M 100, 140, or 200 mg/m^2 with ASCT based on age, cardiac involvement, and renal function. High-risk patients (3 organs involved or advanced cardiac disease) receive 2 cycles of M 40 mg/m^2 without ASCT. Patients with persistent clonal plasma cell disease at 3 months receive 9 months of adjuvant D+T or D alone (if history of deep venous thrombosis or neuropathy). Since September 2002, 39 patients (median age, 58 years; range, 34–73 years; 67% male) have enrolled for a median of 1.5 months (range, 0.5–7 months) from diagnosis. Organ involvement includes 13 (33%) cardiac, 25 (64%) renal (13 with renal only), 13 (33%) liver/GI tract, and 10 (26%) peripheral nervous system. Only 4 high-risk patients have enrolled; all had symptomatic cardiac involvement and died a median of 4 months (range, 2.5–6 months) after M. Thirty-four patients in the low-risk group have been treated, and TRM is 6.4% (2/31), with an additional 3 patients alive < 100 days posttreatment. Four low-risk patients have died of progressive disease (PD) a median of 11.7 months (range, 7–18 months) after M. At 3 months, 19/30 (63%) evaluable low-risk patients had hematologic responses (5 CR, 14 PR) and 11 had stable disease. Twenty patients with persistent clonal disease began adjuvant therapy with D ± T. At 12 months, 11/15 (73%) evaluable patients had responses (5 CR, 6 PR) and 8 (67%) had objective improvement in amyloid-related organ function. Preliminary analysis of serum free light chain data (n = 31) shows an association between a persistently abnormal κ:γ ratio 3 months after treatment and an increased risk of death (relative risk = 1.54; 95% confidence interval = 1.12–2.12; P = .02). Analyses of the prognostic significance of serial troponin and BNP levels and plasma cell cyclin D1 expression are ongoing. In conclusion, risk-adapted dosing of IV M in newly diagnosed AL patients has a low TRM. Adjuvant D ± T is feasible, has moderate toxicity, and has to date benefited 20% (4/20) of patients with persistent clonal plasma cell disease 3 months post-ASCT.

	Adjuvant Therapy* (n = 25 Eligible)	
	Thal/Dex (n)	Dex (n)
Began therapy (n = 5 refused or too ill)	12	8
Completed 9 mos.	4	3
Still on (<9 mos.)	3	2
Reasons for discontinuation	PD (n = 2), pulmonary embolus (1), avascular necrosis hip (1), RSV pneumonia (1)	PD (n = 2), intolerance (1)
Benefit to hematologic response	SD (@3 mos.)→PR (@12 mos.) (n = 1), PR→CR (n = 1)	SD→PR (n = 1), PR→CR (n = 1)

*Dexamethasone (1 to 3 four-day pulses monthly); Thalidomide (50–200mg nightly)

GRAFT PROCESSING

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IDENTIFICATION AND TISSUE DISTRIBUTION OF ANTIGEN-SPECIFIC RECIPIENT ANTI-DONOR CD8+ T CELLS THAT RESIST HEMATOPIETIC ENGRAFTMENT FOLLOWING MHC-MATCHED ALLOGENEIC PROGENITOR CELL TRANSPLANTS

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The role of natural killer (NK) cells in resistance to progenitor cell (PC) engraftment has been extensively studied in experimental bone marrow transplantation (BMT). Although antigen-specific T cells are believed to be the principal effectors resisting engraftment following clinical hematopietic cell transplants (HCT), less is known involving the biology and distribution of these cells following experimental HCT. The present studies established a model of resistance using the well-defined dominant, that is H60 MiHA (in B6 mice primed to BALB.B; B6BALB.B) to precisely identify and track host T cells following BMT into recipients sensitized against donor transplant antigens. We utilized an H60/K^b tetramer and determined the mean (12.2% ± 0.88 of CD8 cells) frequency of circulating CD8+ H60-specific T cells (5.6%–20.5%) in B6 recipients before BMT. Most (> 90%) CD8+ H60+ cells expressed the memory phenotype (CD44+, Ly6C+, CD25/CD69−). Interestingly, the frequency of CD8+ H60+ T cells was higher in the bone marrow than in the blood and splenic compartments, sug-

