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A Trial of Alemtuzumab Adjunctive Therapy in Allogeneic Hematopoietic Cell Transplantation with Minimal Conditioning for Severe Combined Immunodeficiency

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Abstract

For infants with severe combined immunodeficiency (SCID) the ideal conditioning regimen before allogeneic hematopoietic cell transplantation (HCT) would omit cytotoxic chemotherapy to minimize short- and long-term complications. We performed a prospective pilot trial with alemtuzumab monotherapy to overcome NK-cell mediated immunologic barriers to engraftment. We enrolled 4 patients who received CD34-selected haploidentical cells, two of whom failed to engraft donor T cells. The 2 patients who engrafted had delayed T cell reconstitution, despite rapid clearance of circulating alemtuzumab. Although well-tolerated, alemtuzumab failed to overcome immunologic barriers to donor engraftment. Furthermore, alemtuzumab may slow T cell development in patients with SCID in the setting of a T-cell depleted graft.

Keywords

SCID; Alemtuzumab; Haploidentical; Engraftment

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Introduction

Severe combined immunodeficiency (SCID) is a heterogeneous group of genetic disorders with the shared phenotype of profoundly deficient T cells and absent B lymphocyte function, which if untreated leads to early mortality from severe infections. Although progress has been made towards gene therapy for certain SCID genotypes, the majority of patients with SCID require an allogeneic hematopoietic cell transplant (HCT) for curative therapy (1). When an HLA-matched relative is not available, T-cell depleted haploidentical related donors are often used due to their rapid availability (2). However, in certain SCID subtypes, transplantation across major histocompatibility barriers is associated with a high rate of graft rejection unless immunoablative chemotherapy is administered (2). Due to the profound deficiency or complete absence of functional T cells in patients with SCID, this graft rejection is thought to be mediated by natural killer (NK) cells (3-7). Most immunoablative chemotherapeutic agents use DNA alkylation to accomplish removal of host lymphocytes, with the exception of the antimetabolite fludarabine. However, fludarabine does not inhibit NK cell function (8), and fludarabine monotherapy prior to transplant for NK⁺ SCID has been associated with graft rejection (2). In very young infants with non-malignant diseases, such as SCID, avoidance of alkylating forms of chemotherapy is desirable, as these agents can potentially cause significant short- and long-term toxicities, such as endocrinologic dysfunction, short stature, dental abnormalities, and infertility (9, 10). These toxicities are most evident in patients with the T⁻B⁻NK⁺ forms of SCID associated with defects in genes required to repair breaks in DNA (9-11).

Murine models of NK-cell-mediated graft rejection utilize anti-NK monoclonal antibodies (3, 6, 7), for which there are no commercially available human equivalents. Alemtuzumab is a humanized monoclonal antibody against CD52, a molecule expressed on all lymphocyte populations, including NK cells (12). Alemtuzumab is widely utilized as part of the conditioning regimens before un-manipulated T-replete HCT and is thought to improve engraftment (13, 14). However, the optimal dosing of alemtuzumab to achieve complete eradication of NK cells is unknown, and there is no published experience of using alemtuzumab-monotherapy prior to an *ex vivo* T-cell depleted HCT.

To investigate whether this agent could allow HCT for SCID to be successful without chemotherapy, we performed a prospective pilot trial that tested the hypothesis that alemtuzumab as the sole conditioning agent prior to haploidentical HCT in children with NK-positive SCID would facilitate donor T-cell engraftment.

Patients and methods

Study population

Eligible patients presented to the University of California San Francisco (UCSF) Benioff Children's Hospital between July 2007 and September 2009 with a new diagnosis of NK-positive SCID or leaky SCID, as defined by accepted criteria (15, 16), without significant transplacental maternal engraftment (TME) on testing of their peripheral blood mononuclear cells (PBMCs) using a quantitative PCR-based method involving amplification of short tandem repeat (STR) sequences, as previously described (17, 18). All patients had genotypic

confirmation of their diagnosis. All patients treated during this time period enrolled on the trial. The trial was approved by the UCSF Committee on Human Research and informed consent was obtained from the related donors and the parents of the patients in accordance with the Declaration of Helsinki, but was not registered at www.clinicaltrials.gov.

