Gene Therapy for Pediatric Cancer: State of the Art and Future Perspectives

Ettore Biagi, Catherine Bollard, Raphael Rousseau, and Malcolm Brenner*

Center for Cell and Gene Therapy, Department of Pediatrics-Hematology/Oncology, Baylor College of Medicine, Houston, TX 77030, USA

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While modern treatments have led to a dramatic improvement in survival for pediatric malignancy, toxicities are high and a significant proportion of patients remain resistant. Gene transfer offers the prospect of highly specific therapies for childhood cancer. “Corrective” genes may be transferred to overcome the genetic abnormalities present in the precancerous cell. Alternatively, genes can be introduced to render the malignant cell sensitive to therapeutic drugs. The tumor can also be attacked by decreasing its blood supply with genes that inhibit vascular growth. Another possible approach is to modify normal tissues with genes that make them more resistant to conventional drugs and/or radiation, thereby increasing the therapeutic index. Finally, it may be possible to attack the tumor indirectly by using genes that modify the behavior of the immune system, either by making the tumor more immunogenic, or by rendering host effector cells more efficient. Several gene therapy applications have already been reported for pediatric cancer patients in preliminary Phase 1 studies. Although no major clinical success has yet been achieved, improvements in gene delivery technologies and a better understanding of mechanisms of tumor progression and immune escape have opened new perspectives for the cure of pediatric cancer by combining gene therapy with standard therapeutic available treatments.

INTRODUCTION

A multidisciplinary approach combining surgery, chemotherapy, and bone marrow transplantation has led to a dramatic improvement in survival for pediatric malignancy over the past 20 years. Currently, overall 5-year survival rates are more than 75% for children younger than 15 years of age and 77% for 15–19 year olds [1]. But despite these advances in the treatment of pediatric cancer, a significant proportion of patients remain resistant to the standard therapeutic procedures. Moreover, the price of the cure is often unacceptable, and includes not just acute and chronic organ toxicity but most troublingly, an increased risk of secondary malignancy. Hence, new strategies are required to improve the overall survival rate and decrease treatment-related morbidity.

Gene therapy offers the prospect of efficient and highly specific therapy for childhood cancer, and the concept was initially welcomed by investigators and clinicians alike with great enthusiasm mixed with unrealistic expectations. Unfortunately, it has become evident that the complexities of childhood malignancy and the limitations of current gene transfer vectors mean that the successful application of gene transfer technologies for the cure of pediatric malignancy will be a gradual and progressive process over many years. Nonetheless, as we will describe here, gene transfer technologies are already successfully being applied to the treatment of childhood cancer and should increasingly benefit this patient group in the coming years.

Genes may be transferred to cells in vitro with subsequent transfer of the gene-modified cells to the patient, or transfer may take place directly to the target cells in vivo. The gene delivery system, or vector, generally consists of a specific DNA sequence and promoter that drives the expression of the transgene of interest, as well as a polyadenylation signal that stabilizes the specific messenger RNA. The vector usually takes the form of a modified virus, but synthetic nonviral vectors are playing an increasingly important role [2, 3, 4].

There are a number of ways in which these transferred genes may be used for the treatment of cancer. The most obvious, and perhaps the most intellectually appealing, is to transfer corrective genes that will help overcome the genetic abnormalities that have arisen in the precancerous cell and led to the malignant process. Alternatively, it is possible to introduce genes that will render the malignant cell sensitive to small molecules to which it might otherwise be resistant. The third approach is to attack the tumor blood supply with genes that inhibit vascular growth or function. The fourth is to leave the tumor unmodified but to alter normal tissues instead, so that they
Table 1. Advantages and disadvantages of vector systems.

