Clin Exp Med (2003) 3:4–14

REVIEW

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Gene therapy for hematological malignancies

Received: 10 February 2003 / Accepted: 24 February 2003

Abstract Since cancer is the result of genetic mutations, it should be well suited for correction through gene therapy. Hematological malignancies in which human gene transfer has been performed are leukemias, lymphomas, graft-versus-host disease after allogeneic bone marrow transplantation in leukemia, and multiple myeloma. Gene therapy may be used to induce or enhance an antitumor immunological reaction, to correct a genetic defect in the tumor cells, to render the malignant disease more susceptible to conventional therapies, to make the normal host cells more resistant to conventional therapies, or to track cells used for therapy. Gene therapy will probably be most valuable for the eradication of minimal residual disease after the use of conventional therapies.

Key words Review • Gene therapy • Clinical trials • Hematological malignancies

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Introduction

The number of gene therapy clinical trials continues to grow. In November 2002, 636 protocols have been reported, the majority (about 80%) being performed in the United States. About 70% of all the 3,500 patients participating in gene therapy trials world-wide are being treated for cancer (http://www.wiley.co.uk/genmed; The Journal of Gene Medicine Clinical Trials Database). A relatively small number of protocols deals with gene therapy of hematological malignancies. In vivo administration of vectors is more practical and economical, but in hematological malignancies in vitro gene therapy is presently used more frequently because vectors highly selective for tumor cells are still not available.

There have been promising results when applied to in vitro cell cultures and to animal models, but clinical applications have been hindered by several factors. Hematological malignancies are often systemic. This makes in vivo administration of vectors only possible if the vectors are highly selective for tumor cells. The ex vivo methods of gene transfer require specialized cell processing facilities and the transfer efficiencies have to be optimized for most hematological cells. In general, malignant cells have a variety of genetic changes, making correction of the defects difficult. However, hematological malignancies also have advantages concerning gene transfer approaches. Leukemic cells circulate in the blood, thus large numbers of tumor cells can be harvested and sorted for manipulation ex vivo. The efficiency of transduction can easily be monitored in vitro and simple blood tests can be used to monitor, for example, expression of the transgene. Gene therapy is a promising approach to treat hematological malignancies because the molecular basis of these disorders is increasingly understood, therapeutic genes are available, and alternative therapies are often lacking (as for multiple myeloma). The cure rates for leukemia and lym-

phoma are relatively high, but treatments remain toxic, expensive, and ineffective for some patients. These conditions afflict a substantial number of individuals, often in their most-productive years.

Gene therapy may improve the body's ability to fight cancer or make the cancer more sensitive to chemotherapy. It may be used to induce or enhance an antitumor immunological reaction, to correct a genetic defect in the tumor cells, to render the malignant disease more susceptible to conventional therapies, to make the normal host cells more resistant to conventional therapies, or to track cells used for therapy. Hematological malignancies in which human gene transfer has been performed are leukemias, lymphomas, graft-versus-host disease (GvHD) risk after allogeneic bone marrow transplantation (BMT) in leukemia and multiple myeloma. Four gene therapy approaches have been intensively studied in cancer therapy: (1) immunomodulation/tumor vaccines, (2) molecular chemotherapy (suicide genes, gene therapy against drug resistance of tumor cells), (3) introduction of genes triggering apoptosis or reverting malignancy, (4) inactivation of products of oncogenes.

Gene transfer methods into cells

In general, gene therapeutic approaches require efficient gene transfer into host cells and sufficient transgene expression. Although many methods of gene transfer into mammalian cells exist, most do not allow efficient DNA transfer into primary lymphocytes. In contrast to gene transfer into tumor cells and many other cell types, which can be performed using a variety of methods, the efficient expression of foreign DNA in lymphoma cells presents a problem. Improvements in retroviral vector technology make hematopoietic stem cell transduction a distinct reality. Gene therapy vectors can be classified as viral and non-viral vectors. Viral vectors have practical advantages and generally potent gene transfer efficiency, although they can exhibit some harmful antigenicity and/or risks of acquiring novel infectious particles. Of the viral vectors, by far the most important are the human adenoviruses, as reflected by the enormous number of data accumulated [1].

