



## DRUG PROFILE

# Alipogene tiparvec, an adeno-associated virus encoding the Ser<sup>447</sup>X variant of the human lipoprotein lipase gene for the treatment of patients with lipoprotein lipase deficiency

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Amsterdam Molecular Therapeutics BV is developing alipogene tiparvec (Glybera, AMT-011, AAV1-LPLS447X), a Ser<sup>447</sup>X variant of the human lipoprotein lipase (LPL) gene (LPLSer<sup>447</sup>X) in an adeno-associated virus vector, as a potential intramuscular gene therapy for the treatment of LPL deficiency. Familial LPL deficiency is a rare, autosomal-recessive disorder of lipoprotein metabolism that is characterized by severe hypertriglyceridemia with episodes of abdominal pain, acute pancreatitis and eruptive cutaneous xanthomatosis. The lack of functional LPL in patients with LPL deficiency causes an accumulation of triglyceride (TG)-rich lipoproteins in the plasma. The LPLSer<sup>447</sup>X variant is associated with decreased levels of plasma TGs and increased HDL cholesterol concentrations compared with wild-type LPL. Preclinical studies evaluating alipogene tiparvec in a mouse model of LPL deficiency demonstrated a long-term, dose-dependent correction of the lipid abnormalities. The first clinical trials in patients with LPL deficiency appear promising, with a significant decrease in the levels of plasma TGs observed in the first 3 months following the administration of alipogene tiparvec, and an increase in local LPL activity and protein levels observed after 6 months. In addition, a decrease in pancreatitis frequency was observed during a 3-year follow-up period. At the time of publication, a phase II/III trial in patients with LPL deficiency, being conducted to further support the submission of an MAA to the EMEA for alipogene tiparvec, was ongoing. The compound is also under investigation for the treatment of type V hyperlipoproteinemia, Syndrome X and non-alcoholic steatohepatitis.

## Introduction

Lipoprotein lipase (LPL), a member of the lipase family that also includes hepatic lipase, pancreatic lipase and the recently discovered endothelial lipase, is the key enzyme regulating the catabolism of triglyceride (TG)-rich lipoproteins in the circulation [1030566], [1030569]. LPL is synthesized and secreted primarily by adipocytes and by skeletal and cardiac myocytes [1030569]. Active LPL is formed through non-covalent homodimerization of the 448-amino acid LPL protein; the activation of the catalytic center of LPL requires apolipoprotein (apo)CII. LPL interacts with heparan sulfate proteoglycans on the luminal surface of the endothelium, allowing LPL to extend into the plasma in order to sequester TG-rich lipoproteins such as chylomicrons and VLDL particles [1030569]. Lipolysis occurs at the lipid/water interface, where substrate access involves a lipid-binding domain in the distal C-terminal region of LPL [1030574].

Familial LPL deficiency (Online Mendelian Inheritance in Man number: 238600), also known as primary chylomicronemia and type I hyperlipoproteinemia, is a rare autosomal-recessive disorder of lipoprotein metabolism

<b>Therapeutic</b> Alipogene tiparvec
<b>Originator</b> Amsterdam Molecular Therapeutics BV
<b>Status</b> Phase III Clinical
<b>Indications</b> Hypertriglyceridemia, Lipoprotein lipase deficiency, Non-alcoholic steatohepatitis, Syndrome X
<b>Actions</b> Adeno-associated virus-based gene therapy, Anti-inflammatory, Hypertriglyceridemic agent, Lipoprotein-lipase stimulator
<b>Technologies</b> Biological therapeutic, Intramuscular formulation, Viral gene transfer system
<b>Synonyms</b> AAV1-LPLS447X, AMT-010, AMT-011, Glybera

[1030569]. LPL deficiency is characterized by severe hypertriglyceridemia caused by the absence of LPL activity, and consequently leading to the accumulation of TG-rich lipoproteins in the plasma [1030569]. The population frequency of LPL deficiency is 1 per 1 million individuals [1030569], with a carrier frequency of

approximately 1 per 500 individuals. Because of founder effects, the frequency of LPL deficiency is higher in French-Canadians, affecting up to 1 in 5000 individuals in this population [1030570]. Patients with LPL deficiency often exhibit extremely low or absent LPL activity in post-heparin plasma [1030569]. This diagnostic test involves the intravenous administration of heparin to stimulate the release of LPL into the plasma, followed by a measurement of LPL activity. Alternatively, LPL activity can be assayed in adipose tissue. When post-heparin or adipose tissue LPL activity is demonstrated to be severely reduced or absent, LPL deficiency can be confirmed by the identification of mutations in the *LPL* gene by DNA sequencing [1030569].

LPL deficiency typically manifests early in childhood, with repeated episodes of abdominal pain, acute pancreatitis, hepatosplenomegaly, and fat accumulation in the skin (eruptive cutaneous xanthomatosis) and retinal vasculature (lipemia retinalis) [1030569]. The severity of symptoms is proportional to the degree of hyperchylomicronemia, which is affected by dietary fat intake [1030569]. Therefore, the goal of therapy for LPL deficiency is to reduce the risk of life-threatening pancreatitis by increasing local LPL activity [1049166]. The management of LPL deficiency has traditionally consisted of the strict adherence to a low-fat diet (< 15% of total calories), but compliance with such a diet is variable and difficult [1030569]. Potential alternative strategies include the use of a modified Scopinaro's biliopancreatic diversion, which was performed in one patient who could not comply with the diet to cause the almost complete malabsorption of lipids [1049168]. In addition, fibric acid derivatives (fibrates) are often prescribed, but display only modest TG-lowering effects in patients with LPL deficiency [1030571]. Thus, more effective therapeutic strategies are needed for this rare, but potentially fatal, condition.

