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Review

Herpes simplex thymidine kinase (HStk) transgenic donor lymphocytes

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Summary:

Patients with recurrent leukemia after an allogeneic hematopoietic stem cell transplant may be treated with donor lymphocyte infusions (DLI). The transfusion of lymphocytes from the original hematopoietic stem cell donor induces remission in approximately one third of relapsed AML cases and 80% of relapsed CML. DLI may be complicated by delayed and sometimes lethal graft-versus-host disease (GVHD). In an attempt to avoid this complication, several centers have initiated DLI trials in which the infused lymphocytes carry a suicide gene, herpes simplex thymidine kinase (HStk), which confers sensitivity to ganciclovir (GCV). In the event of severe GVHD, administration of GCV should terminate or ameliorate GVHD.
Keywords: donor lymphocyte infusions; retroviral vector; gene therapy

In 1990, Kolb *et al*[1] reported that infusion of lymphocytes from the original bone marrow donor could re-induce remission in patients who relapse after an allogeneic transplant. Subsequently, use of unmanipulated and HStk modified DLI are being evaluated by several investigators.[1–21] Lymphocytes are generally collected from the peripheral blood of the donor by lymphopheresis, purified by Ficoll–Hypaque density gradient centrifugation, and infused as fresh cells into the recipient. Although the effect of cryopreservation on anti-leukemic efficacy is unknown, cells may also be cryopreserved, thawed, and infused at a later date. In general, between 10^6 and 5×10^8 T cells per kilogram recipient weight are infused at one time or at intervals of several days to weeks.

The optimal dose of donor lymphocytes remains unclear.[22] Although it seems intuitive that larger numbers of infused lymphocytes are more likely to reinduce remission, in an EBMT analysis of 258 patients treated with DLI, patients receiving more than 3.0×10^8 mononuclear cells/kg had a lower response rate than those receiving less than 3.0×10^8/kg.[23] This may imply a response plateau or be artifactual since DLI are often given in increments and non-responders would keep receiving periodic infusion resulting in an inverse correlation between lymphocyte dose and response rate. The effect of post-DLI biologic modifiers such as interferon in the development of GVHD or leukemic response rate is unknown. Anecdotal case reports suggest that patients who fail DLI may respond to lymphocytes from the same person after *ex vivo* activation with IL-2 with or without systemic administration of IL-2 and interferon.[24]

Differences in responses between CML, AML and ALL may be due to differences in neoplastic growth kinetics. It may take 1–6 months for donor lymphocytes to induce remission. During this interval, rapidly growing leukemias may simply outgrow the immunologic effects of infused lymphocytes. This is supported by a DLI-induced remission rate for relapsed CML in blast crises of 10–30% compared to 80% for CML in chronic phase. Immunogenic differences between neoplastic clones may also account for differences in response. The immunogenicity of animal tumors can be increased by transduction of the tumor with cytokine genes (IL-2, GM-CSF) or co-stimulatory molecules such as B7–1.[25–28] Although not yet reported, donor lymphocytes could be co-cultured *ex vivo* with irradiated leukemic blasts transduced with cytokines, costimulatory molecules, or other immune stimulants.

The anti-leukemic effect of allogeneic lymphocytes, termed graft-versus-leukemia (GVL) is a double-edged sword since it is also associated with GVHD, the most significant complication contributing to mortality from an allogeneic transplant. There is currently no clear evidence that the lymphocyte subsets or effector pathways involved in GVL are different from those involved in the pathogenesis of GVHD. The beneficial effects of GVL may be maximized while minimizing the risk of severe GVHD by infusing lymphocytes containing an adjustable suicide gene. Several centers have initiated trials using genetically engineered lymphocytes containing the suicide gene, herpes simplex thymidine kinase (HStk).[14–21] Since the HStk death gene is inserted via a retroviral vector, a brief review of retroviral gene therapy follows.

Retroviral vectors

The development of retroviral vectors was the key step that led to the first gene transfer experiments in humans.[29–34] Retroviral vectors have been used for gene transfer into

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human T cells for more than 10 years. Retroviruses are RNA viruses that replicate via DNA proviral intermediates. Retroviruses may be classified by several schema: (1) morphology (ie A, B, C or D-type virions); (2) type of assembly (ie formation within the cytoplasm or at the cell membrane); (3) pathogenicity (ie oncovirinae which cause cancer, lentivirinae which are nononcogenic slow viruses such as HIV, and spumavirinae causing a characteristic vacuolated foamy syncytia of cells); and (4) genetic structure (simple or complex).[35–45] Simple retroviruses (most oncovirinae) express structural genes (gag, pol, and env) involved in virion formation. Complex viruses (lentiviruses, spumaviruses, some oncoviruses) encode virion structural genes (gag, pol, and env) as well as regulatory genes (rev, rex, tat, tax, nif, vpu, etc).

