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Adoptive Immunotherapy with Cytokine-Induced Killer Cells for Patients with Relapsed Hematologic Malignancies after Allogeneic Hematopoietic Cell Transplantation

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Donor leukocyte infusions induce remissions in some patients with hematologic malignancies who relapse after allogeneic hematopoietic cell transplantation (HCT); however, graft-versus-host disease (GVHD) remains the major complication of this strategy. Cytokine-induced killer (CIK) cells are a unique population of cytotoxic T lymphocytes that express the $CD3^+CD56^+$ phenotype and show marked up-regulation of the natural killer cell receptor NKG2D (CD314). CIK cells are non–major histocompatibility complex–restricted and NKG2D-dependent in target recognition and cytotoxicity. We explored the feasibility of ex vivo expansion of allogeneic CIK cells in patients with relapsed hematologic malignancies after allogeneic HCT. Eighteen patients (median age, 53 years; range, 20-69 years) received CIK cell infusions at escalating doses of 1×10^7 $CD3^+$ cells/kg (n = 4), 5×10^7 $CD3^+$ cells/kg (n = 6), and 1×10^8 $CD3^+$ cells/kg (n = 8). The median expansion of $CD3^+$ cells was 12-fold (range, 4- to 91-fold). $CD3^+CD56^+$ cells represented a median of 11% (range, 4%-44%) of the harvested cells, with a median 31-fold (range, 7- to 515-fold) expansion. Median $CD3^+CD314^+$ cell expression was 53% (range, 32%-78%) of harvested cells. Significant cytotoxicity was demonstrated in vitro against a panel of human tumor cell lines. Acute GVHD grade I-II was seen in 2 patients, and 1 patient had limited chronic GVHD. After a median follow-up of 20 months (range, 1-69 months) from CIK infusion, the median overall survival was 28 months, and the median event-free survival was 4 months. All deaths were due to relapsed disease; however, 5 patients had longer remissions after infusion of CIK cells than from allogeneic HCT to relapse. Our findings indicate that this form of adoptive immunotherapy is well tolerated and induces a low incidence of GVHD, supporting further investigation as an upfront modality to enhance graft-versus-tumor responses in high-risk patient populations.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment modality for patients with malignant diseases. Relapse remains one of the leading causes of treatment failure and typically portends a very poor prognosis. Strategies to induce remission after relapse include withdrawal of immunosuppressive medications and/or donor leukocyte infusion (DLI). Such approaches attempt to maximize the graft-versus-tumor effect conferred by donor T cells. Responses are variable, however, and acute graft-versus-host disease (GVHD) is a major cause of treatment failure.

Various forms of adoptive immunotherapy aimed at reducing the incidence of acute GVHD associated with DLI have been explored. These approaches have included escalating doses of T cells, $CD8^+$-depleted DLI, antigen-specific cytotoxic T lymphocytes, and natural killer (NK) cells [1-3].

Cytokine-induced killer (CIK) cells are cytotoxic effector T cells that are readily expandable and express in addition to the T cell marker, CD3+, markers

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typically associated with NK cells such as CD56+ and NKG2D. CIK cells are generated by the in vitro culture of peripheral blood lymphocytes with interferon (IFN)-γ, interleukin (IL)-2, and anti-CD3. T cell expansion and activation occurs, resulting in cytolytic cells that recognize targets through NKG2D [4]. NKG2D is an activating receptor expressed on all NK cells and also serves as a T cell costimulatory molecule that augments cytotoxic and proliferative responses of T cells on encountering an antigen [5,6]. CIK cell–mediated cytotoxicity is major histocompatibility complex–unrestricted and T cell receptor–independent, with target killing occurring through NKG2D-mediated recognition. In preclinical studies, CIK cells have shown potent activity against several tumor cell lines, with a markedly reduced capability to induce GVHD in murine models [4,7]. CIK cells also have been shown to traffic to tumor sites, where they persist for 10-14 days and are associated with a reduced tumor burden. CIK cells generated from patients with acute myelogenous leukemia (AML) have demonstrated cytotoxic activity against both autologous and allogeneic leukemic blasts [8]. In addition, CIK cells show minimal or no cytotoxicity against normal tissues including $CD34^+$ stem cells, do not suppress marrow engraftment in vivo, and induce minimal GVHD in allogeneic models [9]. Compared with $CD3^-CD56^+$ lymphokine-activated killer cells, CIK cells have greater potency against multidrug-resistant tumor cell lines and are more readily expandable [10]. Because of these attributes demonstrated in murine model systems, using human cell lines and fresh tumor samples, we developed conditions for the expansion of CIK cells on a clinical scale under Good Manufacturing Practice conditions. We previously reported on the use of autologous CIK cells [11]. Here we report the results of a Phase I feasibility study in which escalating doses of CIK cells derived from HLA-matched sibling donors were administered to recipients with hematologic malignancies who relapsed after allogeneic HCT.