Donor hematopoietic stem cell collection and manipulation

Patients and potential donors were tested for HLA-compatibility at HLA-A, -B, -C, -DR, and -DQ. For the haploidentical maternal donors, mobilized peripheral blood stem cells (PBSCs) were prepared by CD34 selection using the Baxter Isolex 300i System (Baxter Healthcare, Deerfield, MA) as previously described (2). Cell counting and gating was as previously described (2). The goal for the infused cell dose $>10 \times 10^6$ CD34⁺ cells/kg with 6×10^4 CD3⁺ T cells/kg for haploidentical PBSC. Excess cells were cryopreserved.

Transplant regimen

Patients with NK-positive SCID were originally conditioned with alemtuzumab beginning on Day -8. A test dose of 0.2 mg total was administered and, in the absence of severe allergic reactions, 6 hours later 0.3 mg/kg/day was given for 3 days (total dose 0.9 mg/kg). Premedication with acetaminophen, diphenhydramine, and dexamethasone (0.2 mg/kg) was administered. However, after the first patient successfully achieved donor T cell engraftment, but had very slow recovery of T cell numbers and function (see results section), the protocol was modified to a lower alemtuzumab dose of 0.2 mg/kg/day for 3 days (total dose 0.6 mg/kg/day). Furthermore, we began to measure alemtuzumab levels (prior to first & second doses, 24 hours after the last dose, then on Days 0, 7, 14, 21, and 28 days following HCT), following a standard protocol (19). Serum from 1.5 mL of blood was frozen for shipping. After thawing, the complement was heat-inactivated and the serum was incubated with HUT-78 cells to allow the alemtuzumab to bind to these cells. After several washes, the detection reagent (FITC-labelled polyclonal anti-human IgG Fc domain, Sigma, Poole, UK) was added to the HUT-78 cells. After several more washes, the mean fluorescence intensity (MFI) was measured on a Becton-Dickinson FACS Canto II flow cytometer. These MFI levels were converted to alemtuzumab concentrations using a standard curve developed with known spiked levels of alemtuzumab in control serum samples. Because patients received CD34-selected PBSCs, no pharmacologic GVHD prophylaxis was utilized.

Analysis of engraftment and immunologic parameters

Post-transplant donor chimerism was determined using sorted CD3-, CD19-, and CD14/15-positive cells and STR markers, as above (17, 18). NK cell chimerism was not tested. Sorted cells were tested for purity by flow cytometry, and the inter-assay variation was $\pm 1\%$. Lymphocyte subsets, including naïve and memory markers CD45RA and CD45RO were assessed by flow cytometry and compared to normal ranges for age (20, 21). T-cell function was assessed by ³H-thymidine incorporation in response to phytohemagglutinin (PHA), and reported as a percentage of stimulated immunologically competent control lymphocytes tested simultaneously (Mayo Medical Laboratories, Rochester, MN). T-cell receptor excision circles (TRECs) and T-cell receptor spectratyping were not performed. B-cell function was measured by the ability to produce IgM and IgA within the normal range for

age, presence of appropriate IgM isohemagglutinins (ISH) at 1:8 dilution, and specific antibodies following vaccination, if performed.

Supportive care

Active infections at diagnosis were treated with appropriate anti-microbial therapies that continued until evidence of infection resolution. These included high-dose cotrimoxazole (5 mg/kg/dose of trimethoprim component 4 times daily) for patients with *Pneumocystis jirovecii* pneumonia (PCP). Rotavirus infections were managed with supportive care. Other anti-infective prophylaxis was administered as previously described (2). Acute and chronic GHVD were graded on standard criteria (22, 23).

Statistical considerations

Event-free survival (EFS) and overall survival (OS) were estimated by the Kaplan-Meier method using log-rank tests (NCSS8, Kaysville, UT). Events were defined as a conditioned second HSC infusion or death.

Results

Patient characteristics

Four patients were enrolled, three with newly diagnosed typical SCID and 1 with leaky SCID (UPN1247) (Table 1). All were born prior to the implementation of newborn screening for SCID (24), and were identified due to infections, including vaccine-strain rotavirus (25) (Table 2).