gesting that cells present in this compartment post-BMT may be composed of both migrants and a resident population. To mediate resistance to PC engraftment, effector CD8+ cells must first survive conditioning and the BMT milieu. B6BALB.B mice irradiated at 3, 6, and 9 Gy were analyzed 24 hours later for CD8+ H60-specific T cells in the spleen and BM. Tertramer+ cells were clearly identified in both compartments showing dose-dependent increases. Importantly, 5 days after HCT with 1×10^7 H60 congenic (or BALB.B) TCD BM, PC rejection was evident, as indicated by nondetectable splenic CFU activity in recipients of these allogeneic, but not syngeneic TCD-BM. Notably, tetramer+ cells were again detected in BM and spleen. Interestingly, their frequency was 3fold higher in the marrow (mean, 6%) than in the splenic compartment (mean, 1.7%) in sensitized recipients of either syngeneic or MiHA allogeneic BM. These increases are consistent with the predicted survival advantage of CD8+ memory T cells due to elevated bcl-2 levels. However, resistance did not occur in syngeneic recipients due to lack of activation of H60-specific T cells. These studies conclusively demonstrate that an antigen-specific CD8 T-memory population responsible for resistance survives allogeneic BMT and is present in bone marrow and spleen where resistance is presumed to occur. Studies are underway to functionally and molecularly characterize this MiHA antigen-specific population during ongoing resistance post-HCT.

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IMMUNOLOGICAL AUTOLOGOUS STEM CELL GRAFT ENGINEERING USING COMBINATION G-CSF AND ALDESLEUKIN (IL-2) IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Absolute lymphocyte count (ALC) recovery to $\geq 500/\mu l$ by day 15 post-autologous peripheral blood stem cell transplant (APBSCT) is associated with an improved overall and event-free survival (EFS) for patients with non-Hodgkin's lymphoma (NHL). The benefit of the ALC post-APBSCT is likely due to natural killer (NK) cells, because patients who achieved a normal NK cell count ($\geq 80/\mu l$) on day 15 post-APBSCT (day 15 NK) had an improved EFS compared with those whose day 15 NK was low. Finally, the day 15 ALC correlates to the infused autograft absolute lymphocyte count (A-ALC) and not with total CD34 dose. Thus we hypothesized that a peripheral blood stem cell mobilization regimen would be ideal if it could mobilize CD34+ cells, lymphocytes, and NK cells. A pilot study was undertaken using a sequential mobilization regimen of granulocyte colony-stimulating factor (G-CSF) followed by aldesleukin. Patients received G-CSF at 10 $\mu g/kg/day$, and stem cell collections began on day 5 or later, when the peripheral blood (PB) CD34 cell count was $\geq 10/\mu l$. Once stem cell collection was complete and a minimum of 2×10^6 CD34 cells/kg were collected, patients received 1 subcutaneous injection of aldesleukin and underwent 1 further apheresis the next day collect lymphocytes. The dose levels of aldesleukin were 0, 0.5, 1, 1.5, and 2×10^6 U/m^2. Patients then underwent conditioning for APBSCT and on day 0 received all of the apheresis product, including the extra collection of lymphocytes. PB lymphocyte counts and subset analysis were measured at baseline (before G-CSF), pre-aldesleukin, 1 day post-aldesleukin, and day 15 post APBSCT. A total of 43 patients with NHL were entered in the study. Of these, 26 completed an adequate CD34 collection, received aldesleukin, and underwent an extra apheresis. The results of these 26 patients are presented. The median CD34 collected, A-ALC, autograft NK collected (A-NK), day 15 ALC, and day 15 NK for each dose level are given. The median times to neutrophil and platelet engraftment were 13 and 12 days, respectively. We conclude that aldesleukin up-regulates NK cell numbers. Dose level 3 resulted in the best NK cell apheresis collection, the highest PB NK cell count post-aldesleukin, on day 15 NK, and on day 15 ALC. Only at this dose did all patients achieve a normal day 15 NK cell count. A phase II study is proposed using a sequential regimen of G-CSF followed by aldesleukin at a dose of 1.5×10^6/m^2 to confirm these results. Successful lymphocyte and NK cell engraftment post-APBSCT may become as important as neutrophil and platelet engraftment post-APBSCT.