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<th>Vector</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Current uses</th>
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<tbody>
<tr>
<td>Murine retrovirus</td>
<td><strong>Stable integration into dividing cells</strong>&lt;br&gt;<strong>Minimal immunogenicity</strong>&lt;br&gt;<strong>Stable packaging system</strong></td>
<td><strong>Low titer</strong>&lt;br&gt;<strong>Only integrates in dividing cells</strong>&lt;br&gt;<strong>Limited insert size</strong>&lt;br&gt;<strong>Risk of silencing</strong>&lt;br&gt;<strong>Risk of insertional mutagenesis</strong></td>
<td><strong>Marker studies</strong>&lt;br&gt;<strong>Gene therapy approaches using hemopoietic stem cells</strong>&lt;br&gt;<strong>or T cells, for example, to treat immunodeficiency syndromes</strong>&lt;br&gt;<strong>Transduction tumor cell lines</strong></td>
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<tr>
<td>Lentivirus</td>
<td>Integrates into dividing cells&lt;br&gt;Integrates into nondividing cells&lt;br&gt;Larger insert size than murine retroviruses</td>
<td>No stable packaging system&lt;br&gt;Complex safety issues</td>
<td>No approved trials as yet</td>
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<tr>
<td>Self-inactivating lentiviral vectors (SIN-Lenti)</td>
<td>Incapable of replication post transfection → ? increased safety&lt;br&gt;Stable packaging system</td>
<td>Safety concerns remain</td>
<td>No approved trials as yet</td>
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<td>Adenovirus</td>
<td>Infects wide range cell types&lt;br&gt;Infects nondividing cells&lt;br&gt;High titers&lt;br&gt;High level of expression&lt;br&gt;Accepts 12–15 kb DNA inserts</td>
<td>Highly immunogenic&lt;br&gt;Nonintegrating</td>
<td>Direct in vivo applications&lt;br&gt;Transduction tumor cells</td>
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<tr>
<td>Adeno-associated virus (AAV)</td>
<td>Integrates into dividing cells&lt;br&gt;Infected wide-range cell types</td>
<td>No stable packaging cell line&lt;br&gt;Very limited insert size</td>
<td>Gene therapy approaches using hemopoietic stem cells</td>
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<td>Herpesvirus</td>
<td>High titers&lt;br&gt;Transduces some target cells at high efficiency&lt;br&gt;Accepts large DNA inserts</td>
<td>No packaging cell lines&lt;br&gt;Nonintegrating&lt;br&gt;May be cytotoxic to target cell</td>
<td>Transduction tumor cells&lt;br&gt;Neurologic disorders</td>
</tr>
<tr>
<td>Liposomes and other physical methods using plasmid DNA</td>
<td>Easy to prepare in quantity&lt;br&gt;Virtually unlimited size&lt;br&gt;Limited immunogenicity</td>
<td>Inefficient entry into target cell&lt;br&gt;Nonintegrating</td>
<td>Topical applications&lt;br&gt;Transduction tumor cells</td>
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are more resistant to conventional drugs and/or radiation and thereby increase the therapeutic index. Finally, and perhaps most widely used of all, it may be possible to attack the tumor indirectly by using genes to modify the behavior of the immune system, either by making the tumor more immunogenic, or by rendering host effector cells more efficient.

While each of these approaches has its advantages and disadvantages, at the moment all must be tempered by an appreciation that none of the current vectors with which we work possess the desirable characteristics of high efficiency and specific targeting to the tumor and tumor cell DNA. For many gene therapy approaches, it would also be highly desirable to control the transgene product, something that is not yet readily achievable in humans, and it would also be helpful to further improve the safety of viral vectors. While each of the available vectors has advantages and disadvantages, at present none comes close to meeting the requirements for a truly effective gene therapy vector that could be used in all the approaches outlined above. Instead, the choice of a vector system is based on the “least bad” alternative for the proposed use. An outline of the advantages and disadvantages of each of the vector systems is given in Table 1, while a more detailed account of each of these vectors can be obtained from references [2, 5, 6, 7, 8].

TUMOR CORRECTION

Tumors are increasingly being characterized by their molecular aberrations. Many of these defects involve deletions in molecules important in regulating cell growth, survival, or differentiation, while others lead to the formation of mutant fusion products providing an unwanted gain of function affecting the same critical activities.

