

Gene and cell therapy for relapsed leukemia after allo-stem cell transplantation

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1. ABSTRACT

To control severe GvHD while maintaining strong GvL effects in the context of allo-stem cell transplantation (allo-SCT), a phase I/II clinical trial of infusions of donor lymphocytes transduced with the herpes simplex virus thymidine kinase (TK-DLI) started at the Tsukuba University Hospital. To date, five (2 AML, 2 ALL, and 1 MDS) out of eight patients enrolled in the trial received approximately 7×10^7 transduced cells per kilogram of body weight and four patients showed some clinical responses such inhibition of the leukemic cell proliferation or mitigation of lymph node swelling. Especially, one MDS patient achieved complete remission and has remained in CR for 2 years after the treatment. GvHD developed in two patients (1 acute and 1 chronic) and the acute (grade III) was successfully controlled by administration of ganciclovir without any immunosuppressive drugs. Since HSV-TK as a strong antigen induced CTLs against transduced cells in patients, however, TK-DLI is expected to provide a more effective adoptive immune cell therapy by performance just after allo-SCT where the patient's immune function is severely damaged.

2. INTRODUCTION

Transplantation of hematopoietic stem cells (HSCs) from HLA-matched related donors following both high-dose systemic chemotherapy and total-body irradiation (TBI) is the most effective treatment for patients with hematological malignancies (1-3). The initial rationale of allogeneic stem cell transplantation (allo-SCT) was based on a concept that SCT could provide patients with HSCs to reconstitute their bone marrow hematopoiesis that was devastatingly damaged by such intensive regimens. Recently, allo-SCT is referred to as immunotherapy for leukemia rather than solely a vehicle to delivery intensive therapy because donor lymphocytes transplanted with HSCs function as cytotoxic T lymphocytes (CTLs) against the patient's leukemic cells (4-6). Although only a few successful cases have demonstrated the existence of CTLs against leukemic cells (7), a strong graft-versus-leukemia (GvL) effect demonstrated by indirect evidence that the relapse rate increases if T cells are depleted from transplanted cells or in recipients of identical twin transplants, has made an infusion of donor lymphocytes (DLI) an standard treatment for patients with relapsed leukemia after allo-SCT, especially for those with relapse

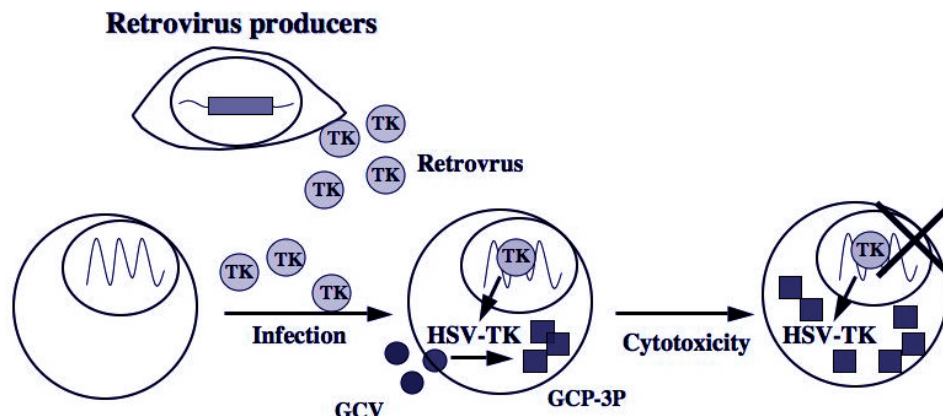


Figure 1. A strategy of TK-DLI using the HSV-TK/ GCV suicide system. Donor T lymphocytes are transduced with the HSV-TK gene using retroviral vectors and infused into a patient with relapsed leukemia. In a situation of no GCV, the cells are expected to function as CTLs against the patient's leukemic cells. In case of occurrence of severe GvHD, GCV is administered into the patient to eradicate the transduced cells. GCV is phosphorylated in only the transduced cells and incorporated into the genomic DNA as a GCV-3P compound, resulting in inhibition of DNA chain elongation and apoptosis of the transduced cells.