METHODS

Eligibility Criteria

Patients with a hematologic malignancy who had relapsed disease after undergoing allogeneic HCT from a matched sibling donor were eligible for this study. Patients with chronic myelogenous leukemia were eligible only if persistent disease was demonstrated after a previous DLI of at least 1×10^8 cells/kg. Patients could not have active GVHD and must have been either receiving no immunosuppressive medication or taking a stable immunosuppressive regimen. Adequate organ function was required, as defined by (1) a serum creatinine level of <2 mg/dL or creatinine clearance of >50 cc/minute and (2) a direct bilirubin level of <3 mg/dL or transaminase values <3 times the upper limit of normal. Exclusion criteria included no active infections. All patients provided written informed consent before enrollment on this clinical trial. The study protocol was approved by the Stanford University Institutional Review Board, and the trial was conducted in accordance with Declaration of Helsinki principles.

Study Design

This was a single institution, open-label, Phase I clinical trial to evaluate the feasibility and safety of allogeneic CIK cells. Although efficacy was not a primary objective of this trial, all patients were followed for objective outcomes. The hypothesis was that human CIK cells expanded ex vivo will retain antitumor activity, but reduce the incidence and severity of GVHD associated with unmanipulated DLI. The primary objectives of this trial were to (1) determine the feasibility of expanding allogeneic CIK cells suitable for clinical application, (2) determine the infusional toxicity of ex vivo expanded allogeneic CIK cells in patients with recurrent or refractory disease after allogeneic HCT, (3) determine the incidence of GVHD after infusion of allogeneic CIK cells, and (4) determine the maximum tolerated dose of allogeneic CIK cell infusion.

In this dose-escalation study, the starting dose of CIK cells was 1×10^7 $CD3^+$ cells/kg, followed by escalation to 5×10^7 $CD3^+$ cells/kg, with a highest planned dose of 1×10^8 $CD3^+$ cells/kg. In the usual Phase I design, the plan was to enroll 3 patients at each cell dose level. If a dose-limiting toxicity (DLT) was not seen in these 3 patients, then the next higher cell dose level was administered. If DLT was seen, then the cohort was expanded to 6 patients. The dose was increased to 5×10^7 expanded cells/kg and 1×10^8 expanded cells/kg in successive escalations based on the absence of significant infusional or other toxicities or GVHD. To progress to the next dose level, there must have been no grade 3 or greater toxicities as defined by the Common Toxicity Criteria and no evidence of acute GVHD beyond grade II.

Donor Apheresis and CIK Cell Expansion

In each case, the matched sibling donor who had donated the graft for the earlier allogeneic HCT was used as the CIK cell donor. The donors underwent unmobilized apheresis, and up to 1 L of autologous plasma was collected from each donor to supplement the expansion culture. Sufficient quantities of cells ($>1.6 \times 10^9$) were collected to allow inoculation of the CIK cell expansion cultures. The cultures were inoculated on the same day as collection, with a minimum of 6.0×10^8 cells and a maximum of 7.5×10^8 cells

added to Aim-V medium (Invitrogen, Grand Island, NY) and supplemented with 5% autologous (donor) heat-inactivated plasma and 2×10^5 IU IFN-γ (Actimmune; InterMune, Brisbane, CA) in a continuous perfusion biochamber maintained at 37° with 20% O_2 and 5% CO_2. During the initial culture activation period, medium perfusion did not occur. This continuous perfusion culture was performed for the first 11 patients, and gas-permeable culture bags were used for all patients thereafter.

On the next day (day +1), expansion was initiated with muronmab-CD3 (Orthoclone, OKT3; Orthobiotech, Raritan, NJ) at 50 ng/mL and IL-2 (Proleukin; Chiron, Emeryville, CA) at 300 IU/mL. Both agents were added to the continuous perfusate of the biochamber with AimV medium containing 5% inactivated autologous plasma and 300 U/mL of IL-2 at a rate of 0.104 mL/minute, equivalent to 150 mL/day. This rate of medium flow resulted in a 50% exchange of the culture volume each day. To assess the accuracy of the perfusion rate, the cultures were sampled on every third day of culture beginning on day +4, and lactate levels were measured as indicators of culture health. If the lactate level measured was >0.8 mg/mL (9 mM), the medium perfusion rate was increased according to prespecified guidelines until the lactate level dropped to <0.8 mg/mL. The period of expansion with IL-2 was maintained for 21-28 days. The static culture bags were fed every 2-3 days, and the volume was increased as needed to maintain the cell density at 2-4 $\times 10^6$/mL.

Samples were removed from each culture bag for detection of *Mycoplasma* by polymerase chain reaction at 5 days before harvest. Additional samples were drawn from each bag after the final addition of medium and IL-2 at 3 days before culture harvest for sterility testing of bacterial and fungal contaminants. On the day of harvest, samples were removed for final release tests, including white blood cell count and viability, Gram staining, endotoxin testing, and T cell content determination. Sterility testing was performed by the Stanford Hospital Clinical Microbiology Laboratory.