Virion structure (Figure 1)

Looking from outside inwards, retroviruses have a phospholipid spherical bilayer containing protruding spikes of envelope glycoprotein. Matrix proteins connect the envelope to the capsid protein icosahedral shell. Within this capsid shell are reverse transcriptase, protease, and integrase proteins and two positive sense RNA strands (positive strand RNA is translated to protein and is transcribed from negative strand DNA).

Genetic structure

Since simple retroviral vectors (oncovirinae) are currently the predominant vehicles for gene therapy trials, this discussion will exclude complex (lentiviruses, spumaviruses) retroviruses. Retroviral genomes are 7–10 kilobases in length.[46] They are composed of *cis* elements that are noncoding areas of the genome necessary for replication and *trans* elements which are coding elements that give rise to proteins (Figure 2).[47]

In the Maloney murine leukemia virus, the retroviral *cis* elements are located in the termini of the viral genome. In general, these elements must be retained in a functional retroviral vector. *Cis* elements at the 5′ end are termed R, U5, PB, and L. The repeat (R) sequence at each end of the genome is involved in transfer of the growing chain during reverse transcription.[48,49] U5 is a unique sequence near the 5′ end that forms stem loops required for initiation of

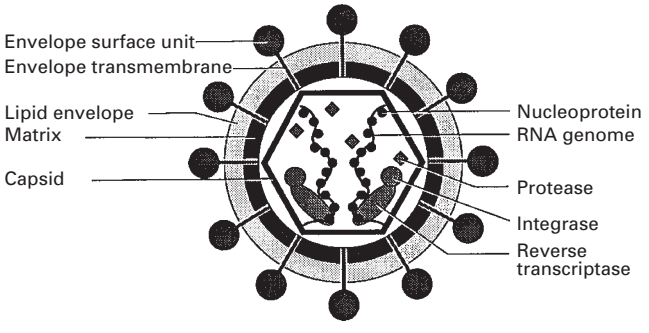

Figure 1 Retroviral morphology.

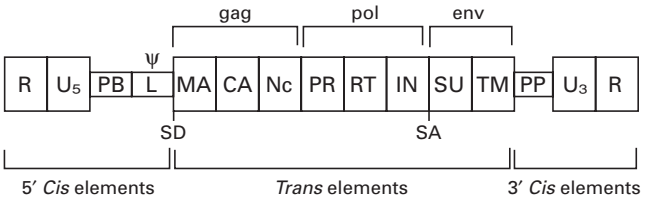

Figure 2 Genetic structure of simple retrovirus: CA, capsid; env, envelop; gag, group-specific antigen; IN, integrase; L, leader; MA, matrix; Nc, nuclear capsid; PB, primer binding site; pol, polymerase; PP, polypurine sequence; PR, protease; R, repeat sequence; RT, reverse transcriptase; SU, surface unit; TM, transmembrane unit; U3, 3 prime unique sequence; U5, 5 prime unique sequence.

reverse transcription.[50] PB is the primer binding site for transfer RNA (t-RNA).[51] All polymerases need a primer to initiate chain elongation. Reverse transcriptase uses t-RNA which binds to the PB site of the retrovirus. L represents the Leader region that is untranslated and contains the splice donor site (SD) for generation of spliced mRNA transcripts, and psi (ψ) the signal for packaging RNA into the virion.[52,53]

Cis elements at the 3′ end are termed PP, U3 and R. The polypurine (PP) region is AG rich and is the primer for plus strand DNA synthesis during reverse transcription. U3 is a unique sequence at the 3′ end that contains transcription enhancer/promotor motifs and transcription start signals which regulate transcription of the integrated provirus.[54,55] Transcriptional control motifs in the U3 region influence the rate of viral transcription.