The treatment-related death of a patient with ornithine transcarbamylase deficiency following adenovirus administration by hepatic artery has led to serious concerns regarding the safety of intravascular adenovirus. However, over 100 cancer patients have been treated with intravascular adenovirus constructs to date with an acceptable toxicity profile [2]. Two cases of induced leukemias in a X-severe combined immunodeficiency trial involving a retroviral vector are currently under investigation [3]. Recently, for safety and practical reasons, the efforts to develop efficient non-viral vectors have increased significantly [4].

Manipulating hematopoietic stem cells

Although gene transfer into hematopoietic stem cells holds a considerable therapeutic potential, clinical trials targeting this cell compartment have achieved limited success. Poor transduction efficiency with gene transfer vectors used in human studies has hindered delivering therapeutic genes to clinically relevant numbers of target cells. One way to overcome the low-efficiency problem is by selecting or expanding the number of genetically modified cells to a suprathreshold level to achieve clinical efficacy [5].

Because of unstable transgene expression, efficient gene transfer to hematopoietic progenitor cells does not ensure long-term gene expression. It would therefore be advantageous if the expression of transgenes could be restored in bone marrow. Transfer of drug resistance genes such as the multidrug resistance (*MDR1*) or mutated dihydrofolate reductase (*DHFR*) genes to hematopoietic cells protects them from the toxicity of anticancer drugs. In addition, transduced cells obtain a selective growth advantage in the presence of anticancer drugs. This can be used to introduce and enrich otherwise non-selectable genes by cotransfer to target cells. With the use of bicistronic vectors, expression and function of therapeutic genes have been increased in tissue culture and in animal models [6].

Hematopoietic stem cells are being increasingly manipulated in order to enhance their therapeutic potential. For example, human immunodeficiency virus (HIV)-infected patients with acute or chronic leukemia, multiple myeloma, myelodysplastic syndrome, Hodgkin's or non-Hodgkin's lymphoma are rarely offered BMT because they are at increased risk of dying from the intense chemotherapy and radiation therapy used for the procedure. A recent trial uses a modified procedure, designed to be less dangerous for such patients. A laboratory manufactured gene designed to obstruct HIV reproduction is inserted into the stem cells, rendering future cells that develop resistance to the virus (protocol number 99-DK-0167). For a review about gene transfer into human stem cells see reference [7].

GvHD versus graft-versus-leukemia – Suicide gene therapy

Clinical data indicate that after allogeneic hematopoietic stem cell transplantation for hematological malignancies, the graft-versus-leukemia (GVL) effect is in large part mediated by the graft-versus-host reaction (GvHR), which also often leads to GvHD. Controlling alloreactivity to prevent GvHD, while retaining GVL, poses a dilemma for the successful treatment of such malignancies. Suicide gene therapy might selectively eliminate alloreactive T cells while sparing non-alloreactive T cells, which can then contribute to immune reconstitution [8].

The suicide gene approach to gene therapy involves the transfer of a gene into cells that can later be used to induce cell death when the cells are no longer needed or are having a deleterious effect on the host. The gene most frequently used is the herpes simplex virus thymidine kinase (*HSVtk*) gene. Cells transfected with the *HSVtk* gene are sensitive to gancyclovir and can be killed by exposure to this drug. This technique has been applied in allogeneic BMT or donor T cell infusion therapy after BMT, as a method of potentially controlling GvHD. Retroviral-mediated gene transfer of the HSV gene into donor T lymphocytes before allogeneic stem cell transplantation was studied in patients [9]. Donor cells may be infused following BMT to induce a GVL response in the case of a relapse or to induce a graft-versus-lymphocyte response in the case of Epstein-Barr virus (EBV)- induced B cell lymphoproliferative disease following transplantation. The aim is to induce an adequate GVL response without grave GvHD. Using T cells transfected with the *HSVtk* gene, one could potentially treat the patient with ganciclovir when the GvHD becomes a problem, killing the lymphocytes inducing GvHD [10].

Immunomodulatory gene therapy

Tumor vaccines

The genetically modulated cancer vaccine represents a classic ex vivo strategy involving the transfection of cytokines or other immunomodulatory factors into cultured, lethally irradiated tumor cells in an effort to stimulate an immune response when the engineered cells are reinjected into the recipient, usually into the skin.

In human hematological malignancies one problem is that the primary malignant cells are highly resistant to transduction by most available vectors [11]. Another problem is that the neoplastic cells of hematological cancers show considerable phenotypic heterogeneity, so that vaccines made from a small proportion of these cells, obtained from a single site, may not express the full array of antigens present in the tumor cell population as a whole [12]. A possible side effect would be that the immune response itself could produce an adverse hypersensitivity reaction [13].

