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MEETING REPORT

RECENT ADVANCES IN GENE THERAPY PROGRAMS

HIGHLIGHTS FROM THE 13TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF GENE & CELL THERAPY

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SUMMARY

This commentary discusses the most significant advances that were presented at the 13th Annual Meeting of the American Society of Gene & Cell Therapy. Many reports focused on clinical trials for the treatment of cancer, genetic disorders, infectious diseases and other pathological conditions. In addition, researchers presented information from preclinical studies conducted for a variety of illnesses and for the improvement of vector design. In this respect, there was a major emphasis on safety issues, such as insertional mutagenesis and host immune responses against viral-derived vector systems.

INTRODUCTION

The 13th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT) was held from May 19 to May 22, 2010, in Washington, D.C., and was very well attended. Investigators from all over the world participated in the ASGCT meeting and presented 913 reports.

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The meeting addressed several issues related to the use of gene transfer technology in clinical trials, genetic immunization programs, preclinical studies for a variety of maladies, improvement of viral and nonviral vector design and engineering of animal and cell culture models to investigate the pathogenesis of a wide variety of human illnesses. This report summarizes the most representative advances discussed at the meeting. First, however, in order to provide a general background on gene transfer technology to the reader, the properties and drawbacks of the main gene delivery systems are summarized in Table I and reviewed elsewhere (1-3).

GENE THERAPY CLINICAL TRIALS FOR CANCER

An impressive number of phase I and phase II human gene therapy clinical trials for the treatment of cancer were presented at this meeting. The types of malignancies treated in these trials comprised glioblastoma (4), colon cancer (5-9), melanoma (6-9), malignancies of the bladder (10, 11), hematological neoplasms (12-15), breast cancer (6-9), ovarian cancer (16-18), prostate cancer (19) and other kinds of solid tumors (6-9, 20-23).

Three patients with glioblastoma were enrolled in a phase I/II clinical trial, which utilized a retroviral-based vector for the delivery of a dominant negative form of the O^6-methylguanine-DNA methyltransferase (*MGMT*) gene, which was termed $MGMT^{P140K}$ (4). The retroviral-encoded mutated $MGMT^{P140K}$ gene was delivered ex vivo into autologous hematopoietic cells, which were subsequently reinfused back into the patients. The genome of these three patients carried an unmethylated MGMT promoter. Several studies indicated that the methylation status of the MGMT promoter has important implications in the prognosis and in clinical responses to therapy in patients with glioblastoma. Specifically, the methylation of the promoter induces an epigenetic silencing of the *MGMT* gene, which, in turn, impairs DNA repair. Thus, MGMT promoter methylation is associated with longer survival in patients with glioblastoma (24-26).

Table I. *Summary of the main gene delivery systems.*

Vector system	Properties	Possible adverse effects in therapy and other disadvantages
Retroviruses	• They can only transduce dividing cells • Relatively high titers (10^6-10^7 tu/mL) • Broad cell tropism • The viral vector integration into the target cell genome leads to stable gene expression • Retroviral transfer vectors can accommodate genetic elements up to 9 kb • Murine and avian retroviral vectors are distantly related to primate retroviruses, reducing the possible interaction with HERVs	• Insertional mutagenesis • Replication-competent retroviruses may be generated by homologous recombination • Complement and other humoral responses neutralize retroviral vector particles in vivo • The in vitro transduction of stem cells requires the addition of growth factors and other nonphysiological growth conditions
Lentiviral vector based on HIV-1	• Dividing and nondividing cells can be transduced: this is appropriate for a variety of applications in stem cell biology • The latest generation of HIV-1-based vectors (FLAP or cPPT) is the best developed system among the various lentiviral vectors in terms of transgene expression, duration and efficiency of cell transduction • Relatively high titers (10^6-10^7 tu/mL) • Pseudotyping with retroviral or VSV-G envelopes confers broad cell tropism • HIV-1-derived vectors can take genetic elements up to 10 kb	• Serum conversion of the subject to HIV-1 • Insertional mutagenesis • HIV-1 is closely related to human retroviruses: this greatly increases the possibility of interaction with HERVs • Homologous recombination may generate replication-competent lentiviruses • Packaging cells express HIV-1 tat and rev regulatory proteins • Complement and other humoral immune responses rapidly destroy HIV-1-based vectors in vivo
FIV- and EIAV-based lentiviral vectors	• Nondividing cells can also be transduced • Broad cell tropism due to pseudotyping with retroviral or VSV-G envelopes • Stable gene expression • Relatively high titers (10^6-10^7 tu/mL) • FIV- and EIAV-based vectors can take genetic elements up to 10 kb • FIV and EIAV are not pathogenic in humans, so the seroconversion of the subject does not pose an issue • FIV and EIAV are distantly related to primate retroviruses, reducing the possibility of interaction with HERVs	• Insertional mutagenesis • Homologous recombination may generate replication-competent lentiviruses • Packaging cells express FIV or EIAV regulatory proteins • The performance of FIV- and EIAV-derived vectors needs to be optimized to the level of the latest generation of HIV-1-based vectors. The required improvements are in terms of transgene expression levels and duration of transgene expression • FLAP or cPPT versions of FIV- and EIAV-based vectors are currently available and have improved transduction efficiency • Studies are in progress to improve transgene expression and duration in target cells