Retroviral *trans*-elements are the protein coding sequences: gag (group-specific antigen), pol (RNA-dependent DNA polymerase), and env (envelope glycoprotein). The gag protein is from the N terminal to C terminal end composed of matrix (MA), capsid (CA), and nucleocapsid (NC) domains.[56] The MA domain determines the site of viral assembly by targeting the protein to the plasma membrane.[57,58] The NC domain of gag binds to the RNA packaging (ψ) motif of the retrovirus to incorporate the retroviral RNA genome within the virion.[59] The pol gene is composed of three enzymes, a protease, reverse transcriptase and integrase.[60] The gag-pol transcript is translated into one protein or two proteins, gag and pol, that arise from ribosomal frame shifting at the time of translation. The viral protease cleaves gag-pol at the time of viral budding into the MA, CA, NC, protease, reverse transcriptase, and integrase proteins. Reverse transcriptase converts the RNA genome into proviral DNA.[61] Integrase forms an integration complex with proviral DNA to insert the provirus into cellular DNA. The envelope protein arises from a spliced transcript occurring between splice donor (SD) and splice acceptor (SA) sites. The envelope protein undergoes proteolysis by cellular proteases into surface (SU) and transmembrane (TM) glycoproteins.[62] The SU protein determines receptor binding affinity and specificity of the virion.

Retroviral life cycle (Figure 3)

Retroviral virion binding occurs via envelope protein to a cellular receptor. Endocytosis of the receptor/virion com-

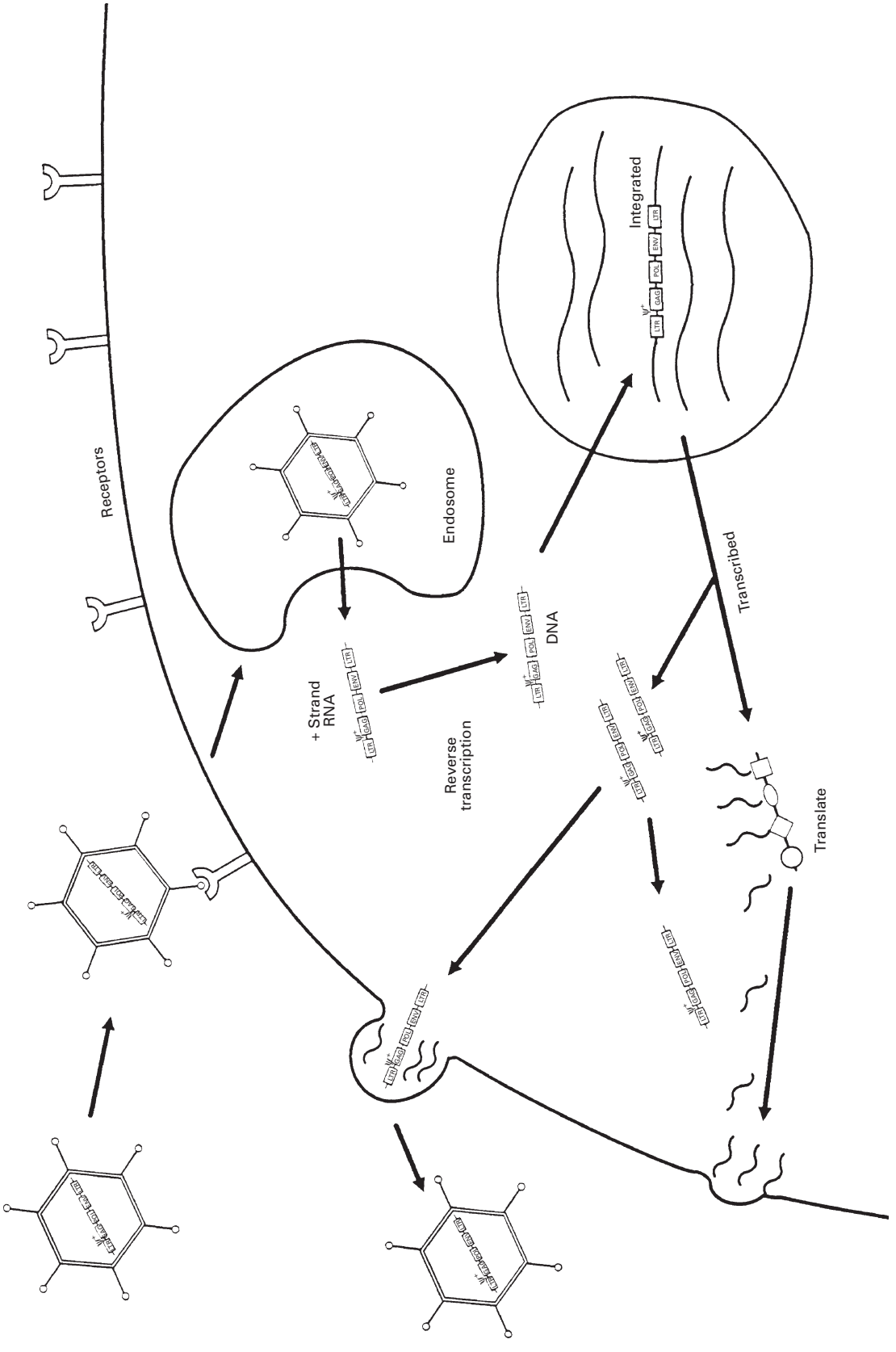

Figure 3 Retroviral life cycle. Endosome compartmentalization may not occur for all retroviruses.

