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Velimogene Aliplasmid Lipid Complex

Prop INN; USAN

Antitumor Gene Therapy

Allovectin-7®

VCL-1005 plasmid DNA formulated with a lipid-based system, DMRIE/DOPE [(±)-*N*-(2-hydroxyethyl)-*N,N*-dimethyl-2,3-bis(tetradecyloxy)- 1-propanaminium bromide/dioleoylphosphatidylethanolamine]

Plasmid encoding the genes HLA-B7 and β_2-microglobulin complexed with a DMRIE/DOPE cationic lipid mixture

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Abstract

The evolution of immunotherapeutic treatments has led to the development of a new class of drugs that have the potential to cure not only congenital genetic disorders but also the acquired genetic defects that likely occur in many malignancies. The discovery of a myriad of genetic abnormalities that lead to a malignant clone of cells has made gene therapy an ideal candidate treatment for cancer. The delivery vector for the gene is an important factor in the development of an effective gene therapy. A vector must be able to overcome cellular barriers, as well as be easily produced in large quantities. Velimogene aliplasmid lipid complex (Allovectin-7®) is an example of a gene therapy that has clinical promise. Limited toxicities related to this drug have been observed to date. It has been studied extensively in the lab and is now being evaluated in clinical trials that are showing promising results, especially in the treatment of melanoma.

Background

The concept of genetic defects leading to human diseases, including hereditary syndromes and cancer, has led to a novel approach to the treatment of a number of disorders. The replacement of defective genes with the correct sequence has the potential to cure many human diseases (1). The term for this is gene therapy – the introduction and expression of recombinant genes in cells for the purpose of treating a disease. Gene therapy has proven to be a difficult task with a number of obstacles to be overcome.

The first step in the advancement of gene transfer is the development of an effective method of delivery. The gene delivery system, or vector, must be able to overcome the physical barriers of the extracellular environment, target the cell of interest, be nontoxic to the host and be an efficiently produced drug. Whether the vector is viral or nonviral, there are several qualities that would make an ideal vector: high concentration that allows many cells to be infected; convenience and reproducibility of production; ability to integrate in a site-specific location on the host chromosome; and a transcriptional unit that can respond to the manipulation of its regulatory elements (2). Despite the fact that no single vector encompasses all these qualities, delivery systems are being developed with these goals in mind.

Another issue in the development of gene therapy is the initial method of integration into the host. Vectors can be delivered either systemically or by direct intralesional injection. Both have advantages depending on the clinical situation. Systemic treatments would be disseminated throughout the body and therefore would be ideal in a patient with metastatic cancer, assuming that it can target the cancer cells. Intralesional injections, however, allow for high concentrations to be given directly into the target site. The main limitation of this method is that sites distant from the lesion would not be affected (3), although systemic responses have been documented with intralesional administration.

A number of viral and nonviral vectors have been developed in an attempt to overcome many of these obstacles. Retroviruses are the most widely used of the viral vectors. Following cellular entry, reverse transcriptase converts viral RNA sequences into DNA, which integrates into the host genome. This process is facilitated by the retroviral enzyme integrase. In order to create a retroviral vector, one must replace the viral protein encoding part of the genome with the therapeutic gene. Recombinant retroviral vectors can be engineered using DNA plasmids encoding certain DNA sequences of the viral genome, as well as the gene of interest. The plasmid

Ragini Kudchadkar, Rene Gonzalez*. Cutaneous Oncology Program, University of Colorado Cancer Center, 1665 N. Ursula Street, Mail Stop F703, P.O. Box 6510, Aurora, CO 80045, USA. *Correspondence: rene.gonzalez@uchsc.edu

is transfected into packaging cells which encode missing complementary viral DNA sequences. The combination of DNA sequences allows for the formation of complete retroviral vectors (2). The gene of interest is thus inserted into a certain area of the recombinant plasmid, which permits the formation of recombinant virus without interference. The major advantage of using this type of vector is that it integrates genes into the host chromosome with long-term stability and permanent expression. Retroviral vectors are limited, however, by their ability to only infect dividing cells, as well as by their risk of insertional mutagenesis, given that they randomly integrate into the genome (5, 6). This was best demonstrated by Fischer *et al.* in 2003. The authors demonstrated uncontrolled clonal proliferation of mature T-cells in 2 patients with X-linked severe combined immunodeficiency after retrovirus-mediated gene transfer into autologous CD34 bone marrow cells. Both patients were documented to have retrovirus vector integration in proximity to a proto-onogene promoter (7). The theoretical potential to cause malignancies is the direct result of the retroviral vectors' risk for insertional mutagenesis.

