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Herpes simplex thymidine kinase gene–transduced donor lymphocyte infusions

Richard K. Burt[a], William R. Drobyski[b,*], Tatiana Seregina[c], Ann Traynor[a], Yu Oyama[a], Carolyn Keever-Taylor[b], Jacob Stefka[a], Timothy M. Kuzel[d], Mary Brush[a], Julianne Rodriquez[a], Willam Burns[b,†], Lucinda Tennant[c], and Charles Link[c]

[a]*Northwestern University School of Medicine, Division of Immunotherapy, Chicago, Ill., USA;* [b]*Medical College of Wisconsin, Bone Marrow Transplantation Program, Milwaukee, Wis., USA;* [c]*Stoddard Cancer Research Institute, Iowa Methodist Medical Center, Des Moines, Iowa, USA;* [d]*Northwestern University School of Medicine, Division of Hematology Oncology, Chicago, Ill., USA*

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Objective. **Donor lymphocytes mediate both a beneficial graft-vs-leukemia/lymphoma (GVL) effect as well as graft-vs-host disease (GVHD), the most dreaded complication of allogeneic hematopoietic stem cell transplantation (HSCT). Transduction of donor lymphocytes with a herpes simplex thymidine kinase (HSVtk) gene prior to infusion confers lethal sensitivity to the anti-herpes drug, ganciclovir (GCV). HSVtk-transduced donor lymphocyte infusions (DLI) have already been used and significant problems have limited the clinical experience to very few patients. To this end, we also report on a study of whether HSVtk-DLI induces GVHD/GVL and if infusion of GCV allows abrogation of GVHD by selective killing of donor lymphocytes.**

Materials and Methods. **Nine patients with relapsed hematologic malignancies after allogeneic hematopoietic stem cell transplantation (HSCT) were infused with HSVtk gene–modified donor lymphocytes. In brief, transgeneic lymphocytes were prepared by 3 days of activation, 1 day of transduction, 6 days of selection with G418, and 2 to 4 weeks of expansion.**

Results. **From 5.0 to 199 $\times$ 10^6 $CD3^+$ DLI were infused. There were no toxicities and no correlation between $CD3^+$ cell dose and either GVHD or GVL was observed. Only one patient who had cutaneous T-cell lymphoma (CTCL) developed GVHD and that same patient is the only patient to have an anti-tumor response. The patient was infused with 23 $\times$ 10^6 $CD4^+$ and 9.7 $\times$ 10^6 $CD8^+$ HSVtk DLI. Following discontinuation of immune suppression and infusion of GCV, GVHD promptly resolved. Although the CTCL relapsed, it has been easily controlled with intermittent topical therapy. One patient with acute myelogenous leukemia (AML) had a remission inversion of undetermined significance. Two patients with AML, one patient with lymphoma, and four patients with chronic myelogenous leukemia (CML) did not respond.**

Conclusion. **HSVtk-DLI may provide an anti-tumor effect in vivo and may induce GVHD that is abrogated by GCV treatment. While technical aspects to improve response need to be perfected, HSVtk-DLI infusion to induce a transient GVL/GVHD may become an effective future therapy to minimize complications of allogeneic HSCT.** **© 2003 International Society for Experimental Hematology. Published by Elsevier Inc.**

Allogeneic hematopoietic stem cell transplantation (HSCT) is the most effective transplant procedure to prevent relapse of hematologic malignancies. In several cases, depending on type and stage of disease, it is unlikely that the "last" malignant cells can be eliminated by intensive chemo-radiotherapy. In relapsed hematologic malignancies occurring after allogeneic HSCT, infused donor lymphocytes are capable of reintroducing remission [1–6]. This immunologic graft-vs-tumor effect, postulated as a mechanism of remission for several hematologic malignancies, has been termed graft-vs-leukemia (GVL) [7,8].

Allogeneic HSCT is, however, complicated by graft-vs-host disease (GVHD), an immune-mediated reaction against

Offprint requests to: Richard K Burt, M.D., Chief, Division of Immunotherapy, Northwestern University Medical Center, Searle Bldg, 320 East Superior, Room 3-489, Chicago, Ill 60611; E-mail: rburt@nwu.edu

*Drs. Burt and Drobyski contributed equally to this work.

†Dr. Burns died Aug. 13, 2001.

