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Feasibility of low dose interleukin-2 therapy following T cell-depleted non-myeloablative allogeneic hematopoietic stem cell transplantation from HLA matched or mismatched family member donors

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Abstract

High relapse rates and infections remain primary causes of failure in non-myeloablative transplantation. Interleukin-2 (IL-2) may stimulate the immune system and improve outcomes. The primary objective of this pilot study was to evaluate the feasibility of administering IL-2 following a T cell-depleted nonmyeloablative hematopoietic stem cell transplant.

Methods—Patients received T cell depleted nonmyeloablative transplant from a matched or mismatched related donor. Those with allogeneic engraftment, < grade 2 acute GVHD at time of study entry, and no severe end organ damage were eligible and received IL2 starting 6 weeks after the first day of stem cell infusion. Patients received 1 mu/m2 daily for 5 days each week for 4 weeks followed by a 2 week rest period for a 6 week cycle to continue for up to 1 year.

Results—Eight patients aged 28–69 were treated. Significant toxicities were limited to GVHD of the skin grade 2 in 3 patients and severe fatigue in 4 patients, limiting the duration of therapy. Two of the 8 patients died of relapsed disease and one from CMV. With a median overall duration of follow up of survivors of 48 months, five patients (63%) remain alive and in continuous complete remission.

Keywords

Non-myeloablative Allogeneic Transplantation; Leukemia /Lymphoma; IL-2; NK cells

Introduction

The graft versus tumor effect from allogeneic hematopoietic stem cell transplantation is due in part to activation of natural killer (NK) cells and other T cells.^{1,2,3,4,5} Many patients still succumb to the disease however, and much room for improvement remains. Due to its

ability to stimulate various aspects of the immune system, including via NK cell activation, IL-2 has been used for the treatment of some malignancies, with or without in vitro activated lymphoid cells (lymphokine activated killer cells or LAK cells).^{6,7,8,9} However very limited studies have evaluated the effect of IL-2 in improving allogeneic immunotherapy.¹⁰ The primary objective of this trial was to demonstrate the feasibility of administering IL-2 following a T cell-depleted, non-myeloablative hematopoietic stem cell transplant.

Patients and Methods

Eligibility

Patients who received a T cell-depleted non-myeloablative allogeneic transplant from a 3-6/6 HLA matched, related donor without active grade 2 transplant related toxicity or overall acute graft versus host disease (aGVHD) grade 2 were eligible. The protocol was approved by institutional review board and all patients provided written consent to participate.

Treatment

We have reported the details of the transplant protocol elsewhere.¹¹ Briefly, patients received 5 days of intravenous alemtuzumab 20 mg/day completing on day 0 and four days of fludarabine 30 mg/m² and cyclophosphamide 500 mg/m² per day. Recipients of 6/6 HLA matched sibling stem cells did not receive any further therapy for aGVHD prophylaxis while patients who received a family member 3-5/6 HLA matched graft received mycophenolate 1 gram twice daily for 60 days following transplantation. Beginning day +1, patients received filgrastim 5 mcg/kg (rounded to nearest vial) till absolute neutrophil count was $> 1 \times 10^9$ /L.

Patients received 1mu/m² IL-2 self administered subcutaneously 5 days per week (M-F) for 4 weeks followed by 2 weeks 'off' to complete a 6-week cycle for a maximum of 1 year. The first dose of IL-2 began 6 +/-2 weeks after their non-myeloablative allogeneic hematopoietic stem cell infusion.

Toxicity and Response

Patients were monitored for toxicity according to the NCI CTC v.3 at least weekly while on therapy. Thereafter, they were formally evaluated at a minimum of 3-month intervals for 2 years and 6-month intervals for 5 years to evaluate toxicities and response.

Immune Reconstitution

In addition to monitoring response, phenotypic recovery of lymphocyte subsets using standard flow cytometry techniques was measured before and after treatment to provide a gross measure of immune recovery. Functional recovery of the NK cells was analyzed as well using our group's previously published flow based assay for this analysis.¹² Briefly, K562 cells and Raji cells were used as positive and negative controls for establishing expected curves for in vitro lysis. The effector cells (PBMC or CD56⁺ cells) were resuspended in RPMI 1640 medium supplemented with 10% FBS. In each assay the total number of target cells was held constant. Three-fold serial dilutions of the effector cells were established. For each dilution, the assays were established in triplicate. For these assays, the percent lysis was measured on the target cells directly as a percentage of 7AAD⁺ cells.

Results

Eight patients aged 28–69 years, 6 HLA matched and 2 HLA mismatched, were enrolled. Four had MDS or leukemia, 2 follicular lymphoma, and 1 each had melanoma or renal cell

carcinoma. This was a high risk, heavily pretreated group. Only half were in a complete remission at the time of transplant, though 6 of the 8 patients (75%) were in remission following transplant but prior to starting IL-2 (Table 1).