For tumor vaccines, vectors require the following features: broad range of tumor cells transduced, high level of gene expression, transient expression adequate for autologous tumors, and long-term expression for allogeneic vaccine lines. Suitable presently available vectors include adenovirus, herpes virus, liposomes, and retroviral vectors for allogeneic cell lines [14].

The ultimate value of tumor immunogens will likely be realized in strategies combining several different immunostimulatory genes representing different components of the immune response. In murine studies, for example, combinations of the T cell-attracting chemokine lymphotactin and the T cell growth factor interleukin-2 (IL-2) can cause regression of pre-established leukemia and neuroblastoma, even when either agent alone is inadequate [15]. The recent availability of vectors capable of transducing primary human leukemic cells with high efficiency will make it possible to test the combined approach in patients with hematological cancers.

Antigens used in gene therapy include CEA, gp100, HLA-B7, HLA-B7/b2m, MART-1, MUC-1, PSA, HLA-A2, HLA-B13, H-2K(k), and others (Wiley Journal of Gene Medicine/ Clinical Trial Database, 2003; http://www.wiley.co.uk/) [16]. For a recent review on the findings emerging from preclinical studies on genetic vaccination for the treatment of cancer we refer to reference [17]. In most clinical trials of tumor vaccines, immunological responses have been found. However, clinical responses have been rare.

Dendritic cells

Dendritic cells (DC), the major class of antigen-presenting cells, are capable of providing all of the signals necessary to activate a de novo immune response. DC are found in the skin and mucous membranes (Langerhans' cells), lymphoid tissue, bone marrow, and circulating blood. Once these cells encounter a potential antigen, they interact with T and B cells to induce the immunological response cascade that is manifest as acquired cellular and humoral immunity. Small numbers of human DC may be isolated directly from blood, skin, or tonsillar tissues. However, considerable numbers of functional DC can be derived in vitro from both CD14+ cells (monocytes) and from CD34+ cells (hematopoietic progenitor stem cells) using appropriate cytokine cocktails. Such DC isolation methods have made it possible to prepare large numbers of DC directly from blood in order to arm them with specific antigens, and return the armed cells to patients. For a review on generation of DC-based vaccines for cancer gene therapy we refer to reference [18].

In a preclinical study, transfection of DCs with the MHC class II transactivator gene *CIITA* led to an increase in antitumoral immunostimulatory capacity of DCs [19].

DC-based cancer vaccines have resulted in long-lived tumor regression and antitumor immunity, although the number of patients treated in each trial was small. Currently, many efforts are being directed towards targeting tumor antigens/genes directly to the ex vivo propagated DCs. The basis for these therapeutic options is the critical role of T cells and additional ways to initiate and to sustain an optimal antitumor T cell response. Clinical phase I/II trials are still at an early stage, but nonetheless have shown some interesting results [20].

Gene-modified cytotoxic T cells

Cytotoxic T lymphocytes (CTL) recognize processed intracellular proteins presented as short peptide fragments (together with MHC molecules) on the cell surface. Therefore, internal proteins unique to the malignant clone may act as tumor-specific antigens for CTL. Hematological malignancies may express a number of tumor-specific proteins, such as immunoglobulin or T cell receptor idiotypes, mutated oncoproteins, or fusion proteins generated by chromosomal translocation, or viral proteins [21, 22].

In one trial, EBV-specific CTL lines were adoptively transferred to patients after marrow allografting to assess the safety and efficacy of this treatment against EBV immunoblastic lymphoma [23]. Since T cells were first marked with the neomycin resistance gene (*neoR*) before they were injected, it was also possible to determine the cell distribution pattern, longevity, and antitumor activity in vivo. In two striking cases, the treatment eliminated biopsyproven immunoblastic lymphoma [24].

One way of enhancing the tumor-specific activity of CTL is to increase the level of cytotoxic cytokines [e.g., tumor necrosis factor (TNF)] they produce at local tumor sites. This approach is being evaluated in studies with tumor-infiltrating lymphocytes (TIL) [25]. This strategy has several inherent difficulties, one being that TILs resist attempts to elicit increased production of cytokines. Nonetheless, a sustained response was noted in one of the first six patients whose TILs secreted transgenic TNF [24]. Genetic modification of peripheral T cells with the *DN-TGF-βR* gene (dominant negative-transforming growth factor-β receptor), a receptor for an immunosuppressive cytokine found at the site of most tumors, enhanced antitumor immunity in mice [26].