The human *LPL* gene is located on chromosome 8p22 and comprises 10 exons [1030572]. Numerous structural mutations in the *LPL* gene have been reported to be associated with a catalytically defective LPL protein [1030569]. Individuals with LPL deficiency may be either homozygous for a single mutation (often associated with consanguinity) or compound heterozygotes [1030569]. Of the approximately 100 loss-of-function *LPL* gene mutations described in humans, the majority are missense or nonsense mutations clustered in the region coded by exons 4, 5 and 6 that forms the proposed catalytic domain of LPL [1030574], [1049169]. Obligate heterozygotes are usually asymptomatic, but can exhibit up to a 50% reduction in post-heparin LPL activity and variable plasma TG concentrations [1030569]. Other candidate genes associated with primary chylomicronemia include *APOA5*, *APOC2* and the recently reported *GPIHBP1* and *LMF1* [1030576], [1030577], [1049171]. Although chylomicronemia is generally primary and familial, the lipoprotein pattern may be produced by several other diseases or metabolic states [1049169].

In contrast to most *LPL* mutations that result in complete or partial loss of LPL function, *LPLSer<sup>447X</sup>* is a common variant of the *LPL* gene that occurs in ~ 20% of the population [1030579]. *LPLSer<sup>447X</sup>* lacks the two C-terminal amino acids (Ser and Gly) of the mature LPL protein. Carriers of the *LPLSer<sup>447X</sup>* variant exhibit decreased TG levels and increased HDL cholesterol concentrations, and have an apparent reduced risk of cardiovascular disease. The exact mechanism whereby *LPLSer<sup>447X</sup>* is apparently cardioprotective remains unclear [1030579]. In addition, *LPLSer<sup>447X</sup>* carriers display enhanced conversion of TG-rich lipoproteins and increased LPL-mediated apoB100 clearance, with a 4-fold increase in preheparin LPL concentration – findings that are consistent with a gain-of-function associated with this *LPL* variant [1030580]. Furthermore, post-prandial apoB48 clearance is enhanced in both *LPLSer<sup>447X</sup>* heterozygotes [1030582] and homozygotes [1030592].

Compared with their non-transgenic litter mates, transgenic mice overexpressing human *LPL* exhibited lower levels of plasma TGs and enhanced clearance and conversion of chylomicrons and VLDL, and resisted the development of hypercholesterolemia when fed a high-cholesterol diet [1030596]. LDL receptor knockout mice, a model of atherosclerosis, developed fatty streaks within weeks of being fed an atherogenic diet; however, the area of the fatty streaks was reduced in mice expressing a human *LPL* transgene [1030597]. Further studies involving *ApoE* knockout mice demonstrated the suppression of plasma TG levels and resistance to atherosclerosis in animals that were engineered to overexpress a human *LPL* transgene [1030598].

Heterozygous *Lpl* knockout (*Lpl<sup>-/-</sup>*) mice survive to adulthood, but display reduced catabolism of VLDL and mild hypertriglyceridemia [526672]. However, the homozygous condition is lethal. Homozygous *Lpl<sup>-/-</sup>* mice die within ~ 18 h of birth because of extreme elevations in chylomicrons that are believed to block the capillaries, causing hypoxia. The muscle-specific expression of human *LPL* in *Lpl<sup>-/-</sup>* mice prevents neonatal death and normalizes lipoprotein levels [526672]. Preliminary proof-of-concept for the use of gene therapy to ameliorate hyperchylomicronemia was provided by studies demonstrating that the adenoviral-mediated transfer of *LPL* rescued *Lpl<sup>-/-</sup>* mice [1030160]. Of particular interest, transfer of the human *LPLSer<sup>447X</sup>* variant to *Lpl<sup>-/-</sup>* mice resulted in more effective rescue, in terms of longevity and lipoprotein metabolism, compared with the transfer of wild-type human *LPL* [1030160]. Similar to humans with LPL deficiency, adult *Lpl<sup>-/-</sup>* mice (rescued on the day of birth by intramuscular administration of recombinant adenovirus-*LPLSer<sup>447X</sup>* vector [1 x 10<sup>8</sup> PFU/mouse]) exhibit low levels of plasma LPL activity, elevated plasma TG concentrations (> 200-fold that of wild-type animals) and low HDL cholesterol (< 10% that of wild-type animals) [1007763], [1030160].

Amsterdam Molecular Therapeutics BV (AMT) is developing alipogene tiparvovec (Glybera, AMT-011, AAV1-LPLS447X), an adeno-associated virus (AAV) encoding the Ser<sup>447</sup>X variant of the human *LPL* gene, as a potential gene therapy for patients with LPL deficiency. Alipogene tiparvovec is administered as a single set of intramuscular injections that is intended to provide the long-term correction of functional LPL deficiency; targeted delivery of the AAV vector genome (comprising the *LPLSer*<sup>447</sup>X variant) to muscle cells leads to the expression and secretion of the functionally active enzyme [1046549]. Preclinical studies are also underway to investigate the potential of alipogene tiparvovec for the treatment of type V hyperlipoproteinemia, non-alcoholic steatohepatitis and metabolic syndrome (Syndrome X) [1048251], [1046549]. In October 2008, the EMEA's Pediatric Committee supported plans to assess alipogene tiparvovec as a treatment for pediatric patients with LPL deficiency [956186]. Alipogene tiparvovec has been granted Orphan Drug status for the treatment of LPL deficiency by the EMEA [527759] and the US FDA [801849]. In November 2009, AMT anticipated that filing for EMEA regulatory approval in Europe would be achieved within 3 months and that approval would be obtained by 2011; in Canada and the US, filing submissions were expected in late 2010 and 2012, respectively [1060497]. At the time of publication, a phase II/III clinical trial (ClinicalTrials.gov identifier: NCT00891306) of alipogene tiparvovec in patients with LPL deficiency was being conducted to support the regulatory filing [1058206].

