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MEETING REPORT

LATEST ADVANCES IN GENE TRANSFER TECHNOLOGY

A SUMMARY OF THE 14TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF GENE & CELL THERAPY

G. Romano

College of Science and Technology, Temple University, Philadelphia, Pennsylvania, USA

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SUMMARY

This commentary highlights the most noteworthy achievements that were discussed at the 14th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT). A certain number of reports focused on various clinical trials for the treatment of cancer, genetic disorders, acquired immunodeficiency syndrome (AIDS) and other pathological conditions. Several preclinical studies were conducted for a wide variety of diseases and for the improvement of vector design. In this regard, particular emphasis was placed on safety issues, such as insertional mutagenesis-induced malignancies and host immune responses to the vector systems.

INTRODUCTION

The 14th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT) was held May 18-21, 2011, in Seattle, Washington, U.S., and was very well attended. Researchers from all over the world participated in this ASGCT meeting and presented a total of 866 reports. This information can be found at the following website: http://www.abstracts2view.com/asgct/sessionindex.php.

Correspondence: Dr. Gaetano Romano, Associate Professor of Biology and Medicine, Director of the Drug Development and Gene Therapy Programs, Center for Biotechnology, College of Science and Technology, Temple University, Bio Life Science Bldg. Suite 456, 1900 N 12th St., Philadelphia, PA 19122, USA. E-mail: gromano@temple.edu.

The ASGCT meeting dealt with several issues related to the use of gene transfer technology in clinical trials, genetic immunization programs, preclinical studies for a wide range of illnesses, 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 diseases. 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 (1-5).

For practical reasons, this report can only summarize the most important progress that was discussed at the ASGCT meeting.

GENE THERAPY CLINICAL TRIALS FOR CANCER

Several phase I and phase II human gene therapy clinical trials for the treatment of cancer were presented at the ASGCT meeting. These gene-based clinical trials dealt with the treatment of a broad range of malignancies, such as human papillomavirus (HPV)-induced cervical carcinoma (6), glioblastoma (7), tumors of the pancreas (8, 9), breast (9), colon (9), prostate (10) and various types of metastatic cancers (11).

HPV is the etiological agent of cervical carcinoma, which is responsible for the death of 270,000 patients per year throughout the world (6, 12, 13). Eighteen subjects were enrolled in a phase I genetic immunization clinical trial (6). The genetic vaccine used in this trial was VGX-3100, which consists of two plasmids, pCon16E6E7 and pCon18E6E7, that encode for the E6/E7 fusion proteins of HPV-16 and HPV-18, respectively. In order to enhance the efficacy of host immune responses to the genetic vaccine, the investigators adopted the following strategies: they optimized the codon/RNA usage for the human system, utilized high-efficiency leader sequences for IgE and delivered the DNA-based immunogen via intramuscular electroporation. VGX-3100 was administered at

Table I. *Summary description 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: this reduces the possible interaction with human endogenous retroviruses (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 non-physiological 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: this reduces 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.
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).	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.

Continued

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

Vector system	Properties	Possible adverse effects in therapy and other disadvantages
Adenoviruses	Broad cell tropism. Large transgenes can be inserted into the vector, provided proper deletion of adenoviral vector (7-8 kb of DNA insert can just be added to the vector). Suitable for immuno-based therapeutic approaches (cancer or infectious diseases).	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 chimeric cell receptors on the surface of these vectors may trigger host immune responses.
Sleaping Beauty (SB) retrotransposon system	It requires a conventional plasmid transfection and has integrating abilities, that 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; FIV, feline immunodeficiency virus; EIAV, equine infectious anemia virus; CsCl, caesium chloride.

three doses of 0.6, 2 and 6 mg at weeks 0, 4 and 12, and each cohort comprised six subjects. Host immune responses were monitored by ELISA, Western blot, T-cell ELISpot and flow cytometry. Overall, the treatments were safe and well tolerated by the subjects. Interestingly, 15 of 18 subjects exhibited various degrees of cytotoxic T-lymphocyte (CTL) immune responses that ranged from 100 to 5,000 spot-forming units (SFU) per million cells. Five of six subjects in the 6-mg dose cohort showed substantial CTL responses with an average of 1,362 SFU per million cells after the third immunization. In the 2-mg dose cohort there were four responders of six with an average of 626 SFU per million cells, whereas in the 0.6-mg cohort there were four responders of six with an average of 497 SFU per million cells. These findings prompted the submission of a phase II clinical trial.