Adverse events to alemtuzumab

One of the 4 patients developed transient rigors and fevers following alemtuzumab. Another patient developed transient bradycardia, felt to be due to the dexamethasone premedication.

Alemtuzumab lymphodepletion efficiency & elimination kinetics

As shown in Figure 1a, the 4 patients who received alemtuzumab all had detectable lymphocytes by the day of transplant, and there was no correlation between fall in absolute lymphocyte count (ALC) and subsequent engraftment, with both UPN1247 and UPN1373 having graft rejection. In 1 patient (UPN 1373), NK cell numbers were measured and found to drop from $196 \times 10^6/\text{L}$ prior to alemtuzumab to a nadir of $38 \times 10^6/\text{L}$ by Day -6, and then rebounded to $139 \times 10^6/\text{L}$ on Day 0. This patient subsequently rejected his graft.

After the first patient had much slower than expected recovery of T cell numbers, we measured plasma alemtuzumab levels (Figure 1b). The alemtuzumab levels on Day 0 ranged from 0.66 – 1.07 mcg/ml, with a drop to 0.36 – 0.47 mcg/ml by Day +7 and 0.02 – 0.24 mcg/ml by Day +14. In all 3 patients, alemtuzumab was no longer detectable by Day +21 post-HCT. Despite this, the 1 patient who did engraft (UPN 1392), had very low absolute T cells ($<100 \times 10^6/\text{L}$ CD3⁺) until 9.5 months after HCT. The 2 patients who rejected their grafts had plasma alemtuzumab levels of 1.07 and 0.78 µg/ml on Day 0, suggesting that this level is insufficient to fully suppress NK cell activity.

T-cell engraftment & reconstitution

The first patient with T⁺B⁻NK⁺ SCID (UPN 1263), conditioned with 0.9 mg/kg total dose of alemtuzumab, demonstrated engraftment of maternal cells by STRs (2% of PBMCs) at Day 28 post HCT. This small degree of maternal cells persisted, but the overall T-cell numbers remained very low for much longer than the historic controls treated on our previous trial (2). We hypothesized that this may have been due to residual circulating alemtuzumab, and so administered 4 donor lymphocyte infusions to “soak-up” the antibody, beginning at 32 days post HCT (26). Nevertheless, his T-cell numbers remained low, so a non-conditioned stem cell boost (20×10^6 CD34⁺ cells/kg with 6×10^4 CD3⁺ cells/kg) was given 17 weeks after initial HCT. Two weeks later, his absolute CD3 count increased 10-fold and subsequently continued to rise to near normal levels by 38 weeks post-HCT with concurrent normalization of the patient's PHA response. His most recent values are shown in Table 3.

However, of the subsequent three patients treated with 0.6 mg/kg total dose of alemtuzumab, only one (UPN 1392) successfully achieved donor T cell engraftment. Similar to UPN 1263, this infant had evidence of donor DNA in the peripheral blood beginning on Day +14, but his T cell numbers remained very low ($<10 \times 10^6$ /L CD3⁺ cells). Therefore, a non-conditioned stem cell boost (13×10^6 CD34⁺ cells/kg, with 6×10^4 CD3⁺ cells/kg) was given 7 weeks after initial HCT. Despite this, the patient's T cells did not increase ($<20 \times 10^6$ /L) and he continued to suffer from rotavirus-associated diarrhea. A second stem cell boost (11×10^6 CD34⁺ cells/kg with 6×10^4 CD3⁺ cells/kg) given at 12 weeks following initial HCT was followed 3 weeks later by Stage IV acute GVHD of the skin. This was refractory to treatment with solumedrol, but was finally controlled with the addition of tacrolimus, daclizumab (27), and mesenchymal stromal cells (MSCs) (28). Chronic GVHD of the skin ensued, which required treatment with systemic immunosuppression until 16.5 months post initial HCT (13.5 months following boost). This patient is now healthy, but his T cell numbers and function remain low (Table 3). The other 2 patients (UPNs 1373 & 1247) had no evidence of donor DNA in the peripheral blood on 3 weekly samples beginning on Day +21 and were determined to have rejected their grafts, prompting full second transplants.