## Synthesis and SAR

Alipogene tiparvovec comprises a recombinant AAV serotype 1 (AAV1) vector expressing the Ser<sup>447</sup>X variant of the human *LPL* gene [1007763]. This gene therapy was first generated using a plasmid system, and was designated as AMT-010 [1031799]. The AMT-010 recombinant AAV1 vector was produced via calcium phosphate-mediated cotransfection of AAV1 helper plasmid (p)DP1 and vector pVD5 or pTRCGW in HEK293 cells [1007763]. Recombinant AAV, pseudotyped with AAV1 capsids and containing AAV2 inverted terminal repeats, was generated using pDP1. An AAV vector encoding the human *LPLSer*<sup>447</sup>X gene was generated with plasmid pTRCGW (comprising the vector cassette) and insertion of the 1.6-kb *LPLSer*<sup>447</sup>X gene in place of the green fluorescent protein (*GFP*) gene, yielding pVD5 [1007763]. Finally, exchange of the ampicillin resistance gene of pDP1 and pVD5 for the kanamycin resistance gene led to the production of pVD20 and pVD23, respectively [1007762]. The transgene cassette within the recombinant AAV vector consists of a CMV promoter, *LPLSer*<sup>447</sup>X complementary DNA, a woodchuck hepatitis virus post-transcriptional regulatory element and a bovine growth hormone polyadenylation sequence [1007762], [1007763]. The vector was purified using anion chromatography, hydrophobic-interaction chromatography, and diafiltration, and then concentrated; viral titer was determined using quantitative PCR [1007762].

Because of limitations with the plasmid transfection system, AMT modified the production of alipogene tiparvovec to use a baculovirus system, which was designated as AMT-011 to differentiate between the two methods of production [1031799]. Recombinant baculovirus seed stocks (P5 for Bac.VD88, Bac.VD84 and Bac.VD43 encoding *Rep*, *Cap* and *LPLSer*<sup>447</sup>X) were used to infect SF+ insect cells in suspension culture, eliminating the transfection process and resulting in a more efficient, large-scale production method [1046178]. The vectors were purified using affinity chromatography [1046178]. A comparison of AMT-010 and AMT-011 in *Lpl*<sup>-/-</sup> mice indicated that the levels of transgene expression and efficacy were similar, irrespective of the production system used [1046178].

## Preclinical development

In an initial study in *Lpl*<sup>-/-</sup> mice, alipogene tiparvovec (5 × 10<sup>10</sup> to 5 × 10<sup>11</sup> genome copies [gc] im; 4 to 40 injection sites) produced a significant increase in LPL protein levels and activity in animals that were injected at 36 sites with a dose of 5 × 10<sup>11</sup> gc or at 40 sites with 4 × 10<sup>11</sup> gc [1007764], [1030162]. Visible hyperlipidemia was completely resolved, and TG levels were reduced by up to 99%. In addition, a 3- to 8-fold increase in HDL cholesterol and a 12- to 19-fold decrease in total cholesterol were observed. The injection of mice at 10-fold fewer sites led to a delay of 1 week in disease resolution, while a 10-fold lower dose was associated with up to a 77% reduction in TG levels [1007764], [1030162]. The effects of gene transfer were sustained for 12 months [1030162].

In order to evaluate the effects of higher dose levels, male *Lpl*<sup>-/-</sup> mice (n = 5 per dose) fed on a standard rodent chow diet received either low- or high-dose alipogene tiparvovec (8 × 10<sup>11</sup> or 8 × 10<sup>12</sup> gc/kg im [36 injection sites, 25 μl/site]) [1007763]. Within 1 week of the high-dose treatment, total plasma TG and total cholesterol concentrations were decreased by an average of 98% (p < 0.001 versus controls) and 92%, respectively. With the low-dose treatment, average reductions in plasma TG and total cholesterol of 73 and 76%, respectively, were observed 4 weeks post-gene transfer. Although the high dose of alipogene tiparvovec normalized plasma free-fatty-acid concentrations (p < 0.001 versus controls), this result was not observed with the low-dose treatment. The decrease in TG concentration with the high-dose treatment was associated with a 5.5-fold increase in HDL cholesterol levels (from 0.2 to 1.1 mmol/l [p < 0.001]; 61% of wild-type murine levels) compared with a 2.5-fold improvement (to 0.4 mmol/l [p < 0.001]; 24% of wild-type murine levels) with the low-dose treatment. The effects on plasma lipids were sustained for > 1 year following a single administration of the vector [1007763].

In the same study, *Lpl*<sup>-/-</sup> mice treated with high-dose alipogene tiparvovec exhibited a significant increase in post-heparin plasma LPL activity of up to 33% compared with wild-type murine levels (week 2 to 10 average = 131 mU/ml; p < 0.001 versus controls and the

low-dose treatment), compared with an increase of 9% of wild-type levels observed with low-dose alipogene tiparvovec [1007763]. Consistent with these findings, LPL protein levels in plasma increased 12-fold with the high-dose treatment ( $p < 0.01$  versus controls and the low-dose treatment) and 2-fold with the low-dose treatment (week 2 to 10 average = 202 ng/ml;  $p < 0.01$  versus controls). At 12 weeks post-injection, the mice were sacrificed. In the mice treated with either the low or high dose of alipogene tiparvovec, LPL protein and activity were detected in the injected gastrocnemius and adductor muscles, but not in the liver, heart or adipose tissue. Both doses of alipogene tiparvovec were associated with a significant increase in LPL activity in injected gastrocnemius muscle tissue compared with tissue in control *Lpl*<sup>-/-</sup> mice. The low-dose treatment resulted in similar levels of LPL activity in injected tissue to those observed in wild-type mice, while the high-dose treatment produced a  $> 11$ -fold elevation in local tissue LPL activity compared with wild-type activity levels. Consistent with these data, a dose-dependent increase in LPL protein was observed in the gastrocnemius muscle of treated mice. At 8 months post-gene transfer, the rate of TG clearance following an intravenous fat load was similar in both wild-type and *Lpl*<sup>-/-</sup> mice treated with high-dose alipogene tiparvovec [1007763]. Overall, these results demonstrated the long-term AAV1-mediated expression of human *LPL*<sup>Ser447X</sup> in the skeletal muscle of *Lpl*<sup>-/-</sup> mice, resulting in the dose-dependent correction of the lipid abnormalities that characterize LPL deficiency. Data from this study have also been reported in an abstract publication [1030163].