Continued

Table I. *(Cont.) Summary of the main gene delivery systems.*

Vector system	Properties	Possible adverse effects in therapy and other disadvantages
Adeno-associated viruses (AAV)	• Nondividing cells can also be transduced. • Broad cell tropism • High titers (10^{10} tu/mL) • Stable gene expression (integration) • Nonpathogenic, nontoxic • Helper viruses no longer required for the production of AAV vectors • Easy purification of AAV vectors with columns (no more CsCl) • Small genome (5 kb)	• Limited capacity to accept foreign genes (about 4 kb) • Insertional mutagenesis • Humoral immune responses neutralize AAV vectors in vivo: for this reason, each AAV serotype has a single use for in vivo gene delivery
Adenoviruses	• Nondividing cells can also be transduced • Transduction efficiency and transgene expression levels are high, but transient • Very high titers (10^{12} tu/mL) • Broad cell tropism • Large transgenes can be inserted into the vector, provided proper deletion of adenoviral vector (7 to 8 kb of DNA insert can be added to the vector) • Suitable for immuno-based therapeutic approaches (cancer or infectious diseases)	• Host immune responses may cause severe adverse effects in patients and depletion of transduced cell populations • Humoral immune responses rapidly neutralize adenoviral vectors in vivo • Adenoviral vectors do not allow for long-term gene expression due to the lack of integration into the cell genome and to host immune responses against transduced cell populations • Not appropriate for the treatment of genetic diseases, as they require long-term transgene expression
Cationic liposomes or DNA-protein complexes	• They are not infectious • Large DNA sequences can be easily inserted into these vectors • Suitable for oligonucleotide delivery • A wide range of cell types can be transfected	• No specific cell targeting • Transfection efficiency can be low • Transient transgene expression • Unmethylated CpG sequences of bacterial plasmid DNA may elicit strong host immune responses • The presence of chimerical cell receptors on the surface of these vectors may trigger host immune responses
Sleeping Beauty retrotransposon system	• It requires a conventional plasmid transfection and has integrating abilities, which allow for long-term transgene expression	• Insertional mutagenesis
Streptomyces bacteriophage integrase ΦC31	• It can be transfected into target cells as a normal plasmid and has integrating abilities that allow for long-term transgene expression	• Insertional mutagenesis

tu/mL, transducing units per milliliter; HERVs, human endogenous retroviruses; VSV-G, vesicular stomatitis virus envelope glycoprotein, FIV, feline immunodeficiency virus; EIAV, equine infectious anemia virus.