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normal host epithelial tissues that is often associated with significant morbidity and mortality [9]. Despite immune suppressive drug prophylaxis and depending on age and extent of HLA matching, on average at least 20% of patients develop severe grade III–IV acute GVHD and 30 to 50% develop extensive chronic GVHD [9]. It is presumed that acute GVHD arises from alloreactive donor T cells. It is also assumed that chronic GVHD arises from infused donor T cells. However, other cell types or T cells arising de novo from the infused allogeneic stem cell compartment may be involved in chronic GVHD. In most cases, nonselective lymphocyte depletion of the allograft can prevent GVHD, but lymphocyte-depleted grafts are accompanied by a loss of GVL effects and increased relapse rate. The unknown identity of most tumor-specific antigens and technical feasibility of generating tumor-specific lymphocytes has limited the practicality of specific immune therapy to induce GVL without GVHD.

Recently, the ex vivo transduction and infusion of herpes simplex thymidine kinase (HSVtk) transgeneic lymphocytes has emerged as a potential method to induce GVL while being able to abrogate GVHD [10–14]. Transgeneic cells have been demonstrated to persist in vivo in the peripheral blood. Bonini et al. have reported undetectable levels of gene-modified cells after ganciclovir (GCV) infusion and resolution of acute GVHD, while Tiberghein et al. have found detectable levels of gene-modified cells after resolution of acute GVHD [15–20]. While these reports have determined that HSVtk transgeneic cells may cause GVHD, and that GVHD may be aborted by infusion of GCV, there are limited data about the anti-tumor effectiveness of HSVtk-transgeneic lymphocytes. Insufficient experience exists to answer several questions: Do in vivo outbreaks of replication competent retrovirus (RCR) occur in patients? Are HSVtk transgeneic cells fully functional in vivo? Do HSVtk lymphocytes maintain potent anti-tumor activity? Do HSVtk-transduced cells home to the involved tumor sites or organ systems? Will disease relapse after GCV infusion, and if so, is it more responsive to traditional medications? Is chronic GVHD mediated by the infused lymphocytes or are other cells or mechanisms involved? In order to help answer these questions, we performed HSVtk-transgeneic donor lymphocyte infusions (DLI) to attempt remission of relapsed hematologic malignancies.

Methods

Patient population

Eligible candidates were patients at least 2 years of age with relapsed chronic myelogenous leukemia (CML), acute lymphoid leukemia (ALL), acute myelogenous leukemia (AML), or lymphoma after allogeneic bone marrow transplant (BMT). The protocol was approved by the US Food and Drug Administration (FDA) under IND 6206, and the Institutional Review Boards and Biosafety Committees of the Iowa Methodist Medical Center, Medical College of Wisconsin, and Northwestern University Medical Center.

Procedure for harvesting donor lymphocytes

Lymphocytes from the original bone marrow or peripheral blood stem cell (PBSC) donor of a patient who relapsed after allogeneic HSCT were collected by 5- to 10-liter lymphopheresis using a Cobe Spectra (Cobe, Lakewood, CO, USA) or Fenwal (Baxter, Chicago, IL, USA) apheresis instrument. Red blood cells (RBC) were removed from the mononuclear product by Ficoll gradient separation. A sample of 0.5×10^6 cells was analyzed by flow cytometrics for CD4, CD8, CD3, CD19 markers. If the cells were not scheduled to undergo ex vivo transduction within 48 hours of apheresis, the mononuclear product was cryopreserved.

Retroviral vector design and production

LTKOSN is a retroviral vector derived from the Moloney murine leukemia virus (MOMLV) by cloning a polymerase chain reaction (PCR)-amplified 1179-bp HSVtk open reading frame into LXSN backbone [21,22]. The HSVtk gene is expressed from the viral long terminal repeat (LTR) and a bacterial neomycin resistance (neo^r) gene transcribed from an internal SV40 promoter. A sense clone was sequenced through both strands of the HSVtk open reading frame and found to be as predicted. LTKOSN retroviral vector producer cell line (VPC) was established by transduction of PA317 packaging cell line as described previously [12]. VPC clone LTKOSN.1 was found to produce the highest titer, 1×10^6 colony-forming units (cfu)/mL. This VPC clone was subjected to quality control testing required by the FDA. LTKOSN.1 Master Cell Bank, Working Cell Bank, and clinical grade production lot supernatants were manufactured in accordance with principles of current Good Manufacturing Practice. Certified clinical-grade LTKOSN.1 retroviral supernatants used for ex vivo donor lymphocyte transductions had titers 3 to 10×10^5 cfu/mL.