More recently, interest has increased in transducing T cells with antibody molecules that become linked to the gamma or zeta chain of the T cell receptor, so when antigen is engaged, the T cell is activated and – hopefully – kills the target cell to which it is bound [27]. For a review on T cell receptor gene therapy we refer to reference [28].

Cytokine gene therapy

While administration of recombinant cytokine(s) can produce to some extent antitumor effects or immunological changes in patients, the toxic reactions preclude the use of large amounts of the recombinant proteins. Therefore, researchers attempted to transfer cytokine gene(s) into tumors and to secrete the cytokine into the vicinity of tumors. Induction of tumor-specific CTLs and generation of potent cytotoxicity are the purpose. It is important in the concept of cytokine gene transfected cancer vaccines that cytokines are produced at high concentrations local to the tumor cell. Thus, proinflammatory side effects of systemic administration can be avoided. Furthermore, the paracrine way much more closely resembles the natural cytokine physiology. More than 60% of recent cytokine-supported clinical cancer gene therapy trials use IL-2 [29]; in others, IL-4, -7, and -12, interferon (IFN)-γ; granulocyte/macrophage colony-stimulating factor (GM-CSF) [30], or TNF are administered individually or in combination [31]. In a physiological immune response, cytokines are not produced singly, but in succession, and multiple cytokines may be required to ensure an effective response. Several studies have reported greater therapeutic activity using vaccines consisting of tumor cells transduced with multiple genes when compared with single gene vaccines [32].

Results of preclinical studies in human myeloma cell lines suggest potential utility of tricistronic viral vectors co-expressing IL-12 and CD80 (B7-1) [33]. For a review on GM-CSF as part of vaccine strategies we refer to reference [34]. For a review on immunomodulatory gene therapy of hematological malignancies in general we refer to reference [35].

Drug resistance gene therapy

Resistance to therapeutic agents remains a significant obstacle to overcome in order to improve the outcome of hematological malignancies. Despite the majority of patients showing a good response to induction chemotherapy, as the disease progresses most patients become resistant to therapy and classically cross-resistance to a number of classes of drugs is noted. Emerging new therapies for chemotherapy resistant cancer using adenoviral vectors are reviewed by Nemunaitis and Cunningham [36].

A better understanding of the molecular events responsible for the development of drug resistance in cancer cells has emerged in recent years. It is now established that tumor cells can acquire drug resistance by alterations of pathways involved in the regulation of apoptosis and that failure to activate this pathway in cancer cells may confer resistance to chemotherapy. This resistance to drug-induced apoptosis is likely to play an important role in tumors that are refractory to chemotherapy. The identification of points in the apoptotic pathway at which dysregulation occurs opens up new therapeutic opportunities in situations where conventional cytotoxic chemotherapy approaches fail. Although these gene therapy based strategies are still in their infancy, they will likely lead to more-effective treatments for human cancers [37].

Induction of tumor cell apoptosis

Components of the signaling network of apoptosis (programmed cell death), which include ligands such as CD95,

TNF, and TNF-related apoptosis-inducing ligand, as well as downstream molecules, such as caspases, Bcl-2 family members, and inhibitor-of-apoptosis proteins, which trigger and regulate apoptosis, are important targets for the development of cancer gene therapy.

The introduction of tumor suppressor genes, such as *p53*, into tumor cells can result in apoptosis (programmed cell death) and block tumor growth. Additionally, the introduction of *p53* into tumor cells is synergistic with chemotherapeutics such as cisplatin. Also another tumor suppressor gene, *BRCA1sv*, was used in clinical studies [38].

Gene therapy combined with chemotherapy/chemoprotection

One of the main barriers to more-efficacious use of modern chemotherapeutic agents is the collateral toxicity exhibited in normal, highly proliferative tissues, primarily the hematopoietic, gastrointestinal, and pulmonary tissues. Drug resistance compounds this problem. A gene therapy approach combines two strategies to confer chemoprotection to vulnerable tissues whilst sensitizing malignant tissues. O6-alkylguanine-DNA alkyltransferase (ATase) can confer protection against O6-alkylating agents. Mutant forms of ATase are resistant to the effects of soluble analogues of O6-alkylation such as O6-benzylguanine [39].