The potential of alleviating hypertriglyceridemia of a different etiology was investigated using *APOE2* knock-in mice, a murine model of familial dysbetalipoproteinemia (type III hyperlipoproteinemia associated with increased plasma concentrations of remnant lipoproteins) [1007760]. Alipogene tiparvovec ( $8 \times 10^{12}$  gc/kg im [4 injection sites, 50  $\mu$ l/site]) was administered to female *APOE2* knock-in mice expressing human *APOE2*, but deficient for endogenous *ApoE* ( $n = 6$ ), and fed a standard rodent chow diet. At 3 weeks following treatment with alipogene tiparvovec, fasting plasma TG and total cholesterol concentrations were decreased by 27% ( $p = 0.05$ ) and 18% ( $p = 0.05$ ), respectively; HDL cholesterol levels were unchanged [1007760].

The overexpression of *LPL* has been associated with protection against atherosclerosis in LDL receptor knockout mice [1030597]; thus, the hypothesis that alipogene tiparvovec would decrease hyperlipidemia and atherosclerosis was tested [1030157]. Alipogene tiparvovec ( $8 \times 10^{12}$  gc/kg im [4 injection sites, 50  $\mu$ l/site]) was administered to female LDL receptor knockout mice ( $n = 10$ ) fed a standard rodent chow diet. At 4 weeks post-treatment, the diet of the mice was changed to a high-fat, atherogenic Western-type diet (15% cacao butter and 0.25% cholesterol) for 12 weeks; animals

were then sacrificed and atherosclerosis was assessed. Although alipogene tiparvovec therapy produced a 48% reduction in fasting plasma TG levels ( $p < 0.0001$  versus controls), there was no evidence of an effect on atherosclerosis. However, an analysis of homogenates demonstrated that significant increases in TG (40%) and total cholesterol (24%) in muscle tissue were accompanied by significant decreases in TG (20%), total cholesterol (22%) and free cholesterol (24%) in liver tissue [1030157].

The efficacy of alipogene tiparvovec was also assessed in a large-animal model of LPL deficiency, *Lpl*<sup>-/-</sup> cats [1007761], [1030163], [1031777], [1031779], [1031780]. *Lpl*<sup>-/-</sup> cats are homozygous for the LPL Gly<sup>412</sup>Arg mutation, display no detectable LPL protein or activity, and exhibit hypertriglyceridemia (plasma TG levels  $> 10,000$  mg/dl) with clinical symptoms of LPL deficiency, such as pancreatitis [1007761]. The administration of alipogene tiparvovec ( $5 \times 10^{11}$  to  $2 \times 10^{12}$  gc/kg im) to *Lpl*<sup>-/-</sup> cats induced a  $> 98\%$  reduction in plasma TG within 1 week [1030163]. However, by 3 weeks post-treatment, TG levels returned to baseline, and *LPL* expression was reduced substantially. This effect was attributed to the fact that residual LPL protein mass is not evident in *Lpl*<sup>-/-</sup> cats, making it likely that the administration of alipogene tiparvovec evoked an immune response [1030163]. Similar results were also noted in two further reports describing the effects of alipogene tiparvovec ( $1 \times 10^{11}$  to  $1 \times 10^{12}$  gc/kg im [2 to 50 injection sites]) in *Lpl*<sup>-/-</sup> cats [1031779], [1031780]. At doses of  $> 5 \times 10^{11}$  gc/kg, plasma TG levels were reduced by 99.9% and, within 3 to 7 days of treatment, plasma lipemia was no longer visible [1031779], [1031780].

Subsequently, low- and high-dose alipogene tiparvovec ( $1 \times 10^{11}$  and  $1.7 \times 10^{12}$  gc/kg im, respectively [10 injection sites]) was administered to male *Lpl*<sup>-/-</sup> cats ( $n = 2$  per dose) fed a commercial cat-food diet [1007761]. Within 1 week of high-dose treatment, plasma TG and total cholesterol concentrations were reduced from baseline by an average of 99 and 74%, respectively ( $p = 0.029$  and  $0.039$ , respectively, based on average reductions versus baseline in weeks 1 to 2), and plasma HDL cholesterol levels were elevated by 27% ( $p = 0.0004$  versus baseline). Low-dose alipogene tiparvovec had no effect on plasma lipid levels, and the improvements in plasma lipid concentrations observed with the high-dose treatment were transient, reverting to baseline levels within 3 weeks. Within 1 week of treatment, a dose-dependent increase in post-heparin LPL protein levels was observed ( $p < 0.0001$  versus baseline for both doses), and was accompanied by a dose-dependent increase in LPL activity ( $p = 0.015$  and  $0.016$  for low- and high-dose treatment versus baseline, respectively). However, at 2 to 3 weeks following treatment, plasma LPL activity and protein levels had returned to baseline levels. Loss of efficacy was associated with the presence of anti-LPL antibodies, which were first detected at week 3 post-treatment [1007761].

To determine if efficacy was improved by inhibiting the immune response to the expressed human LPL protein, alipogene tiparvovec ( $1 \times 10^{11}$ ,  $5 \times 10^{11}$  or  $1 \times 10^{12}$  gc/kg im [10 injection sites]) was administered in combination with cyclophosphamide (100 to 200 mg/m<sup>2</sup>/week po) to *Lpl*<sup>-/-</sup> cats (n = 2 to 5 per dose) fed a commercial cat-food diet [1007761], [1031777]. Within 1 week of treatment, the two highest doses of alipogene tiparvovec reduced plasma TG levels by > 99.9%, and the lower dose reduced plasma TG levels by 96%; changes in plasma TG levels correlated with a decrease in the levels of total cholesterol and an increase in HDL cholesterol. Despite the improvement in short-term efficacy following the co-administration of alipogene tiparvovec with cyclophosphamide, plasma LPL levels decreased to baseline levels within 2 to 3 weeks of treatment with both of the higher two doses. However, in two out of the three cats that received the lowest dose of alipogene tiparvovec in combination with higher doses of cyclophosphamide (150 to 200 mg/m<sup>2</sup>/week po), an anti-LPL antibody response was not detected; post-heparin plasma LPL levels and LPL activity decreased over time, but were not reduced to less than background levels (p = 0.007 and 0.043, respectively, based on average reductions versus baseline in weeks 2 to 8) [1007761], [1031777]. No positive effects on pancreatitis, pancreatic function or histology were evident following the treatment of *Lpl*<sup>-/-</sup> cats with alipogene tiparvovec [1007761].