Procedure for lymphocyte HSVtk transduction

Fresh or previously cryopreserved and thawed mononuclear cells were washed in 500 mL normal saline followed by one wash with X-VIVO 15 (Bio Whittaker, Walkersville, MD, USA) containing 1% human serum albumin (HSA) (Baxter, Chicago, IL, USA) and 1% penicillin/streptomycin. Cells were pelleted at 1250 rpm for 10 minutes and 3×10^8 cells resuspended in 50 mL X-VIVO 15 with 1% HSA. Activation to induce lymphocyte mitosis was done over 3 days at 37°C in 5% CO_2 using 1 ucg/mL OKT3 monoclonal antibody (Ortho Biotech, Raritan, NJ, USA) and 200 U/mL interleukin-2 (IL-2) (Chiron, Emeryville, CA, USA) at a cell concentration of 1×10^6 cells/mL. Activated cells were pelleted at 1250 rpm for 10 minutes and resuspended in phosphate-free RPMI-1640 with 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA) and 1% penicillin/streptomycin. After 12 hours, cells were pelleted and resuspended in viral supernatant with 10 ucg/mL protamine sulfate, 200 U/mL IL-2, and 1.0 ucg/mL OKT3 at a concentration of 0.5×10^6 cells/mL. After a 12-hour incubation, the phosphate depletion and supernatant transduction procedures were repeated twice. Transduced cells were selected by incubation in X-VIVO 15 with 1% penicillin/streptomycin with 200 U/mL IL-2 and 0.3 mg/mL G418 for 6 days at 37°C and 5% CO_2. Viable cells were separated by Lymphoprep (Gibco, Grand Island, NY, USA) and expanded over 2 to 4 weeks in X-VIVO 15 with 1% penicillin/streptomycin with 200 U/mL IL-2. After expansion, the following tests were performed: Gram stain, bacterial endotoxin, fungal and mycoplasma cultures, PCR for percent transduced cells, flow cytometry for CD3, CD4, CD8, and CD19, PCR for RCR outbreak, and RCR coculture. Cells were washed

and resuspended in Plasmalyte A (Baxter, Chicago, IL, USA) with 2% HSA prior to infusion.

Replication competent retrovirus testing (Mus dunni—PG-4assay)

RCR S^+L^- assays were conducted on donor peripheral blood lymphocytes (PBL) transduced with HSVtk and on PBL of patients after HSVtk donor lymphocyte infusion. Cells were cocultured with *Mus dunni* cells for two weeks (four passages). After two weeks, supernatants were collected, centrifuged, and placed on PG-4 cells. The supernatant was removed 18 hours later and D10 (DMEM, 10% fetal bovine serum, L-glutatmine) added. After four to seven days, PG-4 cells were observed for development of foci. MMLV 4070A was used as the positive control and *Mus dunni* cells alone were used as the negative control.

Retroviral envelope gene expression (RCR detection by PCR)

Genomic DNA was isolated using the Genomic DNA Purification Kit (Sigma, St. Louis, MO, USA). MLVENV-F (5′-ACCTGGAGA-GTCACCAACC-3′) and MLVENV-R (5′TACTTTGGAGAGGT-CGTAGC-3′) were designed to amplify a 411 base-pair fragment of the envelope gene. The PCR reaction was 3 minutes at 94°C followed by 30 cycles of 94°C for 20 seconds, 68°C for 1 minute, and 72°C for 1 minute with a final extension of 10 minutes at 72°C. The reaction mix was 500 mg of genomic DNA (sample), 25 pmoles of each primer, 1 × PCR buffer, 0.2 mM dNTPs, 1.25 mM $MgCl_2$, and 1.25 U Taq. Nontransduced lymphocytes and a sample containing no genomic DNA were used as negative controls. One hundred fg of pPAM3 was used as a positive control for each sample [23]. Five hundred ng of each A375 LTKOSN.1 dilution of genomic DNA (1×10^{-4}) was used as additional controls. PCR product from blood lymphocytes and controls were transferred to membrane using a slot blot. The envelope probe was labeled with (^{32}P) dCTP by the random priming technique (Boehringer Mannheim, Mannheim, Germany). The blots were hybridized overnight at 42°C in Hybridisol (Oncor, Gaithersburg, MD, USA) and washed.