Myeloid & B-cell engraftment & B-cell reconstitution

Significant myeloid engraftment was not seen in any patient (Table 3). All surviving patients continue to require routine immunoglobulin replacement.

Clinical Outcomes

Three of the four patients are alive with a follow-up of 4 - 5.6 years. All are growing well without significant late effects, excepting the first patient who rejected his initial HCT (UPN1373) received cyclophosphamide (60 mg/kg $\times$ 2 days) and melphalan (70 mg/m² $\times$ 2 days) followed by unmodified BM from a 9/10 (A-allele) mismatched URD. He developed moderate but reversible sinusoidal obstruction syndrome, and achieved donor T-cell engraftment without donor myeloid or B cells. He also has developmental and speech delay. The second patient (UPN1247) received melphalan (70 mg/m² $\times$ 2 days), thiotepea (10 mg/kg), fludarabine (40 mg/m² $\times$ 5 days), and thymoglobulin (3.5 mg/kg total dose) followed by CD34-selected PBSCs from an 8/10 (B- & DQ-antigen) mismatched URD. He engrafted

donor T-cells and myeloid cells, but developed pulmonary infections with non-tuberculous mycobacteria and *Aspergillus fumigatus*, and eventually expired during treatment of refractory autoimmune hemolytic anemia.

Discussion

In this pilot trial, the use of alemtuzumab as the sole conditioning agent prior to haploidentical HCT in children with NK-positive SCID did not successfully facilitate donor T-cell engraftment and was associated with prolonged T-cell lymphopenia in the engrafted patients. The graft rejection rate seen (2 of 4 patients) was similar to that reported by us (2) and others (29, 30) for patients with NK⁺ SCID undergoing non-conditioned haploidentical HCT with no pre-transplant evidence of maternal chimerism. Of note, the engrafted patients failed to reconstitute B cells and remain IVIG-dependent. This failure to achieve complete immunologic reconstitution is typical in cases of B-negative SCID that do not receive cytotoxic chemotherapy designed to ‘make space’ in the bone marrow (2) and we do not believe is an adverse effect of the alemtuzumab-based conditioning.

One possible explanation for the failure to achieve T-cell engraftment may be that the dose of alemtuzumab chosen (0.6 mg/kg total) for the final 3 patients was too low. For T-cell replete HCTs, alemtuzumab has been studied as part of the pre-transplant conditioning, with total doses in the “low” range of 0.6 mg/kg (14), “medium” range of 1.5 mg/kg (13) or 1.8 mg/kg (54 mg/m²) (31), or “high” range of 3-6 mg/kg (32). However, in all of these trials, alemtuzumab was always combined with other lymphocytotoxic chemotherapeutic agents, making it possible that none of these doses of alemtuzumab are sufficiently NK-cell-suppressive to serve as monotherapy for conditioning prior to an HLA-mismatched donor HCT. The *in vitro* data support this hypothesis. Penack, *et al.* report that only 20-30% of NK cells became apoptotic when exposed to concentrations of alemtuzumab ranging from 0.1 to 10 mcg/ml (12), while Stauch, *et al.* reported a 40% apoptosis rate in the same range of alemtuzumab concentrations, and only a 25% drop in NK-mediated cytotoxicity (33). This concentration was similar to the levels of alemtuzumab that we measured in the peripheral blood for at least 14 days following a dose of 0.6 mg/kg. We conclude that either higher doses of alemtuzumab, or combination therapy with other anti-NK-cell agents, may be necessary to prevent graft rejection in NK⁺ SCID patients undergoing HLA-mismatched HCT.

A concern about utilizing higher doses of alemtuzumab is that the 2 patients who did achieve engraftment had very slow T-cell recovery compared to patients in our preceding trial, where the median time to developing a CD4 count of greater than $200 \times 10^6/L$ was 1.2 months, and the latest was 3.7 months (2). The only other published experience of using alemtuzumab in the *ex vivo* T-cell depleted setting used a total dose of 0.8 mg/kg (on Days -12 to -8), with 5×10^4 CD3⁺ cells/kg given with the graft (34). One possible explanation for very slow T cell recovery may be that the residual circulating alemtuzumab destroyed *de novo* T cells as they were produced. In adult T-cell-replete transplants, utilizing a total dose of approximately 0.83 mg/kg, alemtuzumab is generally undetectable in the serum by about 6 days post-HCT (35). In our 3 patients who received a total of 0.6 mg/kg of alemtuzumab, we found that the level of circulating alemtuzumab immediately prior to stem cell infusion