In a further experiment, alipogene tiparvovec ( $5 \times 10^{11}$  gc/kg im [2, 10 or 50 injection sites]) was administered to *Lpl*<sup>-/-</sup> cats to investigate the effect of varying the number of injection sites; local doses ranged from  $4 \times 10^{10}$  to  $1 \times 10^{12}$  gc per site. A total of 50 injection sites was determined to be optimal because the therapeutic levels of TG reductions persisted until week 4; with fewer injection sites, TG reductions were maintained for only 1 week [1007761], [1031780].

To support the initiation of a clinical trial, primary skeletal cell cultures were prepared using muscle tissue obtained from patients with LPL deficiency (n = 5; LPL deficiency confirmed to be of genetic origin) [1007762]. Prior to tissue extraction, none of the patients exhibited LPL catalytic activity, and LPL protein levels ranged from 20 to 100% of normal levels. At 48 h post-infection with alipogene tiparvovec ( $1.6 \times 10^4$  gc/cell), *LPL*<sup>ser447X</sup> was detected in the culture medium at levels ranging from 60 to 200% of an infected control-cell culture derived from a healthy individual. LPL activity levels in the medium of patient-derived cultures ranged from 22 to 90%, and immunohistochemistry demonstrated that ~ 40% of cells expressed LPL. In the majority of cultures, supplementation with wild-type adenovirus led to an increase in transgene expression [1007762]. Similar data have also been reported in conference abstracts [513227], [1031779], [1031780], [1031790].

## Toxicity

Safety studies were conducted in male and female C57BL/6 mice administered a low, medium or high dose of alipogene tiparvovec ( $1 \times 10^{11}$ ,  $1 \times 10^{12}$  or  $1 \times 10^{13}$  gc/kg im

[2 injection sites, 25 µl/site]) [1007762]. Mice were sacrificed (n = 6 per time point) at 7, 28 or 90 days post-treatment, and blood and various organ and tissue samples were obtained for toxicological analyses. No deaths or significant changes in overall health or food intake between the dose groups were reported. However, a reduction in body-weight gain (of ~ 30% compared with control mice) was observed by day 90, predominantly in female mice administered high-dose alipogene tiparvovec. Changes in hematological and clinical biochemical parameters were considered to be 'minor and sporadic' for all three doses. A macroscopic examination of organs did not reveal any effect of alipogene tiparvovec treatment at any time point. However, microscopically, transient lymphoid hyperplasia was observed in the spleen of animals that received high-dose alipogene tiparvovec on days 7 and 28. In the control and high-dose animals, grade 1 myositis was noted at the injection sites on day 7; histology of the injection area was normal on day 28. At day 90, the control and low-dose groups displayed normal histology, while all animals in the high-dose group had grade 2 myositis at the injection sites; this myositis was characterized by multifocal-to-diffuse muscle fiber degeneration associated with a regeneration of fibers and chronic infiltration of inflammatory cells. An analysis of plasma samples obtained between 4 h and 7 days post-administration indicated that the levels of murine serum amyloid A, an inflammatory response marker, were elevated at 4 h, but declined rapidly thereafter [1007762].

Alipogene tiparvovec ( $1 \times 10^{11}$ ,  $1 \times 10^{12}$  or  $1 \times 10^{13}$  gc/kg im) was administered to female CD1 mice (n = 29 to 31 per dose group) 4 weeks prior to mating; dams were sacrificed at gestation day 18 [1030165], [1031785]. In maternal tissues, the concentrations of vector DNA increased dose dependently, with peak levels observed in injected muscles of the high-dose group. Other tissues contained < 1% of the values observed in muscle. None of the fetuses (total n = 52; n = 6 to 20 per dose group; n = 16 in the high-dose group) displayed vector DNA levels that were significantly greater than background values. No mortalities or prominent clinical signs were observed, and treatment was not associated with changes in body weight, food consumption, organ weight or gross necropsy. Measures of reproduction, including numbers of pregnant females, implantation sites, live and dead fetuses, resorptions, post-implantation loss and sex ratio, were similar for all dose groups. Treatment was not associated with any fetal abnormalities. In a second experiment, alipogene tiparvovec ( $1 \times 10^{13}$  gc/kg im) was administered to pregnant mice at gestation day 0, 3 or 6, and animals were then sacrificed on gestation day 18. As assessed by the levels of humanized renilla GFP, none of the fetuses contained vector DNA. Only the placenta of animals injected at day 6 produced a positive GFP signal and, interestingly, a signal was detected only in the maternal side of the placenta [1030165], [1031785].

In *Lpl*<sup>-/-</sup> mice (n = 5 per dose) that received alipogene tiparvec (8 × 10<sup>11</sup> or 8 × 10<sup>12</sup> gc/kg im [36 injection sites, 25 µl/site]), no increase in the concentrations of creatine phosphokinase (CPK), a marker of muscle injury, was observed in the 12 weeks following treatment [1007763]. No signs of local toxicity were detected in the muscles of treated mice in response to the AAV infection, transgene expression or overexpression of *LPL*. Transient levels of serum amyloid A were detected shortly after gene transfer [1007763].