Detection of HSVtk gene in LTKOSN.1-transduced donor lymphocytes and patients blood samples

Semi-quantitative PCR with a sensitivity of 0.5% was used on peripheral blood mononuclear cells to detect retrovirus. PCR primers (JMTKO1 5′ TAT AGA CGG TCC TCA CGG GAT 3′; JMTKO3 5′ TCA TGC TGC CCA TAA GGT AT 3′) were designed to amplify a 388 base-pair fragment of the TK gene. The reaction mix contained 500 ng of genomic DNA sample. A375 NV cells and a sample containing no genomic DNA were used as negative controls. One hundred fg of pLTKOSN.1 was used as a positive control for each sample. For the lymphocyte samples, 500 ng of each A375 LTKOSN.1 dilution of genomic DNA (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) was used as additional controls. PCR product from lymphocytes and controls were transferred to membrane using a slot blot. A TK probe was labeled with (^{32}P) dCTP by the random priming technique (Boehringer Mannheim).

Treatment of graft-vs-host disease

GVHD that is refractory to oral corticosteroids and cyclosporine would be treated by withdrawal of immune suppression and infusion of gancyclovir 5 mg/kg intravenously every 12 hours for 5 days.

Results

Safety of treatment with gene-modified lymphocytes

A screening for RCR involving PCR amplification for MOMLV envelope gene from genomic DNA samples was performed on all HSVtk-transduced donor lymphocytes prior to their infusion into patients. No RCR was detected in any of preinfusion samples by PCR (Table 1). According to FDA requirements, 1% of HSVtk-transduced donor lymphocytes were tested for RCR using *Mus dunni* amplifications followed by S^+L^- assay. All samples tested were negative for presence of RCR (Table 1). The only adverse reaction potentially associated with HSVtk gene–modified donor lymphocyte infusion was observed in patient 13 on the day of the second infusion of donor lymphocytes. The patient had developed a fever and chills several hours after cell infusion was completed. The reaction corresponded to grade 2 toxicity. None of the other patients experienced any adverse reaction associated with gene-modified cell infusions. Patients receiving HSVtk-transduced donor lymphocytes were monitored for presence of RCR in peripheral blood cells at several time points after infusion. All blood samples from all patients tested were negative for RCR (Table 2). Infused lymphocytes had a viability of greater than 85%.

Clinical outcomes

Fifteen patients were enrolled in the study. Three patients died or were deemed ineligible due to disease progression before receiving transgeneic cells. These patients received all available standard treatments including chemotherapy

Table 1. Replication-competent retrovirus testing on preinfusion samples of HSVtk-transduced donor lymphocytes

Patient Number	Test: PCR for env gene — Infusion	PCR for env gene — Results	*Mus dunni* cocultivation followed by S^+L^- assay — Infusion	*Mus dunni* cocultivation followed by S^+L^- assay — Results
1	1	Negative	1	ND*
	2	Negative	2	ND
3	1	Negative	1	ND
5	1	Negative	1	Negative
	2	Negative	2	Negative
6	1	Negative	1	ND
	2	Negative	2	ND
8	1	Negative	1	Negative
9	1	Negative	1	Negative
	2	Negative	2	Negative
11	1	Negative	1	Negative
12	1	Negative	1	Negative
13	1	Negative	1	Negative (Supernatant)
	2	Negative	2	Negative (Cells)

Patients are listed in order enrolled in study. The missing numbers represent patients who did not receive HSVtk-DLI either because the cells could not be expanded or the patient died before receiving HSVtk-DLI.

*ND-Not Done; *Mus dunni* cocultivation followed by S^+L^- assay not performed in these samples because not initially required for lot release.