Culture Conditions and Culture Harvest

Cultures were maintained in either Aastrom Replicell biochambers (Aastrom Biosciences, Ann Arbor, MI) or Baxter LifeCell culture bags (Baxter Healthcare, Deerfield, IL) at 37°C and 5% CO_2 over 21-28 days, as indicated. Cultures for patients 1-11 were expanded in Aastrom Replicell biochambers modified to allow 10-15 L of medium perfusion per biochamber over the duration of the culture. Each biochamber was inoculated with 750×10^6 peripheral blood cells collected by apheresis and cytokines added as described earlier. On day +4 of incubation, continuous perfusion of the culture began with fresh medium supplemented with IL-2 at 300 IU/mL. The infusion rate was gradually increased over the course of the culture based on lactic acid levels in medium perfused from the biochambers. Cultures for patients 12-18 were expanded in LifeCell culture bags once the Aastrom Replicell biochambers were discontinued for this potential indication. Cultures were inoculated in multiple 1-L LifeCell bags, each containing 200 mL of medium with 4×10^8 peripheral blood cells. Cytokines were added as described earlier. On day +4 and every third day thereafter, cultures were assessed for cell density and viability. Additional culture medium supplemented with sufficient IL-2 to maintain ≥300 IU IL-2/mL was added to LifeCell bags to adjust cell density to $\geq 2 \times 10^6$ and 4×10^6 viable cells/mL. If cell density did not require additional volume, then IL-2 alone was added at 300 IU/mL of culture volume. When bag volume exceeded 500 mL, the contents were transferred to a 3-L LifeCell bag and maintained as before until harvest.

After 21 or 28 days, cells cultured in biochambers were collected in an Aastrom cell processor and washed with Normosol-R (pH 7.4; Baxter Healthcare, Deerfield, IL) supplemented with 1% human serum albumin (CLS Behring, King of Prussia, PA or Telacris, Research Triangle Park, NC). Cells cultured in LifeCell bags were pooled, volume-reduced, and washed with Normosol plus 1% haemophilus selective agar. Harvested cells were concentrated to 200-300 mL for infusion and maintained at ambient temperature until infusion. Cells were infused within 6 hours of harvest and were not cryopreserved.

Samples also were obtained for phenotyping and cytotoxicity assessments. The infused cell dose was based on total $CD3^+$ cell content and adjusted to the cohort dose.

Cell Analysis and Cytotoxicity Assays

Cell counts were performed on an impedance counter (Beckman Coulter, Brea, CA) and viability by Trypan blue exclusion. Cell phenotypes were assessed by flow cytometry of inoculating cells and harvested cell cultures using monoclonal antibodies to CD3 (clone HIT-3a) and CD56 (clone NCAM) for CIK cell enumeration. Additional phenotyping included CD4 (clone RPA-T4), CD8 (clones HIT-8A and SK1), and CD314 (clone 1D11). $CD314^+$ designates *KLRK1*, the gene encoding NKG2D.

Killing of target cell lines, including SUDHL-4, OCI-Ly8, DB, and Jurkat, by CIK cell cultures for patients 1-16 was assessed by chromium release assays as described previously [11]. For patients 17 and 18, cytotoxicity against the target cell lines was assessed using the ApoLogix carbofluorescein polycaspase detection assay (Cell Technology, Mountain View, CA) according to the manufacturer's directions.

CIK Cell Infusion

CIK cells harvested from the expansion cultures and meeting the release criteria were infused within 6 hours of harvest. The cells were infused through a central venous catheter or peripheral intravenous line of at least 18 gauge and were infused over a period of 30 minutes. Electrocardiogram tracings were obtained before the CIK cell infusion, and vital signs were monitored every 30 minutes after infusion for at least 2 hours.

Study Assessments

Postinfusion toxicity evaluations were performed in the outpatient setting on days +1, +3, +7 +14, +21, and +56. Variable number tandem repeat analysis was performed before infusion and at days +7, +21, and +56 after infusion of CIK cells. Positron emission tomography scanning and/or bone marrow biopsy evaluation were performed at regular intervals after CIK cell infusion to assess clinical responses. These tests were performed on days +30 and +60, at a minimum, after CIK infusion.

