Purpose of review
The present review summarizes the clinical development of adeno-associated viral vector (AAV1)-lipoprotein lipase (LPL) S447X gene therapy (alipogene tiparvovec) for lipoprotein lipase deficiency. Lipoprotein lipase deficiency is a rare inherited disease characterized by severe hypertriglyceridaemia, chylomicronaemia and risk of recurrent pancreatitis or other complications. AAV1-LPL S447X gene therapy is based on the rationale that by adding episomal copies of functional LPL genes into muscle cells lacking active LPL, metabolic function could be improved or restored.

Recent findings
AAV1-LPL S447X is a nonreplicating and nonintegrating AAV of serotype 1 designed to deliver and express the human LPL gene variant S447X. The clinical development programme for AAV1-LPL S447X consisted of two observational studies, three open-label interventional studies and one case note review analysis. Intramuscular administration of AAV1-LPL S447X was generally well tolerated and was associated with reduction in overall pancreatitis incidence and signs of clinical improvement up to 2 years after administration. Results of interventional studies suggest that markers of postprandial metabolism could be more accurate than fasting plasma triglyceride concentration to monitor the effect of AAV1-LPL S447X.

Summary
The overall benefit–risk ratio of AAV1-LPL S447X gene therapy appears positive to date, particularly for the patients presenting the highest risk of complications.

Keywords
gene therapy, hyperchylomicronaemia, hypertriglyceridaemia, lipoprotein lipase, LPL deficiency

INTRODUCTION
Gene therapy is part of the broad family of oligonucleotide-based treatments which also include antisense therapy and chimeroplasmy amongst others. Clinically, gene therapy aims at introducing competent genes into an individual’s cells to treat disease. The technology is complex, but its basic principle is simple: using a DNA-based agent that encodes a therapeutic protein to supplement a dysfunctional gene. Currently, human gene therapy targets somatic cells and uses nonintegrating agents, so that the effects of the treatment will be restricted to the individual, will not be transmitted to the offspring and will not integrate into the recipient’s genome. Gene therapy is performed either ex vivo, by using living cells as vehicles to transport the genes, or in vivo by direct transfer of genes into the patient’s tissues, using artificial or biological shuttles (Fig. 1) [1**,2**]. In all cases, a delivery system using viral or nonviral (biological, physical or chemical) vectors is required to assure the delivery of DNA [3*]. Since the first Food and Drug Administration-approved trial in 1990 [4], several gene therapy clinical trials have been designed and conducted worldwide with variable success, most of them targeting cancer or, less often, cardiovascular or Mendelian genetic diseases. Several extremely severe inborn errors of lipid metabolism are documented and by principle gene replacement therapy could apply to almost all of them.

GENE THERAPY STRATEGIES IN CLINICAL LIPIDOLOGY
Several proteins and enzymes involved in lipid metabolism are targets for the development of oligonucleotide-based treatments. Chimeroplasmy, a technique using synthetic RNA-DNA oligonucleotides

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Curr Opin Lipidol 2012, 23:310–320
DOI:10.1097/MOL.0b013e3283555a7e
(chimeric oligonucleotides) to induce single base pair changes in genomic DNA, has been tested in animal models to induce single-nucleotide conversion in the apolipoprotein E (APOE) gene with the intent to treat APOE4-related diseases or to prevent coronary and peripheral atherosclerosis caused by type III dysbetalipoproteinaemia, a form of severe hypertriglyceridaemia associated with the APOE2-E2 genotype [5]. Antisense technology, the most common form of oligonucleotide-based treatments, uses oligonucleotides (DNA, RNA or a chemical analogue) that will bind to a specific messenger RNA (mRNA) in order to physically obstruct translation and thus attenuate the levels of the corresponding protein [6,7]. Several antisense treatments developed to treat common cardiometabolic diseases target apolipoproteins or enzymes involved in lipoproteins pathways, such as apo B-100, apo B-48, apo(a), DGAT1, CETP or apo C3 [8–10]. Antisense therapy and related techniques do not aim at introducing coding genes into the cells which is a process that specifically characterizes gene replacement therapy.

The first gene replacement therapy trial in clinical lipidology was conducted in 1994 and utilized an ex-vivo approach [11]. It was aimed at treating the homozygous form of familial hypercholesterolaemia (HoFH) by introducing functional copies of the LDL receptor (LDLR) into the liver. Autologous hepatocytes were biopsied, transduced with retroviruses containing LDLR cDNA and transplanted into the liver of HoFH patients. Although the treatment was well tolerated, the efficacy was not impressive and the potential of in-vivo gene therapy in HoFH is now under investigation [12]. Hypercholesterolaemia is obviously not the only lipid target for gene therapy, and attention has recently focussed on the development and assessment of in-vivo gene therapy for severe hypertriglyceridaemia due to lipoprotein lipase deficiency (LPLD, type I hyperlipoproteinaemia).