of chronic myelogenous leukemia (CML) (8-12). Infusions of a large number of donor lymphocytes have proven to restore the full donor chimerism and produce long-term complete molecular genetic remissions in many CML relapsed cases because CML is more sensitive to DLI compared to other hematological malignancies. However, 50-60% of patients treated with DLI developed a possible adverse effect such as graft-versus-host disease (GvHD) resulting in a considerable amount of transplant-related mortality (TRM) (13). Although steroid (prednisolone) is used as first-line therapy against severe GvHD, the combination therapy with various immunosuppressive agents such as cyclosporine, anti-thymocyte globulin (ATG), and mycophenolate mofetil (MMF) is not frequently unsatisfactory in steroid-resistant GvHD (14). One of effective strategies to prevent the occurrence of severe GvHD while maximizing GvL effects is the escalating dose regimen (EDR) in which the number of donor cells infused into the patients increases in a step-wise manner until the disease responds to DLI or GvHD occurs (15). Indeed, the EDR can significantly reduce the rate of severe GvHD with equal GvL effects for CML patients. Since the strategy takes much time to acquire GvL effects, however, the EDR cannot be easily employed as a treatment for other acute leukemia with rapid progression. Another approach to decrease the risk of GvHD is to infuse specific effector lymphocytes that proliferate in response to leukemic cells into patients (16, 17). However, infusions of such cells show less significant GvL effects than do those of heterogeneous lymphocytes because most of T cells exerting GvL effects recognize the patient's allo-antigens.

3. CONTROL OF SEVERE GvHD USING THE SUICIDE GENE

To overcome the problems, a strategy of genetic manipulation of donor lymphocytes using retroviral vectors expressing the herpes simplex virus thymidine kinase (HSV-TK) gene has been devised and tested in clinical trials (18-22). HSV-TK converts the prodrug ganciclovir

(GCV) to its monophosphate intermediate derivative that is further phosphorylated to di- and triphosphate (GCV-3P) compound by cellular kinases (23). The GCV-3P is incorporated into DNA during the cell division, resulting in inhibition of DNA chain elongation. In the trial, donor lymphocytes are transduced with the HSV-TK gene using retroviral vectors and infused into the patients (Figure 1). In a situation without GCV, the transduced lymphocytes are expected to function as CTLs against the patient's leukemic cells. If severe GvHD occurs, GCV is administered into the patient to eradicate the transduced cells. In 1997, the Italian group reported successful cases of the gene therapy (TK-DLI) (18). They performed TK-DLI for 23 high-risk patients with hematological relapse after allo-SCT and reported clinical results of 17 patients who were alive more than 30 days after receiving the therapy. The number of transduced cells infused, although it varied among patients, was approximately 4×10^7 per kilogram of body weight. Eleven patients (65%) experienced substantial clinical benefits, resulting in 6 complete remissions (2 CML, 1 AML, 2 NHL, and 1 multiple myeloma; 35%) and 5 partial responses (2 CML, 1 AML, 1 NHL, and 1 multiple myeloma; 29%). Four patients with GvHD (3 acute and 1 chronic) received GCV administrations, resulting in elimination of the transduced cells and control of severe GvHD.

Based on successful results of the clinical trial, they have extended the strategy to haplo-SCT for hematologic malignancies (24). Haplo-SCT is the last option for patients who lack an HLA-identical donor but it remarkably increases the rate of morbidity and mortality due to severe GvHD. Transplantation of hematopoietic progenitor cells ($CD34^+$ cells) obtained from haplo-identical donors followed by infusions of lymphocytes transduced with HSV-TK gene in an incremental manner (TK add-back) would help rapid immune recovery to protect from viral infection and relapse, and control severe GvHD by administration of GCV if it occurs. Eight patients with high-risk hematologic malignancies who underwent

Table 1. Clinical protocol of TK-DLI in Tsukuba university hospital

Title	Infusions of donor lymphocytes transduced with the herpes simplex virus thymidine kinase gene into patients with relapsed leukemia after allogeneic stem cell transplantation
Population	Patients >2years of age with relapsed hematologic malignancies after allo-SCT
Sample size	Five patients for 3 years
Treatment	Infusion of 1×10^8 transduced cells per kilogram of body weight
	Infusion of GCV (5mg/kg) twice a day for 7 days at severe GvHD (grade III)
End Point	
1 st	Safety, GvL effects, Control of GvHD by administration of GCV
2 nd	Adverse effects, Overall survivals, relapse
3 rd	Immunological study (CTLs against TK-expressing cells)