RESULTS

Patient Characteristics

Eighteen patients received CIK cell infusions at 3 dose levels (Table 1). The median age was 53 years (range, 20-69 years). Diagnoses included non-Hodgkin lymphoma (NHL; n = 5), AML (n = 3), multiple myeloma (MM; n = 3), chronic lymphocytic leukemia (CLL, n = 2), acute lymphoblastic leukemia (ALL; n = 2), myelodysplastic syndrome (MDS; n = 2), and Hodgkin's disease (HD; n = 1). All patients had relapsed after allogeneic HCT using a matched sibling donor. Twelve patients had received a reduced-intensity conditioning (RIC) regimen, and 6 patients had received a myeloablative regimen. The median time from HCT to relapse was 12 months (range, 3-142 months), and the median time from relapse to CIK infusion was 4 months (range, 1-34 months). With the exception of 1 patient with CLL and 1 patient with MDS, all patients received some form of cytoreductive therapy (including chemotherapy, corticosteroids, surgical resection, or DLI) before CIK infusion. At the time of CIK infusion, 10 patients (56%) were in complete remission (CR), 4 patients were in partial remission (PR), 1 patient with acute promyelocytic leukemia (APL) had a molecular relapse that failed to respond to DLI, and 3 patients had progressive disease.

Cell Characterization and Expansion

Table 2 specifies the viability and cell counts of the 18 harvested products after expansion and activation that were prepared under Good Manufacturing Practice conditions. The median viability of the cultured products was 86.5% (range, 70%-95%) with a median 12-fold (range, 4- to 91-fold) expansion of $CD3^+$ cells and a median 31-fold (range, 7- to 515-fold) expansion of $CD3^+CD56^+$ cells. The median $CD3^+CD314^+$- or NKG2D-expressing population was 53% (range, 32%-78%). Figure 1 is a contour plot from the product of a donor, depicting the expansion of $CD3^+$ cells

Table 1. Patient Characteristics

Patient	Dose Level	Age, Years	Diagnosis	HCT Preparative Regimen	Time from BMT to Relapse, Months	Treatment before CIK	Time from Relapse to CIK, Months	Disease Status at CIK
1	1	39	AML	ABL	3	MEC	1	CR3
2	1	52	NHL	RIC	8	Prednisone	2	CR3
3	1	45	APL	RIC	3	DLI × 2	10	Molecular relapse
4	1	33	NHL	ABL	140	IFRT throat	4	CR3
5	2	55	MM	RIC	3	Bortezimib/dex, thal/dex	11	PD/refractory
6	2	44	MM	RIC	20	Thal/dex	9	PR2
7	2	28	HD	RIC	11	Bortezimib, MOPP, gemcitabine	5	CR3
8	2	45	MDS	ABL	12	HiDAC + idarubicin	2	CR3
9	2	60	MM	RIC	24	Thal/dex	14	PD/refractory/2 relapse
10	2	52	NHL	RIC	18	Lymph node resection	2	CR3
11	3	55	AML	ABL	29	MEC	3	CR2
12	3	56	NHL	RIC	6	Lymph node resection	4	CR4
13	3	57	CLL	RIC	24	None	6	PR2
14	3	69	CLL	RIC	30	XRT	5	PR4
15	3	53	ALL	ABL	42	Imatinib	4	CR2
16	3	53	NHL	RIC	3	RTX, XRT	34	CR4
17	3	20	ALL	ABL	9	HCVAD × 2, XRT	4	CR2
18	3	68	MDS	RIC	3	None	*	PD

ABL indicates myeloablative; MEC, mitoxantrone, etoposide, and cytarabine; IFRT, involved field radiotherapy; dex, dexamethasone; thal, thalidomide; MOPP, mechlorethamine, vincristine, procarbazine, and prednisone; HiDAC, high-dose cytarabine; XRT, radiotherapy; RTX, rituximab; HCVAD, cyclophosphamide, vincristine, doxorubicin, and dexamethasone; PD, progressive disease.
*This patient had persistent disease after allogeneic HCT.

Table 2. Cell Expansion and Characterization

Total Harvested Cells	Median	Range
Viability	87%	70%-95%
% $CD3^+$ cells	97%	46%-100%
$CD3^+$ cell count	7.5×10^9	1.9×10^9-1.2×10^{10}
CD3 fold expansion	12	4-91
% $CD3^+CD56^+$	11%	4%-44%
$CD3^+CD56^+$ cell count	1.2×10^9	2.9×10^8-6.2×10^9
$CD3^+CD56^+$ fold expansion	31	7-515
% $CD3^+CD314^+$	53%	32%-78%
$CD3^+CD314^+$ cell count	5.4×10^9	1.5×10^9-1.2×10^{10}

expressing $CD56^+$ and $CD314^+$ over the 21-day culture period.

Cytotoxicity assays were performed with the expanded CIK cells against 4 tumor targets: SUDHL-4, OCI-Ly8, DB, and Jurkat. Approximately 40%-60% specific killing at an effector-target ratio of 40:1 was observed against all 4 cell lines, with the greatest percentage of killing seen against Jurkat, a T cell leukemia target (Figure 2). Because of ineffective ^{51}Cr labeling or lack of cell expansion, cytotoxicity was not assessed in all donors against all 4 cell lines. The numbers of donors tested against each cell line were 16 for SUDHL-4, 17 for OCI-Ly8, 15 for DB, and 16 for Jurkat.