Alkylating agents such as carmustine (BCNU), temozolomide and O^6-benzylguanine (O6BG) have been used to silence MGMT expression in preclinical studies conducted in dogs and nonhuman primates. Unfortunately, the preclinical use of the aforementioned alkylating agents was associated with deleterious effects on the hematopoietic system. In this respect, the purpose of this phase I/II gene-based clinical trial consisted of circumventing the harmful effects of alkylating agents in the hematopoietic compartment. Patients underwent radiation therapy, followed by granulocyte colony-stimulating factor-induced mobilization, apheresis and treatment with 600 mg/m^2 BCNU. They then received infusion of the gene-modified autologous hematopoietic cells. Preinfusion colony-forming units showed that the mutated *MGMT^{P140K}* gene was present in 70.6%, 79.0% and 74.0%, respectively, of cell fractions in patients 1, 2 and 3. BCNU administration was well tolerated by the patients. Real-time polymerase chain reaction (RT-PCR) detected the presence of proviral copies in peripheral blood mononuclear cells (PBMCs) and sorted granulocytes after the engraftment. The number of proviral copies per cell ranged from 0.33 to 0.83. Immunocytochemical analysis confirmed the cytoplasmic expression of mutated *MGMT^{P140K}* gene in PBMCs. Colony-forming unit gene marking detected the mutated *MGMT^{P140K}* gene in CD34-selected hematopoietic cell fractions that ranged from 28.5% to 47.5%. This analysis was conducted 2 months postintervention. The patients received one cycle of O6BG and temozolomide after the infusion of gene-modified autologous hematopoietic cells. So far, no evidence for toxic effects in the hematopoietic compartment was observed among the patients. In conclusion, insertion of the retroviral-encoded mutated *MGMT^{P140K}* gene into autologous hematopoietic cells conferred enhanced tolerance for BCNU treatment.

Nineteen patients with advanced solid tumors were enrolled in a phase I clinical trial based on cancer genetic immunization (6). The malignancies treated in this clinical study comprised four cases of colon cancer, three cases of melanoma, two cases of lung cancer, two cases of breast cancer, four cases of neuroendocrine cancer and four cases of other kinds of neoplasms. A plasmid termed TAG vaccine was used as a genetic vector for the simultaneous expression of two gene-based vaccines: transforming growth factor-β_2 (TGF-β_2) antisense complementary DNA (cDNA) and the granulocyte–macrophage colony-stimulating factor (GM-CSF). Malignant cells and/or tissues were removed from the patients and treated to generate a single cell suspension. TAG vaccine was delivered into cancer cells by electroporation. Autologous TAG vaccine-expressing malignant cells were then irradiated and infused on a monthly basis into patients though intradermal injections in the following two dosing cohorts: 10^7 cells/injection and 2.5×10^7 cells/injection. This treatment was given for 4 months. In the course of therapy, no grade 3 or 4 treatment-related toxic effects were observed among the patients. Sixteen subjects showed stable disease in a 3-month period. Interestingly, 1 patient with melanoma had a complete response to treatment and was still in complete response 18 months after the first injection.

Bacillus Calmette-Guérin (BCG)-based immunotherapeutic approaches constitute the standard treatment for patients with high-risk nonmuscle invasive bladder cancer. Unfortunately, BCG immunotherapy is not effective in treating more advanced malignancies of the bladder; hence, novel therapeutic approaches must be developed. Preclinical studies in animal models indicated that adenoviral-encoded CD40L (AdCD40L) triggered systemic immunity in local and invasive tumors of the bladder (10, 27). These findings prompted the use of AdCD40L in clinical trials. Eight patients with invasive forms of bladder cancer participated in a phase I/II clinical trial utilizing AdCD40L. Patients underwent three cycles of Clorpactin WCS-90 bladder washes, which were followed by AdCD40L intrabladder fusion. The treatment was well tolerated by all patients. Adverse effects were limited to the momentary distress associated with Clorpactin WCS-90 bladder washes. Biopsy analysis showed an efficient adenoviral vector delivery and massive intrabladder T-lymphocyte infiltration. In addition, histological assessment indicated that AdCD40L administration effectively reduced the tumor mass (10). In conclusion, local AdCD40L injection was safe in patients, increased T-lymphocyte intrabladder infiltration and reduced the tumor mass.

CG-0070 consists of a replication-competent oncolytic adenovirus that carries recombinant GM-CSF cDNA, which is under the control of the human E2F-1 promoter. This promoter becomes transcriptionally active in cells that have flaws in the retinoblastoma signaling pathway. Such a feature confers tumor specificity to the human E2F-1 promoter-driven gene expression pattern (11, 28). Thirty-five patients with superficial bladder cancer who had failed previous BCG therapy were enrolled in a phase I clinical trial that utilized CG-0070 (11). CG-0070 was infused into patients via intravesical (IVE) instillation at doses that ranged from 10^{12} to 3×10^{13} viral particles (vp) in the bolus regimen, whereas the CG-0070 dose range was from 10^{12} to 10^{13} vp in patients who underwent multiple IVE injections. The multidose treatments were conducted at two schedules: 1) weekly IVE injections x 6; 2) one IVE injection every 4 weeks x 3. Transitory adverse effects were confined to the bladder and comprised hematuria, bladder spasms, pain, urgency and nocturia. Evidence of fever, fatigue, chills and myalgia was observed in 20% of the subjects. A 46% complete response rate was observed among the patients. Analysis of urine showed high GM-CSF levels and a deferred second peak of CG-0070 genome in most of the study participants. The deferred occurrence of CG-0070 genome in the urine samples is indicative of adenoviral infection and replication.

