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Cellular Suicide Therapy of Malignant Disease

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ABSTRACT

Adoptive cellular therapy is developing as a supplement or alternative to chemotherapy and/or radiation for malignant disease. Our focus is two ongoing clinical studies with transgeneic (genetically altered) cellular therapy; one uses allogeneic (from another person) lymphocytes to treat leukemia, and the second uses xenogeneic (from another species) fibroblast cells genetically altered to contain a toxin-producing suicide gene to treat ovarian cancer.

Allogeneic donor lymphocyte infusions (DLI) are known to induce remission of hematologic malignancies. However, the toxicity associated with DLI is related to graft-versus-host-disease, which is due to donor lymphocytes attacking normal tissue in the recipient. Therefore, we have taken the approach of infusing DLI that have been modified to contain a latent suicide gene to treat leukemia.

To treat ovarian cancer, we used xenogeneic nonimmune fibroblast-derived cells to deliver a tumor-directed cytotoxic gene to carcinoma cells. These cells release *HStk* transgene retroviruses that in turn transduce replicating tumor cells but not quiescent epithelium, rendering the tumor selectively susceptible to ganciclovir-mediated killing.

These initial trials summarize the early stage of allogeneic/xenogeneic adoptive cellular therapy for cancer, and although the data are limited, it is encouraging to see some patients with evidence of antitumor responses. Advances in our understanding of the basic science of these treatments, together with improvements in the technology of vector design, will be required to streamline these methodologies into broader application. *The Oncologist* 2000;5:68-74

INTRODUCTION

Adoptive cellular therapy is developing as a supplement or alternative to chemotherapy and/or radiation for malignant diseases. A variety of immune and nonimmune cells are being used for this purpose (Table 1). Allogeneic donor lymphocyte infusion (DLI) can induce complete responses in patients who have relapsed hematologic malignancies (lymphoma, leukemia, myeloma) after allogeneic bone marrow transplant [1, 2]. If DLI engraftment occurs, treatment may be complicated by graft-versus-host disease (GVHD), which results from the allogeneic-immune competent cells reacting against a patient's normal organs. Trials are ongoing with lymphocytes that are genetically modified with a suicide gene before they are administered [3-5]. Genetic modification allows their in vivo destruction if severe GVHD occurs.

Trials using xenogeneic cells are usually designed to introduce a suicide gene into neighboring tumors by infusing a nonimmune cell, usually of mouse fibroblast lineage [6, 7]. These xenogeneic cells may also function as a potent adjuvant to induce immunologic rejection of the tumor.

We will focus on two of our ongoing clinical studies with transgeneic cellular therapy. One uses transgeneic (genetically altered) allogeneic lymphocytes (from a brother or sister) to treat leukemia. The other trial uses xenogeneic (mouse) fibroblast cells genetically altered to contain a toxin-producing suicide gene to treat patients with ovarian cancer.

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Table 1. Cells for adoptive therapy of cancer

Immune-nonspecific cellular therapy

- Buffy-coat DLI
- T cells
- NK cells
- Dendritic cells

Immune-target-specific cellular therapy

- TSA-primed T cells
- TSA-loaded dendritic cells
- TSA-transduced dendritic cells
- T cells designed with specific T-cell receptors for TSA

Nonimmune

- Xenogeneic vector-producing cells, i.e., genetically modified murine fibroblasts

We, therefore, need to briefly explain the concepts involved in genetically altering cells to contain a suicide gene.

Rationale of a Suicide Gene

To selectively kill a cell at some future time while avoiding injury to other cells within the body, a nonhuman suicide gene may be incorporated into the chromosome of the infused cells. The most commonly used suicide gene is the herpes viral gene, herpes simplex thymidine kinase (*HStk*) [8, 9].

Cells containing *HStk* may be selectively targeted for killing by infusion of ganciclovir (GCV). GCV is nontoxic unless it is metabolized. It is a poor substrate for human thymidine kinase but is metabolized to monophosphate GCV (MP-GCV) by herpes thymidine kinase at GCV concentrations easily attainable in serum. Normal human cellular enzymes convert MP-GCV to triphosphate-GCV, which is incorporated into DNA and RNA, resulting in DNA and RNA chain termination and cell death. Therefore, cells containing *HStk* may be selectively targeted for killing by infusion of GCV.