plex, release of the viral core, and reverse transcription results in generation of double stranded DNA (provirus). Integration of proviral DNA into the genome followed by transcription and translation generates more infectious virions that are released by budding from the cell membrane.

Generation of retroviral vectors for gene therapy

Generation of a retroviral vector for gene therapy requires: (1) a plasmid vector containing a retroviral provirus with a selectable marker; (2) a helper virus in a packaging cell line; (3) transfection of the packaging cell with plasmid vector; and (4) selection of a vector producing cell (VPC) line.

Restriction enzymes are available to clone genes into DNA but not RNA. Therefore, developing a retroviral vector begins with a plasmid containing the retroviral provirus DNA. *Trans*-elements (gag-pol and env) are spliced out and the gene(s) of interest (transgene) is cloned into the plasmid. Large quantities of plasmid containing the retroviral construct can be rapidly produced in and isolated from bacteria. These plasmids can then be used to transfect a packaging cell line by physical methods such as calcium phosphate precipitation or lipofection to make a stably transfected vector producing cell (VPC) line (Figure 4). A packaging cell line contains helper retrovirus integrated into DNA. This helper virus contains *trans*-elements (gag, pol, env) but is lacking some *cis*-elements including the packaging (ψ) signal. Without the packaging signal, the helper retrovirus cannot be packaged into a virion. Therefore, a packaging cell line produces virions lacking vector RNA. Selectable markers allow for isolation of stably transfected packaging and vector producer cells.

Stable transfection of a packaging cell line results in a vector producing cell line (Figure 4). The VPC can be subcloned and titered for the highest titer producer cells. A plasmid containing the retroviral vector and transgene is tranfected into an ecotropic vector packaging cell which produces a retrovirus capable of infecting only mouse cells. Supernatant from this cell line is then used to transduce an amphotropic packaging cell which produces a retrovirus capable of infecting both murine and non-murine cells. This circuitous route is employed because the titer of retrovirus produced by VPC is higher if it is transduced with a retroviral vector compared to transfection with a plasmid.

Transduction of lymphocytes with a retroviral HStk vector construct (Figure 5)

Supernatant from vector producing cells can be cryopreserved and at a later time thawed to transduce target cells (lymphocytes). Efficient transduction is dependent on several factors: (1) retroviral titer, (2) cell mitosis, (3) retrovirus/lymphocyte contact, (4) retroviral half-life, and (5) retrovirus receptor density on the cell surface. The titer of retroviral supernatant is usually considered the most important limiting factor for transduction. The higher the titer, the more efficient the transduction. However, retroviral titers obtained directly from VPC rarely exceed 10^6 to