FIGURE 1. Ex-vivo and in-vivo gene therapy. Ex-vivo approach (left) has been used to treat the homozygous form of familial hypercholesterolaemia (HoFH). Autologous hepatocytes were biopsied, transduced with retroviruses containing the LDL receptor (LDLR) cDNA and transplanted into the liver of HoFH patients. LPLD gene therapy (right) is based on the in-vivo administration of LPL transgene and capsid in the muscles of the lower limb.
chylomicronaemia and severe hypertriglyceridaemia. The prevalence of severe hypertriglyceridaemia is estimated at 1:600 individuals in North America, whereas that of LPLD (type I hyperlipidaemia) is 1–2:10^6 [14^*,15]. Thus, the majority of individuals with chylomicronaemia do not have LPLD but rather exhibit a combination of genetic susceptibility and secondary causes of hypertriglyceridaemia.

LPLD is a rare autosomal recessive disease. It may be diagnosed during adulthood only, although it often presents in infancy or childhood with severe abdominal pain, repetitive colicky pains, repeated episodes of pancreatitis and failure to thrive [15–17,18^*]. On physical examination, eruptive xanthomas, lipaemia retinalis and hepatosplenomegaly can be present. Laboratory investigation reveals lactic plasma which often interferes with the determination of several biochemical, enzymatic and electrolyte activities [18^*]. LPLD is associated with a number of complications, one of the most serious being acute pancreatitis [19], which can be life-threatening and recurring, sometimes leading to chronic pancreatic insufficiency. The risk of pancreatitis varies according to the cause of chylomicronaemia and the presence of additional genetic or environmental risk modifiers [15–17,18^*].

Fasting plasma triglyceride in LPLD may exceed the normal values of 1.7 mmol/l by 10-fold to 100-fold. Classical disease management in LPLD aims at maintaining the triglyceride concentration below 20 mmol/l (2000 mg/dl), a level at which the risk of pancreatitis is considered reduced. Treatment of LPLD patients currently consists of severe reductions in dietary fat to less than 20% of caloric intake [15]. Compliance with this dietary regimen is very difficult, and even with good compliance, the diet is often not sufficiently effective at reducing chylomicronaemia and triglyceride levels. Hence, LPLD patients remain at increased risk for pancreatitis. Currently, no triglyceride-lowering drug such as fibrates or niacin or any specific therapy is available to modulate the course of the illness, so these patients are at high risk of morbidity and mortality [18^*,19–21]. Enzyme replacement therapy is not expected to be effective, because of the short intravascular half-life of the LPL protein (approximately 15 min). Therefore, gene therapy was proposed as potential treatment for LPLD.

LIPOPROTEIN LIPOASE DEFICIENCY-CAUSING GENES AS TARGETS FOR GENE REPLACEMENT THERAPY

Familial chylomicronaemia is caused by the loss-of-function mutations in the LPL gene or by mutations in genes coding for proteins or enzymes directly affecting LPL activity, such as apolipoprotein C-II (APOC2), lipase maturation factor 1 (LMF1), glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1) or apolipoprotein A-V (APOA5) [14^*,22^*]. APOC2 is the key cofactor for LPL, whereas LMF1 is a transmembrane protein involved in LPL maturation. LPL is mainly produced in adipocytes, skeletal muscle cells and cardiac muscle cells. After intracellular dimerization, LPL is secreted and transported to the luminal side of the blood capillaries where it is bound to the endothelium through heparan-sulphate proteoglycans [13]. It has been suggested that LMF1 and APOA5 facilitate the interaction of APOC2 with LPL on the capillary endothelium and that GPIHBP1 facilitates the binding of LPL to the chylomicrons through a mechanism involving APOA5 and possibly LMF1 [22^*,23–25]. Through these interactions, LPL clears dietary triglyceride loaded in chylomicrons after a meal, whereas in the fasting state, when chylomicrons are usually absent, LPL predominantly acts on triglyceride circulating liver-derived VLDL. Because of the lack of LPL activity, patients with LPLD are almost never in a fasting metabolic state. Loss-of-function mutations in the genes coding for any of the LPL cofactors can cause LPLD and chylomicronaemia, and recessive forms of familial chylomicronaemia due to mutations in the APOC2, APOA5, GPIHBP1 and LMF1 genes have been reported [14^*]. By principle, LPL and LPL co-factors constitute potential targets for gene replacement therapy. Not all loss-of-function mutations in LPL, LMF1 or other genes coding for LPL co-factors are associated with the LPLD phenotype however [22^*,26].