In *Lpl*<sup>-/-</sup> cats, plasma CPK levels were elevated significantly following the administration of alipogene tiparvec (1 × 10<sup>11</sup> or 1.7 × 10<sup>12</sup> gc/kg im [10 injection sites]) [1007761]. Levels of CPK peaked at weeks 4 and 3 for the lower and higher doses, respectively (p < 0.0001 versus background), but returned to baseline by week 8. The coadministration of alipogene tiparvec (1 × 10<sup>11</sup>, 5 × 10<sup>11</sup> or 1 × 10<sup>12</sup> gc/kg im) and cyclophosphamide (100 to 200 mg/m<sup>2</sup>/week po) was also associated with significant increases in plasma CPK levels, with peak concentrations observed at weeks 3 to 4 (p < 0.01). At week 4, *Lpl*<sup>-/-</sup> cats that did not generate an immune response to treatment demonstrated increases in plasma CPK levels that were smaller, but still significant, compared with the increases observed in animals with an immune response (p = 0.041) [1007761].

## Metabolism and pharmacokinetics

To assess vector biodistribution, male and female C57BL/6 mice (n = 5 per time point) were administered alipogene tiparvec (1 × 10<sup>11</sup> or 1 × 10<sup>13</sup> gc/kg im) and sacrificed at 7, 28 or 90 days post-dosing [1007762]. An analysis of samples from the high-dose group indicated that although viral DNA was present in whole blood until day 28, clearance from plasma occurred within 3 to 4 days. On day 7, vector DNA was detected mainly in the injected muscles, spleen, liver, inguinal lymph nodes and bone marrow; levels declined over time and, by day 90, vector DNA was detected only at the injection sites, in the liver and in the inguinal lymph nodes. The low-dose group exhibited a similar biodistribution to the high-dose group, but with proportionally lower levels of vector sequence. An analysis of male and female gonads from the high-dose group revealed that the levels of vector DNA changed from < 19,000 copies/µg on day 7 to between < 10 and < 550 copies/µg on day 90. In the low-dose group, vector DNA was present on day 7 in the gonads, at levels just exceeding background values (< 10 copies/µg), but in most cases vector DNA was undetectable beyond this time point. An assessment of antibody responses revealed that high titers of anti-AAV1 antibodies were produced following treatment; however, a trend toward a lower response was observed with the lowest dose tested, particularly in female mice. Anti-LPL antibodies were not detected [1007762].

In male *Lpl*<sup>-/-</sup> mice, alipogene tiparvec (8 × 10<sup>11</sup> or 8 × 10<sup>12</sup> gc/kg im [36 injection sites, 25 µl/site]) did not evoke an immune response. However, an anti-AAV1

response was observed in similar studies using a similar dose (unpublished data cited in [1007763]).

The administration of alipogene tiparvec (1 × 10<sup>11</sup> or 1.7 × 10<sup>12</sup> gc/kg im [10 injection sites]) to *Lpl*<sup>-/-</sup> cats resulted in a dose-dependent anti-LPL antibody response; irrespective of the dose level, the peak antibody-binding capacity was reached at 6 weeks post-dosing [1007761]. Although *LPL* expression deteriorated by week 2, significant levels of anti-LPL antibodies were not evident until week 3. LPL activity was completely abrogated by the immune response in all except one of the treated animals. The coadministration of alipogene tiparvec (1 × 10<sup>11</sup>, 5 × 10<sup>11</sup> or 1 × 10<sup>12</sup> gc/kg im) and cyclophosphamide (100 to 200 mg/m<sup>2</sup>/week po) did not prevent the development of anti-LPL antibodies in *Lpl*<sup>-/-</sup> cats that received either of the two highest doses of gene therapy [1007761]. However, the combination of cyclophosphamide and the lowest dose of alipogene tiparvec was not associated with an anti-LPL response [1007761], [1031777]. The anti-LPL response in *Lpl*<sup>-/-</sup> cats was attributed to species differences between the human and feline enzyme [1007761]. Similar data have also been presented in further publications [1030163], [1031777], [1031779], [1031780].

A phase I/II clinical trial of alipogene tiparvec (1 × 10<sup>11</sup> or 3 × 10<sup>11</sup> gc/kg im) assessed the excretion and shedding of the AMT-010 vector in patients with LPL deficiency. Vector sequences were transiently detected in all body fluids tested (ie, serum, saliva, urine, semen and muscle biopsies), but high levels were only detected persistently in injected muscles [1030166], [1031798]. Clearance of the vector from the serum, where concentrations were highest, occurred at a rate of 1 to 2 logs/week; urine was the first bodily fluid to clear, at 1 week following treatment. For short time periods, low levels of vector (a maximum of 25 to 28 copies/µg DNA) were present in the semen [1030166], [1031798]. Although no T- or B-cell responses to the LPL transgene product were observed in this trial, a dose-dependent T-cell response to the AAV1 capsid was detected in four patients, with an increase in anticapsid IgG3 levels following vector injection [1045360]. CD4<sup>+</sup> and, to a lesser extent, CD8<sup>+</sup> T-cells were activated, resulting in the production of TNFα and IFNγ [1045360]. A comparison of these data was to be made with a second clinical trial that included investigative arms of alipogene tiparvec (as AMT-011) monotherapy and in combination with immunosuppression to attempt to limit the T-cell responses observed in the AMT-010 trial [1031799]. However, at the time of publication, no comparative data were available.

## Clinical development

### Phase I/II

The clinical program for alipogene tiparvec began with an observational clinical trial and a phase I/II trial of AMT-010 [1043696], [1045471]. In the observational trial (PREP-01), baseline TG data were obtained from patients (n = 18) with LPL deficiency and a history of pancreatitis [1043696]. Diagnosis of LPL deficiency was performed on

the basis of known mutations, an LPL activity of < 20% and an LPL mass of > 5%, a median TG of > 10 mmol/l, and no apoCII deficiency; subsequently, eligible patients were enrolled in the phase I/II trial. All patients adhered to a low-fat (< 20 to 25% of total calories) and alcohol-free diet during both trials [1043696].