If hematopoietic stem cells could be rendered resistant to cytotoxic drugs, it may enable them to resist the myelosuppressive effects of cytotoxic drugs during cancer therapy. Because drugs could be given in higher doses for longer periods, more patients might be cured of their malignant disease.

Various drug resistance genes have been shown to protect hematopoietic stem cells in animal models. These include the multidrug resistance 1 gene (*MDR1*), dihydrofolate reductase (*DHFR*), and methylguanine methyltransferase (*MGMT*). Several clinical trials have evaluated the feasibility of hematopoietic protection using MDR1-expressing vectors in adult cancer patients [40].

Protocols are attempting to enhance marrow protection during chemotherapy by transducing bone marrow or peripheral blood-derived stem cells with the multiple-drug resistance gene (*MDR1*).

Dihydrofolate reductase (DHFR) is an enzyme that is protective against methotrexate toxicity. Mice reconstituted with DHFR gene-modified bone marrow cells were subsequently shown to be protected against trimetrexate-induced neutropenia and reticulocytopenia [41].

A number of drug resistance genes, whose products include mutant forms of enzymes that confer resistance to chemotherapeutic drugs, are discussed in a review by Banerjee and Bertino [42].

These strategies have the possible risk of transferring drug resistance genes to neoplastic cells, which inevitably contaminate the stem cell graft and produce a drug-resistant relapse. It may be that higher doses of chemotherapy may not translate into higher response rates, also toxic side effects may be dose-limiting in organs that are not reached by the gene therapy.

Oncolytic viruses

Although the concept of using viruses as anti cancer agents is not new, recent advances in molecular biology, genetics, and virology have enabled investigators to engineer viruses with greater potency and tumor specificity. Further enhancements involve arming these viruses with therapeutic transgenes, and combining the traditional modalities of chemotherapy and radiation therapy with oncolytic viral therapy in the hope of reducing the chance of developing resistant tumor cell clones. Another approach to augmenting the antineoplastic effect of these viruses involves modulating the immune response to minimize antiviral immunity, while at the same time maximizing antitumor immunity. A better understanding of mechanisms that viruses use to overcome cellular defenses to achieve robust replication within the cell will lead to the development of oncolytic viruses with better tumor specificity and reduced toxicity. Initial clinical studies have shown that oncolytic viral therapy for metastatic disease is safe and well tolerated. In addition, using similar genetic modification strategies, these viruses have demonstrated antineoplastic effects in humans similar to those seen in preclinical animal models [43].

Attenuated measles virus (MV) has therapeutic potential as a replicating oncolytic virus in models of non-Hodgkin's lymphoma and myeloma. At least in vitro, MV has intrinsic specifity to lyze transformed cells [44, 45].

Reoviruses infect and kill cancer cells with an activated *Ras* or *Ras*-signaling pathway, while sparing normal cells [46]. They have been shown to effectively destroy many different types of neoplastic cells, including lymphoid malignancies [47]. Mutants of human adenovirus 5 (Ad5) with enhanced oncolytic activity were isolated by using a procedure termed bioselection [48].

Both replication-incompetent (rAd.p53, e.g., SCH58500) and replication-selective (dl1520, Onyx-015, CG7870) oncolytic adenoviruses, by intravascular administration, have been tested in clinical trials [2].

The current and potential clinical applications/limitations are discussed for oncolytic viruses from the herpesvirus, adenovirus, picornavirus, rhabdovirus, and paramyxovirus families in the reviews by Bell et al. [49] and by Ring [50].

Adhesion molecules

Changes in the myeloma cell adhesion molecule profile correlate with the egress of tumor cells into the peripheral blood in progressive disease and plasma cell leukemia [51].

Gene marking

Marker trials have been initiated that use two neo gene markers (distinguishable by size) in two related retroviral vectors in each patient. By doing this, marrow purging versus no purging, or two different purging techniques, can be compared in the same patient [52]. Double marking is also used to determine reconstitution of ex vivo-manipulated hematopoietic stem cells. Although gene marking provides no immediate beneficial effects to the patient, much of the information gained from these trials is valuable for improving therapies that use autologous stem cell transplantation as a method for eradicating the tumor.