RATIONALE FOR LPL GENE THERAPY

To date, more than 70 LPL gene mutations have been described, most of them associated with loss of catalytic function. The most prevalent loss-of-function mutations in the LPL gene, such as the N291S and D9N variants, are defective alleles associated with significant residual LPL activity even amongst homozygotes [27,28]. These mutations are associated with increased cardiometabolic risk and they variably affect the triglyceride-rich lipoproteins’ catabolic pathways and networks. These common variants constitute a susceptibility to chylomicronaemia and pancreatitis only when other genetic or secondary factors interfering with LPL activity, increasing the enzyme workload or saturating the LPL catalytic site are present. Only mutations associated with complete loss of LPL activity (null alleles) underlie true LPLD and familial chylomicronaemia.

The rationale for LPL gene therapy is based on the strategy of adding extra copies of functionally
potent enzyme into the muscle tissue of affected patients, specifically homozygotes or compound heterozygotes for LPL mutations causing LPLD. LPL gene replacement therapy is not suitable for patients with familial chylomicronaemia due to mutations in other genes, such as APOC2, LMF1, APOA5 or GPIHBP1. Thus, all patients should be genotyped prior to treatment.

ALIPOGENE TIPARVOVEC (AAV1-LPLS447X)

Gene therapy

Adeno-associated viral vector (AAV)1-LPLS447X is a pharmacological agent developed by Amsterdam Molecular Therapeutics (now uniQure) for the treatment of LPLD [29]. The product is a nonreplicating and nonintegrating AAV of serotype 1 designed to deliver and express the human LPL gene variant S447X, a naturally-occurring gain-of-function LPL variant, found in 20% of Caucasians. LPLS447X is a ‘gain-of-function’ mutation associated with increased turnover of triglyceride-rich particles and lower rate of cardiovascular disease (Fig. 2) [30].

AAV1-LPLS447X was initially produced using plasmid-based transient transfection and human embryonic kidney (HEK293) cells as a production platform. As the plasmid-based production process was not amenable to large-scale production, this method was replaced by baculovirus-based production in insect cells. AAV1-LPLS447X produced in this manner is named alipogene tiparvovec.

Alipogene tiparvovec formula is a sterile solution presented as single use vials. Each vial contains 3 x 10^{12} genomic copies (gc) of AAV1-LPLS447X in 1 ml of a phosphate-based formulation buffer containing 5% sucrose. It is injected under spinal anaesthesia at multiple sites in the muscles of the lower limbs at a dose of 1 x 10^{12} gc/kg body weight. Once transduced, vector genomes persist in muscle cells as episomal concatemers (Fig. 3), thus mediating long-term expression following a single (one-time) administration. Alipogene tiparvovec was designated orphan drug in the European Union in 2004 and in the USA in 2007.

PRECLINICAL STUDIES

Proof of principle for the treatment of LPLD with AAV1-LPLS447X has been analysed in two studies using LPL-deficient (LPL/−) mice and cats, respectively [31,32]. Comparable to humans, LPL−/− cats develop xanthomas, lipaemia retinai and controversially discussed pancreatitis. AAV-mediated delivery of LPLS447X in LPL−/− mice and cats has been shown to result in complete resolution of lipaemia and efficient reduction in plasma triglyceride concentration; in LPL−/− mice, efficacy lasted for the lifetime of the animal. The pharmacokinetic profile of AAV1-LPLS447X was investigated in cats, mice and rabbits. General toxicity studies were conducted in mice. Overall, the preclinical studies have shown that biologically active LPL is produced in the muscle following AAV1-mediated gene transfer [31,32].

REVIEW OF ALIPOGENE TIPARVOVEC

Clinical development

The clinical development programme for alipogene tiparvovec consisted of two observational studies (Preparation-01 and Preparation-02) and three open-label interventional studies: CT-AMT-010-01, CT-AMT-011-01 and CT-AMT-011-02 (Table 1) [21,33,34,35,36]. Another study, CT-AMT-011-03 was performed as a case note review analysis to provide data on the incidence and the nature of pancreatitis and abdominal pain episodes in LPLD patients and to establish the efficacy of alipogene tiparvovec.

Preparation-01 and Preparation-02 studies were observation studies evaluating for up to 18 months the baseline triglyceride levels, the maximum effect of a controlled low-fat diet on fasting plasma triglyceride levels and the occurrence of pancreatitis or other complications reflecting the morbidity of LPLD. During the course of the observation studies, the patients received counselling by a dietician and were instructed to comply to a diet in which the fat content did not exceed 20% of the total daily caloric intake and no more than 55 g/day. The majority of the participants were previously followed in a lipid clinic and results of these observational studies showed no incremental triglyceridaemia (TG)-lowering effect of the tight nutritional follow-up during observation.

