

Oral Presentations

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. Tetramer⁺ 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 $\geq 500/\mu\text{l}$ 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 ($\geq 80/\mu\text{l}$) 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 $\mu\text{g}/\text{kg}/\text{day}$, and stem cell collections began on day 5 or later, when the peripheral blood (PB) CD34 cell count was $\geq 10/\mu\text{l}$. 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/m². 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 $1.5 \times 10^6/\text{m}^2$ to confirm these results. Successful lymphocyte and NK cell engraft-

ment 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	$7.36 \times 10^6/\text{kg}$	$0.57 \times 10^9/\text{kg}$	$0.09 \times 10^9/\text{kg}$	744/ μl	194/ μl
1	0.5×10^6 IU/m ²	5	$4.37 \times 10^6/\text{kg}$	$0.47 \times 10^9/\text{kg}$	$0.09 \times 10^9/\text{kg}$	310/ μl	92/ μl
2	1×10^6 IU/m ²	5	$4.87 \times 10^6/\text{kg}$	$0.67 \times 10^9/\text{kg}$	$0.10 \times 10^9/\text{kg}$	700/ μl	120/ μl
3	1.5×10^6 IU/m ²	5	$6.96 \times 10^6/\text{kg}$	$0.38 \times 10^9/\text{kg}$	$0.11 \times 10^9/\text{kg}$	1380/ μl	472/ μl
4	2×10^6 IU/m ²	6	$3.73 \times 10^6/\text{kg}$	$0.43 \times 10^9/\text{kg}$	$0.10 \times 10^9/\text{kg}$	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 SPICE VARIANT

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NKG2D is an activating receptor that is expressed on CD8⁺ T cells and $\gamma\delta$ 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)-I 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 pR3 vectors. Human CD8⁺ T cells were transduced and directed against human cell line targets in Cr⁵¹ 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 isoform 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.

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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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