Adenoviral vectors are another commonly used viral vector. Adenoviruses are a family of double-stranded DNA viruses that can infect dividing and nondividing cells. They also differ from retroviral vectors in that they do not integrate into host DNA. They are replicated by extrachromosomal elements in the nucleus of the host cell, thus eliminating the possibility of mutagenesis (2, 3). However, transgene expression declines after 2 weeks, which limits their use. Another limitation is that they activate a host immune response consisting of inflammation and activation of neutralizing antibodies, as well as a cellular host response, which together can cause a clinically significant inflammatory process (8).

Nonviral vectors are another promising method of delivering gene therapy. Nonviral techniques use naked DNA delivered by either a physical or a chemical method (9). Physical methods include electroporation, gene gun, ultrasound and hydrodynamic pressure. Electroporation involves the use of electric fields to permeate the cell; the gene gun uses DNA-coated gold particles to penetrate the cell membrane; and ultrasound makes the membrane more permeable, which allows access to the vector. Large-volume injection of DNA is used with hydrodynamic pressure delivery. In contrast, chemical carriers include cationic lipids, polymers and peptides that are complexed to plasmid DNA (9). Nonviral vectors are not incorporated into the genome and expression is transient. They are advantageous because of their ease of production and administration (9, 10).

Velimogene aliplasmid lipid complex (Allovectin-7®) is an example of a nonviral vector which has clinical promise. It is a DNA plasmid gene transfer product consisting of the human leukocyte antigen HLA-B7, cloned from a human B-cell library, combined with chimpanzee β_2-microglobulin (11). The 4853-base pair DNA plasmid uses a backbone of an eukaryotic expression vector (pBR22) into which the genes containing human major histocompatibility class (MHC) class I HLA-B7 and β_2-microglobulin are inserted. There is co-expression of both genes due to their separation by an internal ribosomal entry site. A single respiratory syncytial virus (RSV) promoter controls co-expression. The plasmid DNA is combined with a cationic lipid (DMRIE/DOPE) to form a DNA-liposomal complex that facilitates transfection into the cell (11, 12) (Fig. 1).

A number of preclinical trials indirectly support Allovectin-7® as a viable method for killing malignant cells. Mutations in the β_2-microglobulin gene have been related to decreased tumor cell expression of MHC class I proteins. A number of cancers, including melanoma, head and neck cancer and renal cell carcinoma, have been shown to have decreased expression of class I MHC proteins needed for the presentation of tumor-associated antigens (13, 14). HLA class I antigens regulate the proliferation of malignant cells, as well as the lysis of malignant cells via natural killer cells (14). Abnormalities in class I antigen expression may affect the ability of host immune cells to recognize these abnormal cells and eliminate them. Therefore, gene therapy could potentially correct the expression, thus allowing the host immune system to appropriately recognize and remove these cells from circulation (15).