Gene therapy could in principle be used to replace an inactive gene with an active one, or to neutralize the behavior of a gain of function mutation. In adults, such efforts have been made, apparently with some success, in the treatment of p53 deficient head and neck tumors and carcinoma of the bronchus [9, 10, 11]. No equivalent pediatric trials have been reported, and formidable problems remain before this approach can be fully exploited. For example, it will be necessary to get a corrective gene into an extremely high proportion of malignant cells, although it has been suggested that there is some form of
uncharacterized bystander effect on nontransduced tumor. Secondly, targeting to metastatic tumor will usually be necessary. Thirdly, correction of a single defect may be inadequate to actually kill the tumor cells, leaving instead a collection of “\(n-1\) cells” (where \(n\) is the number of mutations required for malignancy to occur) capable of undergoing another mutation to restart the malignant process. Finally, since many of the mutations observed are gain of function and/or have a dominant phenotype, introduction of a wild-type gene alone is insufficient. Instead, the mutant gene or its products must be neutralized using strategies that include introduction of ribozymes or antisense RNA or siRNA that are proving troublesome to develop [12, 13].

Hence, exploitation of the tumor correction approach will require significant improvements in vector efficiency and targeting, and until these come to pass, the development of novel rationally targeted small molecules will likely dominate this approach.

**PRODRUG-METABOLIZING ENZYME (PDME)**

Introduction of a gene encoding an enzyme which metabolizes an otherwise inert molecule into a cytotoxic agent has frequently been used in tumor gene therapy. Although the herpes simplex thymidine kinase-ganciclovir system has been most widely applied, there are in fact more than 20 such PDME systems currently in various stages of development and/or clinical trials [14, 15]. For all of these, the concept is that the gene encoding the prodrug-metabolizing enzyme is expressed in the cancer cell, and metabolizes a small molecule to an active moiety which then kills the tumor cell directly. The molecule may also diffuse either through intercellular gap junctions or in the extracellular space and destroy adjacent tumor cells. In this way, transduction of even a small proportion of tumor cells can produce a large bystander effect on adjacent tumor tissue. This in turn compensates for the low efficiency of transduction achieved by currently available vectors and may help to destroy a large tumor burden.

**Pediatric clinical studies of genetic transfer of PDME**

Brain tumors were an attractive initial target for PDME gene therapy. Since the tumors seldom metastasize, the goal of the therapy is the local eradication of the tumor. Hence, the major limitation of PDME, that it requires local inoculation of a tumor with the vector encoding the gene, does not represent a major disadvantage. A number of adult studies have been performed using retroviral and, more recently, adenoviral vectors [16, 17, 18], but to date only one pediatric study has been reported in patients with recurrent or progressive supratentorial brain tumors [19]. Twelve children were enrolled and after tumor resection they were treated with instillation in the tumor bed of retroviral producer cells generating particles encoding HSV-\(tk\) followed by ganciclovir administration. Unfortunately, disease progression was seen in 10 of 11 patients, although one patient remained free of progression for 18 months.

More recently, a study has been performed on patients with retinoblastoma [20, 21], which is also a highly localized tumor that is conventionally treated by enucleation and/or chemoradiotherapy. Enucleation is obviously disabling and deforming, and if the tumor is bilateral it leads to blindness. The alternatives of chemotherapy and radiotherapy are less mutilating but both are associated with secondary malignancies. In a study at Baylor College of Medicine, Hurwitz and colleagues are injecting bilateral retinoblastomas with adenovirus type 5 encoding the thymidine kinase gene, followed by administration of ganciclovir. Two of the first three patients have shown substantial tumor responses with the Ad-\(tk\) and both are disease free, with retained vision, at up-to-one-year post therapy. This trial is now accruing patients with monolateral retinoblastoma.

**Future trends in PDME therapy**

Other suicide gene therapies are being evaluated. Amongst the most developed of these is the cytosine deaminase system, which converts fluorocytosine to fluorourouracil [22]. There are, however, concerns that this suicide system may be less potent than the \(tk\)-ganciclovir prodrug system. Other molecules which metabolize drugs or trigger apoptotic pathways within tumor cells are also being considered. Perhaps the most important future trend is to attempt to enhance the bystander effect. At present, this is mediated predominantly by transfer of the small molecule cytotoxic drug from cell to cell. However, it is apparent that at least part of the bystander effect is dependent on an immune response generated to the lysed tumor. Hence, the bystander effect in immunocompromised animals has been observed to be substantially less than in those with intact immune systems. Investigators are now attempting to combine PDME genes with sequences encoding a variety of immunostimulatory molecules (see section “Gene Modification of The Immune Response”), including but not limited to Interleukin 2, Interleukin 12, and GM-CSF [23, 24, 25, 26, 27, 28]. Data from these studies are yet to be evaluated. Efforts are also being made to generate vectors, which can selectively divide in malignant cells (conditionally replication-competent vectors) and may therefore spread their encoded PDME genes throughout the tumor bed [29].