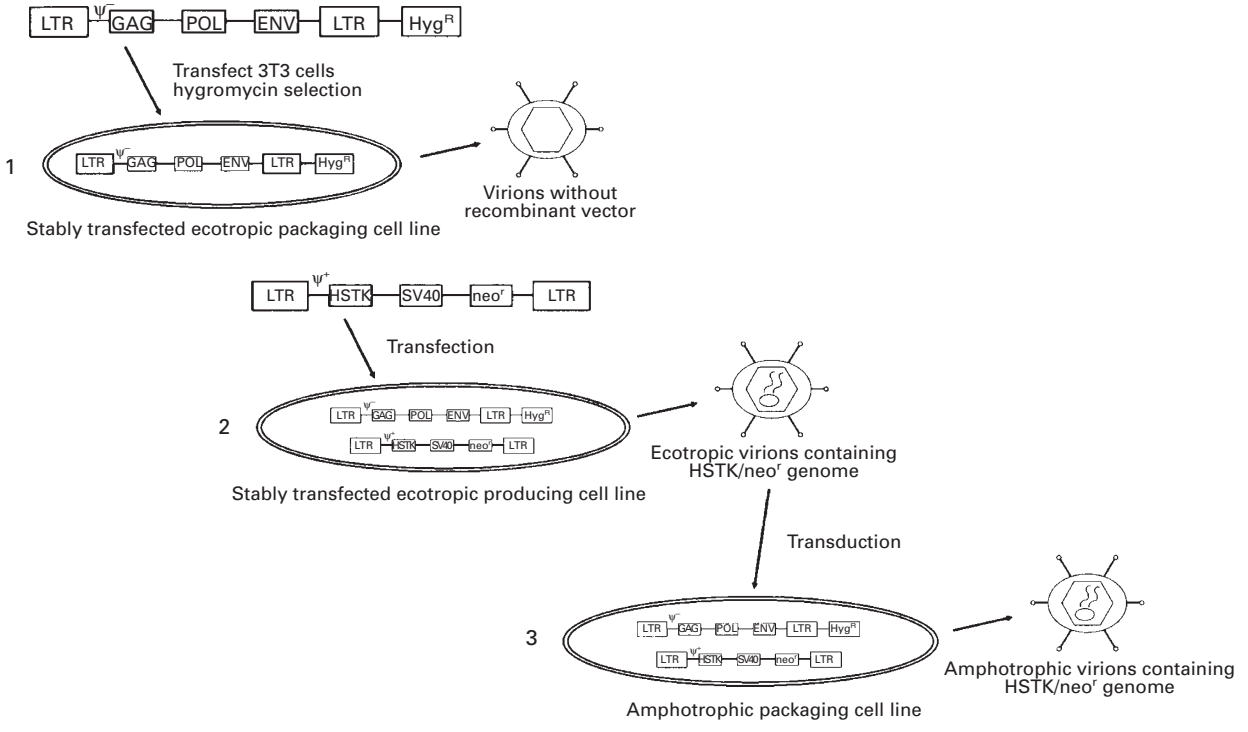

Figure 4 Generation of vector producing cell line. env, envelop; gag, group-specific antigen; HSTK, herpes simplex thymidine kinase; Hyg^r, hygromycin resistance; LTR, long terminal repeat; pol, polymerase; ψ^+ (psi), packaging motif present, ψ^- (psi), packaging motif absent; SV40, simian virus promoter; neo^r, neomycin phosphotransferase (confers resistance to G418, a neomycin analog).

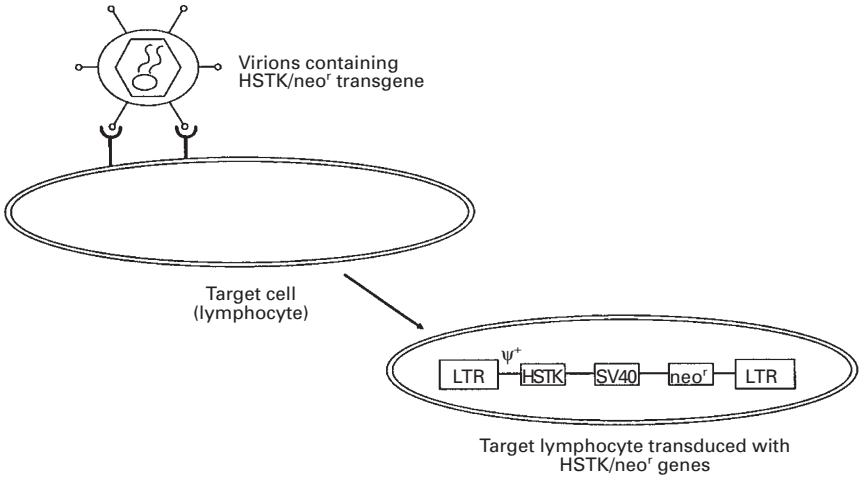

Figure 5 Transduction of lymphocytes. HSTK, Herpes simplex thymidine kinase; LTR, long terminal repeat; ψ^+ (psi), packaging motif present; SV40, simian virus promoter; neo^r, neomycin phosphotransferase (confers resistance to G418, a neomycin analog).

10^7 infectious virion particles/ml. Attempts to concentrate amphotrophic retrovirus by centrifugation of supernatant have generally been unsuccessful due to virion lysis. Some investigators have circumvented this by using hybrid virions called pseudotypes which are composed of envelope glycoprotein from one virus and genome from another. For example, vesicular stomatitis virus (VSV) pseudotype virions are composed of a VSV envelope and retroviral genome.[63] VSV pseudotype viron titers may be as high as 10^{10} infectious virion particles/ml.