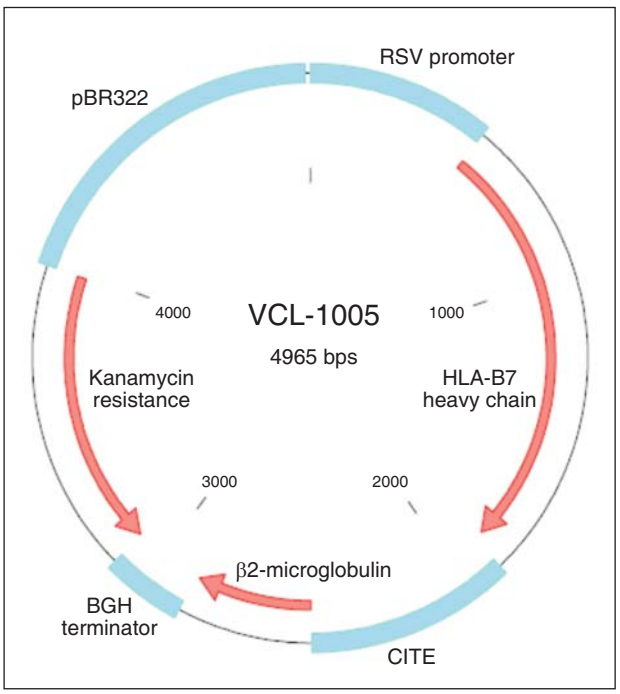

Fig. 1. Allovectin-7® expression vector (courtesy of Vical, Inc.). The RSV-LTR promoter drives the expression of two cDNAs, HLA-B7 heavy chain and β_2-microglobulin. The two genes are separated by CITE, an internal ribosomal entry site which permits co-expression of the genes from a single promoter in eukaryotic cells. Bovine growth hormone (BGH) transcription terminator and polyadenylation signal, BCH P(A) terminator, terminate transcription. The kanamycin resistance gene is expressed only in the bacterial hosts.

In the early 1990s, Plautz *et al.* evaluated the antitumor activity of intratumoral gene transfer against adenocarcinoma and fibrosarcoma xenografts in mice. They demonstrated that the transfer of allogeneic MHC class I genes into murine tumor cells resulted in immunological rejection of transfected tumor cells (16). The physiology behind this rejection was mediated by $CD8^+$ cytotoxic T-lymphocytes (11, 16). The authors concluded that the expression of the foreign histocompatibility protein stimulated an immune response that triggered a cytokine cascade and antitumor response against both transfected and nontransfected murine tumor cells (16, 17).

Pharmacokinetics and Metabolism

The metabolism of Allovectin-7® was first evaluated in mice. Plasmid DNA complexed with cationic lipids was delivered i.v. to mice and evaluated at different time points up to 6 months postinjection (18). Plasmid DNA was rapidly degraded, with a half-life of < 5 min, and was no longer detectable in peripheral blood of mice in 1 h. However, intact plasmid DNA was present in the major organs (lung, liver, spleen, kidney, heart, marrow and muscle) at 24 h after administration. PCR could detect plasmid in all tissues at 7 and 28 days. After 6 months, the only organ with detectable plasmid was muscle (11, 18). The cytofectin component was only evaluated in organs and not in plasma. At 20 h after administration, most of the lipid was present in the liver, with lowest levels in brain, muscle and blood. This highlights the fact that Allovectin-7® is metabolized and retained differently by different organs. This fact may affect which type of patient would benefit the most from treatment. In patients with metastatic disease to the liver, Allovectin-7® would potentially be more effective given that it is retained by the liver for greater periods of time. Only 17% of the radiolabeled lipid was detected in urine 4 days after administration (19), demonstrating greater metabolism via the liver than the kidney.

Safety

Animal studies have not demonstrated toxic side effects for Allovectin-7®. Repeated i.v. administration of up to 10 μg of plasmid DNA for 14 days had no pathological effects in mice and only incidental effects on clinical chemistry and hematology. The results were similar in nonhuman primates after repeated i.v. administration at a cumulative dose of 720 μg, with no effects seen on clinical chemistry, hematology or gross pathology (19).

Human trials have shown, as animal studies predicted, that Allovectin-7® is very well tolerated overall and associated with minimal toxicities. Most of the toxicity is related to complications arising from injection, and no major toxicities have been reported to date. Phase I trials in humans reported no grade 3 or 4 systemic toxicities. Pain and/or bleeding at the injection site, fatigue, anemia and nausea were the most common grade 1/2 side effects seen (21). Injection site-related complications such as pain, hemorrhage and pneumothorax were seen in other phase I studies (22). Hypotension has been seen with intratumoral administration of Allovectin-7® and is thought to be related to a vagal response to the injection (22, 23). Antibody responses have been observed in patients' serum positive for antinuclear antibodies (ANA), although systemic manifestations have not been seen (21).

Thus, in rodents, nonhuman primates and humans, Allovectin-7® appears to be extremely well tolerated. Long-term toxicities have not been seen thus far, although it should be mentioned that human clinical work is still in the early stage.