PDME has also effectively been used as a means of controlling T cell therapies. For example, graft versus host disease may occur when donor T cells are given to patients after allogeneic stem cell transplantation in an effort to treat tumor relapse (graft versus tumor effect) or post-transplant infections. Several groups have infused donor T cells transduced with the HSV-\(tk\) gene and reported successful abrogation of GvHD after treatment with ganciclovir [30, 31]. More recently efforts have been made to induce expression of the death signal, Fas, in donor T cells. An inducible construct is used in which Fas expression
occurs only in the presence of an orally administered small molecule (rapamycin or its analogues) that dimerises two individually inactive components of a Fas transcriptional regulator, leading to expression of the Fas receptor and cell death on exposure to the ligand [32].

As T cell therapies for cancer become more widespread, these suicide mechanisms will become extremely important in ensuring that the regimens are acceptably safe.

**ANTIANGIOGENESIS GENE THERAPY**

Because angiogenesis is a prerequisite for the development of metastatic disease for solid tumors, and probably for leukemias and lymphomas as well, an attack on newly formed blood vessels may help impede the spread of the disease. A number of different large and small molecule inhibitors are currently under study and some of these are suitable for a gene therapy approach [33]. For example, endostatin, a 20-kilodalton fragment of collagen XV111 can efficiently block angiogenesis, but the recombinant protein is difficult and expensive to produce and is somewhat unstable. Delivery of endostatin in murine tumor models by several different vector systems has been able to overcome this limitation and has proved extremely promising [34, 35, 36]. Similarly, angiostatin, a fragment of plasminogen, also functions as a large molecule inhibitor of vessel growth and impedes metastatic tumors. This too can be transferred (eg, by adeno-associated virus vector) to produce benefit in animal models of malignant brain tumors [37, 38].

Much remains to be learned about the most appropriate route and cell of delivery of angiogenesis inhibition, but as with any protein-based therapeutic, gene transfer should allow a continual delivery of the drug rather than the peak and trough concentrations that result from most forms of injection, and may thereby produce a more sustained and effective response.

**CYTOTOXIC DRUG RESISTANCE GENE TRANSFER**

The concept of dose intensification has long been current in modern oncology, including pediatric oncology. In other words, it is believed that giving more of a cytotoxic drug over a longer period will cure a higher proportion of patients. While there are many obvious exceptions to this rule, for many pediatric malignancies it is clear that failure to tolerate chemotherapy in the intended doses correlates well with an increased risk of relapse. For that reason, there is an interest in using genes which will protect normal tissues while leaving malignant cells vulnerable to destruction. By increasing the therapeutic index in this way, it is hoped that more drug can be administered and a higher percentage of patients cured.

There are many different candidate drug resistance genes to be transferred, but perhaps the most widely studied is the human Multidrug Resistance-1 (MDR-1) gene. The gene product acts as a drug efflux pump and prevents accumulation of toxic small molecules, including a range of cytotoxic drugs such as mitoxantrone and daunorubicin. The primary toxicity of many of these cytotoxic drugs is on hematopoietic progenitor cells. Retroviral-mediated gene transfer of drug resistance genes into hematopoietic stem cells has, until recently, been difficult to accomplish. The incorporation of fibronectin together with hematopoietic growth factors into the transduction regimen, together with repeated cycles of gene transfer, has allowed a significant proportion of hematopoietic cells to be protected with expression levels adequate to reduce the sensitivity of these stem cells to chemotherapeutic agents [39]. Several other drug resistance genes are currently under study and may soon join MDR-1 in clinical trial. These include the bacterial nitroreductase gene (which protects against drugs such as thiopeta) [40] and dihydrofolate reductase mutants which protect against methotrexate/trimetrexate [41].