Principles of Gene Therapy

Several physical and chemical methods are available to introduce DNA into cells, but most clinical gene therapy protocols use viruses to transfer DNA into cells. Viruses may be altered so that the viral backbone with essential transcriptional regulatory element(s) remains intact, while the viral genes responsible for replication are replaced by genes (transgenes, such as *HStk*) that the investigator desires to introduce into a cell. The method for incorporating genes into a cell using a disabled replication-incompetent retrovirus is termed transduction.

Retroviral vector is harvested from the supernatant of vector-producing cells (VPCs). In our allogeneic gene-modified DLI protocol, supernatant from these VPC cells is used to transduce the target cells (donor lymphocytes) ex vivo. A unique feature of retroviruses is integration of the vector containing the transgene into a cell's chromosome. When the cells divide, all progeny or daughter cells will contain the transgene. For Moloney murine leukemia retrovirus-based vectors, as used in our current studies, integration into cellular DNA only occurs at the time of mitosis [10]. Therefore, dividing tumor cells or normal cells induced into mitosis would be susceptible, while normal tissue in G_0 would be resistant to retroviral transduction.

Hematologic Malignancies and Allogeneic Adoptive Cellular Therapy

The effectiveness of allogeneic hematopoietic stem cell transplantation is in large part due to adoptive transfer of donor immune cells, conferring a potent graft-versus-malignancy effect. Despite similar chemotherapy-conditioning regimens, an autologous transplant has a higher relapse rate than an allogeneic transplant. This allogeneic graft-versus-malignancy effect has been demonstrated for a variety of hematologic malignancies, including leukemia, lymphomas, and multiple myeloma, and may be abrogated by lymphocyte depletion of the graft. In fact, allogeneic hematopoietic stem cell transplantation is a form of allogeneic adoptive immunotherapy. The chemotherapy not only helps to decrease tumor burden but causes recipient immune suppression allowing engraftment of donor-derived hematopoietic cells. For patients who relapse after allogeneic transplantation, remission may be induced without chemotherapy simply by modulating allogeneic immunity. In patients with chronic myelogenous leukemia (CML), lymphomas, multiple myeloma, and acute leukemia, remission may follow withdrawal of immunosuppressive medications or, if disease persists, infusion of white blood cells from the original donor (i.e., donor lymphocyte infusion) [1, 2].

The optimal DLI dose is unknown. Donor lymphocytes may be infused on one occasion or multiple intervals over several days to weeks at a dose of 10^6 to 5×10^8 nucleated cells/kg. Donor lymphocyte infusions appear to work best against slower growing malignancies, such as low-grade lymphomas, multiple myeloma, and CML in chronic phase. Responses may take two to six months to be appreciated. The contribution of T, natural killer (NK), dendritic, or other cellular subsets to remission induction remains unclear. Toxicities of DLI are related to GVHD, especially liver and gastrointestinal failure and/or cutaneous scleroderma-like symptoms. If relapse is associated with return of autologous hematopoiesis, another manifestation of DLI-induced GVHD is marrow aplasia and pancytopenia. These

symptoms may be lethal and refractory to reinstitution of immunosuppressive medications.

A patient cured of leukemia by DLI may still die of DLI-induced GVHD. One approach to avoid this complication is to infuse lymphocytes that recognize only tumor-specific antigens (TSA). Unfortunately, most TSA are unknown and generation of immune-competent TSA-specific lymphocytes is technically difficult. Therefore, several investigators have taken the alternative approach of infusing nonspecific DLI that have been modified to contain a latent suicide gene (e.g., *HStk*) [3-5].