Toxicity

While tolerated well overall, fatigue and listlessness were noted problems with this dose of IL-2 given in this manner following transplantation. Seven of the 8 patients completed at least 2 cycles, though 1 patient withdrew after cycle 1 and 3 patients withdrew after the 2 cycles (3 months) due to excessive fatigue and 1 withdrew after 6 cycles due to persistent skin GVHD. One further patient had early progressive disease after 2 cycles of therapy. Though only 2 completed the maximum 8 cycles of therapy over the 1 year period, only 1 patient was unable to tolerate at least 2 cycles (3 months) of therapy. Both patients with a mismatched donor tolerated only 2 cycles of therapy, though 1 remains in a long term remission.

In addition, 1 patient developed drug resistant CMV which resulted in his demise and 1 other had CMV reactivation. One patient had bacteremia, 1 non-GVHD related grade 3 diarrhea, and one grade 2 cough while on therapy. Importantly, occurrence of acute GVHD was limited in this pilot study. One patient had overall grade 1 and two experienced overall grade 2 GVHD involving only the skin. No patient had severe gut or liver GVHD.

Durability of Response

As noted from the disease type and status prior to transplantation, this group of subjects was at high risk for progression of their disease (Table 1). Of the 2 with persistent disease following transplant and prior to the start of IL-2 therapy, the one with lymphoma attained a CR while the one with renal cell carcinoma maintained a prolonged stable disease state. With long term follow up, 3 patients progressed, one with leukemia, one with AML arising from myelofibrosis, and one with renal cell carcinoma. The remaining five patients (63%) remain alive and in continuous complete remission with a median follow up of survivors of 48 months (range 39–56 months). This includes both patients with follicular lymphoma, 2 with leukemia/MDS, and the 1 patient with melanoma. The median number of prior regimens in these 5 patients was 3. While the patient with melanoma had a long history of disease and 3 prior remissions and the patient with MDS entered transplant in early first remission, the 2 with lymphoma had not attained prior remissions and the other patient with leukemia experienced only a 7 month first remission duration. Thus, despite most subjects completing a limited number of cycles of IL2, the durability of response is encouraging in this pilot study.

Immune Recovery

Table 2 reveals that lymphocyte recovery following 2 cycles of therapy (7 of 8 completed at least this many cycles) was variable in the subjects enrolled. In fact, the 2 with early progression had some of the highest measured absolute lymphocyte, CD4, CD8, or NK cell counts. The small size of the series prevents formal statistical analyses to correlate phenotypic recovery with outcomes. Using the flow based assay we've previously described¹², the *in vitro* analysis of NK cell function revealed patients had very poor function 6 weeks following transplantation and just before IL2 therapy, in which there was no ability for the NK cells to lyse target cells even at high effector to target ratios (Figure 1). Six months following therapy, there was a modest improvement in function in many patients, with further improvements noted by 1 year of recovery, a point beyond which most subjects were still exposed to IL2.

Discussion

Though helpful in offering transplant to a broader array of patients, improvements in T cell depleted, non-myeloablative allogeneic therapy must address continued concerns over relapse and infectious toxicities. We have previously reported concerns in this setting, noting only a 31% 1 year survival rate following T cell depleted non-myeloablative therapy for haploidentical transplanted, high risk subjects such as those included on this pilot study. In our prior work we noted 50% of patients died of relapsed disease and 23% from severe infections, highlighting the need for improved immune reconstitution.¹¹ Unmanipulated donor lymphocyte infusions have some benefit, but at the risk of serious GVHD and low response rates in the face of active disease.^{13, 14} Improvements may come in the form of therapies designed to stimulate cellular subsets present in the graft that may provide a graft versus tumor effect without a heightened risk of other toxicities. Mere proliferation of lymphocytes is not adequate to ensure improved reconstitution though, as the cells must be functional. As one of the few readily available agents with reported ability to induce secretion of cytokines, impact the function of cytotoxic T lymphocytes, and expand NK cells, IL-2 has been an attractive agent to assess for clinical impact as an anticancer agent in the transplant setting.^{15,16,17,18} This pilot study suggests it is feasible to infuse limited doses of IL-2 following T cell depleted, nonmyeloablative allogeneic transplantation as 7 of 8 patients tolerated at least 2 cycles of therapy delivered in this manner and GVHD remained at acceptable levels with no occurrence of severe gut or liver GVHD. Prolonged exposure at this dose and in this setting was poorly tolerated however as the most common reason for early withdrawal was extreme fatigue. It is intriguing that the long term follow up of these subjects indicates nearly 2/3 of these high risk subjects remain as long term remitters. Prior studies in the autologous setting have noted improved lymphocyte recovery and documented increased cytokine release, though there has been limited clinical benefit of delivering IL2 following high dose therapy^{19,20,21}. In a modified autologous setting with transient allogeneic lymphocyte boosts combined²², data has been somewhat more encouraging in a small report noting safety and potential clinical benefit, indicating the allogeneic setting may offer enhanced opportunities for the activated immune system to target the cancer cells.