A phase I clinical trial for the treatment of six patients with recurrent gynecological cancer utilized an adenoviral-derived vector system, which encodes for the thymidine kinase suicide gene and the somatostatin receptor type 2 (16). This adenoviral vector was termed Ad5.SSTR/TK.RGD and was administered into patients via intraperitoneal injections in combination with the prodrug ganciclovir. Ad5.SSTR/TK.RGD was injected into patients at doses between 10^9 and 10^{12} vp/injection. After Ad5.SSTR/TK.RGD injection, patients underwent ganciclovir treatment for 14 days. Ascitic fluid, serum and other specimens were taken to evaluate adenoviral-mediated gene transfer efficiency, host immune responses and viral shedding. Side effects in patients were limited to moderate abdominal pain. Unfortunately, three patients exhibited signs of disease progression, whereas the other three patients showed stable disease.

ONYX-015 is a replication-competent oncolytic adenovirus that has already been used in many human gene therapy clinical trials for the

treatment of different types of tumors (3, 20, 29). While the use of ONYX-015 was safe in patients (3, 20, 29), its clinical benefits were moderate (20). For this reason, a new oncolytic adenovirus was engineered and termed VRX-007. This novel replication-competent oncolytic adenovirus bears an overexpression system for the adenovirus death protein (ADP) E3-11.6K. After being tested in several preclinical studies, VRX-007 is finally being studied in a phase I clinical trial in advanced cancers (20). The first patient, with recurrent squamous carcinoma of the tongue, was treated with a single intratumoral injection of 2×10^8 vp. No treatment-related toxicity was reported in the patient. A reduction of the hard nodular mass and disease stabilization were observed 8 days postintervention.

GM-CSF may trigger host antitumor immune responses though two mechanisms: 1) recruitment of natural killer cells; 2) activation of $CD8^+$ cytotoxic T lymphocyte-mediated tumor-specific immune responses. In this respect, adenovirus-induced oncolytic tumor cell lysis may represent a powerful contributor in activating the antigen-presenting cell pathway for the destruction of the tumor cells and/or tissues. The most common adenovirus oncolytic systems are based on serotype 5, which utilizes the cellular receptor CAR. This receptor, however, may have highly variable levels of expression on the membrane of malignant cells (3, 21). A number of preclinical studies indicated that there are practical advantages in constructing a hybrid adenoviral serotype 5 fiber with the knob of serotype 3 (3, 21), which was termed 5/3 (21). Such a 5/3 capsid-modified oncolytic adenovirus showed an enhanced efficiency in targeting human cancer cells in several preclinical studies. After improving the targeting efficacy, the hybrid oncolytic adenovirus was engineered to encode for the GM-CSF gene *CSF2*. This new hybrid oncolytic adenovirus was termed Ad5/3-D24-GMCSF and was utilized in a phase I clinical trial for the treatment of 21 patients with advanced and recurrent solid tumors (21). Following intratumoral Ad5/3-D24-GMCSF injection, patients showed grade 1-2 adverse effects that resembled the symptoms of a flu-like illness. Adenoviral-related biological activity was detected in 62% of treated patients (13 of 21). CT scan showed substantial tumor mass decrease in eight subjects, who also exhibited signs of adenoviral biological activity.

GENE THERAPY CLINICAL TRIALS FOR AIDS

Some gene-based clinical trials for the treatment of AIDS were discussed at the ASGCT meeting (30-32). These clinical trials utilized lentiviral vector systems based on the human immunodeficiency virus type 1 (HIV-1) (2).