Dose Level	IL-2 Dose	# Patients	CD34	Total A-ALC	Total A-NK	Day 15 ALC	Day 15 NK
0	0	5	7.36×10^6/kg	0.57×10^9/kg	0.09×10^9/kg	744/μl	194/μl
1	0.5×10^6 IU/m^2	5	4.37×10^6/kg	0.47×10^9/kg	0.09×10^9/kg	310/μl	92/μl
2	1×10^6 IU/m^2	5	4.87×10^6/kg	0.67×10^9/kg	0.10×10^9/kg	700/μl	120/μl
3	1.5×10^6 IU/m^2	5	6.96×10^6/kg	0.38×10^9/kg	0.11×10^9/kg	1380/μl	472/μl
4	2×10^6 IU/m^2	6	3.73×10^6/kg	0.43×10^9/kg	0.10×10^9/kg	787/μl	192/μl

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INHIBITORY EFFECTS OF CYTOTOXICITY OF CYTOKINE ACTIVATED AND EXPANDED HUMAN CD8+ T CELLS BY A HUMAN NKG2D ALTERNATIVE SPLICE VARIANT

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NKG2D is an activating receptor that is expressed on CD8+ T cells and γ&δ T cells where it up-regulates activation through T-cell receptor (TCR) costimulation. NKG2D is triggered by ligands that are structurally related to major histocompatibility complex (MHC)-1 and expressed by tissues early in ontogeny, stress, and transformation. The function of NKG2D on natural killer (NK) cells and CD8+ T cells is mediated by 2 distinct pathways of signaling through its association with DAP10 and DAP12. One functionally distinct human NKG2D isoform has been identified. In evaluating human NKG2D gene expression by reverse-transcription polymerase chain reaction (RT-PCR) in CD8+T cells derived from 7 different donors, we consistently observed 2 distinct bands differing by 218bp. Cloning and sequencing of both PCR amplicons revealed a previously described alternatively spliced NKG2D variant from inclusion of intron 6. Intron 6 inclusion introduces a stop codon, resulting in a truncated protein product lacking the entire extracellular domain. To determine the functional consequences of truncated NKG2D expression, we isolated and cloned both full-length and truncated NKG2D into pRJ3 vectors. Human CD8+ T cells were tranduced and directed against human cell line targets in Cr^{51} cytotoxicity assays. We found that expression of the full-length NKG2D resulted in a ~15% increase in cytotoxicity, whereas expression of the truncated NKG2D resulted in ~70% reduction in cytotoxicity. Results were compared to CD8+ T cells transduced with an empty vector. To further elucidate the role of each NKG2D isoform, we coexpressed full-length and truncated NKG2D with DAP-10 or DAP-12. Coexpression of full-length NKG2D with either DAP10 or DAP12 resulted in a comparable 25% cytotoxicity versus NKG2D alone. Coexpression of full-length and truncated NKG2D also resulted in reduction of up to 65% coexpression of the truncated NKG2D isoform with DAP10 or DAP12 did not alter the reduction in cytotoxicity observed with truncated NKG2D alone. We also performed experiments using murine CD8+ T cells with human cell line targets. Overexpression of full-length NKG2D resulted in a > 32% increase in cytotoxicity, whereas coexpression of full-length NKG2D and DAP10 resulted in a 42% increase in cytotoxicity. Collectively, these results demonstrate that the NKG2D isform lacking the extracellular domain unexpectedly inhibits cytotoxicity in human CD8+ T cells. Also of interest is whether variability in expression of the truncated NKG2D isoform influences the cytotoxic potency of activated CD8+ T cells expanded from different donors.

GVH/GVL

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RABBIT IGG LEVELS IN PATIENTS RECEIVING THYMOGLOBULIN AS PART OF CONDITIONING BEFORE UNRELATED-DONOR ALLOGENEIC STEM CELL TRANSPLANTATION

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