Antiangiogenic gene therapy

Targeting angiogenesis represents a new strategy for the development of anticancer therapies [53]. Increased angiogenesis in human myeloma bone marrow has been observed. Low microvessel density has been identified as a favorable prognostic feature [54, 55]. Anti-angiogeneic drugs such as thalidomide and vascular endothelial growth factor inhibitors are in clinical trials.

Antisense oligonucleotides

The use of antisense oligonucleotides aims to block target proteins specifically. Antisense oligonucleotides are short DNA or RNA fragments consisting of a sequence complementary to a mRNA. When such an oligonucleotide is introduced into/synthesized in a cell it forms a double strand together with the mRNA, blocking the reading of the mRNA at the ribosomes. The complexes are rapidly destroyed by RNAse H. Hence the production of a specific gene product/protein can be blocked.

Gene therapy, such as *bcl-2* antisense oligodeoxynucleotide G3139 (G3139), may make cancer cells more sensitive to chemotherapy drugs. Combining more than one drug with G3139 may kill more cancer cells. Patients with refractory or relapsed acute myeloid leukemia or acute lymphoblastic leukemia are treated with G3139 plus fludarabine and cytarabine (http://clinicaltrials.gov/ct/; database by the National Institutes of Health). Various other oncogenes (among them c-*raf*-1, proteinkinase-C, and H-*ras*) are being studied as targets for antisense oligonucleotide therapy in clinical phase I and II trials.

Manipulation of telomeric activity

Reactivation of telomerase maintains telomere function and is considered critical for immortalization in most human cancer cells. Elevation of telomerase expression in cancer cells is highly specific: transcription of both RNA (hTR) and protein (hTERT) components is strongly upregulated in cancer cells relative to normal cells. Therefore, telomerase promoters may be useful in cancer gene therapy by selectively expressing suicide genes in cancer cells and not normal cells. One example of suicide gene therapy is the bacterial nitroreductase (*NTR*) gene, which bioactivates the prodrug CB1954 into an active cytotoxic alkylating agent [56].

In hematopoietic carcinogenesis, gene expression for suppressors of telomeric activity, such as TRF and TIN2, is decreased. Although mechanisms regulating telomere length are poorly understood in human hematopoietic cells, these genes might hold promise for gene therapy [57].

RNA therapeutics

RNA-based therapeutics that can inhibit gene expression, block protein function or induce potent immune (CTL) responses have all entered clinical trials. Several phase I and II clinical trials have been initiated using a class of therapeutic RNAs called trans-cleaving ribozymes, which target pathogenic RNA for destruction, in a small number of patients with cancer. In these studies the ribozymes have been delivered to the patients either by gene therapy methods or by direct injection of a synthetic ribozyme. Recently, trans-splicing ribozymes have been generated that can amend mutant transcripts associated with many cancers (mutant p53 tumor-suppressor transcripts) in mammalian cell lines [58].

Transfecting DCs with mRNA-encoding (tumor) antigens is yet another way to load DCs with antigens [59]. mRNA can be isolated directly from tumor cells or synthesized in vitro from complementary DNA templates. DCs transfected with mRNA-encoding specific antigens or total tumor-derived RNA elicited potent CTL responses and tumor immunity in mice [60], and DCs generated from healthy volunteers or from cancer patients transfected with tumor RNA stimulated CTL responses in culture [61]. Biologically active RNA can be amplified from microscopic amounts of tumor tissue to provide a virtually inexhaustible amount of antigen from practically every patient. Cancer vaccination with tumor RNA-transfected DCs may consti-

tute a highly effective and broadly applicable treatment for patients with recurring cancer. For a review on clinical applications of RNA we refer to reference [62].

Treatment of cachexia

Cachexia is a common manifestation of late stage, also of hematological, malignancy and is characterized by anemia, anorexia, muscle wasting, loss of adipose tissue, and fatigue. To date, there are no effective therapies. In dogs, intramuscular injection of a growth hormone-releasing hormone-expressing plasmid resulted in anabolic responses without adverse effects [63].