Table 2. Replication-competent retrovirus testing in HSVtk-transduced donor lymphocyte recipients on postinfusion blood samples

Patient #	Infusion #	Day after infusion	PCR for MoMLV env gene result
1	1	14, 49,87, 108, 142, 164	Negative
3	1	28, 57, 3, m0, 6 m0	Negative
5	1	17	Negative
	2	9,23	Negative
6	1	28, 4 mo	Negative
	2	28, 74	Negative
8	1	24, 52, 89	Negative
	2	6,29, 57	Negative
9	1	29, 56	Negative
	2	19, 26, 34, 40, 4 mo, 6 mo, 9 mo	Negative
11	1	0, 7, 14, 21, 28, 56, 3 mo, 6 mo	Negative
12	1	20, 41, 3 mo	Negative
13	1	0, 29, 57	Negative
	2	0,26	Negative

Patients are listed in order enrolled in study. The missing numbers represent patients enrolled but who did not receive HSVtk-DLI either because the cells could not be expanded or the patient died before receiving HSVtk-DLI.

while awaiting lymphocyte transduction and expansion. In three more patients, lymphocytes could not be successfully expanded ex vivo. Nine patients have received at least one infusion of HSVtk-transduced donor lymphocytes. Of the treated patients, four were in blast crisis or progressed within weeks after HSVtk-DLI infusion. These patients rapidly progressed and in some cases were treated with chemotherapy and/or nontransduced DLI, making any response to DLI difficult to document. Of the remaining five patients, one with relapsed leukemia had a questionable response (remission inversion) and three treated for CML in chronic phase did not respond; only one patient who had cutaneous T-cell lymphoma (CTCL) responded.

In all three relapsed chronic-phase CML patients, HSVtk-transgeneic cells were undetectable in the peripheral blood within weeks of infusion. The patients were subsequently treated with nontransduced fresh DLI. Since it may take months for a clinical GVL effect, it is impossible to determine if HSVtk-DLI or nontransduced DLI contributed to remission. One patient with AML who had also received chemotherapy may have had a partial response, defined as a remission inversion, to HSVtk-DLI. The final patient, with CTCL, had both an anti-tumor response and GVHD that resolved within 3 days of starting intravenous ganciclovir (Fig. 1).

The CTCL patient was a 26-year-old female with tumor-stage disease that was refractory to psoralen ultraviolet A (PUVA), interferon, and CHOP (cyclophosphamide, adriamycin, vincristine, prednisone) and 9-aminocaptothecan chemotherapy. She entered a complete remission following HLA-matched sibling HSCT. Nine months after HSCT, disease relapsed. Immune suppression was discontinued, and donor lymphocytes were collected by lymphopheresis and transduced with retroviral vector containing HSVtk. Due

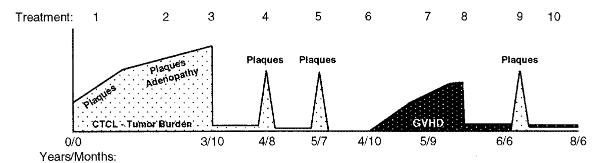

Figure 1. Eight years of treatment for patient with CTCL. Y-axis represents either increasing disease progression or increasing extent of cutaneous GVHD. Numbers across top represent: 1, PUVA; 2, cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) and 9-aminocaptothecan; 3, allogeneic HSCT; 4, withdrawal of immunosuppression; 5, infusion of HSVtk transgenic donor lymphocytes; 6, untreated limited cutaneous chronic GVHD (cGVHD); 7, cyclosporine and corticosteroids for extensive cutaneous cGVHD; 8, withdrawal of immunosuppression and infusion of ganciclovir; 9, limited cutaneous CTCL plaques; 10, plaques easily controlled with topical therapy.

to remission of CTCL following withdrawal of immune suppression, HSVtk-DLI were cyropreserved. One year later CTCL relapsed and the cryopreserved lymphocytes (seven billion HSVtk transgeneic lymphocytes) were thawed and infused into the patient. Disease regressed and several months later histologically confirmed cutaneous GVHD developed and progressed despite PUVA, cyclosporine, and corticosteroids (60 mg oral daily). While HSVtk donor lymphocytes became undetectable in peripheral blood, sites of cutaneous GVHD were HSVtk^{+} by PCR (Fig. 2). Infusion of GCV (5 mg/kg) resulted in rapid resolution of GVHD. For three years since GCV infusion, the patient has been without GVHD despite remaining off immunosuppression, remains HSVtk PCR negative, and while CTCL recurred it