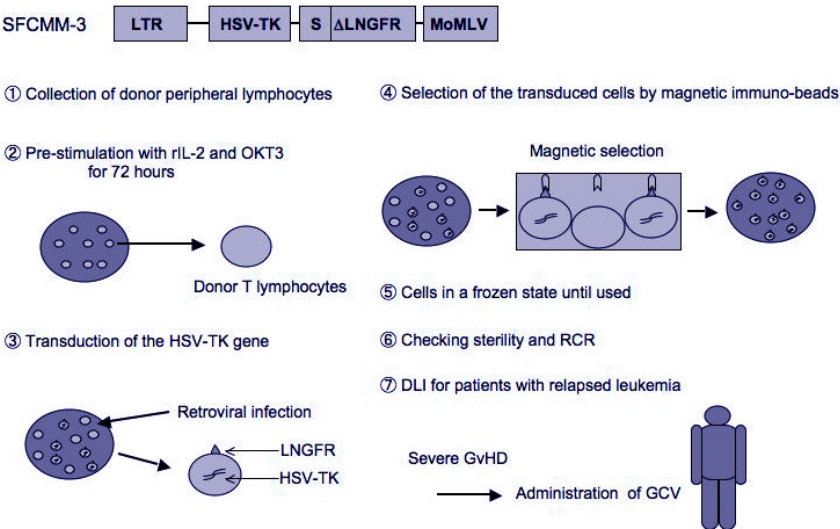


Figure 2. A structure of the retroviral vector SFCMM-3 and a Tsukuba TK-DLI protocol.

haplo-SCT were enrolled; three patients received 1×10^6 and five did 1×10^7 of transduced cells per kilogram of body weight. Although no immune reconstitution was observed in patients who received 1×10^6 , three out of 5 patients with infusions of 1×10^7 recovered full immune reconstitution and showed significant reduction of the incidence of viral infection. Especially, two patients out of these three have been free from leukemic relapse. Regarding GvHD occurrence, one patient out of the three developed acute GvHD (grade II) that was quickly controlled by administration of GCV. Given that an effective dose to reconstitute the full immune function and prevent the relapse is 1×10^7 per kilogram of body weight, a phase III multi-center trial in which patients undergoing haplo-SCT are infused with the similar dose of transduced cells several times in certain intervals is under way in Europe.

4. TK-DLI IN THE TSUKUBA HOSPITAL

In collaboration with Dr. Bordignon at the H. S. Raffaele Institute, we started a phase I/II clinical trial of TK-DLI for patients with relapsed leukemia after allo-SCT at the Tsukuba University Hospital in 2004 (Table 1). A retroviral vector used is the SFCMM-3 that expresses both HSV-TK and nerve growth factor receptor (NGFR) genes and a working process of transduction into peripheral mononuclear cells is shown in Figure 2 and 3. Peripheral mononuclear cells collected from donors by apheresis (CS3000plus; Baxter Corp, IL) are maintained in RPMI-

1640 medium with 3% autologous serum in gas-permeable culture bags (GT-T610; Takara Bio, Japan) and pre-stimulated with a high dose of recombinant human interleukin-2 (600U/ml, Proleukin^R; Chiron, CA) and anti-CD3 antibody (Orthoclone OKT3 Injection; Ortho, NJ) for 72 hours. For transduction, cells are suspended with the viral supernatant at 5×10^6 /ml, transferred into small bags with tolerance to centrifugation (Cryocyte Frysebeholder-50ml; Baxter), and then centrifuged at 2000g for 2 hours using the bucket-type centrifuge (MX301; TOMY, Japan). At 72 hours after two rounds of transduction, cells are stained with anti-NGFR antibody and magnetic immune-beads (DynaBeads M450, sheep anti-mouse IgG; Invitrogen, CA) to isolate transduced cells with Isolex 50 (Baxter). Isolated cells are cultured to expanded for additional 3 to 5 days and stocked in -150°C until used. In this trial, patients are supposed to receive transduced cells at 1×10^8 per kilogram of body weight in a single infusion. To prepare such a large number of cells sterily, we have developed the culture system allowing performance of all procedures from collection of donor mononuclear cells to infusion into patients in bags (Figure 3). In particular, the automatic cell manipulator, CyteMate (Nexell Therapeutics Inc.) enabled us to wash and concentrate a large number of cells in a relatively short time (one liter in a hour).