The phase I/II, proof-of-concept, open-label, dose-escalation clinical trial (CT-AMT-010-01), investigated a low or mid dose of alipogene tiparvovec ( $1 \times 10^{11}$  or  $3 \times 10^{11}$  gc/kg im, at multiple body sites as 40 or 60 injections of 500  $\mu$ l each, respectively) in patients ( $n = 8$ ) with complete LPL deficiency (compound heterozygotes) [800876], [1030707], [1043696], [1045471]. At 12 weeks post-injection, all patients demonstrated a reduction in median TG levels (27 and 41% for low- and mid-dose treatments, respectively) compared with baseline ( $p < 0.007$ ) [1030707]. The average median fasting TG level from 3 to 12 weeks post-injection was reduced compared with during the observational period ( $p < 0.01$ ) [1043696]. Three individuals in the mid-dose group and one in the low-dose group achieved the primary outcome measure of either median fasting plasma TG levels of  $\leq 10$  mmol/l or a reduction in plasma TG levels of 40% [1030707]. In the mid-dose group, a significant increase in local LPL activity and protein levels was observed in muscle homogenates between 26 and 36 weeks post-injection [1030707]. Furthermore, at 6 months post-injection, vector DNA and *LPL*Ser<sup>447</sup>X expression was detected in muscle tissue [1043696]. However, at 18 to 31 months post-treatment, improvements in plasma TG levels were diminished (ie, plasma TG levels were non-significant versus baseline) [1030707]. Despite this result, the incidence of pancreatitis decreased from 0.49 (in PREP-01) to 0.04 episodes per year in those individuals treated with alipogene tiparvovec and followed for up to 3 years ( $p < 0.05$ ) [1043696]. Follow-up of this interventional trial was to continue for 5 years post-dosing [1043696].

Further clinical trials of alipogene tiparvovec have been conducted using AMT-011 [1031799]. In a phase I/II, open-label, dose-escalation trial (CT-AMT-011-01), patients with partial LPL deficiency (a condition that frequently occurs in patients with type V hyperlipoproteinemia) received a mid or high dose of alipogene tiparvovec ( $3 \times 10^{11}$  or  $1 \times 10^{12}$  gc/kg im [mid-dose,  $n = 2$ ; mid-dose plus immunosuppression and high-dose plus immunosuppression,  $n = 4$  and  $8$ , respectively]) [798182], [1031508], [1031799]. Prior to treatment, the patients were enrolled in an observational trial (PREP-02); all patients displayed high fat levels and experienced recurrent pancreatitis episodes [911243]. From 2 to 12 weeks post-injection, most patients experienced a > 40% decrease in fasting plasma TG levels [1031508]. All patients reported a gain of energy, and in the two patients enrolled with diabetes mellitus, insulin resistance was reduced, enabling the patients to use less medication for their diabetes [911243], [948672], [1019043]. Fat accumulations in the skin or retinal vasculature of

patients were diminished or disappeared [911243], [948672], [1019043]. Approximately 4 months post-injection, TG levels typically increased toward pretreatment values, although this increase correlated with altered lipoprotein characteristics and lower TG levels in the chylomicron fraction [1031508]. Longitudinal follow-up was planned for 15 years [1031508].

Combined data obtained from the two observational periods and from the > 1 to 3.5 years of the CT-AMT-010-01 and CT-AMT-011-01 clinical trials indicated that the overall incidence of pancreatitis was reduced from 0.33 (expected mean incidence based on observational trials) to 0.06 episodes per patient per year ( $p < 0.05$  post- versus pre-alipogene tiparvovec treatment) for both interventional trials (total  $n = 22$ ). Compared with the values for TG levels that were obtained from patients in the observational studies, a reduction in fasting TG levels ( $p < 0.01$ ) was observed over 3 to 12 weeks post-injection and, in addition, a dose-range effect was evident [1054484]. Long-term follow-up from the CT-AMT-010-01 and CT-AMT-011-01 trials demonstrated that 4 out of 22 patients experienced a pancreatitis attack ( $n = 1$  immediately after injection;  $n = 3$  during long-term follow-up) [1060497].

### Phase II/III

At the time of publication, a phase II/III, open-label, single-group clinical trial (NCT00891306; CT-AMT-011-02) was recruiting patients with LPL deficiency (expected  $n = 8$ ). High-dose alipogene tiparvovec ( $1 \times 10^{12}$  gc/kg im) was to be administered with the immunosuppressants mycophenolate mofetil (2 g/day po, day -3 to week 12), cyclosporine (3 mg/kg/day po, day -3 to week 12) and methylprednisolone (1 mg/kg, single iv bolus). The primary endpoint was a reduction in TG concentrations at week 12. Secondary endpoints included reductions in chylomicrons and/or the chylomicron-TG ratio at 12 weeks, the biological activity and expression of the *LPL*Ser<sup>447</sup>X transgene product, the safety profile, and the shedding of viral vector at week 14. At the time of publication, recruitment and dosing of patients ( $n = 5$ ) had been completed [1058206]; however, results were not yet available.

### Side effects and contraindications

In the CT-AMT-010-01 clinical trial, alipogene tiparvovec was well tolerated [1019043], [1030707], [1043696], [1045471]. No treatment-related serious adverse events were identified, and no DLTs or clinically significant abnormalities in laboratory measurements, physical findings or other parameters were identified [948672], [1045471]. A total of 15 serious adverse events were reported by four individuals, but none of these events was considered to be treatment related [1043696]. Minor discomfort at the injection site occurred for approximately 1 day following administration [1043696], but muscle function tests and fat content, as assessed by MRI, were unaffected by alipogene tiparvovec [1030707]. However, one patient who

received a mid-dose treatment of alipogene tiparvec (3 x 10<sup>11</sup> gc/kg im) developed a transient increase in serum CPK levels (a 2-fold increase that was not considered to be an adverse event) at 4 weeks post-injection. This CPK elevation coincided with a loss of transgene expression, and was suggestive of T-cell-mediated destruction of transduced muscle cells [1045360], [1045471].

The CT-AMT-011-01 clinical trial was completed by all patients, and alipogene tiparvec was reported to be well tolerated [1019043], [1031508].