CIK Administration and Clinical Responses

The CIK cell doses administered based on $CD3^+$cells/kg were 1×10^7 in 4 patients, 5×10^7 in 6 patients, and 1×10^8 in 8 patients. The median follow-up duration from the time of CIK infusion was 20 months (range, 1-69 months), and the median event-free survival (EFS) and overall survival (OS) from the time of CIK infusion were 4 months and 28 months, respectively. Of the 12 patients who received CIK cells while in CR, 11 patients relapsed, with a median time to relapse of 6 months (range, 2-37 months). Patient 3 had a molecular relapse of APL at day +93 after nonmyeloablative HCT. She had previously received 2 DLI infusions without response. This patient achieved a molecular response approximately 4 weeks after CIK infusion. The median follow-up time for all patients from relapse after allogeneic HCT was 29 months, (range, 5-74 months), whereas the median EFS and OS from the time of relapse after allogeneic HCT were 13 months and 37 months, respectively. Table 3 summarizes patient outcomes.

Of the 18 patients enrolled on this trial, 5 achieved or maintained CR for more than 1 year after CIK infusion, with 1 patient remaining in CR at 32 months after CIK infusion. Four of these 5 patients had a lymphoid malignancy, and 1 patient had AML. The patient with

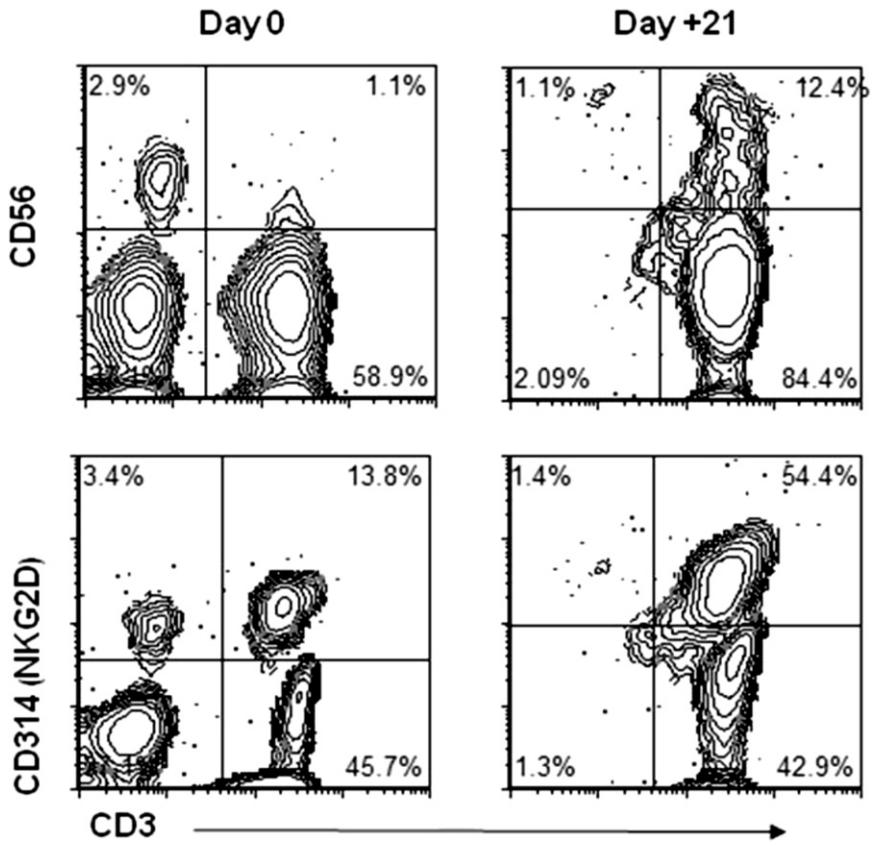

Figure 1. Fluorescence-activated cell sorting analysis of peripheral blood mononuclear cells after 21 days of culture, illustrating the increase in the number of cells expressing $CD3^+C56^+$ and $CD3^+CD314^+$ phenotypes.