There are two major problems with using transfer of drug resistance genes. The lack of targeting of current vectors means that they may transduce malignant cells as well as normal cells, and therefore increase the resistance of both to the cytotoxic drug. Moreover, while it may be possible to protect a significant proportion of marrow stem cells, secondary toxicities to other organ systems such as skin, lung, and gut will rapidly become evident as doses are escalated because these tissues are much less readily protected than hematopoietic stem cells.

**GENE MARKING AND PEDIATRIC MALIGNANCIES**

The principle of gene marking is the transfer of a unique DNA sequence (eg, a nonhuman gene) into a host cell (eg, T cell, hematopoietic stem cell, etc) allowing the gene or the gene product to be easily detected, thereby serving as a marker for these labeled cells [42].

In all these studies, gene marking is not intended for direct therapeutic benefit, but rather to obtain information regarding the biology and function of adoptively transferred cells.

**Gene marking for autologous stem cell transplantation**

By marking stem cells prior to stem cell infusion, it has been possible to determine if contaminating malignant cells in the stem cell harvest contribute to relapse following autologous stem cell transplant [43]. The hematopoietic stem cell (HSC) product is marked at the time of harvest with murine retroviral vectors encoding the neomycin resistance gene. Then, at relapse, it is possible to detect whether the marker gene is present in the malignant cells. Since 1991, studies have been initiated using this approach in a variety of malignancies treated by autologous HSC transplantation [43, 44, 45, 46] including acute myeloid leukemia (AML), chronic myeloid...
leukemia (CML), acute lymphoblastic leukemia (ALL), neuroblastoma, and lymphoma.

In pediatric patients, receiving autologous BMT as part of therapy for AML, four of twelve patients who received marked marrow relapsed. In three of the four patients, detection of both the transferred marker and the tumor-specific marker in the same cells at the time of relapse provided unequivocal evidence that the residual malignant cells in the marrow were a source of leukemic recurrence [42]. These marking studies also provided information on the transfer of marker genes to normal hematopoietic cells and showed that marrow autografts contribute to long-term hematopoietic reconstitution after transplant [47]. Long-term transfer for more than 10 years has been seen in the mature progeny of marrow precursor cells, including peripheral blood T and B cells and neutrophils [48].

Gene marking of T cells

Several studies have also shown the feasibility of gene marking cytotoxic T lymphocytes (CTL) to track their expansion, persistence, and homing potential to the sites of disease [49, 50, 51]. For example, gene marking of Epstein Barr virus (EBV)-specific CTL for the prophylaxis and treatment of lymphoproliferative disorder posthematopoietic stem cell transplant has demonstrated persistence of gene marked CTL to 78 months post-infusion [52]. In addition, as described below, gene-marked EBV-CTL given as treatment for relapsed Hodgkin disease have been shown to traffic to tumor sites [51].

GENE MODIFICATION OF THE IMMUNE RESPONSE

Rendering the tumor more immunogenic

One of the most striking observations of the past 10 years has been the demonstration that human tumors widely express tumor associated or tumor specific antigens. Moreover, even if these are internal antigens, they may be processed and presented by the tumor cell and become targets for the immune response. These antigens may be particularly prevalent on pediatric malignancies which frequently express oncofetal or developmental antigens not present in the mature child or which may express antigens directly relating to the genetic lesions that have caused the tumor [3, 53].

One of the most commonly used approaches to cancer gene therapy is the attempt to enhance the immunogenicity of these weak tumor antigens and to amplify the scanty T cell precursors capable of recognizing them. An immune response to any antigen has a number of different phases. These include antigen processing and presentation, chemoattraction of T cells to the site of the presented antigen, the costimulation of any T cell which engages the antigen with its specific receptor and the amplification of the immune response so generated. Each of these stages is the primary responsibility of one or more of a range of secreted chemokines and cytokines or of cell-bound receptor-ligand systems. It has become apparent that the forced expression of one or more of these agents within a tumor cell is capable of greatly enhancing the immune response to the weak tumor antigens that cell may express. The immune response so generated may then be effective elsewhere in the body against nontransduced cells. This immunologic bystander effect is an important consideration, since the inefficiency of vectors, currently available, makes the probability of transducing all tumor cells in a patient exceedingly remote. Hence, by transducing even a small proportion of cells, it may be possible to use the efficient targeting mechanisms of the immune system to ensure that the response affects the bulk of tumor cells, including those that were not genetically modified.