So far, nine transduction procedures have been done for eight enrolled patients and cells ranging from 4.4×10^9 to 2.4×10^{10} was prepared (Table 2). The

Table 2. Patients enrolled in Tsukuba TK-DLI Trial

UPN	Diagnosis	Age, Sex	# of prep (/kg)	NGFR+	# of infused (/kg)	GvHD
1	MDS (RAEB)	42, M	1.0x10 ¹⁰ (1.8x10 ⁸)	93.5%		
2	ALL (Ph1+)	15, F	4.6x10 ⁹ (1.2x10 ⁸)	97.8%		
3	AML	60, M	1.0x10 ¹⁰ (2.3x10 ⁸)	97.2%	3.8x10 ⁹ (7.7x10 ⁷)	acute (grade III)
4 ¹	ALL	20, M	0.1x10 ⁹ (0.2x10 ⁷)	37.7%		
5 ¹	ALL		4.4x10 ⁹ (8.8x10 ⁷)	93.1%		
6	MDS (RAEB)	58, M	2.4x10 ¹⁰ (3.1x10 ⁸)	95.1%	9.7x10 ⁸ (9.5x10 ⁷)	chronic
7-1	ALL	14, M	7.9x10 ⁹ (2.6x10 ⁸)	94.9%	2.0x10 ⁹ (6.7x10 ⁷)	
-2					5.0x10 ⁹ (8.5x10 ⁷)	
8-1	AML	46, M	1.8x10 ¹⁰ (2.3x10 ⁸)	90.7%	9.0x10 ⁹ (8.5x10 ⁷)	
-2					9.0x10 ⁹ (8.5x10 ⁷)	
9-1	ALL	50, M	7.2x10 ⁹ (1.8x10 ⁸)	90.7%	4.4x10 ⁹ (8.6x10 ⁷)	
-2					2.1x10 ⁹ (4.1x10 ⁷)	

¹an identical patient

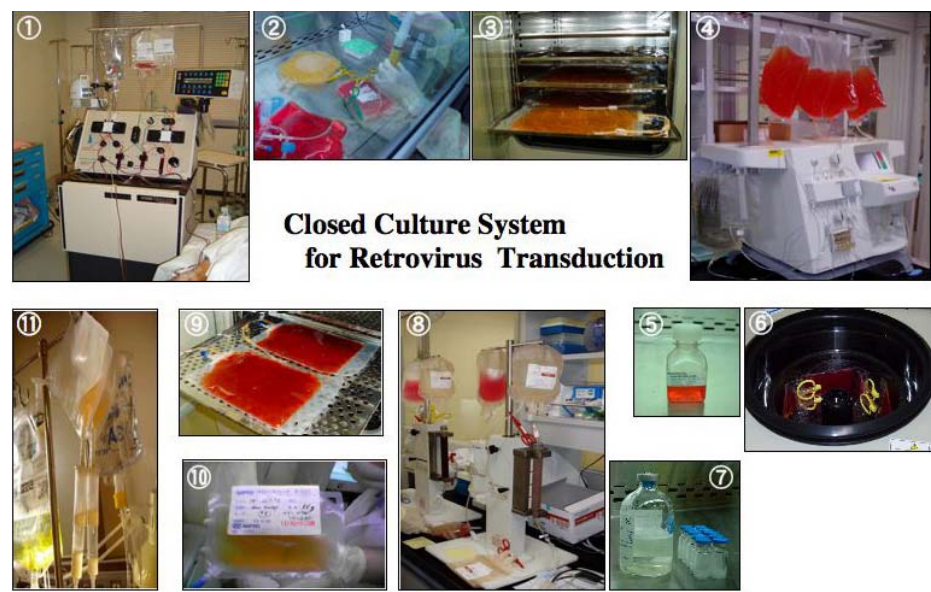


Figure 3. The closed culture system for transduction into peripheral lymphocytes. Collection of donor lymphocytes using CS3000plus (1), cell culture in gas-permeable culture bags (2, 3), cell washing using CyteMate (4), SFCMM-3 virus sup (5), transduction by spinoculation (6), anti-NGFR antibody (7), isolation of transduced cells using Isolex 50 (8), culture and collection of transduced cells (9, 10), and infusion into patients (11).