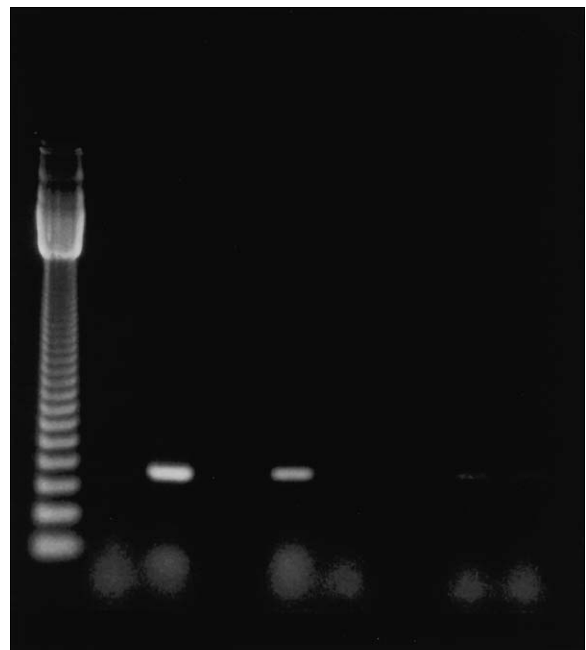

Figure 2. PCR for HSVtk DNA in blood and skin of CTCL patient from left to right, DNA ladder. Column 1 = negative control; Column 2 = positive control; Column 3 = empty lane; Column 4 = biopsy of skin with chronic GVHD; Column 5 = peripheral blood mononuclear cells; Column 6 = empty lane; Columns 7 and 8 = biopsy of normal skin.

Table 3. Number and type of HSVtk-transduced donor lymphocytes infused and persistence in peripheral blood

Patient #	Infusion #	% of $HSVtk^+$ cells in infusion sample	# of $CD3^+/CD8^+/CD4^+$ cells × 10^6 cells/kg infused	Days after infusion	% $HSVtk^+$ cells in blood
1	1	>80%	7.1/6.3/0.6	7–35	<1%
	2	>80%	58/52/1.4	7–35	<1%
				5 mo, 6 mo	ND
3	1	85%	32/9.7/23	7–42	<1%
				48, 57, 3 mo, 7 mo	ND in blood but positive in skin
5	1	~85%	5.0/0.3/4.5	10	3.8%
	2	~85%	5.0/0.3/4.5	17	2.1%
				9	3%
				16	1%
				23	<1%
6	1	~85%	N/A	7	<1%
	2	~85%		14–28	ND
				48, 74	ND
8	1	~85%	10/3.2/6.4	10–18	ND
				24–38	<1%
				52–89	ND
	2	~85%	100/58/20	1	ND
				7	<1%
				14, 21, 29, 36, 43, 56	ND
9	1	~85%	10/1.2/8.7	7–56, 3 mo	ND
	2	~85%	27/5/21	26, 34, 40, 3 mo, 6 mo, 9 mo	ND
11	1	~85%	94/92/28	0, 7, 14, 21, 28, 36, 45, 57, 3 mo, 6 mo	ND
12	1	~85%	9.9/2.1/8.1	6–20, 27, 41, 3 mo	ND
13	1	~85%	65/57/6.5	1	2%
				8, 15, 22, 29, 36, 44, 57	<1%
					ND
	2	~85%	199/160/37	Preinfusion	ND
				6	<1%
				14, 21, 26	ND

Patients are listed in order enrolled in study. The missing numbers represent patients enrolled but who did not receive HSVtk-DLI either because the cells could not be expanded or the patient died before receiving HSVtk-DLI. Mo = month, ND = not detectable.

has been easily controlled with intermittent topical nitrogen mustards and local radiation to a single knee plaque.

Discussion

In general, allogeneic HSCT, which provides an immunologic anti-tumor response from allogeneic lymphocytes, is the most effective method for preventing relapse of hematologic malignancies. It is, however, complicated by significant lymphocyte-mediated morbidity and mortality from GVHD. Infusion of donor lymphocytes genetically modified to contain a suicide gene to abort refractory GVHD has been suggested as a method to improve the safety of allogeneic HSCT. The laboratory and clinical feasibility of this approach has already been reported [10–14]. There are a limited number of clinical cases supporting the in vivo effectiveness of HSVtk lymphocytes [15–20]. However, limited effectiveness was suggested by Champlin et al., who reported no GVHD in 23 patients treated with HSVtk DLI [24].