Four patients with glioblastoma participated in a phase I/II clinical trial to assess the hematopoietic polyclonality following retroviral-mediated gene transfer of mutant methylated-DNA–protein-cysteine methyltransferase (*MGMT* P140K) into autologous hematopoietic stem cells (7). Many studies showed that the methylation status of the MGMT promoter has significant implications in the prognosis and clinical response to therapy in patients with glioblastoma (14-17). Specifically, the methylation of the promoter induces an epigenetic-mediated silencing of the *MGMT* gene, which weakens DNA repair efficacy of the cell. Consequently, MGMT promoter methylation is associated with longer survival in patients with glioblastoma (14-16). Alkylating agents such as carmustine (BCNU), temozolomide (TMZ) and O^6-benzylguanine (O6BG) have been used to silence *MGMT* expression in preclinical studies conducted in animal models in nonhuman primates (17). Regrettably, the use of the alkylating agents was associated with detrimental effects on the hematopoietic compartment. This phase I/II gene-based clinical trial aimed at limiting the harmful effects of alkylating agents in the hematopoietic system (7). Patients received two cycles of BCNU, O6BG and TMZ chemotherapy, followed by nine cycles of combination O6BG and TMZ chemotherapy. Transient increases in both circulating retrovirally transduced white blood cells and granulocytes were observed in patients after each treatment, whereas no side effects were reported.

The vaccinia virus WR strain was used as an oncolytic virus in a phase I clinical trial for the treatment of 4 patients with metastatic breast cancer, 10 patients with colorectal cancer and 2 patients with pancreatic cancer (9); in total, 16 patients were enrolled in this clinical trial. The replication cycle of the vaccinia virus was rendered tumor-specific by removing the thymidine kinase (*TK*) and vaccinia growth factor genes. These two factors are required for vaccinia virus replication in normal and nondividing cells, whereas the virus can replicate per se in malignant cells, as they harbor activation mutations in E2F and/or EGFR pathways. Modified vaccinia virus was administered with an initial dose of 3×10^7 plaque-forming units (PFU) and a maximum dose of 3×10^9 PFU. Minor adverse effects were observed, including fever, swelling, erythema at the site of

injection, diffuse rash not related to replicating vaccinia virus and thrombocytopenia. There was no indication of viral shedding, while evidence of viral replication was only observed in tumor biopsies. Interestingly, the virus did not diffuse to normal skin tissues.

Twelve patients with metastatic castration-resistant prostate cancer participated in a phase I/II clinical trial that utilized the BPX001 vaccine (10). This vaccine consists of a formulation based on autologous dendritic cells electroporated with a plasmid-encoded inducible prostate-specific membrane antigen (PSMA). AP1903 is the recombinant form of CD40 and has the function of activating BPX001 following lymph node migration. This vaccination protocol proved safe in humans and resulted in strong host immune responses against PSMA.

An oncolytic poxvirus, JX-594, was used in a phase II clinical trial for the treatment of patients with refractory metastatic cancers (11). JX-594 was constructed to be replication-specific for cancer cells and also encodes for granulocyte–macrophage colony-stimulating factor (GM-CSF), which has the functions of stimulating the shutdown of the tumor vascular system and eliciting antitumor immune responses. Adverse effects were limited to flu-like symptoms in patients. Remarkably, viral replication was only observed in neoplastic tissues.

GENE THERAPY CLINICAL TRIALS FOR GENETIC DISORDERS

A good number of gene therapy clinical trials for the treatment of genetic disorders were discussed at this meeting, which dealt with the treatment of Wiskott-Aldrich syndrome (WAS) (18), Leber congenital amaurosis (19), lipoprotein lipase (LPL) deficiency (20), chronic granulomatous disease (21) and limb girdle muscular dystrophy (22).

Ten patients with WAS participated in a phase I clinical trial conducted in Germany (18). WAS is a rare X-linked recessive primary immunodeficiency characterized by dysfunction of the immune system (23). A gamma murine leukemia virus (MLV)-derived vector system encoding for the functional copy of the recombinant *WAS* gene was used in this clinical trial. Eight patients exhibited a reconstitution of the hematopoietic system 3.5 years after the gene-based intervention. Unfortunately, 1 patient developed a T-lineage acute lymphoblastic leukemia (T-ALL) 489 days after the intervention (18). Leukemic sample analysis revealed a dominant clone containing one retroviral vector integration site 15.33 kb upstream of the *LMO2* locus. On the other hand, this patient underwent remission following chemotherapy.