transduction efficiency was approximately 20% and the purity of NGFR-expressing cells isolated using Isolex 50 exceeded 90% in all the cases except for UPN 4. All samples cleared the safety tests including cell viability, sterility, existence of replication competent retrovirus (RCR), and sensitivity to GCV. Five out of 8 patients (2 AML, 2 ALL, and 1 MDS) were treated with TK-DLI and three (UPN 7, 8, and 9) among them received the infusions twice (Table 2). The number of cells infused, although it varied among patients, was approximately 8.7×10^7 per kilogram of body weight. Four patients showed some clinical responses such as inhibition of leukemic cell proliferation, mitigation of lymph node swelling, and lowering the values of tumor markers. Especially, a MDS patient (UPN 6) achieved complete remission and has remained in CR for 2 years after the treatment. Regarding GvHD occurrence, a patient (UPN 3) developed the grade III GvHD due to severe liver dysfunction that was successfully controlled by administration of GCV without any immunosuppressive drugs. Another patient (UPN 6)

showed chronic GvHD with precordial erythema that has been observed without any treatments. No adverse effects related to gene therapy have been observed.

5. PROBLEMS IN TK-DLI

Although TK-DLI proved to be clinically beneficial, it also has several critical problems. One of the major limitations of TK-DLI is considered to be the strong immunogenicity of the viral protein, HSV-TK. Two reports revealed that infusions of the transduced cells into immune-competent patients resulted in the development of an immune response to TK-derived epitopes (25, 26). Once cytotoxic T lymphocytes (CTLs) against cells expressing HSV-TK are developed in patients, the transduced cells infused would be eradicated from the patient body in no time at all. Indeed, HSV-TK-expressing cells in our patients without any GvL effects had a very short time to survive in the patient's peripheral blood, which was measured by quantitative PCR (TaqMan PCR).

Interestingly, the Italian group observed that patients who received infusions of transduced cells at the immunosuppressed condition, e.g. soon after stem cell transplantation, caused less development on such an immune response. These results suggest that TK-DLI is the most suitable therapy in the context of allo-SCT from partially mismatched or unrelated donors, where the risk of severe GvHD is particularly high, and patients are profound immunodeficient.

Another problem is weaker immune response of cultured cells against allo-antigens compared with that of primary lymphocytes. In general, *in vitro* culture to manipulate donor lymphocytes genetically impairs their immune functions (27, 28), which may explain why a few patients developed severe GvHD despite infusions of a large number of donor cells in our trial. An improved culture condition could preserve the T-cell repertoire and their immune functions (29).

6. CONCLUSIONS

The clinical trial confirmed the safety and therapeutic effects of the suicide-gene transduced lymphocytes for relapsed leukemia after allo-SCT. Furthermore, acute GvHD could be controlled by administration of GCV without any immunosuppressive drugs. However, rapid disappearance of transduced lymphocytes was also observed in patients without any clinical benefits. Since the suicide gene derived from viruses elicits immune responses in patients as a strong antigen, it is likely that CTLs against HSV-TK eradicated transduced cells soon after infusions. While an approach to the problem is to use the suicide genes of human origin instead (29, 30), an alternative is to combine TK-DLI with allo-SCT in which the patient's immune function is severely damaged to impair T cell priming against foreign antigens (24).

With further modifications including vector constructs (31, 32), culture conditions (33), and the timing of infusions, the suicide-gene strategy would offer the safe and effective immune cell therapy for patients with hematologic malignancies.

7. ACKNOWLEDGMENT

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Abbreviations: allo-SCT: allogeneic stem cell transplantation; GvL: graft versus leukemia; GvHD: graft versus host disease; DLI: donor lymphocyte infusion; GCV: ganciclovir; HSCs: hematopoietic stem cells; TBI: total-body irradiation; CTLs: cytotoxic T lymphocytes; CML: chronic myelogenous leukemia; TRM: transplant-related mortality; ATG: anti-thymocyte globulin; EDR: escalating dose regimen; HSV-TK: herpes simplex virus thymidine kinase; NGFR: nerve growth factor receptor; rIL-2: recombinant human interleukin-2

Gene and cell therapy for relapsed leukemia

Key Words: Gene Therapy, Retroviral Vector, Suicide Gene, Graft-Versus-Leukemia, Donor Lymphocyte Infusion, Review

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