Allogeneic hematopoietic transplantation is the perfect venue for allogeneic immunotherapy because the transplant-conditioning regimen causes recipient immunosuppression and allows for donor engraftment. *HStk*-transduced allogeneic cells have been infused after relapse for remission induction and at the time of initial transplant as a fail-safe therapy for GVHD [3-5]. In current clinical trials, the transduction of lymphocytes with a retroviral *HStk* construct is limited by low efficiency, with only 1% to 10% of cells being transduced. Therefore, the retroviral construct not only contains *HStk* but also a selectable marker that allows isolation of the transduced cells before infusion. In an Italian trial [3], the selectable marker is the low-affinity truncated nerve growth factor receptor (NGFR). This marker allows for rapid separation by flow cytometry. In our trial [5] and a study by *Tiberghien* and colleagues [4], the selectable marker is *neo*^{r}, a gene that allows for positive selection of cells in culture with the antibiotic G418. A flow diagram of the current protocol is shown in Table 2 and Figure 1.

These trials, although reporting only limited numbers of patients, demonstrate in vivo survival of transgeneic cells, remission of hematologic malignancies, development of GVHD, and termination of acute GVHD after infusion of GCV. However, in a case reported by *Bonini* and colleagues [11], one patient with chronic GVHD had only a partial remission to GCV accompanied by incomplete clearance of *HStk* transgeneic cells from the peripheral blood. This partial remission may indicate relative resistance of *HStk* transgeneic cells to in vivo killing by GCV for chronic GVHD relative to acute GVHD. Alternatively, the mechanisms and cells involved in initiation and progression of acute and chronic GVHD may differ.

In these trials, two major concerns persist. The transgeneic cells, depending on method of selection and ex vivo culture

Table 2. Protocol schematic

Allogeneic bone marrow transplant followed by relapse

↓

Apheresis of original transplant donor and transduction of donor lymphocytes with suicide (*HStk*) vectors

↓

Administration of cytoreductive therapy to recipient, if clinically indicated

↓

Infusion of *HStk* donor lymphocytes

↓

Weekly grading of GVHD and leukemia response

↓

Development of clinically significant GVHD. Treat with standard therapy (corticosteroids and cyclosporine)

↓

Infusion of GCV for progressive or unresponsive GVHD

Dose Administration

Patient population	$CD3^+$ lymphocytes/kg recipient weight
HLA-identical sibling	0.1 to 2.5×10^8
HLA-mismatched related or HLA-unrelated donor	1×10^7

GCV = ganciclovir; *HStk* = herpes simplex thymidine kinase; GVHD = graft-versus-host disease.

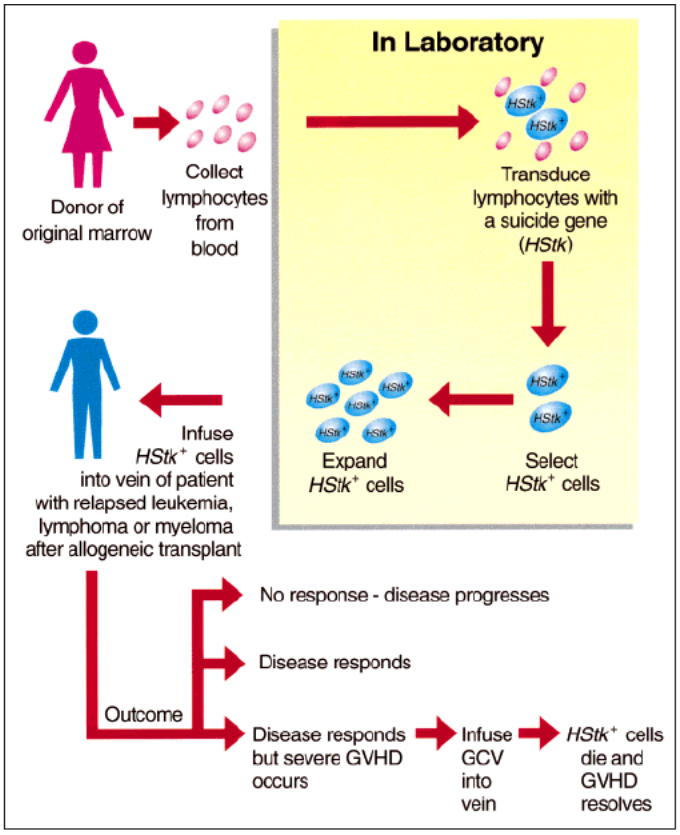

Figure 1. Transgeneic donor lymphocyte infusion to treat patients who relapse after allogeneic transplant. GCV = ganciclovir; **HStk** *= herpes simplex thymidine kinase; GVHD = graft-versus-host disease.*