Clinical Studies

The first study using nonviral gene transfer was performed by Nabel *et al.* and published in 1993. They injected a plasmid containing HLA-B7 (without β_2-microglobulin) into tumor nodules in 5 HLA-B7-negative metastatic melanoma patients (17). One patient was found to demonstrate regression of both injected nodules, as well as regression of distant sites of metastasis.

A follow-up study by the same group (24) evaluated 10 patients with metastatic melanoma using the same methodology, except that Allovectin-7® (HLA-B7 with β_2-microglobulin gene) was used in this study. Doses of 3-300 μg Allovectin-7® were injected into metastatic nodules. Local inhibition of tumor growth was seen in 2 patients. T-cell migration into treated tumor lesions was demonstrated as well, although increased cytotoxic T-lymphocytes against autologous tumor cells were not identified in peripheral blood.

Rini *et al.* (21) evaluated Allovectin-7® in a phase I trial in renal cell carcinoma patients. Fifteen patients with histologically confirmed, HLA-B7-negative metastatic renal cell carcinoma received intratumoral injections of escalating doses. Protein expression of the HLA-B7 gene was confirmed in 8 of 14 patients. This study demonstrated the viability of gene transfer; however, no significant clinical responses were seen.

To expand upon these trials, Stopeck *et al.* (22) conducted a study with Allovectin-7® in 17 HLA-B7-negative patients with metastatic melanoma. Intralesional injections of 10-250 μg were administered. Half of the patients (7 of 14) showed a > 25% reduction in the size of injected nodules and 1 patient had a complete response. Of note, the post-treatment tumor biopsies demonstrated detectable plasmid DNA in two-thirds of the patients.

Based on the above data, several phase II trials were performed with Allovectin-7®. Stopeck *et al.* published the results of a phase II trial that included 52 patients with metastatic melanoma. Patients received 4 injections weekly for 4 weeks followed by a 4-week observation period. If there were no signs of progressive disease, the patients could then receive 2 additional weekly injections (weeks 8 and 9). If the disease continued to be stable or improved, patients were eligible to receive an additional course of treatment. Nine of 51 patients showed an over-

all tumor response. Interestingly, 2 patients had a systemic partial response to treatment. Overall, 1 patient had a local complete response, 3 had local partial responses and 5 minor responses were documented (25).

In 2001, Gleich *et al.* published the results of a phase I/II trial using HLA-B7/lipid complex in patients with advanced squamous cell carcinoma of the head and neck (23). Patients received 2 biweekly intratumoral injections of 10 or 100 μg of Allovectin-7® followed by 4 weeks of observation. Patients with stable or improved disease were eligible for additional cycles. Sixty-nine patients were treated and 23 had stable disease or a partial response after the first cycles. Response time varied from 21 to 106 weeks.

High-dose Allovectin-7® was tested in a study by Richards *et al.* (26). A dose of 2 mg, over 200-fold higher than that previously studied, was injected weekly for 6 weeks. One hundred and twenty-seven patients were studied. Approximately 12% of patients (15 patients) had an objective response, with a median duration of response of 12.7 months. Median time to progression was 1.6 months and overall survival was 21.3 months. No increased toxicities were noted with the higher doses used in this study.

In 2006, Gonzalez *et al.* (27) published the results of a phase II trial that evaluated the efficacy of 10 μg of weekly intralesional Allovectin-7® for a total of 6 weeks of treatment in 77 patients with metastatic melanoma. Seven patients had a complete or partial response to therapy and the median duration of response was 4.8 months.

Ongoing trials continue to evaluate Allovectin-7® in the clinical setting. Vical is sponsoring a phase III clinical trial comparing 2 mg of intralesional Allovectin-7® with standard chemotherapy, dacarbazine or temozolomide, in patients with stage III or IV melanoma. This trial is currently accruing patients and the results are eagerly awaited (28).

Drug Interactions

There are no significant drug interactions documented in the literature to date. Theoretically, the use of steroids would blunt the immune response to tumor cells that may be stimulated by the use of Allovectin-7®. Therefore, immunosuppressive drugs should be used with caution in these patients. Patients on immunosuppressants or anti-HIV agents have been excluded from all the clinical trials.

Source

Vical, Inc. (US).

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