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Alipogene Tiparvovec

Prop INN

Gene Therapy
Treatment of Lipoprotein Lipase Deficiency

AAV.LPL
AAV1-LPL^{S447X}
AMT-010
AMT-011
Glybera®

Adeno-associated virus serotype 1 (AAV1) vector expressing the S447X variant of the human lipoprotein lipase (*LPL*) gene produced using a plasmid system (code AMT-010) or a baculovirus system (code AMT-011)

Recombinant adeno-associated virus serotype 1 (AAV1) vector expressing the S447X variant of the human lipoprotein lipase (*LPL*) gene

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Abstract

Familial lipoprotein lipase (LPL) deficiency is an inherited condition involving autosomal recessive mutations in the *LPL* gene, which provides an enzyme responsible for the breakdown of lipoproteins to release fats that the body uses for energy or puts into storage. It is therefore characterized by hypertriglyceridemia and the absence of LPL activity. Familial LPL deficiency patients can control associated symptoms (which include abdominal pain, acute and recurrent pancreatitis, eruptive cutaneous xanthoma and an enlargement of the liver and spleen) and blood triglyceride levels with a fat-free diet; however, to date, no pharmacotherapeutic option exists for this condition. Amsterdam Molecular Therapeutics has developed a novel adeno-associated virus (AAV)-based gene therapy expressing the S447X variant of the human *LPL* gene, with the aim of correcting LPL activity in these patients. This new candidate, known as alipogene tiparvovec (AAV1-LPL^{S447X}), is presently in preregistrational studies.

Background

Lipoproteins normally carry fat molecules from the intestine into the bloodstream. As lipoproteins are broken down, they release fats that the body uses for energy or puts into storage. The enzyme lipoprotein lipase (LPL) hydrolyzes plasma triglycerides and very low density lipoproteins (VLDL) (1). Familial LPL deficiency, also known as chylomicronemia syndrome and type I hyperlipoproteinemia, is an inherited condition involving autosomal recessive mutations in the *LPL* gene, which prevents the enzyme from breaking down lipoproteins effectively. As a result, fatty substances build up in the bloodstream, leading to multiple signs and symptoms including inflammation of the pancreas (pancreatitis), abdominal pain, enlargement of the liver and spleen (hepatosplenomegaly) and small yellow skin lesions called eruptive xanthomas (www.ncbi.nlm.nih.gov/medlineplus). The only treatment is a fat-free diet.

Amsterdam Molecular Therapeutics (AMT) has developed a novel adeno-associated virus (AAV)-based gene therapy expressing the S447X variant of the human *LPL* gene, known as alipogene tiparvovec (AAV1-LPL^{S447X}). This therapy is transported into the cell nucleus upon administration, leading to the production of viable LPL protein, with the overall aim of increasing lipolytic function of the skeletal muscle tissue. This gene therapy can be produced using a plasmid (mammalian cell line HEK 293) or baculovirus system using insect cells (2), with the code name for the drug designated according to the system used: AMT-010 (plasmid system) and AMT-011 (baculovirus system). The therapy was assigned orphan drug status by the European Medicines Agency (EMEA) in 2004 and by the U.S. Food and Drug Administration (FDA) in 2007; it is currently in preregistrational studies (3, 4).

Preclinical Pharmacology

Initial studies were conducted in an adult murine model of LPL deficiency: LPL knockout (*Lpl*$^{-/-}$) mice displaying grossly elevated plasma triglyceride levels

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(> 200-fold) and very low high density lipoprotein (HDL) cholesterol (< 10% of normal). Single i.m. injections of alipogene tiparvovec (8 x 10^{11} or 8 x 10^{12} genome copies [gc]/kg) resulted in dose-dependent expression of human LPL protein and LPL activity (maximum of 33% of normal murine levels) in postheparin plasma. Furthermore, visible hyperlipidemia was resolved within 1 week; plasma triglyceride levels were reduced to near normal levels (from 99.0 to 1.8 mmol/l), and plasma HDL cholesterol was increased by 6-fold (from 0.2 to 1.1 mmol/l). Following an i.v. lipid challenge 8 months after administration of alipogene tiparvovec, effective clearance of plasma triglycerides was seen. The gene therapy also reversed abnormal muscle morphology observed in *Lpl*$^{+/-}$ mice, with a beneficial effect on plasma lipid levels for over 1 year (5-7).

The ability of alipogene tiparvovec to correct severe hypertriglyceridemia was further demonstrated in *LPL*$^{-/-}$ cats, which demonstrate plasma triglyceride levels of > 10,000 mg/dl and clinical symptoms similar to LPL deficiency in humans, including pancreatitis. I.m. injection of alipogene tiparvovec (1 x 10^{11}-1.7 x 10^{12} gc/kg) provided resolution of visible plasma lipidemia and reduced plasma triglyceride levels by > 99%. This was observed within 3-7 days after administration of 5 x 10^{11} gc/kg or more. Interestingly, the combined use of cyclophosphamide (given orally up to a dose of 200 mg/m^2/week) significantly improved the level and duration of alipogene tiparvovec efficacy in this model due to an immune response against human LPL (7-11).