time, may be less immune-competent than fresh donor lymphocytes, meaning that cell dose required to induce remission may be different for transgeneic compared with fresh donor lymphocytes. Also transgeneic cells contain nonhuman suicide (i.e., *HStk*) and selectable (i.e., *neo*^{r}) genes. Proteins from these genes may be recognized as foreign and rejected by the recipient's immune system, causing disappearance of the transgeneic cells. Rejection of transgeneic cells has been reported in patients with AIDS who were administered genetically modified cells [12]. We and others have seen in vivo persistence of transgeneic cells for up to 12 months in patients undergoing transplant for hematologic malignancies. Therefore, the immunogenicity of transgeneic lymphocytes will probably vary by protocol and depend on suicide gene and selectable marker, patients' disease, and degree of immune suppression of the recipient before adoptive cellular therapy.

Solid Tumors and Xenogeneic Adoptive Cellular Therapy

Allogeneic lymphocytes are a known potent antileukemic therapy, but for solid malignancies, evidence neither supports nor disproves an antitumor effect of allogeneic immune cells. We, therefore, chose xenogeneic (mouse) nonimmune fibroblast-derived cells to deliver a tumor-directed cytotoxic gene (*HStk*) to carcinoma cells [7]. The infused cells are mouse fibroblast VPCs that release *HStk* transgeneic retroviruses. These retroviruses can transduce replicating tumor cells but not quiescent epithelium, rendering the tumor selectively susceptible to GCV-mediated killing.

Animal Models

HStk VPC implantation is designed to localize VPCs that produce *HStk* retrovirus near tumor implants. Transduction of malignant cells by VPCs and prodrug (GCV) selection should then induce tumor regression. The implantation of VPC to effectively deliver genes into tumor cells was first demonstrated in a brain tumor model by engrafting VPC into glioma tumors of rodents [13]. Antitumor efficacy of the *HStk*/VPC system was confirmed in a rat model of glioblastoma multiforme [14, 15] and in colorectal metastasis to the liver [16]. In these rodent models, gene transfer into solid tumors from the VPC was significant and resulted in tumor regression after administration of GCV.

Human Clinical Trials

Several clinical trials have been performed using retroviruses and the *HStk* VPC/GCV xenogeneic system, including one ex vivo gene-transfer approach for ovarian cancer, one in vivo gene-transfer trial for leptomeningeal carcinomatosis, and several in vivo gene-transfer trials for the treatment of brain tumors and melanoma. In the ex vivo *HStk*/GCV trial for the treatment of ovarian cancer, women with recurrent ovarian cancer receive i.p. infusions of a human ovarian tumor cell line (PA-1) transduced by the *HStk* gene [17]. The PA-1 cell line was derived from the ascites fluid of a patient with ovarian teratocarcinoma. Only one study was attempted for leptomeningeal disease. A single patient with leptomeningeal carcinomatosis underwent the direct injection of *HStk* VPC into the subarachnoid space [18]. The trial was discontinued secondary to a meningeal reaction that occurred to the murine VPC.

The first human trial of murine VPC used multiple stereotactic injections to introduce murine *HStk* VPC into the enhancing portion of brain tumors. Patients received stereotactic injections of *HStk* VPC into a portion of the tumor followed by GCV. Antitumor activity was observed in selected local tumor deposits [6]. Magnetic resonance imaging (MRI) scanning revealed three patients with significant decreases in their tumor size. Biopsies of responding lesions demonstrated an inflammatory reaction with a mild lymphocytic infiltrate, tumor cell necrosis, and foci of viable tumor. Interestingly, despite some evidence of antitumor responses, only a very low degree of gene transfer could be documented. A second brain tumor trial was reported by *Klaztmann* and colleagues [19]. Murine VPC-producing *HStk* retroviral vectors were injected into the tumor margin after surgical debulking of recurrent glioblastoma. Seven days later, patients were treated with GCV. All twelve patients were treated without side effects. Median survival was 206 days, with 4 of 12 patients surviving longer than 12 months. One patient was still alive at 2.8 years after the procedure without evidence of progression by MRI. One other study was performed on patients with melanoma with noncentral nervous system malignancy [20]. Eight patients were treated by the direct injection of murine packaging cells that produced *HStk* vector. The total cell dose ranged from 8×10^7 to 12.5×10^8 cells injected directly into tumors. Inflammatory reactions were common immediately after xenogeneic VPC injection. A limited antitumor effect with some areas of local necrosis was noted on biopsy samples. The lack of more significant efficacy was attributed to poor gene transfer.