Forty-three patients with AIDS were enrolled in a phase II gene therapy clinical trial that used an HIV-1-based vector carrying 937 base pair antisense RNA against the HIV-1 envelope (30). This lentiviral vector system was termed VRX-496 and was utilized for the ex vivo transduction of autologous $CD4^+$ T lymphocytes. The VRX-496 gene-modified cells were subsequently divided into aliquots of 10 billion cells each. These aliquots were named lexgenleucel-T. The patients received various doses of lexgenleucel-T. Thirteen patients received 4 doses every 2 weeks, 11 patients received 8 doses every 2 weeks, 8 patients received a bolus injection of 1 dose, 6 patients received a bolus injection of 2 doses and 5 patients received a bolus injection of 3 doses. All the patients enrolled in this trial tolerated the treatment quite well. Three patients who received a bolus injection of one dose showed a considerable and constant increase in $CD4^+$ T cell counts, 6-12 months postintervention. Two of these patients were selected to receive a further dose of lexgenleucel-T after a year from the last injection. $CD4^+$ T-cell counts increased in each of these two subjects by 68% and 83%. Transient reductions in the viral load were reported in the single bolus cohort in a postintervention time period comprised between 2 and 6 months. Overall, the treatment was safe in patients and bolus dosing appears to provide better immunological responses than repeated lexgenleucel-T dosing.

Lexgenleucel-T-induced host humoral immune responses against the envelope of VRX-496 were evaluated in a cohort of 48 patients with AIDS who participated in a phase II clinical trial (31). As mentioned, VRX-496 is an HIV-1-derived lentiviral vector system in which the HIV-1 core was pseudotyped with the vesicular stomatitis virus envelope glycoprotein (VSV-G) (1, 2, 30, 31, 33). This clinical study is tackling one of the major obstacles for the effective application of gene therapy programs in the clinical setting (1, 2). Host humoral immune responses may easily neutralize viral-derived vector particles, and therefore pose a substantial limit to the clinical benefits of gene-based interventions (1-3). Ten patients enrolled in this clinical trial tested positive for circulating immunoglobulins against the VRX-496 envelope. This test was carried out 1.5 years after the lexgenleucel-T infusion. Remarkably, the presence of circulating immunoglobulins to the VRX-496 envelope did not reduce the presence of VRX-496 gene-modified autologous $CD4^+$ T lymphocytes (lexgenleucel-T) among the patients who seroconverted to the VSV-G protein. Some of these patients received additional lexgenleucel-T infusions and, interestingly, no adverse effects were reported. Moreover, VSV-G immunoglobulin class analysis revealed the presence of IgG antibodies, whereas immunoglobulins of the IgE class were never reported. Taken together, these findings exclude the eventuality of anaphylaxis as a consequence of VSV-G seroconversion in patients in this clinical trial. Another positive aspect of this clinical study is that host immune responses against VRX-496 envelope did not have a detrimental effect on the efficacy of lexgenleucel-T infusions in patients.

GENE THERAPY CLINICAL TRIALS FOR GENETIC DISORDERS

A number of phase I and II human gene therapy clinical trials that dealt with the treatment of various genetic disorders were also presented at this ASGCT meeting. The genetic disorders included Wiskott-Aldrich syndrome (WAS) (34), muscular dystrophy and other muscle diseases (35-38), adenosine deaminase deficiency (39) and Leber's congenital amaurosis (40). The gene delivery systems used in these clinical trials were based on retroviral vectors, adeno-associated viral (AAV)-based vectors and nonviral-mediated gene transfer.

Two patients with WAS participated in a phase I human gene therapy clinical trial, which used a retroviral-derived vector encoding for the recombinant and functional human *WAS* gene (34). This clinical study has also addressed the critical issue of retroviral-induced insertional mutagenesis. Autologous $CD34^+$ hematopoietic stem cells were removed from the subjects, transduced ex vivo with the retroviral vector system carrying the functional *WAS* gene and then gene-modified autologous $CD34^+$ hematopoietic stem cells were re-