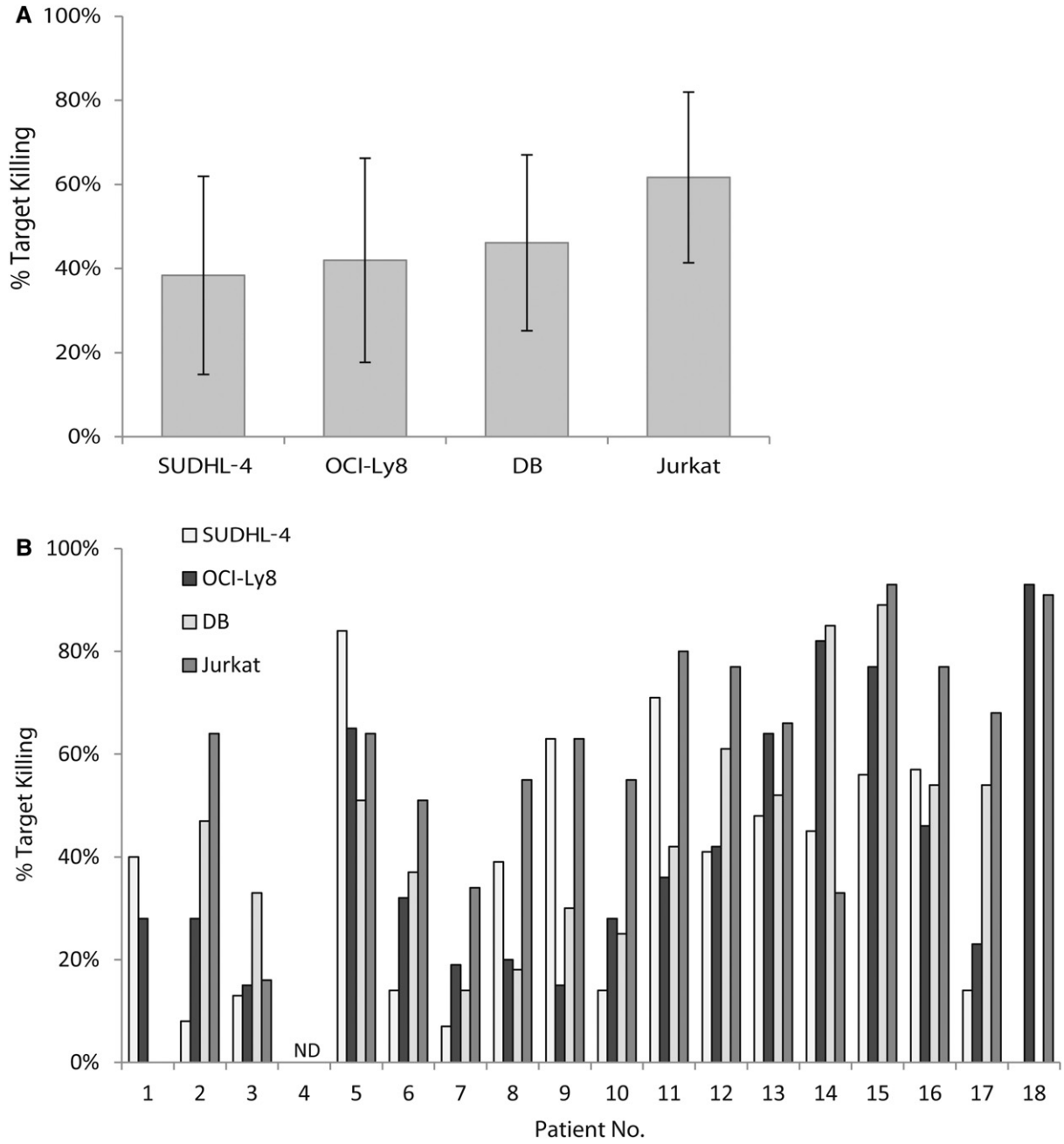

Figure 2. (A) In vitro cytotoxic activity of CIK cells by specific tumor target. Killing of target cell lines including SUDHL-4, OCI-Ly8, DB, and Jurkat at an E:T ratio of 40:1. (B) In vitro cytotoxic activity of CIK cells by each patient against 4 tumor targets—SUDH-L4, OCI-Ly8, DB, and Jurkat—at an E:T ratio of 40:1.

the longest CR after CIK infusion was a 33-year-old man with multiply relapsed mantle cell lymphoma. This patient underwent myeloablative allogeneic HCT in 1995 while in second CR. Twelve years later, he had an isolated relapse in a supraglottic node, for which he received involved field radiation therapy followed by CIK infusion. At the time of this report, he had maintained CR for 32 months. Another notable case was a 39-year-old man with primary refractory AML who relapsed 3 months after myeloablative allogeneic HCT. He received salvage chemotherapy followed by CIK infusion. His remission lasted 20 months until disease relapse, and he died soon after. A long-term remission was seen in a 56-year-old woman who underwent autologous HCT in 1995 for relapsed diffuse large cell lymphoma (DLCL). She relapsed 3 years later and received salvage chemotherapy followed by RIC allogeneic HCT. She experienced relapse in the left axilla 5 months later, and underwent an excisional biopsy followed by CIK infusion. She subsequently sustained a CR for 37 months. A 68-year-old woman with MDS received a CIK infusion approximately 6 months