Genetic modification of tumor cells

A number of different agents have been successfully utilized in animals, including chemokines such as lymphotactin [54], agents which enhance antigen presentation such as GM-CSF [55, 56, 57], and cytokines that enhance CD4 cell activity (eg, TNF and Interleukin 7) [58, 59], increase expression of class I MHC antigens (eg, gamma Interferon) [60], or amplify T cell responses (eg, Interleukin 2) [61]. Additionally, efforts have been made to express costimulator molecules on tumor cells, including CD40 Ligand [62, 63, 64, 65, 66] and B7.1 [67], or intercellular adhesion molecules such as ICAM 1 and ICAM 3 [68].

Source of cells

The cells used for gene-modified tumor immunotherapy may be derived either from the patient themselves or from an allogeneic cell line grown in culture. Each of these approaches has reciprocal advantages and disadvantages. For example, an autologous cell line, unlike an allogeneic cell line, will almost certainly express the MHC antigens that are present elsewhere in the patient. The heterogeneity of human tumors means that this may not be so for an allogeneic tumor cell line. An autologous cell line will also express these tumor associated antigens in the context of the patient’s own MHC molecules, and so will be recognized by the host immune system. An allogeneic cell line will likely only do this if the antigens on that cell line are taken up by host antigen presenting cells and subsequently presented to the host immune system (cross priming). The clear advantages of allogeneic tumors are that they are much more readily obtained in quantity than autologous tumors, which may be difficult to harvest in adequate numbers to generate a vaccine. Allogeneic cells are also much easier to standardize, since the level of transgene expression will be constant and will not vary from patient to patient. This makes the design and interpretation of clinical trials much simpler. Finally, from a practical point of view, should a tumor vaccine be promising in early phase clinical study, the development of an allogeneic vaccine would be substantially
facilitated, since the material could be manufactured, tested, and stored in bulk rather than generated as an individualized therapy for each patient in a large study. For the moment, it seems reasonable to study both autologous and allogeneic tumor cells and to decide which approach is optimal when more information is available.

**Pediatric clinical studies with gene modified tumor cells**

Neuroblastoma cells were transduced with adenoviral vectors so that they expressed the interleukin 2 gene. Both autologous and allogeneic studies were instituted. In the autologous trial, patients received up to 8 injections of their own tumor cells subcutaneously. More than half the patients produced specific antibody and a specific cytotoxic T cell response directed against the autologous neuroblasts. Of 10 patients, 5 had clinical tumor responses including one complete and one very good partial response [69]. In the allogeneic study, however, the immunizing cell line induced no evident specific immune response [69]. Of note, in both studies a significant number of children showed good tumor responses on subsequent treatment with low dose oral etoposide. This interaction between genetic immunotherapy and low dose chemotherapy has subsequently been observed in a number of adult tumor vaccine studies, and likely represents a genuine interactivity between these treatment modalities that may usefully be exploited for therapeutic benefit in the future.

A subsequent clinical study in neuroblastoma was based upon animal data showing that the combination of lymphotactin (Lptn), a T cell chemokine, and interleukin 2 (IL-2), the T cell growth factor, accelerated and augmented the immune response to a neuroblastoma cell line [54]. Accordingly, patients received either an autologous vaccine or an allogeneic one expressing both IL-2 and Lptn [71]. In the allogeneic group, it was possible for the first time to observe specific antitumor immune responses to the immunizing cell line, and two patients entered complete remission, which was durable in one. In the autologous study, the results did not appear to be measurably superior to interleukin 2 alone. Hence, in the allogeneic setting at least, there is preliminary evidence that the combination of two agents acting at different phases of the immune response may be superior to a single agent. If these results are confirmed they may increase the feasibility of utilizing allogeneic vaccines with the considerable simplification in protocol development that would result.

In hematologic malignancy, a Phase I study of autologous acute myeloblastic leukemia cells engineered to secrete GM-CSF has recently commenced and this study is now being extended to pediatric AML [57].