We suggest that NK cells may be a prime reason for this potential benefit. Expansion and activation of natural killer cells, a cellular subset noted to recover early and be affected by IL-2, is purported to have direct graft versus tumor capabilities following allogeneic transplantation though they do not appear to cause aGVHD.^{12,23} We have recently reported on the successful enrichment of DLIs for NK cells with infusion of high doses being safe in this setting. The function of the selected and infused NK cells often remains limited however.¹² Notably, Rubnitz have reported that low dose peri-NK cell infusion of IL2 was associated with a significant increase in the number of NK cells within a month of infusion and possibly improved NK cell function as well.²⁴ In the feasibility study reported here, we assessed the numbers and functionality via lysis of NK cell targets seen at 3 and 6 months to investigate the utility of interleukin-2 in improving the function of the NK cells that are recovering and noted a pattern of improved NK cell absolute number recovery and functional capability. Future larger studies are required to see if this improvement correlates to outcomes and what the specific activation/inhibition signals are for NK cells so we can more effectively choose donors. An alternative explanation for the encouraging outcome of patients on this pilot study is the ability of IL-2 to increase regulatory T cells (Tregs), or cytotoxic T cells, cell types purported to minimize GVHD yet allow the immune system to maintain antitumor and anti-viral fighting capabilities. This cellular subtype deserves further attention in future studies^{25,26,27}. Continued efforts to better understand the aspects of immune recovery affected by IL-2 in vivo is warranted in future studies using this agent.

This study has shown feasibility of delivering this agent in this setting, allowing future comparative studies to assess whether recovery of these cellular subsets is occurring simply as a result of ‘tincture of time’ or if the administration of IL2 allows enhanced immune phenotypic recovery, functional ability, and clinical outcome.

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Figure 1.

Functional Analysis of NK cells measured as the median percent lysis ($\pm$ SD) of target cells of patients treated in this pilot study pre and post exposure in vivo to IL-2.

Panel A: NK function was monitored in a single patient at 2 weeks after having received IL2 therapy, at 2 months post-IL2 therapy, and again at 6 months post-IL2 therapy (as labeled).

Panel B: NK function was monitored in 4 patients at 5 to 7 months post-IL2 therapy.

Panel C: NK function was monitored in 4 patients at 8 to 10 months post-IL2 therapy. Poor function early in recovery is noted, with subsequent progressive improvements in function over time and with further exposure to IL2. Whether improved function is related to IL2 exposure or other aspects of recovery is not able to be answered in this pilot study.

Table 1
Patient characteristics, toxicity and response to IL2 post nonmyeloablative therapy

Patient	Diagnosis	Age at Entry	# Prior Regimens	Duration of Last Remission	Disease Status at TP	HLA	Disease Status at Entry	# Cycles completed	Reason for Stopping	Toxicity (Grade)	Best Response	Duration of Response	Overall Survival
1	Melanoma	54	3	70 mo	CR3	6/6 sibling	CR4	8	N/A	Fatigue (2), cough (2),	CR4	50 mo +	50 mo +
2	AML	61	1	N/A	CR1	6/6 sibling	CR1	2	Relapse	N/A	CR1	3 mo	17 mo
3	AML	28	2	7 mo	CR1	6/6 sibling	CR2	8	N/A	Staph bacteremia (3)	CR2	56 mo +	56 mo +
4	Follicular Lymphoma	46	4	N/A	SD	6/6 sibling	SD	2	Fatigue	Fatigue (3)	CR1	39 mo +	39 mo +
5	AML/Myelofibrosis	73	3	12 mo	CR1	6/6 sibling	CR2	1	Fatigue	CMV reactivation (3), Fatigue (3), diarrhea (3)	CR2	38 mo	39 mo
6	Follicular Lymphoma	53	3	N/A	SD	6/6 sibling	CR1	6	GVHD	GVHD skin (1)	CR1	48 mo +	48 mo +
7	Renal Cell Cancer	39	2	19 mo	SD	4/6 son	SD	2	Fatigue	Fatigue (3), CMV in colon/blood (3), GVHD skin (2)	SD	9 mo	11 mo
8	MDS	57	2	N/A	CR1	4/6 daughter	CR1	2	Fatigue	GVHD skin (2), Fatigue (3)	CR1	46 mo +	46 mo +
N/A Not applicable													
TP Transplant													
HLA Human Leukocyte Antigen													

Table 2

Lymphocyte subset recovery before therapy and 2 cycles of cycles of IL-2

Patient	Response Duration	Assessment relative to IL-2 Exposure		Absolute CD3	Absolute CD4	Absolute CD8	Absolute NK cells	Absolute NKT cells
		pre	post					
1	50+ months	pre	post	58	31	14	2	22
				36	0	0	146	0
2	3 months	pre	post	1710	166	833	437	75
				987	168	323	304	4
3	56+ months	pre	post	0	0	0	0	0
				0	0	0	0	0
4	39+ months	pre	post	13	0	0	178	0
				44	3	1	412	0
5	38 months	pre	post	234	17	39	577	9
				196	18	19	635	1
6	48+ months	pre	post	426	168	24	289	12
				223	0	0	549	1
7	9 months	pre	post	199	179	19	29	7
				349	84	256	64	8
8	46+ months	pre	post	86	3	0	270	2
				1322	348	0	708	12