Mutations in the *RPE65* gene lead to Leber congenital blindness (24, 25). Adeno-associated virus type 2 (AAV2) vectors were previously used in a phase I clinical trial for the subretinal delivery of the functional copy of the *RPE65* gene (19, 25). The safety of AAV2-encoded *RPE65* readministration was assessed in two patients with Leber congenital amaurosis 2 years from the first intervention (19). The subretinal readministration was carried out at a dose of 1.5×10^{11} vector genomes (vg) per patient. The intervention was well tolerated by both patients. There was no sign of inflammation and no evidence of CTL immune responses in these subjects.

Patients with LPL deficiency are not able to hydrolyze triglycerides, which therefore accumulate in the circulation (20). Life-threatening pancreatitis is a clinical manifestation of this genetic disorder. Five patients with LPL deficiency were enrolled in a phase I clinical trial that utilized a nonviral vector, alipogene tiparvovec, for the delivery of the functional copy of the *LPL* gene. The patients were administered alipogene tiparvovec i.m. at a dose of 10^{12} genome copies/kg. Patients underwent a test meal supplemented with a [^{3}H]-palmitate tracer to monitor postprandial chylomicronemia, which was very poor prior to the gene-based intervention. Conversely, after the clinical trial, all patients exhibited a remarkable improvement in handling chylomicronemia (20).

A retroviral-based vector encoding for recombinant gp91-phox was used in a phase I clinical trial conducted in Germany for the treatment of two children with chronic granulomatous disease (21). This disorder can be responsible for life-threatening paraparesis and/or respiratory insufficiency. Unfortunately, one patient developed insertional mutagenesis-related myeloid dysplasia 2.5 years post-intervention and is currently under treatment.

Limb girdle muscular dystrophy type 2C, or γ-sarcoglycanopathy, is an incurable inherited autosomal recessive disorder (22, 26). Nine nonambulatory patients with limb girdle muscular dystrophy type 2C were enrolled in a phase I clinical trial for the safety assessment of dose escalation of the AAV1-encoded human γ-sarcoglycan gene (*SGCG*), which was under the control of the human desmin promoter (22). Patients were divided into three cohorts and treated with the following doses of AAV1: 3×10^9 vg, 1.5×10^{10} vg and 4.5×10^{10} vg. The gene-based treatment was safe, as no severe side effects were observed in this trial. All patients tested seropositive for AAV1. In particular, one patient developed a CTL immune response against the capsid of AAV1. Immunohistochemistry analysis of biopsy samples showed expression of the recombinant *SGCG* gene in the tissues of five patients.

GENE THERAPY CLINICAL TRIALS FOR AIDS

One phase I human gene therapy clinical trial for the treatment of nine patients with AIDS was discussed at this meeting (27). These patients were on HAART and aviremic, but exhibited $CD4^+$ T-lymphocyte counts below 500 cells/mm^3. Interestingly, this trial adopted a nonviral vector system for the delivery of zinc finger nuclease, which was designed for the destruction of the chemokine CCR5 receptor in autologous $CD4^+$ T lymphocytes. $CD4^+$ T cells were extracted from the patients, transduced ex vivo, expanded and then reinfused into patients. Patients were divided into three groups of three subjects each and CCR5-depleted T cells were administered at three doses: 1×10^{10}, 2×10^{10} and 3×10^{10} total cells per patient. The procedure was safe and well tolerated by patients. A significant increase in $CD4^+$ T-lymphocyte counts was observed among all the subjects who participated in this clinical trial.

GENE THERAPY CLINICAL TRIALS FOR CARDIOVASCULAR DISORDERS

The outcomes of three gene-based clinical trials for the treatment of cardiovascular diseases were also presented and discussed at the ASGCT meeting (28, 29, 31).

A phase I/IIa gene therapy clinical trial was conducted in Japan for the treatment of 12 patients with peripheral arterial disease (28). A

Sendai virus-derived vector encoding for the human *FGF2* gene was utilized in this trial in a four-dose escalation: 5×10^7, 2×10^8, 1×10^9 and 5×10^9. Each dose was administered to a group of three patients diagnosed with critical limb ischemia. No gene transfer-related adverse effects were reported among the patients, and they exhibited significant improvement in limb functions.