Table 3. Patient Outcomes

Patient	Dose Level	Diagnosis	Best Response	Time from CIK to Relapse	Alive	Cause of Death	GVHD	Adverse Events	Follow-up Post-CIK, Months
1	1	AML	CR	19 months	No	Relapse			21
2	1	NHL	PR/SD	13 months	No	Relapse			30
3	1	AML	CR	4 months	No	Relapse		Ventricular arrhythmia grade 4	46
4	1	NHL	CR/SD	Remission	Yes				32
5	2	MM	PD	9 days	No	Relapse			1
6	2	MM	CR	6 months	No	Relapse		Ventricular arrhythmia grade 3, ALT grade 3	28
7	2	HD	CR	6 months	Yes		Chronic limited		70
8	2	MDS	CR	4 months	No	Relapse	Acute grade 2 skin		13
9	2	MM	PD	9 days	No	Relapse			14
10	2	NHL	CR	12 months	Yes				59
11	3	AML	CR	2 months	No	Relapse		Grade 3 ALT, AST	24
12	3	NHL	CR/SD	37 months	Yes				43
13	3	CLL	SD	2 months	Yes				20
14	3	CLL	PD	15 days	Yes				18
15	3	ALL	CR/SD	2 months	Yes				18
16	3	NHL	CR/SD	10 months	Yes				13
17	3	ALL	CR/SD	8 months	Yes		Acute grade 2 skin, hepatic		9
18	3	MDS	CR	Remission	Yes				3

PD indicates progressive disease; SD, stable disease; ALT, alanine aminotransferease; AST, aspartate aminotransferase.

after RIC allogeneic HCT for persistent disease. A bone marrow biopsy performed at 3 months after CIK infusion showed no evidence of MDS.

Four patients (2 with MM and 2 with CLL) progressed rapidly within 2 months after CIK infusion. All 4 of these patients had active disease at the time of CIK infusion despite receiving cytoreductive therapy before CIK infusion.

With a median follow-up time of 20 months (range, 1-69 months) for all patients, 10 patients are alive at the time of this report. Of these, 2 remain disease-free and 8 are alive with relapsed disease after CIK infusion. Eight patients have died, with progressive disease being the primary cause of death.

Chimerism and Serum Immunophenotyping

Eleven patients retained full donor chimerism after relapse from allogeneic HCT, and their status did not change after CIK infusion. Five patients exhibited mixed donor chimerism (defined by >5% and <95% $CD3^+$ cells) at the time of CIK infusion. Of these 5 patients, 1 patient with MDS who had mixed chimerism with 82% donor $CD3^+$ chimerism at the time of CIK infusion converted to full donor chimerism showing 100% $CD3^+$ cells at approximately 2 months after CIK infusion. This patient received the highest CIK dose level. The mixed donor chimerism status of the other 4 patients remained stable. Chimerism data was not obtained on 2 patients.

GVHD and Adverse Events

Acute GVHD occurred in 2 patients. One of these patients, at the second dose level, developed grade II skin GVHD at approximately 98 days after CIK infusion. The GVHD resolved with topical corticosteroids alone. At the third dose level, 1 patient developed grade II hepatic GVHD with a concomitant skin rash on the trunk and bilateral upper extremities at approximately 7 weeks after CIK infusion. A skin biopsy and liver biopsy were performed, and results from both organs were equivocal for either GVHD or a drug reaction. The patient was treated with corticosteroids, tacrolimus, and mycophenolate mofetil and achieved partial resolution of GVHD. Chronic GVHD occurred in only 1 patient. This patient developed limited chronic GVHD on day +123 after CIK infusion, manifesting as joint stiffness and aching that responded to oral corticosteroids, which were eventually discontinued.

One patient at the first dose level experienced a transient grade 3 ventricular arrhythmia that resolved spontaneously without intervention during CIK cell infusion. At the second dose level, 1 patient also experienced syncope at approximately 3 weeks after CIK infusion. A comprehensive investigation of the etiology of the syncope revealed inducible sustained ventricular tachycardia during cardiac electrophysiologic studies, which led to placement of an implantable defibrillator at 1 month after CIK infusion. The dysrhythmia did not recur after placement of the device. This patient also experienced a transient rise in hepatic transaminase levels that resolved without intervention. Because of these 2 DLTs, the cohort was expanded to a total of 6 patients. No other DLTs were seen at this dose level, and thus we proceeded to the third dose level of 1×10^8 $CD3^+$ cells/kg. To date, 8 patients have been infused at this dose, and no DLTs have been observed in these patients.

DISCUSSION

Relapse remains one of the leading causes of treatment failure after allogeneic HCT and typically carries a poor prognosis. DLI is a strategy offered to relapsed patients in this situation, but this form of adoptive immunotherapy can incur significant toxicity, with acute GVHD and marrow aplasia as the leading causes of nonrelapse mortality after DLI [12]. Furthermore, although DLI has been extremely effective in treating chronic myelogenous leukemia, this treatment modality has been less effective in the treatment of other hematologic malignancies [13-15].