The best anti-tumor effects of DLI are manifested in slower progressive diseases such as low-grade lymphomas, myeloma, and chronic myelogenous leukemia [6]. One patient with AML in this study achieved an inversion (i.e., the subsequent remission was of longer duration than the prior remission) following chemotherapy and HSVtk-DLI. The significance of this response is questionable. A second patient with CTCL demonstrated cutaneous tumor regression with infusion of HSVtk-DLI. CTCL is an indolent lymphoma and, like other slower-growing hematologic malignancies, is probably more susceptible to an immunologic anti-tumor effect. In fact, the CTCL initially entered remission after only withdrawal of immune suppression. The transgeneic HSVtk lymphocytes were, therefore, cryopreserved and not infused until 12 months later, when CTCL relapsed. Subsequently, the patient developed GVHD unresponsive to cyclosporine and oral corticosteroids that rapidly remitted upon GCV infusion. GCV-resistant GVHD has been reported by others [25], but despite remaining off all immune suppression for 3 years, the GVHD has never recurred. After infusion of GCV, small-volume CTCL cutaneous lesions have persisted, but in contrast to prior chemotherapy refractory disease, lesions have been easily controlled with intermittent topical therapy.

Table 4. Clinical outcome of infused allogeneic HSVtk transgeneic cells

Patient	Disease and stage at time of DLI	Date of HSVtk infusion	HSVtk-DLI tumor response (GVHD)	Outcome
1	AML—chemo-refractory	1st 1-5-98 2nd 3-30-98 matched sib	Possible—disease inversion—no GVHD	Chemotherapy-refractory AML with relapse within one month of salvage chemotherapy. Treated chemotherapy and HSVtk-DLI with a 2-month remission. Treated with chemotherapy and 2nd HSVtk-DLI with a 3-month remission. Taken off study and treated by 2nd allogeneic HSCT but died of intracranial bleed related to cyclosporine-associated hypertension and thrombocytopenia.
3	CTCL	11-30-98 matched sib	Yes—regression of skin lesions—GVHD responded to GCV	CTCL resistant to PUVA, topical mustards, CHOP, and 9-aminocamptothecan. Relapsed after allogeneic HSCT. Regression of skin lesion following HSVtk-DLI. Developed cyclosporine- and corticosteroid-refractory chronic cutaneous GVHD that promptly resolved with infusion of GCV. Since GCV, CTCL plaques have responded to only intermittent topical therapy. Remains alive.
5	CML-BC	1st 3-20-98 2nd 5-27-98 I antigen mismatched unrelated	No	Entered blast crisis only 8 days after 1st HSVtk-DLI, transient remission after treated with vincristine and prednisone. 2nd HSVtk-DLI infused 2 months later but within 5 weeks entered 2nd blast crisis. Taken off study. Died awaiting unrelated HSCT.
6	CML-CP	1st 6-11-98 2nd 10-8-98 matched sib	No	HSVtk-DLI undetectable in blood within one week of infusion. 2nd HSVtk-DLI also undetectable in blood after 1 week. Patient taken off study and nontransduced fresh DLI infused. Alive in complete remission.
8	AML 1st	1st 7-24-98 2nd 7-26-99 matched sib	No	Unable to determine DLI anti-tumor response since received chemotherapy after both DLI infusion. In complete remission at time of autopsy. Cause of death was end-organ dysfunction.
9	CML-CP	1st 9-14-98 2nd 12-23-98 matched sib	No	No response to 1st or 2nd HSVtk-DLI. Treated with unmanipulated DLI off study. Now in remission with chronic GVHD.
11	CML-CP	4-19-99 unrelated donor	No	Developed rash that on biopsy was lichen planus before HSVtk-DLI infused. Rash responded to steroids. Developed chronic GVHD but not clear if due to HSVtk-DLI or pre-DLI lichen GVHD rash. Never given ganciclovir. In remission on Gleevec.
12	AML—refractory	12-23-98 unrelated	No	Died progressive disease 3 months after HSVTK-DLI.
13	Lymphoma in relapse	1st 7-14-99 2nd 10-29-99 matched sib	No	Disease progressed 2 months after HSVtk-DLI. Entered CR after chemotherapy. Relapsed and received HSVtk-DLI followed by radiation and chemotherapy. Died of relapsed disease 7 months later.