It has also proved feasible to express costimulator molecules such as CD40, CD40 Ligand, or B7.1 on primary tumor cells surfaces. We are currently using a combination of CD40 Ligand and IL-2 gene transfer into pediatric acute lymphoblastic leukemia cells in an effort to generate an antitumor immune response in patients with high-risk disease who have entered remission. To date, this study has proved to be safe and has generated antileukemia immune responses. Because these patients are treated in remission, we do not yet know whether there has been any antileukemia activity.

In conclusion, therefore, genetic modification of tumor cells appears safe and is capable of generating specific humoral and cellular antitumor cytotoxic responses. There have been at least some tumor regressions and the approach is now being evaluated in a wider range of tumors and in a larger number of patients.

**PEDIATRIC CANCER THERAPY WITH GENE MODIFIED T CELLS**

**Prophylaxis and treatment of Epstein Barr virus (EBV)-associated posttransplant lymphoproliferative disorder (EBV-PTLD)**

Several studies have suggested the feasibility and apparent clinical efficacy of adoptive transfer of cytotoxic T-cells (CTL) directed at viral or tumor antigens [50, 72, 73, 74]. By using gene-marked cells in these studies, it has not only been possible to determine the survival and homing of the infused T-cells, but also determine if they mediate adverse effects such as GvHD [75, 76].

For example, Epstein Barr virus (EBV)-associated posttransplant lymphoproliferative disorder (PTLD) is a complication due to proliferation of EBV-infected B cells and occurs in 5–30% of patients receiving T-depleted marrows from mismatched family or unrelated donors. Several groups have investigated the feasibility of generating donor-derived EBV-specific CTL to treat this disorder [77, 78, 79, 80]. Our group generated EBV-specific T cell lines from donor lymphocytes and used them as prophylaxis and treatment for EBV-PTLD in patients post HSCT [77]. Over a 7-year period, 56 pediatric patients who received a T cell-depleted HSCT were given EBV-CTL prophylactically. The first 26 patients enrolled on to the study received CTL marked with the neomycin resistance gene. None of the 56 patients who received the EBV-CTL developed PTLD compared with an incidence of 11.5% in a comparable group who did not receive CTL [81]. Using conventional PCR and real-time PCR, the marker gene was identified in the peripheral blood for at least 78 months post CTL. [82]. Three patients who declined or were ineligible for our prophylaxis study were treated for established EBV lymphoma. The EBV-specific CTL therapy induced sustained remission in 2 patients, but the third patient treatment failed and was found to have an antigen-loss mutant in her EBV lymphoma cells [83].

These studies are now being extended to patients receiving solid organ transplants. Pediatric populations are particularly susceptible to PTLD after solid organ transplant, because children are much more frequently EBV-seronegative at the time of transplant [84].
Adoptive immunotherapy for EBV-positive Hodgkin disease

EBV-positive lymphoma cells in posttransplant lymphoproliferative disease, express a wide range of EBV encoded antigens and are readily susceptible to immunotherapy. What of the malignant cells of Hodgkin disease and Nasopharyngeal cancer, which express a more restricted pattern of antigens? More than 80% of children with EBV-associated Hodgkin disease can be cured, but treatment for those who relapse is limited. Moreover, long-term follow-up studies of Hodgkin disease survivors show greatly increased risks of second malignancy [85]. Nonfatal sequelae of therapy, such as altered somatic growth, infertility, and restrictive lung disease can also seriously affect the quality of life of the survivors [86]. It is therefore desirable to develop novel therapies that could improve disease-free survival in relapsed/refractory patients and reduce long-term complications.

We have treated 13 patients with EBV+ Hodgkin disease using EBV-specific CTL. Five patients with minimal residual disease postautologous bone marrow transplant remain well for 2–21 months post CTL infusion [87], and mixed tumor responses in 6 patients. Of 8 patients treated with active disease, injection of EBV-specific gene-marked CTL showed gene-marked CTL within tumor [51] and in peripheral blood for up to 9 months following infusion [87].