In general, a type C retrovirus cannot cross an intact nuclear membrane. During mitosis the nuclear membrane breaks down. Therefore, cells must be induced to divide to allow retroviral integration into the genome. An exception to this are lentivirinae (eg HIV) which can integrate into the DNA of a nondividing cell due to a nuclear localization signal in the HIV matrix protein.[64,65] However, for safety reasons and due to initially low lentiviral titers most gene therapy trials use oncoviridae vectors. The plasma membrane of cells and retroviral virions have negative charges and are ionically repulsed. Electrostatic charges may be neutralized with positively charged agents such as polybrene or protamine to enhance cell and virion contact and increase efficiency of transduction. Contact may also be increased by gentle centrifugation of virion supernatant over target cells.[66] Retroviral infectivity has a half-life of 8 h at 37°C, therefore, fresh supernatant is often replaced every 12 h for several cycles to increase transduction efficiency. Finally, different retroviruses utilize different receptors for cellular entry.[67] The HIV receptor is the CD4 molecule; the amphotrophic receptor and the gibbon ape leukemia virus (GALV) receptor are phosphate transporters, the murine ecotropic receptor is a cationic amino acid transporter; the avian leukosis sarcoma virus (ALSV) receptor is the low density lipoprotein receptor. Retrovirus entry into cells may be increased by upregulating target cell receptor density. For example, use of phosphate depleted medium increases both amphotrophic and GALV receptor expression.[68]

Human transgenic lymphocytes

The first human gene transfer experiments in lymphocytes were done to correct genetic defects. Hypoxanthine phosphoribosyltransferase deficiency is associated with a devastating disease known as Lesch–Nyhan syndrome. HPRT cells are sensitive to HAT (hypoxanthine, amethopterin, thymidine) media. A retrovirus construct containing the human HPRT gene was transfered into a Lesch–Nyhan cell line correcting *in vitro* HAT sensitivity.[31] Similarly, retroviral transduction of ADA deficient SCID T cells using human ADA cDNA reversed *in vitro* hypersensitivity.[33] *In vivo* human ADA expression was demonstrated in nonhuman primate lymphocytes after *in vitro* retroviral gene transduction of autologous primate hematopoietic cells.[69] These data and others led to the first human gene transfer trial that attempted to correct a genetic disease, ADA deficiency.[70] Lymphocytes from patients with ADA deficient SCID were obtained by apheresis, transduced with an ADA retroviral vector, culture-expanded, and reinfused into the patient. Results from this study demonstrated persistence of gene modified T cells in the peripheral blood for a period of years without any apparent adverse effects.

Retroviral transduction of lymphocytes has also been used to insert a gene to mark trafficking and survival of lymphocytes *in vivo*. The marker gene has generally been neo^r which encodes neomycin phosphotransferase confering resistence to G418. The first clinical gene transfer experiment conducted by Rosenberg *et al*[71] at the NIH employed a neo^r containing retrovirus for transduction of tumor infiltrating lymphocytes. Transgenic lymphocytes were recovered in the peripheral blood up to 189 days after infusion as well as from some tumor biopsy specimens. Rooney *et al*[72] infused retroviral transduced virus-specific donor T cells into patients with EBV-related lymphoproliferation following allogeneic marrow transplantation. The transgenic cells persisted beyond 10 weeks and were associated with resolution of lymphadenopathy and disappearance of B symptoms (fever). Riddell *et al*[73] marked CD8$^+$

HIV-specific cytotoxic T cells (CTL) with a retrovirus encoding genes (HyTK) that permit both positive (hygromycin phosphotransferase) and negative (thymidine kinase) selection. The HIV-specific autologous CTL disappeared rapidly when infused into HIV positive patients. Disappearance was accompanied by anti-Hy and anti-TK CTL responses. Therefore, the recipient's immune system may eliminate genetically altered cells which express foreign proteins.

Suicide gene/prodrug combination

Approximately 20% of human gene therapy protocols involve the use of a suicide gene/prodrug combination usually ganciclovir/herpes simplex thymidine kinase.[74] These combinations are based on insertion of a gene for an enzyme (HStk) that metabolizes an inert prodrug (GCV) into a lethal metabolite. Ganciclovir is a poor substrate for human thymidine kinase but is monophosphorylated by the herpes viral thymidine kinase. Human cellular enzymes then triphosphorylate ganciclovir which is incorporated into DNA and causes cell death.