was similar (median of 0.78 +/- 0.21 mcg/ml) to that reported in adults (median of 1.0 +/- 0.5 mcg/ml) (35). However, following infusion of selected CD34⁺ cells, the elimination kinetics of alemtuzumab were relatively slow. It took between Day +7 (median of 0.4 +/- 0.06 mcg/ml) and Day +14 (median of 0.04 +/- 0.12 mcg/ml) for the level to drop below that of 0.1 mcg/ml, the concentration required for antibody-dependent cellular cytotoxicity of peripheral blood mononuclear cells (36). We hypothesize that this slow elimination of circulating alemtuzumab is due to the low numbers of donor T cells administered with the graft, so that only a small amount of the residual alemtuzumab is “soaked up” by CD3⁺CD52^{high} cells. Nevertheless, in all 3 patients, alemtuzumab was no longer detectable in the patients' serum by Day +21 post-HCT, suggesting that this was not the sole explanation for prolonged T-cell lymphopenia.

An alternate hypothesis may be that residual circulating alemtuzumab at the time of donor stem cell infusion may result in clearance of a subset of donor CD34⁺ cells. It has been shown that CD52 is expressed on the majority of CD34⁺ cells in a PBSC collection (37). CD52 is expressed on virtually all CD34⁺/[CD38/Lin]^{lo} cells, and this CD34⁺/CD52⁺ subset is able to generate multi-lineage human cell engraftment in NOD/SCID-IL2RG^{null} mice (38). Gene expression data also suggests that CD52 is highly expressed on HSCs (39, 40). As lineage-specific markers increase on CD34⁺ cells, CD52 mRNA and surface protein expression decrease on cells undergoing myeloid differentiation, and increase on those undergoing lymphoid commitment (39-41). The degree of CD52 expression on non-fractionated CD34⁺ cells appears to be only 25-50% of the CD52 intensity reported for normal T- and B-cells (37, 42). The level of CD52 antigen expression on the cells affects the binding affinity to monoclonal antibodies, so that cells with higher levels have increased susceptibility to cytolysis (42). Preliminary evidence in human HSC-xenografted mice demonstrates drops in human chimerism after anti-CD52 treatment, suggesting a potential direct effect of alemtuzumab on HSCs (43). However, in the setting of a classic T-cell-replete donor cell infusion, residual circulating alemtuzumab likely causes preferential depletion of CD52^{high} T- and B-cells, which immediately “soaks up” most of the circulating alemtuzumab so that it is not at a sufficient concentration to destroy the CD34⁺/CD52^{low} lymphoid progenitors. This likely also explains why alemtuzumab can be added *ex vivo* directly to a T-replete stem cell product without damaging the product's ability to engraft (44). However, in the setting of a CD34-selected transplant, the infused product generally has extremely low numbers of CD52^{high} T- and B-cells. Therefore, the residual alemtuzumab is not “soaked-up” and may persist at levels high enough to cause clearance of CD34⁺/CD52^{low} lymphoid progenitors. The initial wave of naïve T-cells seen in the first several months following T-cell depleted haploidentical HCT may originate from donor CD34⁺/CD52^{low} lymphoid progenitors infused at the time of transplant. If these cells are destroyed around the time of infusion by residual alemtuzumab, then this pool of lymphoid progenitors would need to be replenished from very early pluripotent HSCs before thymopoiesis can begin, which could explain the significantly delayed T-cell recovery seen in our patients. One approach to minimizing the suppression of donor CD34⁺/CD52^{low} cells at the time of HCT would be to move the administration of alemtuzumab to an earlier time point, so that sufficient time has passed to allow the residual antibody to be cleared from the circulation. However, this may also result in a rebound in NK cell levels, as seen in patient

UPN 1373. Other potential approaches would be to increase the dose of HSCs, the dose of T-cells (either viable or potentially irradiated) administered at the time of HCT to serve as a more effective “sink” for the residual alemtuzumab, or to pre-treat the infused HSCs with a non-inhibitory anti-CD52 monoclonal antibody that would interfere with alemtuzumab binding.