In our trial, we inject murine VPC-producing *HStk* vectors directly into the peritoneal cavity of patients with ovarian cancer who have failed standard therapies (Fig. 2). Patients with relapsed or refractory disease have a uniformly fatal outcome. Ovarian cancer has an interesting natural history: even in its advanced stages, ovarian tumors tend to remain confined to the abdomen for

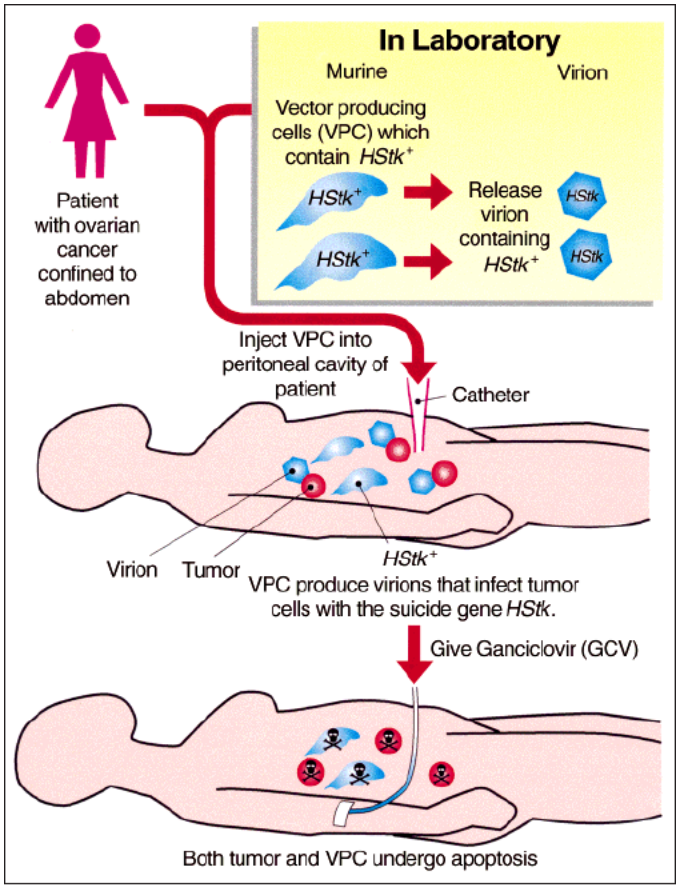

Figure 2. Xenogeneic/*HStk *therapy for patients with ovarian cancer. VPC = vector producing cells; HStk = herpes simplex thymidine kinase.

extended periods of time. This biology makes consideration of local i.p. *HStk* VPC therapy possible.

Patients undergo minor surgery to place a plastic catheter into the abdomen and then receive an injection of *HStk* VPCs into the peritoneal cavity through the catheter. Two weeks after injection of the VPC, the patients receive GCV by i.v. infusion. Patients only received one cycle of cells (10^6 to 10^8 xenogeneic VPC/kg) in this dose-escalation protocol. The infusion was well tolerated with no grade 3 or 4 toxicities noted. VPC were present as determined by polymerase chain reaction, up to seven days but not later. One patient had the complete resolution of a 2 cm mass on computerized tomography (CT) scan but still had an increased CA125 level in her peripheral blood. Three patients (one each) had a partial, minor, or mixed response. Only very low-level gene transfer to carcinoma cells was demonstrated.