Alloreactivity of HStk transduced lymphocytes

HStk transduced lymphocytes using a retroviral vector containing both the HStk and neo^r genes appear to retain alloreactivity.[14,20,75] PHA and CD3 stimulated T cells co-cultured on irradiated VPC or incubated with VPC supernatant and subsequently selected in G418 for 1 week retained normal allogeneic reactivity. However, concern persists that long-term *ex vivo* propagation and expansion of lymphocytes may adversely affect function and anti-tumor immunogenicity.

Clinical trials using HStk modified lymphocytes

Bordignon, Tiberghien and Link have published results utilizing HStk retrovirally transduced lymphocytes to modulate graft-versus-host disease (GVHD) (Table 1). In addition, the pharmaceutical company Chiron has initiated a multicenter study providing HStk transduced lymphocytes for treatment of hematologic malignancies that relapse after allogeneic transplantation. Bordignon *et al*[15] and Bonini *et al*[16] proposed the first clinical study using HStk gene modified lymphocytes as a method to modulate GVHD after allogeneic bone marrow transplantation. Three goals were defined in the original clinical protocol: safety of infusing donor lymphocytes transduced with a suicide retroviral vector; *in vivo* survival and immunologic potential of donor lymphocytes; and *in vivo* effect of ganciclovir on GVHD. The retroviral vector contained two transgenes, a HStk/neo^r fusion gene and a truncated nonfunctional form of the low affinity receptor for nerve growth factor (NGFR). The NGFR gene permitted rapid *in vitro* immunoselection of transduced cells. A further benefit was that this marker allowed *ex vivo* selection, detection, and characterization of the transduced cells.

Patients with leukemia who relapsed or developed Epstein–Barr virus-induced lymphoma after T cell-depleted BMT were administered transduced donor lymphocytes.[15] No toxicity or complication was observed that could be attributed to the gene transfer procedure. Transduced cells were present in the blood more than 12 months after the infusion of the HStk modified cells. Five patients demonstrated anti-tumor responses. Two patients developed acute GVHD and one patient developed chronic GVHD. Biopsy of GVHD lesions revealed the presence of the HStk transgene by PCR. In the two patients who developed acute GVHD prompting infusion of ganciclovir both had elimination of transduced cells resulting in normalization of elevated liver enzymes and regression of cutaneous signs of GVHD. A third patient developed signs of chronic GVHD involving skin, lungs and the mouth. After treatment with ganciclovir for 7 days, the patient had significant improvement of clinical signs with a reduction but not elimination of circulating transgenic cells. Two of the patients who received ganciclovir had achieved leukemic remission prior to the administration of the drug and remained in full remission thereafter. During treatment with ganciclovir no significant toxicity was observed.

Following T cell-depleted BMT, Tiberghien *et al*[18,21] administered transgenic donor T cells containing the HStk and neo^r genes to three patients at high risk for GVHD (unrelated donor or related donor with recipient over 40 years old or female donor to male recipient). 2×10^5 gene modified T lymphocytes/kg were infused. No acute toxicity was observed. PCR analysis demonstrated survival of the gene modified cells for at least 60 days confirmed both by sensitivity to ganciclovir and resistance to G418. Two patients developed GVHD and both had complete

Table 1 Gene therapy protocols using HSTK transduced donor lymphocytes for patients with hematologic malignancies

Investigator/Ref.	*Transgene(s)*	*HStk promotor*	*Results*
Bonini *et al*[16]	HStk/neo^r fusion gene and NGFR	retroviral LTR	5/8 patients show anti-tumor response
Bordignon *et al*[15]			3/8 patients develop GVHD
			2/3 with GVHD have a complete response to GCV
			1/3 with GVHD have a partial response to GCV
Tiberghien *et al*[18]	HStk/neo^r	retroviral LTR	2/3 patients develop GVHD
			2/2 with GVHD have complete response to GCV
Link, Drobyski, Burt *et al*[20,76]	HStk/neo^r	retroviral LTR	4 patients infused
			1/4 anti-tumor response
			0/4 GVHD

responses after administration of ganciclovir. As in the Bordignon trial, remission of GVHD following ganciclovir infusion indicates that the HStk transgene is expressed *in vivo*.

In the protocol by Link *et al*[20] patients greater than 2 years of age with relapsed hematologic malignancies after allogeneic BMT are potentially eligible. Patients are administered HStk gene modified donor lymphocytes in dose ranges from 0.1 to 2.5×10^8 T cells/kg for recipients of HLA sibling grafts or 0.01 to 1.0×10^8 T cells/kg for recipients of HLA mismatched or unrelated marrow grafts. In the absence of significant GVHD, no ganciclovir is administered. Patients in whom GVHD is progressive or unresponsive to conventional therapy are treated with ganciclovir twice daily by i.v. infusion. *In vitro* transduction of lymphocytes is by activation with interleukin-2 and OKT-3 followed by three 12 h exposures to retroviral supernatant with protamine sulfate at a multiplicity of infection (MOI) of 1:2–5. Selection of transduced lymphocytes is in G418 for 6 days. Subsequently, transgenic modified lymphocytes are expanded *ex vivo* 50- to 100-fold over 3–6 weeks.