## Patent summary

AMT first claimed for therapeutics containing *LPLSer*<sup>447X</sup>, nucleic acids encoding *LPLSer*<sup>447X</sup> or gene-therapy vectors encoding *LPLSer*<sup>447X</sup> in WO-00100220; this application was granted in Europe as EP-01200117, which was due to expire in June 2020. By November 2009, a US application for an equivalent to WO-00100220 had not been submitted.

AMT has also claimed the use of *LPLSer*<sup>447X</sup> therapeutics (eg, gene-therapy vectors) for treating non-alcoholic steatohepatitis in WO-2005123117; the European equivalent, EP-01761273, was due to expire in June 2025. By November 2009, a corresponding US patent application (US-20080280823) was still pending grant.

Several claims relating to the production of AAV vectors (potentially including alipogene tiparvec) in insect cells have also been filed by AMT, including WO-2007046703, WO-2007148971 and WO-2009014445.

## Current opinion

Familial LPL deficiency is an autosomal recessive disorder of lipoprotein metabolism that is characterized by severe hypertriglyceridemia and results in recurrent and potentially life-threatening pancreatitis. The severity of symptoms is proportional to the degree of hyperchylomicronemia, which is affected by dietary fat intake. Therefore, the goal of therapy for LPL deficiency is to reduce the risk of life-threatening pancreatitis by lowering chylomicronemia. The treatment of LPL deficiency has traditionally involved strict adherence to a low-fat diet; however, compliance with such a diet is variable and difficult. Fibrates are only modestly effective in patients with LPL deficiency. Even with aggressive management, plasma TG concentrations in patients with LPL deficiency often remain > 10 mmol/l – a threshold for which the risk of acute pancreatitis becomes clinically significant [1030571]. Thus, there is a need for more effective therapeutic strategies to be developed for the treatment of LPL deficiency.

Alipogene tiparvec is an AAV-based gene therapy for the delivery of *LPLSer*<sup>447X</sup> to muscles in order to enhance TG metabolism in patients with LPL deficiency. A single administration of alipogene tiparvec resulted in the long-term complete correction of hypertriglyceridemia in *Lpl*<sup>-/-</sup> mice. Preclinical toxicity and biodistribution studies have led to regulatory approval of this gene-therapy vector for clinical testing in patients with LPL deficiency. In short- to medium-term clinical trials (up to 3 years), alipogene tiparvec appeared to be safe and tolerable, with evidence for a decrease in the incidence of pancreatitis. However, the long-term efficacy and safety of alipogene tiparvec in humans remains to be determined. One major concern for AAV-based gene therapies is the potential for the development of an immune response; whether this response would limit the long-term expression of LPL is unknown [1045360]. To counteract this immune response, patients involved in the current phase II/III clinical trial of alipogene tiparvec were to be administered immunosuppressants.

LPL deficiency is a rare condition, affecting only an estimated 4000 patients globally, according to AMT [751002]; thus, alipogene tiparvec would target only a small niche market. In October 2009, AMT established an agreement with the company Progenika Biopharma SA to develop and commercialize a diagnostic tool, LPLChip, that would enable the efficient diagnosis of patients with complete or partial LPL deficiency [1058206]. Whether alipogene tiparvec would be effective in other, more common forms of hypertriglyceridemia that may be associated with partial LPL deficiency remains to be determined. AMT has initiated a separate preclinical development program to evaluate alipogene tiparvec in type V hyperlipoproteinemia, which is estimated to affect approximately 100,000 patients in Europe and North America [1046549]. However, patients with chylomicronemia caused by inherited defects in genes other than *LPL*, such as *APOA5* and *APOC2*, might not be expected to benefit from alipogene tiparvec therapy.

In addition, preclinical studies were underway to determine whether alipogene tiparvec could be a potential treatment for non-alcoholic steatohepatitis; in a murine model of atherosclerosis, the administration of alipogene tiparvec caused a reduction in hepatic fat content.

Alipogene tiparvec could become the first commercially available AAV-based gene therapy, and provides proof-of-principle that such AAV-vector design and technology may be applicable to other rare genetic disorders, such as acute intermittent porphyria, primary hyperoxaluria and apoAI deficiency, for which effective treatments do not currently exist.

## Deals

### Amsterdam Molecular Therapeutics BV

In April 2001, AMT licensed the AAV technology, on which the *LPL* gene therapy is based, from the German Cancer Institute [419907].

In August 2001, AMT signed an exclusive sublicense and research agreement with Xenon Pharmaceuticals Inc, providing AMT with access to technology and IP related to *LPL* [419907].

### Xenon Pharmaceuticals Inc

By August 2001, Xenon Pharmaceuticals had exclusively licensed technology and IP related to *LPL* from the University of British Columbia [419907].

## Development status

Developer	Country	Status	Indication	Date	Reference
Amsterdam Molecular Therapeutics BV	Canada	Phase III	Lipoprotein lipase deficiency	07-MAY-09	1006971
Amsterdam Molecular Therapeutics BV	Netherlands	Discovery	Hypertriglyceridemia	21-MAY-09	1048251
Amsterdam Molecular Therapeutics BV	Netherlands	Discovery	Syndrome X	21-MAY-09	1048251
Amsterdam Molecular Therapeutics BV	Netherlands	Discovery	Non-alcoholic steatohepatitis	21-MAY-09	1048251

## Associated patent

**Title** *LPL* variant therapeutics.

**Assignees** Amsterdam Molecular Therapeutics BV; Academisch Ziekenhuis bij de Universiteit van Amsterdam [Universiteit van Amsterdam]; University of British Columbia

**Publication** WO-00100220 04-JAN-01

**Inventors** Hayden MR, Kastelein JJP.

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798182 **Amsterdam Molecular Therapeutics announces intention to launch an Initial Public Offering (IPO) on Euronext Amsterdam.** Amsterdam Molecular Therapeutics BV *PRESS RELEASE* 2007 May 22

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