Gene therapy for leukemia

BMT has improved the prognosis of patients with chronic myeloid leukemia (CML), but not all patients are suitable for BMT. In those patients who undergo BMT, the 3-year disease-free survival is only around 65%. Therefore, other therapies are needed. Genetically modified autologous CML cells may be used to enhance the T cell response. It is likely that T cells present in most CML patients are anergic to autologous CML cells because of the lack of appropriate costimulatory molecules, such as the B7.1 molecules on leukemic cells. Therefore, T cell recognition of leukemic cells only leads to partial T cell activation, with subsequent development of anergy. T cell activation may be achieved using genetically manipulated irradiated leukemic cells, which are now equipped with costimulatory molecules and IL-2 as stimulator, and any T cell clones resulting will react with the native CML cells, since T cell recognition does not need either IL-2 or costimulatory molecules. The advantage of this approach is that the response, which will probably be polyclonal, can be achieved without knowledge of the leukemic antigen. Unfortunately, gene transfer efficiency is extremely low in CML, due to the low number of cells in the cell cycle [64]. For a review on gene therapy for patients with indolent B cell malignancies we refer to reference [65].

In B cell chronic lymphocytic leukemia (B-CLL), T cells do not generally develop a clinically significant response against B-CLL cells. Buhmann et al. [66] have shown that CD40 activation of B-CLL cells might reverse T cell anergy against the neoplastic cell clone, although the character of the immune response depends on the MHC background on which the CLL or tumor antigens are presented. It is feasible to transfer a gene into the leukemia cells that encodes a stable and active form of CD154 that can trigger activation of leukemia cells via its interaction with CD40. This approach has the potential for not only activating the leukemia cells that have taken up the CD154 gene but also bystander leukemia B cells that have not. The results of a phase 1 study of Ad(adenovirus)-CD154-infected CLL cells in the treatment of patients with CLL were encouraging. Changes in the bystander leukemia cells in vivo and significant increases in the numbers of circulating T cells were observed. These results have stimulated the design and implementation of phase II trials that examine the effects of administering multiple doses of Ad-CD154-infected autologous leukemia cells [67]. Infusion of Ad-CD154-transduced leukemic B cells was well tolerated by the patients, and biological and clinical responses were observed. Furthermore, preliminary data suggest that this approach can enhance antibody dependent cellular cytotoxicity and thereby augment the activity of antitumor monoclonal antibody therapy [68].

In the treatment of children with acute lymphoblastic leukemia (ALL), an alternative therapeutic approach is required for children with very early bone marrow relapse or T cell relapse, especially when finding a suitable donor for allogeneic BMT is a problem. Borgmann et al. [69] proposed inducing a specific cellular immune reaction after autologous BMT by vaccination with human leukocyte antigen (HLA) B13-transfected autologous leukemic cells in children with a high risk of relapse of ALL. Specific CTL have been expanded in vivo by low-dose IL-2 [69].

Gene therapy for multiple myeloma

Multiple myeloma (MM) is an incurable illness with a median survival of about 30 months. Many patients will have primary refractory or relapsing disease in whom survival is short despite all current therapies [70]. Gene transfer is increasingly applied in MM. Most of the gene therapy strategies currently investigated in MM are based on immunotherapy, since myeloma cells express tumor-associated antigens (TAAs) and both allogeneic and autologous immune responses have been shown [71].

Studies aimed at enhancing the immunogenicity of myeloma cells apply ex vivo transfection and readministration of irradiated autologous myeloma cells as vaccine, using genes encoding cytokines, costimulatory and MHC molecules. Retroviral-mediated gene transfer into human myeloma cells is feasible [72, 73].

Kopantzev et al. [74] transduced murine myeloma cell lines with a retroviral vector expressing the human IL-2 gene. They observed that intravenous immunization of mice with irradiated, IL-2-secreting cells led to significant protection from administration of parental myeloma cells.

Eight MM patients participated in a phase I trial evaluating the feasibility and safety of subcutaneous vaccination with adenovector engineered IL-2-expressing autologous plasma cells. Vaccines were well tolerated with only minor systemic symptoms reported [75].

Turner et al. [76] used a tumor cell vaccine consisting of a myeloma cell line (MPC11) transfected with IL-12 and GM-CSF genes using particle-mediated gene transfer in the

poorly immunogenic murine myeloma MPC11 model. Injection of this vaccine induced tumor rejection in 60% of mice, whereas control MPC11 provided no protection. Furthermore, this group confirmed this efficacy for a multidrug-resistant isogenic subline of MPC11-cells, suggesting that p-glycoprotein-mediated multidrug-resistant does not interfere with lysis by CTLs. Since selection of myeloma cells with multidrug-resistant phenotype by chemotherapy is a frequent problem, this approach may be attractive in the treatment of chemotherapy resistant MM [77].