Prior to infusion into the patient, transduced lymphocytes are analyzed for function and phenotype. It has been previously reported that HStk transduced lymphocytes maintain proliferative responses and allogeneic reactivity.[14,75] We have found that proliferative responses to IL-2/OKT-3 or PHA were decreased when compared to fresh lymphocytes but no different than control untransduced lymphocytes cultured under the same conditions. Therefore, *ex vivo* culture, not transduction *per se*, diminishes but does not eliminate proliferative responses. Whether or not propagation and expansion of lymphocytes for 3–6 weeks *ex vivo* will diminish their anti-leukemic effectiveness is unknown. Following activation, transduction, selection and culture expansion, the percentage of cells that are $CD4^+$, $CD8^+$, or NK vary widely, while B cells are usually undetectable. The effect of infusing varied $CD4^+$, $CD8^+$ or NK percentages in the transgenic lymphocyte population on leukemia remission and/or GVHD is unknown.

In our trial using HSTK transduced donor lymphocytes, we have also noted persistence of transduced lymphocytes for more than 6 months. In the limited number of patients receiving chemotherapy, the transduced lymphocytes persist after chemotherapy,[76] perhaps suggesting that many of the transgenic lymphocytes are not in cycle during exposure to chemotherapy. Theoretically, chemotherapy with resulting neutropenic fever and cytokine release may push resting HStk transduced lymphocytes towards activation and into cell cycle.

Safety concerns

HStk transduced lymphocytes present two major safety concerns: insertional mutagenesis and production of replication competent retrovirus (RCR). Mutagenesis may arise by chance insertion of the retrovirus within a suppressor oncogene resulting in disruption of a normal apoptotic or anti-proliferative signal. Alternatively, the retrovirus may insert upstream of an oncogene resulting in LTR promotor driven transcription of the oncogene. To date, no human malignancies or evidence for insertional mutagenesis have been reported. However, use of a suicide (HStk) containing construct should allow for prodrug (ganciclovir) killing of any somatic malignant clone arising by insertional mutagenesis. Germline mutagenesis could result in transgene presence in every cell within the body. This would obviate the ability to selectively kill only the malignant clone by prodrug infusion. Transduction of the germ line could cause mutagenesis and/or teratogenesis. This would be unlikely in the BMT setting since a consequence of transplant conditioning regimens is infertility. Furthermore, in current HStk retroviral lymphocyte protocols, transduction is performed *ex vivo*.

Generation of RCR has the potential to infect other cells within the body and to increase the likelihood of insertional mutagenesis. Replication competent retroviruses may arise by homologous recombination with other cellular retroviral sequences. RCR is ruled out by both *mus dunni* assays and PCR for envelope gene prior to release of supernatant for *ex vivo* lymphocyte transduction. PCR is performed prior to infusion of transduced lymphocytes. Finally, if HStk transduced lymphocytes should undergo homologous recombination after infusion into the patient, any infectious virions generated should be lysed by human complement.

Future directions

Limitations of current clinical gene therapy protocols include low efficiency of transduction, failure to regulate transgene transcription, and requirement for *ex vivo* selection. Increasing efficiency of transduction is essential to decrease the time and expense required to generate transgenic cells. Currently, cells require drug selection *ex vivo* with G418 to select for the gene modified cells, or alternatively a marker gene is expressed and subsequently processed as a transmembrane protein that can be used for antibody-mediated selection. Improvements resulting in high tranduction percentages could eliminate the need for *ex vivo* selection. Retroviral constructs with alterations in the U3 transcriptional control motifs may allow for more selective or targeted control of transcription as well as higher rates of transcription.

The ability to create genetically modified lymphocytes may ultimately lead to strategies that do not require pharmacologic immunosuppressive treatment for GVHD. This could broaden the use of unrelated and related mismatched transplants and allow for new applications of allogeneic transplantation such as treatment of autoimmune diseases.[77] Genetically engineered lymphocytes may also allow for allogeneic lymphocytes to supplement or replace chemotherapy as a conditioning and/or anti-leukemic induction agent.

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