Our Phase I study demonstrated the feasibility and safety of allogeneic CIK cell infusions in patients with relapsed hematologic malignancies. This Phase I dose-escalation trial reached a planned maximum cell dose of 1×10^8 $CD3^+$ cells/kg as the maximum tolerated dose. We observed only 2 cases of grade II acute GVHD that responded to topical or systemic corticosteroids and 1 case of limited chronic GVHD. The most serious adverse events seen were at the lowest cell dose. Two patients developed transient ventricular dysrhythmias, the etiology of which was not elucidated.

The low incidence of GVHD in this clinical study parallels the low incidence seen in preclinical models. We previously demonstrated that the adoptive transfer of allogeneic CIK cells in a rodent model induced minimal GVHD [9]. Using bioluminescence imaging, we showed that luciferase-expressing CIK cells generated from splenocytes exhibited traffic patterns similar to those of conventional T cells. However, compared with the conventional T cells, the CIK cells infiltrated GVHD target tissues much less, demonstrated a slower division rate, were less susceptible to apoptosis, and produced high amounts of IFN-γ, a cytokine known to confer a protective effect against acute GVHD [16].

Because this was a Phase I feasibility study in a very heterogeneous patient population, it is difficult to draw conclusions regarding efficacy. In the 12 patients who received CIK infusions while in CR, the median time to progression was 6 months. This duration of remission is notable, considering that this was a high-risk population with the most durable remissions observed in patients with lymphoid malignancies. From the time of CIK infusion, the median EFS and OS were 4 months and 28 months, respectively. Although almost all of the patients in this study underwent some form of cytoreduction before CIK infusion, 5 patients had a longer time to progression/relapse after CIK infusion compared with the time to progression/relapse immediately after allogeneic HCT. CIK cell infusion had no affect on patients with rapidly progressive disease at the time of infusion. The affect of CIK infusion on donor chimerism could not be assessed in most patients, because 11 patients already exhibited full donor chimerism at the time of CIK infusion. However, 1 of 5 patients with mixed chimerism converted to full donor chimerism by approximately 2 months after CIK infusion without the development of GVHD.

A previous Phase I study of similar design also described clinical responses in patients who had received allogeneic CIK infusions after post-HCT relapse. Introna et al. [17] administered allogeneic CIK infusions in 11 patients with hematologic malignancies and reported CR in 3 patients, with 1 remission lasting for more than 2 years at the time of the report. Two of the 3 patients with CR converted to full donor chimerism but also developed extensive cutaneous GVHD that commenced shortly after the documented clinical response. Unlike our study, in which patients received only 1 CIK cell infusion, most patients received multiple sequential infusions, with cell doses ranging from 3×10^6 to 15×10^6 $CD3^+$ cells/kg.

In terms of feasibility of culture and expansion, we generated a median 12-fold expansion of $CD3^+$ cells and 31-fold expansion of $CD3^+CD56^+$ cells, similar to the expansion data reported by Introna et al. [17]. In addition, we characterized the percentage and number of $CD3^+CD314^+$ cells, with 53% of cells expressing this phenotype after culture. We also confirmed in vitro antitumor activity against various tumor targets, as reported previously by our group and others [9,17,18].

Other groups have manipulated CIK cells with the intent of improving specificity and enhancing cytotoxicity against various tumor targets. The cytotoxicity of CIK cells was significantly increased against B-NHL targets when cocultured with the anti-CD20 antibodies, rituximab, and GA101 [19]. Other investigators have incorporated bispecific antibodies with the goal of redirecting CIK cells and increasing killing against primary ovarian cancer cells and against B cell ALL [20,21]. The expansion of cord blood–derived CIK cells represents another promising source of adoptive immunotherapy with potential application for patients with relapsed malignancies after umbilical cord blood transplantation [22]. We also have used CIK cells to deliver an oncolytic virus to the tumor bed in a preclinical model with remarkable efficacy [23]. This approach is being developed for clinical translation.

In conclusion, the present study shows the feasibility and safety of allogeneic CIK cell infusion in patients with relapsed hematologic malignancies. Although CIK cells require expansion under specific culture conditions, we and other groups have demonstrated the feasibility of this approach and have obtained similar results in terms of low observed toxicity and high cell yields. Although assessing clinical responses in this setting is difficult given the variable and high-risk nature of this patient population, it should be noted that several responses extended past 1 year in this high-risk patient population. As seen with DLI,

CIK cells appear to induce the longest duration of response in patients who had minimal residual disease at the time of infusion. Determining the comparable efficacy of CIK cells and DLI in the setting of relapse is difficult, but our data and those of others have confirmed lengthy clinical responses accompanied by a low incidence of acute and chronic GVHD compared with DLI [17]. Thus, our results suggest that CIK cells exert a biological effect and warrant further investigation. We are currently conducting 2 follow-on studies with CIK cell infusion after allogeneic HCT with RIC. One of these studies involves high-risk patients with MDS who received CIK cells preemptively on day +42, and a second trial is open to patients with CLL who demonstrate mixed chimerism. In this second trial, both chimerism and molecular disease burden will be assessed.

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