Patients are listed in order enrolled in study. The missing numbers represent patients enrolled but who did not receive HSVtk-DLI either because the cells could not be expanded or the patient, died before receiving HSVtk-DLI. AML = acute myelogenous leukemia, BC = blast crisis, CHOP = cyclophosphamide, adriamycin, vincristine, prednisone, CML = chronic myelogenous leukemia, CP = chronic phase, CR = complete remission, CTCL = cutaneous T-cell lymphoma, DLI = donor lymphocyte infusion, GCV = ganciclovir, GVHD = graft versus host disease, HSVtk = herpes simplex thymidine kinase, PUVA = psoralen ultraviolet A, sib = sibling.

Our results reaffirm the ability of HSVtk gene–modified lymphocytes to retain anti-tumor efficacy and induce GVHD that can be terminated with GCV. Our study also demonstrates the limited effectiveness of HSVtk-transduced lymphocytes using early-generation retroviral constructs and transduction methodology to induce either GVHD or GVL, which is emphasized by failure of three chronic-phase CML patients to enter remission with HSVtk cells. Reasons for a poor anti-tumor response in CML patients include: 1) immune-mediated rejection or in vivo apoptosis of the transgenic cells, 2) sub-therapeutic dose of infused cells, 3) skewed phenotype of transgeneic cells, and 4) loss of transgeneic lymphocyte immunogeneicity. In vivo immune rejection of HSVtk-hydromycin transgeneic cells has been previously reported [26]. Although in this trial, support of rejection by HSVtk or neo CTL precursor frequency was not performed, inability to detect peripheral blood transgeneic cells may have been related to rejection. Transcriptional silencing of the transgene may occur in transduced T cells by methylation; however, PCR of lymphocyte DNA would

still detect the transgene independent of methylation. Although cultured ex vivo in relatively low-dose IL-2, infusion-related growth factor withdrawal may have initiated apoptosis independent of immune-mediated rejection. Inability to detect peripheral blood HSVtk^{+} cells may also be due to sequestration of transgeneic cells in target organs. For example, the CTCL patient with chronic GVHD was HSVtk PCR negative in the peripheral blood, while the skin biopsy was positive for HSVtk at sites of GVHD. Repeated infusions and higher HSVtk-DLI doses may have precipitated more frequent anti-tumor responses.

Ex vivo culture of lymphocytes, whether transduced or control cells, may result in diminished alloreactivity and/or altered T cell function [27]. This is supported by tritiated thymidine proliferative response in mixed lymphocyte culture. Both cultured nontransduced control lymphocytes and HSVtk lymphocytes had diminished proliferative responses (data not shown) indicating a loss of immune effectiveness related to the prolonged ex vivo culture, usually 4 to 6 weeks, required for activation, transduction, selection, and expansion of transgeneic cells. The time required for ex vivo expansion varied between donor samples, which could have impacted on T cell functionality. However, since only one patient had an unquestionable GVHD and anti-tumor response, no correlation could be drawn from this study between ex vivo culture duration and clinical responses. Of interest, the one CTCL patient with an anti-tumor response was infused with a predominance of CD4^{+} cells (Tables 3 and 4). Selection and expansion of transduced clones may also lead to T cell repertoire skewing [28,29]. Phenotype and repertoire skewing of HSVtk-DLI may affect anti-tumor response.

This study indicates that HSVtk-DLI may provide an anti-tumor effect in vivo, but it is less effective than unmanipulated DLI since only 1 of 9 patients developed GVHD and CML patients who failed to respond to HSVtk DLI responded to unmanipulated DLI. The development of GVHD and GVL in only one patient, as well as the inability to generate HSVtk-DLI ex vivo from some patients, indicates that technical aspects involved in gene-manipulated lymphocyte infusion need to be perfected before larger studies can be entertained. In specific, methods of improving transduction efficiency to decrease culture duration may improve immune effectiveness of infused cells and markedly decrease the time, expense, and expertise required for prolonged tissue culture. Improvements in gene therapy technology that occurred since design of this trial include: 1) vectors that do not require ex vivo toxic drug selection but may be rapidly selected by flow cytometry [18], and 2) lentiviral vectors that require only minimal activation (G0 to G1) for integration [30]. Despite current technical limitations, with improvements in suicide vector technology and transduction methodology HSVtk transgeneic cells may overcome the major toxicity of allogeneic HSCT while preserving the anti-tumor effectiveness of donor lymphocytes.

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