Li et al. [78] investigated vaccines consisting of a retroviral-transduced myeloma cell line (B9BM1) expressing single or combinations of transgenes (GM-CSF, IL-12, CD80, Flt3L) in a mouse model. They demonstrated that the combined use of GM-CSF, IL-12, CD80 is superior to the use of any single gene product in the protection against subcutaneous challenge with parental tumor cells.

Gene transfer of B7-1 (CD80) and B7-2 (CD86), which are important costimulatory molecules for CTLs (by binding to CD28), induced potent antitumor responses in a variety of tumor models [79]. Myeloma cells often show little or no expression of B7 antigens. Tarte et al. [80] were able to demonstrate stimulation of allogeneic CD8+ T cell proliferation after retroviral transduction of myeloma cells with the *B7-1* gene. For one patient with advanced disease, *B7-1* gene transfer made it possible to amplify autologous CTLs that killed autologous myeloma cells in an HLA class I-restricted manner. These results suggest that *B7-1* gene transfer is of great promise in the immunotherapy of MM.

Dotti et al. [81] tested the hypothesis that expression of CD40 ligand (CD154) in the region of a myeloma cell vaccine might trigger tumor-specific immunity by recruitment of antigen-presenting cells. Mice were inoculated subcutaneously with two poorly immunogenic murine myeloma cell lines (MPC-11 and S107) mixed with irradiated CL7.1 fibroblasts that had been retrovirally transduced to express the CD40 ligand. For both cell lines, co-injection with mCD40 ligand and fibroblasts significantly retarded tumor growth. Thus, CD40 ligand transfection may play a role in the immunotherapy of MM.

Furthermore, DC are very attractive targets for transfection of *TAA* genes. Retroviral transduction of DC precursors of myeloma patients with scFv sequences is currently under investigation [82]. For a detailed review about DNA vaccination against multiple myeloma we refer to reference [82], and about gene therapy and immunotherapy for myeloma in general to references [83, 84].

Gene therapy for lymphoma

Idiotypic determinants are unique to each tumor, and the prospect of preparing individual protein vaccines for patients has made the application to the clinic difficult and expensive. Recently, since genes encoding immunoglobulin variable regions have been well characterized and custom DNA vaccines can be made rapidly, DNA immunization was proposed as an attractive alternative to protein immunization against B cell lymphoma [85].

Transfer of genes encoding IL-2, IL-12, IFN-α, IFN-γ, and GM-CSF into lymphoma cells has shown first experimental results in vivo [86]. In a phase I clinical trial, autologous cytokine-induced killer cells transduced with the gene encoding IL-2 were infused into patients with metastatic disease. Treatment was well tolerated and resulted in significant elevation in the serum levels of IFN-γ, GM-CSF, and transforming factor-beta. One patient with lymphoma achieved a complete response with this treatment [29]. For a review about gene therapy for B cell lymphomas see references [87, 88].

Concluding remarks

Biological effectiveness of gene transfer was shown in the following studies: induction or amplification of a tumor-specific immune response to tumor vaccination, some tumor regression or stable disease following transfer of suppressor genes (p53), and reduction of GvHD after allogeneic hematopoietic transplantation as a result of a transfer of the HSV*tk* suicide gene transfer into allogeneic donor lymphocytes after treatment with gancyclovir. To date, tumor regression has only been exceptionally a result of clinical gene therapy trials.

Technical problems still to be solved include transfection efficiency in vivo, level and stability of expression of the therapeutic gene, regulation of gene expression, targeted transfer in vivo, production of viral vectors on an industrial scale, and immunogenicity of vectors/therapeutic gene products.

Since tumor cells can be obtained easily in large quantities, hematological malignancies are an optimal target of gene therapeutic approaches using in vitro modulation of autologous tumor cells. Hematopoietic stem cell gene therapy offers an opportunity to widen the anticancer therapeutic index. Recently, many innovative and exciting genetic targets have been recently identified.

It is expected that major improvements will be achieved in the near future. The future of cancer treatment could be customized treatment based on the molecular properties of the tumor, combining conventional and novel therapeutics.

Acknowledgements We apologize to those colleagues whose work could not be cited due to space limitations.

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