Future trends in the development of gene-modified CTL

Although these results have been promising and there have been tumor responses, these have been partial, or often transient, and no patient with aggressive relapsed Hodgkin disease has been cured. This may be due to a lack of specificity of the EBV-specific CTL for the immunosubdominant LMP1 and LMP2 antigens that are all present on the Hodgkin tumor. In addition, the tumor secretes immunosuppressive cytokines and chemokines which affect CTL function and antigen presenting cell activity [88]. Gene transfer can be used to overcome both types of problems. By using dendritic cells transduced with adenoviral vectors encoding LMP2, it has proved possible to generate CTL that have high cytolytic activity in vitro to LMP2-positive targets when compared to conventional EBV-CTL [89, 90].

Although such specific cells may be more effective, there is a concern that the CTL will remain vulnerable to the immunosuppressive cytokines secreted by the Hodgkin Reed-Sternberg cell. The cytokine, which has the most devastating effects on CTL proliferation and function, is transforming growth factor-beta (TGFβ) [91, 92]. This cytokine is secreted by a wide variety of childhood tumors, and allows the tumor to escape the immune response [93]. To overcome this capacity to inhibit the EBV-CTL, we transduced CTL from patients with relapsed EBV-positive Hodgkin disease with a retrovirus vector expressing a dominant-negative TGFβ type-II receptor (DNR). This prevents formation of the functional trimeric receptor. Cytotoxicity, proliferation, and cytokine release assays showed that exogenous TGFβ had minimal inhibitory effects on DNR-transduced CTLs [94]. This combination of tumor-specific and tumor-resistant CTL may prove highly effective for therapy.

Adoptive immunotherapy for EBV-positive Nasopharyngeal carcinoma

Despite the good overall survival rates following conventional therapy for this disease in children, follow-up reports have shown substantial longer-term treatment-related morbidity and mortality [95, 96], including growth hormone deficiency, hypothyroidism, pulmonary fibrosis, and secondary malignancies [96, 97]. Although EBV-CTL have been used in this disease, [98] with limited success, we are using the same approaches described for Hodgkin disease to treat these tumors as well.

Chimeric T cells for tumor therapy

Primary T cells genetically modified to express chimeric receptors derived from antibodies and specific for tumor or viral antigens have considerable therapeutic potential. Chimeric T cell receptors allow the recognition specificity of T lymphocytes to extend beyond classical T cell epitopes by transducing cells with genes that encode the variable domain of a tumor-specific monoclonal antibody (MAb) (ScFv) joined to a cytoplasmic signaling domain. This strategy can therefore be applied to every malignancy that expresses a tumor-associated antigen for which an MAb exists [99, 100]. Unlike conventional T cell receptors, these chimeric receptors will be active even if the tumor cells are class-1-MHC negative.

Neuroblastoma is the commonest extracranial solid tumor of children, and is often resistant to conventional treatments. A high proportion of tumors express tumor-associated antigens such as GD2, L1-CAM, and N-CAM. CD8+ve CTL clones genetically modified to express the CE7R chimeric immunoreceptor which consists of an extracellular L1-CAM-specific single-chain antibody, transmembrane CD4, and T cell CD3-complex zeta chain, is currently being investigated in a clinical trial [101]. However, chimeric receptor signaling produces only limited activation of the T cells, and we are currently exploring an alternative approach to increase the in vivo functionality of the cells [102, 103]. We have transduced EBV-specific (not primary) T cells with GD2-specific chimeric receptor genes. In vitro, we have shown that these cells can be expanded and maintained long term in the presence of EBV-infected B cells. While they recognize EBV-infected targets through their conventional T cell receptor and thereby become activated, they are also able to recognize and lyse tumor targets through their chimeric receptors. Several cycles of virus target → tumor target → virus target can be demonstrated ex vivo, implying that EBV-specific T cells expressing chimeric antitumor receptors may represent a new source of effector cells that would
persist and function long term after their transfer to cancer patients [104].

CONCLUSION

We have far to go before gene therapy of pediatric malignancy can truly be said to have made a major impact on these diseases. Nonetheless, over the past decade, these new techniques have produced unequivocal tumor responses even in advanced disease. As we continue to make incremental advances in the application of these approaches, we can expect to see gene therapy increasingly supplement and perhaps even eventually supplant conventional cancer therapeutics.

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* Corresponding author.
E-mail: mbrenner@bcm.tmc.edu
Fax: +1 832 825 4668; Tel: +1 832 824 4671
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