In conclusion, targeting of NK cells with alemtuzumab (at a total dose of 0.6 mg/kg) prior to T-cell depleted haploidentical HCT for patients with NK⁺ SCID was insufficient to reliably facilitate donor T-cell engraftment. While alemtuzumab monotherapy has the potential to have significantly less long-term toxicities than classic cytotoxic chemotherapeutic agents, more patients will need to be studied in order to determine if an optimal immunoablative dose prior to haploidentical HCT can be found. In addition, strategies to overcome the slow T cell recovery seen with alemtuzumab prior to T-cell depleted transplant will need to be developed. Given the rarity of SCID, efforts to perform these trials via a multi-center consortium, such as the Primary Immune Deficiency Treatment Consortium (16), may generate more rapid results that improve the outcomes of these patients.

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Figure 1a. ALC following alemtuzumab

*UPN1263 got a higher dose of alemtuzumab (0.3 mg/kg)

Figure 1b. Alemtuzumab levels after T-cell depleted HCT

Note: Analysis not performed in all patients

Table 1

Patient characteristics at time of HCT

UPN	Age at HCT	Gender	Ethnicity	Gene Mutation	CD3	CD4	CD8	CD19	CD16/56	PHA	TME	Indication for HCT
					(× 10 ⁶ /L)							
1263	8 mo	M	Navajo	Artemis (c.597C>A)	82	82	0	0	245	1%	0%	PCP
1373	8 mo	M	Middle Eastern	RAG-1 (c.2210G>A)	2	1	1	0	38	0%	0%	Rotavirus
1392	2 mo	M	Navajo	Artemis (c.597C>A)	32	31	1	0	105	0%	1%	Rotavirus
1247	10 yr	M	Asian	IL2RG (c.678C>T); leaky	263	132	131	291	376	17%	0%	Multiple [#]

UPN, Unique patient number; PHA, Phytohemagglutinin; TME, Transplacental maternal engraftment; RAG, recombinaase-activating gene; PCP, *Pneumocystis jiroveci* pneumonia

[#]Life-long problems with recurrent otitis media, pneumonias, flat warts, zoster, & tinea capitis

Table 2

HCT features & clinical outcomes

UPN	Conditioning	Donor	HSC Source	CD34 Dose ($\times 10^6/\text{kg}$)	CD3 Dose ($\times 10^4/\text{kg}$)	Additional Cells	Clinical Outcome	F/U (years)
1263	Alentuzumab (0.3 mg/kg $\times 3$)	7/10 Mother	PBSC (CD34 Selected)	25	6	q3 week DLI (n=4) starting D+34; Boost D+117	Alive & Well	5.6
1373	Alentuzumab (0.2 mg/kg $\times 3$)	6/10 Mother	PBSC (CD34 Selected)	29.7	6	Full HCT D+74	Alive & Well	4.1
1392	Alentuzumab (0.2 mg/kg $\times 3$)	7/10 Mother	PBSC (CD34 Selected)	30.2	6	Boosts D+50 & D+86	cGVHD, now Alive & Well	4.0
1247	Alentuzumab (0.2 mg/kg $\times 3$)	5/10 Mother	PBSC (CD34 Selected)	19	6	Full HCT D+139	Dead (Refractory AIHA)	1.4

UPN, Unique patient number; HSC, hematopoietic stem cells; F/U, follow-up; PBSC, peripheral blood stem cells; DLI, donor lymphocyte infusion; cGVHD, chronic graft-versus-host-disease; AIHA, autoimmune hemolytic anemia

Table 3**Immunologic outcomes of engrafted patients**

UPN	Time to Reach (months)			At last follow-up					
	CD4 >200 × 10 ⁶ /L	CD4 >400 × 10 ⁶ /L	PHA >50%	CD4 (× 10 ⁶ /L)	CD4 / CD45RA +	PHA	CD19 (× 10 ⁶ /L)	Donor CD19	Donor CD14/15
1263	8.9	12	8.9	350	6%	100%	0	0%	0%
1392	38.4	NR	4.3	248	47%	100%	0	0%	0%