The mechanism of the tumor rejection in the VPC *HStk* system is thought to be delivery of the suicide gene to the neighboring tumor cells, but not all of the tumor's cells must contain *HStk* to be killed by GCV [8, 14]. The mechanism of this "bystander tumor kill" is not yet completely understood. Mechanisms related to gap junction passage of phosphorylated GCV between transduced and nontransduced epithelial cells have been proposed [21, 22], and a role for connexins has been strongly suggested [23]. The bystander effect does not appear to involve generalized nonspecific cellular toxicity to normal tissues surrounding these *HStk*-treated tumors, perhaps because of the quiescent G_0 state of normal tissue. For metabolic cooperation to play a significant role, the data suggest that at least 1% to 5% of the tumor must express *HStk*. This creates an important unanswered question, since the human trial data, even in patients with evidence of response, have not shown convincing data for gene transfer efficiency >1%.

Another possible mechanism of tumor kill is immunologic hyperacute rejection of murine xenografts. Strong immunologic barriers to xenotransplants can destroy a transplanted solid organ within minutes, a process termed hyperacute rejection. The hyperacute rejection model of xenograft survival is typically a vascularized xenograft directly exposed to blood serum [24]. Research has demonstrated that hyperacute rejection of porcine xenografts transplanted into baboons occurs secondary to porcine α(1,3)galactosyltransferase (GT) gene expression and αgal presentation [24, 25]. The enzyme α(1,3)GT is expressed in mice (the species of origin for VPCs used in the above trials) but not in Old World monkeys, apes, or humans [26]. The α(1,3)GT gene is not active in humans due to the presence of two base pair frameshift mutations [27]. Anti-αgal antibody present in the human serum can recognize this epitope [26]. In fact, preexisting human antibody against αgal represents almost 1% of total human antibody [28] and is the basis for complement-mediated hyperacute rejection [29]. Human anti-αgal antibodies are thought to arise in response to αgal structures on the surface of normal gastrointestinal flora.

Our laboratory and others have demonstrated that murine retroviral VPCs and the viral vectors they produce express αgal and therefore are lysed by antibodies and complement within 30 min after being exposed to human serum [30-32], but not after exposure to i.p. ascites. The effect of this serum inactivation on VPC and retroviruses is due to gal expression on the cells [31-33]. The hyperacute rejection process of the murine cell in vivo may induce a bystander immunologic reaction against tumor cells. Further studies to delineate these complex interactions between murine cell xenografts and the human immune system are under way. Our group has recently designed a phase II protocol to delineate immune phenomena from effects of metabolic cooperation (Table 3). In this study, analysis for antitumor response will be performed before and after the administration of GCV.

Table 3. Protocol schematic for phase II trial of murine VPC for ovarian cancer

Study day -30 to -1
Patients found to be eligible for protocol
↓
Study day -7 to -1
Laparotomy or laparoscopy with tumor biopsy
Surgery may include tumor debulking and/or the lysis of adhesions
Placement of peritoneal dialysis catheter
↓
Study: Cycle 1: Day 0
Injection of *HStk* VPCs
↓
Study Days 7 and 14
Peritoneal washing
Evaluate nature of immune response and look for *HStk*-positive tumor cells

Study Day 27
Evaluate for response
Key point: determination of antitumor efficacy before the initiation of GCV (laparoscopy and/or CT scan and CA125)
↓
Study Days 28-41
Treat with prodrug GCV
↓
Study Day 48
Evaluate for response: determination of antitumor efficacy from GCV (laparoscopy and/or CT scan and CA125)
↓ Progressive disease — **Off study**
↓ Responsive or stable disease — **Study: Cycle 2: Day 0**

VPC = vector producing cells; *HStk* = herpes simplex thymidine kinase; GCV = ganciclovir.

Summary

The future of medicine is cell and gene therapy. These initial trials summarize the early stage of allogeneic/xenogeneic adoptive cellular therapy for cancer. Although these data are limited, it is encouraging to see some patients with evidence of antitumor responses. Ultimately, advances in our understanding of the basic science of these treatments and improved technology in vector development will be required to streamline these methodologies into broader application.

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