A study in LDL receptor knockout (*Ldlr*$^{-/-}$) mice receiving i.m. injections of alipogene tiparvovec (8 x 10^{12} gc/kg) and subsequently fed a Western diet demonstrated long-term transgene expression and a reduction in fasting plasma triglycerides. Fasting plasma triglyceride levels were shown to decrease by 48% 16 weeks posttreatment. Further analysis of muscle and liver tissue homogenates revealed an increase in lipid content of injected muscles along with a significant decrease in triglycerides (-20%) and free cholesterol (-24%) in liver homogenates. In contrast, alipogene tiparvovec did not affect total cholesterol levels or atherosclerotic lesion area (12).

Further studies have been carried out in a mouse model of type III dyslipidemia, which presents clinically with xanthomata and is associated with an increased risk of cardiovascular disease. In mice expressing human apolipoprotein E2 (apoE2) but deficient in endogenous protein (apoE2KI), single i.m. injections of alipogene tiparvovec (8 x 10^{12} gc/kg) produced a marked increase in postheparin human LPL protein levels, which was accompanied by a 20% reduction in fasting plasma triglyceride levels and a 2-fold increase in the rate of triglyceride clearance (13).

Safety

Additional investigations have assessed germline transmission of alipogene tiparvovec and its embryotoxicity. Following i.m. injections of the gene therapy into the hind legs of mice 4 weeks prior to mating at 1 x 10^{11}, 1 x 10^{12} and 1 x 10^{13} gc/kg, fetuses were assessed at gestational day 18. It was confirmed that fetuses did not exhibit vector DNA levels despite a dose-dependent increase in vector DNA in the tissues of the dams (including the placenta). Moreover, embryological development and general well-being and fertility of the dams were not affected during treatment (14).

In vivo safety and biodistribution studies have also been completed in wild-type mice. I.m. administration of 1 x 10^{11}, 1 x 10^{12} and 1 x 10^{13} gc/kg did not cause any deaths, significant changes in overall health or food consumption or organ pathology. Dose-dependent, reversible spleen hyperplasia and myositis at injection sites were the most consistently reported toxic effects. Biodistribution data showed short-term vector leakage from injection sites into the circulation, followed by liver-mediated clearance. Persistence of vector DNA was limited to the injected muscle and draining lymph nodes (15, 16).

Clinical Studies

The feasibility of the clinical use of the gene therapy was initially tested using skeletal muscle biopsies from patients with genetic LPL deficiency. Myosin-positive cells were infected with alipogene tiparvovec (1.6 x 10^4 gc/cell) and immunoreactive human LPL^{S447X} was detected in the culture medium, as was LPL production (15, 17).

To date, preliminary clinical findings have been presented for LPL-deficient patients presenting with hypertriglyceridemia and recurrent pancreatitis involved in phase I/II clinical trials in The Netherlands (receiving low and high doses of alipogene tiparvovec: 1 x 10^{11} and 3 x 10^{11} gc/kg, respectively; n = 4 in each dose group). Analysis of serum, saliva, urine, semen and muscle biopsies via polymerase chain reaction (PCR) indicated an effective biodistribution of alipogene tiparvovec. Vector sequences were shown to be only transiently detected in these studies, with highest levels detected in patient serum. Persistent high levels of vector sequences were only detected in injected muscle (18, 19). No acute toxicity has been observed related to alipogene tiparvovec injection; however, CD8$^+$ T-cell responses to capsids were apparent in these patients following injection with multiple serotypes and in at least two different tissue types. Importantly, no T-cell response has been observed to the LPL transgene product (20). The gene therapy was shown to be safe and well tolerated at these doses, with a significant reduction in median triglyceride levels in this patient cohort (below the target value of 10 mmol/l, or a 40% reduction posttreatment). Long-term expression of the therapeutic gene was also observed in 2 patients from the lower dose group, with a reduction in triglyceride levels coinciding with the expression of active LPL in the injected muscle at 26-32 weeks after administration (21).

Interim results from a pivotal phase II trial have also been presented. In this study, patients with LPL deficiency were administered alipogene tiparvovec at doses of 3

x 10^{11} and 1 x 10^{12} gc/kg with or without immunosuppression. Treatment was well tolerated and fat levels were reduced in all but 1 case; all patients reported increased energy. Only one episode of pancreatitis was seen soon after injection (22, 23).

Source

Amsterdam Molecular Therapeutics (NL).

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