Previous preclinical studies indicated that stromal cell-derived factor 1 (SDF-1) plays an important role in wound healing in several types of organs, including heart failure (29, 30). The plasmid JVS-100 was used in a phase I clinical trial for the delivery of the recombinant gene that encodes for SDF-1 (29). Seventeen patients diagnosed with class III heart failure caused by myocardial infarction were enrolled in this clinical trial. JVS-100 was administered to patients as three doses: four patients received 5 mg, six patients received 15 mg and seven patients were treated with 30 mg. No adverse effects were observed in the patients. Bioactivity was determined by measuring modifications from baseline values in the following features: echocardiographic parameters comprising left ventricular volume and ejection fraction, cardiac perfusion determined by SPECT imaging and clinical parameters that comprised the New York Heart Association (NYHA) functional classification system, 6-minute walk distance test and assessment of quality of life. Some clinical improvement was observed in three patients in the 5-mg cohort and in four patients in the 15-mg cohort. The assessment of other clinical data is still pending.

Thirty-nine patients with advanced heart failure were enrolled in a phase II clinical trial, which utilized AAV1-encoded sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2) (31). Decreased levels of SERCA2 activity are associated with pathophysiological abnormalities that lead to heart failure (31, 32). Viral vectors were administered via intracoronary injection, and 14 patients received placebo. No adverse effects were observed in this clinical trial, indicating that the gene-based intervention was safe in humans. In addition, SERCA2 enzyme replacement was to some extent beneficial in terms of clinical outcome, symptoms and functional status, as indicated by the response to therapy of treated patients versus the placebo group control (31).

SUMMARY OF VARIOUS PRECLINICAL STUDIES

Several preclinical studies for cancer gene therapy focused on the use of recombinant T-cell receptors that recognize certain types of tumor-associated antigens, which can then be used to engineer T lymphocytes for targeting specific malignant tissues (33-36). For instance, a group of investigators utilized the recombinant T-cell receptor specific for the antigen encoded by the protein SSX2 gene (*SSX2*), which is commonly expressed in various types of tumors (33). This T-cell receptor was isolated from natural T lymphocytes obtained from tumor-infiltrated lymph nodes of two patients with melanoma. A retroviral-based vector system was used to transduce the *SSX2*-specific recombinant T-cell receptor into peripheral blood lymphocytes for preclinical studies.

Non-viral-mediated gene transfer is attracting a great deal of interest in terms of gene therapy applications (1-5). As anticipated, a number of human gene therapy clinical trials are currently relying on this technology (6, 10, 20, 27, 29). Non-viral-based vector systems require combination with physical methods of gene delivery (1-5). Interestingly, one of the most applied physical modalities of gene delivery is based on electroporation, as evidenced by both clinical trials and preclinical studies (6, 10, 37-43).

Zinc finger nucleases are another important emerging tool in the sector of non-viral-mediated gene transfer and may find useful applications for the treatment of genetic disorders, infectious diseases, cancer and neurological illnesses (1, 4, 27, 44-50). As previously discussed, a zinc finger nuclease was utilized to suppress CCR5 expression in a phase I clinical trial for the treatment of nine patients with AIDS (27).

Gene transfer technology is also used to reprogram the epigenetic state of somatic mammalian cells into so-called induced pluripotent stem (iPS) cells (51, 52). Studies are currently ongoing for the production of iPS cells via transient expression of pluripotent factors such as octamer-binding protein 4 (Oct-4), transcription factor SOX-2, Krueppel-like factor 4 and proto-oncogene c-Myc (51-53), as integrating vector systems may be responsible for insertional mutagenesis events, which in turn may result in the establishment of a malignant cell phenotype (4, 51-53).

Lastly, several reports addressed the issue of improving the gene manipulation of adult stem cells derived from various tissues. Naturally, particular emphasis was placed on stem cells of the hematopoietic compartment.

CONCLUSION

Each year, the field of gene therapy is consistent in reporting a certain degree of progress in terms of applications in clinical trials and improvement of vector design. However, successful gene-based interventions in the clinical setting still require a major effort in the matter of safety and vector design improvement. A major safety issue is still related to insertional mutagenesis-induced malignancies. In this respect, two gene therapy clinical trials conducted in Germany reported the unfortunate onset of hematological neoplasia in two patients (18, 21).

As it stands, gene transfer technology is very useful to engineer animal and cell culture models for the study of a wide variety of human maladies and to implement stem cell research.

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