injected back into the patients. The clonal repertoire of gene-modified autologous hematopoietic cells of these two patients was examined by linear amplification mediated PCR (LAM-PCR). This analysis reported 5,709 and 9,538 unique retroviral vector integration sites within the genomes of gene-modified hematopoietic cells of patient 1 and 2, respectively. Cellular samples were obtained from bone marrow, peripheral blood and lymph nodes. The latest LAM-PCR time points were taken 892 and 891 days postintervention in patient 1 and 2, respectively. Interestingly, the reconstitution of the hematopoietic system exhibited polyclonal blood cell populations in both patients. In addition, quantitative PCR and LAM-PCR amplicon retrieval frequency examination of single clonal input showed that the most active blood cell clones bared retroviral vector integrations in formerly described common integration sites (CIS) inside the target cellular genome (34, 41). The most recurrent CIS involved the following gene loci: *MDS1-EVI1*, *LMO2*, *PRDM16* and *CCND2* (the number of integration sites per gene loci are listed in Table II) (34). Retroviral integration sites, either proximal or inside the *LMO2* and *CCND2* gene loci, were more preponderant in lymphoid cells, whereas retroviral integration sites affecting either the *MDS1-EVI1* or *PRDM16* gene loci were more prevalent in myeloid cells. Albeit retroviral vector integrations occurred in the proximity of cellular proto-oncogenes, no development of overriding clones was seen in the hematopoietic system of the two patients who participated in this clinical trial. The last follow-up was conducted 2 years after the gene-based intervention. Further follow-up will be made for both patients to examine the profile of clonal composition of gene-modified blood cells.

A phase I human gene therapy clinical trial enrolled three patients with a genetic disorder named limb-girdle muscular dystrophy (LGMD) type 2 (35). This illness derives from a deficiency of α-sarcoglycan (α-SG), which consists of a sarcolemmal transmembrane protein that confers membrane stability. Sadly, this genetic disorder leads to a severe form of muscular dystrophy (42). This trial used an adeno-associated viral type 1 (AAV1)-based vector to express the functional human sarcoglycan (hSGCA) gene, which was driven by a truncated muscle creatine kinase (tMCK) promoter (35). The AAV1-encoded transgene expression was tested in biopsies taken from the three patients 3 months after the intervention. Western blot assays revealed an average 4- to 5-fold increase in α-SG expression levels in all the specimens. Tissue section immunostaining showed counts of α-SG-expressing fibers of 57%, 69% and 62%, respectively, in patients 1, 2 and 3. Another three patients were admitted in a second phase I clinical trial, which relied upon the same vector system and modality of administration as in the first. These three patients were injected with 3×10^{11} AAV1-derived vector genome copies in a side of the exterior digitorum brevis (EDB) muscle, while saline solution was injected into the other EDB muscle side. Biopsies were taken from both EDB muscle sides 6 months after the intervention. Transgene-specific PCR analysis found the presence of α-SG expression in the EDB side that was injected with AAV1-encoded hSGCA in all three subjects. In contrast, the control side always tested negative by transgene-specific PCR analysis. Patients 1 and 2 showed a twofold increase in α-SG expression in the treated EDB muscle side, along with an enhancement in average muscle diameter from 28.2 ± 11.1 to 52.2 ± 13.1 mm. Interestingly, a twofold increase in α-SG expression is in the range of physiological levels of expression present in normal muscle tissues. Unfortunately, patient 3 did not respond to therapy due to preexisting host immunity to AAV1. Overall, these findings look promising for the establishment of gene therapy programs for the treatment of LGMD type 2, as long as the subjects are not seropositive for AAV1 (1, 35, 43).

Table II. *List of retroviral vector common integration sites (IS) within main gene loci in two patients with Wiskott-Aldrich syndrome in a clinical gene therapy trial (34).*

Gene loci bearing IS	Number of IS per each gene loci	
	Patient 1	Patient 2
MDS1-EVI1	81	94
LMO2	13	29
PRDM16	10	28
CCND2	11	18

CONCLUSIONS

In spite of substantial progress reported every year in the field of gene transfer technology, several issues related to vector design and safety concerns remain to be addressed to develop more effective gene therapy clinical trials and genetic immunization programs. As mentioned, major safety issues concern insertional mutagenesis and host immune responses to viral-derived gene delivery systems. The effort of gene therapists to solve these problems is admirable. However, we are still far away from defining the optimal conditions for successful gene-based interventions in the clinical setting. However, gene transfer technology may provide reliable applications for the engineering of animal and cell culture models for the study of a wide variety of human maladies.

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DISCLOSURES

The author states no conflicts of interest.

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