The Preparation-01 and CT-AMT-010-01 studies were executed in the Netherlands with all subsequent studies conducted in Canada. CT-AMT-010-01 was the first clinical study of AAV1-LPLS447X (produced using the plasmid-based production in HEK293 cells; this product was termed AMT-010) [33]. The CT-AMT-010-01 open-label, dose-escalation, interventional study was conducted in a single centre and involved a total of eight LPLD patients enrolled in the Preparation-01 study. Participants were administered AAV1-LPLS447X (AMT-010) at a dose of 1 x 10^{11} gc/kg (four participants) or 3 x 10^{11} gc/kg body weight (four participants). Efficacy was assessed using data from the first 12 weeks after dosing. Long-term efficacy follow-up consisted of data collected over a period of 26 weeks and up to 3 years after dosing. Over the first 12 weeks...
after dosing, all participants showed a reduction in median fasting plasma triglyceride levels, and three participants showed a reduction of greater than 40%. Three years after dosing, all of the participants showed triglyceride levels around or above baseline, indicating that the administration of AAV1-LPLS447X had induced a transient reduction of plasma triglyceride, which at the time was hypothesized to be related to an immune response to AAV1-capsid proteins [34]. The treatment was well tolerated and there were no drug-induced serious adverse events (SAEs).

Following the CT-AMT-010-01 study, 22 Canadian adult LPLD patients with a history of pancreatitis participated in the Preparation-02 observation study. Amongst them, 14 eligible patients were

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**FIGURE 2.** Schematic illustration of AAV-LPL<sup>S447X</sup> structure and administration. (a) The transgene expression cassette carries a CMV (cytomegalovirus) immediate early promoter that drives the expression of the human LPL gene variant LPL<sup>S447X</sup>. The cassette also contains a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), a bovine growth hormone polyadenylation (pA) sequence, and the entire cassette is flanked by adeno-associated virus (AAV) of serotype 2 (AAV2)-derived inverted terminal repeats (ITR). The AAV-LPL<sup>S447X</sup> cassette encoding AAV2 genome is packaged within capsids of serotype 1 (AAV1) which transduce skeletal muscle cells more efficiently than AAV2. (b) Alipogene tiparvovec is injected under spinal anaesthesia at multiple sites in the muscles of the lower limbs. In the myocytes, the expression cassette codes for the gain-of-function S447X variant of LPL. This variant presents two amino acid truncation compared to the wildtype.
enrolled in a pivotal, open-label, dose-escalation study (CT-AMT-011-01) [21,35]. CT-AMT-011-01 was designed to evaluate the safety of alipogene tiparvovec (i.e. AAV1-LPLS447X produced using baculovirus-based production in insect cells) and its effect on fasting triglyceride (primary objectives), as well as the long-term expression of LPLS447X in the muscle tissue and occurrence of LPLD comorbidities. Participants were assigned to three arms of treatment. Cohorts 1 (n = 2) and 2 (n = 4) received $3 \times 10^{11}$ gc/kg of alipogene tiparvovec, while cohort 3 (n = 8) received $1 \times 10^{12}$ gc/kg. Cohorts 2 and 3 also received immunosuppressants from the time of alipogene tiparvovec administration and continued for 12 weeks. The immunosuppressants regimen consisted of cyclosporine A

**FIGURE 3.** Mechanism of action. (a) Following the intramuscular administration of alipogene tiparvovec (A), the cassette and vector are internalized (B) and AAV-LPLS447X genomes persist in muscle cells as episomal monomers or concatemers (C). Once produced by the muscle cells, it is expected that LPL is secreted and translocated to muscle blood capillaries in the vicinity of the injection sites (D). (b) Once translocated to the luminal surface of endothelial cells (E), LPL is bound to the endothelium through heparan-sulphate proteoglycans. Results of interventional studies suggest that chylomicrons could become smaller (or less buoyant) following alipogene tiparvovec treatment. Significant improvement of postprandial clearance of the chylomicron particles has also been observed.
(3 mg/kg/day) and mycophenolate mofetil (2 g/day) initiated at the time of alipogene tiparvovec administration and maintained for 12 weeks thereafter. A combination of cyclosporine and an antiproliferative (mycophenolate mofetil) was chosen, because it has been widely used in graft rejection and chronic inflammatory diseases, is well tolerated, and is not associated with a significantly increased rate of infections [37]. Immunosuppressants for 12 weeks and alipogene tiparvovec treatment were generally well tolerated without any acute detrimental effects. Three to 12 weeks after alipogene tiparvovec administration, all but two participants demonstrated significantly reduced median plasma triglyceride compared to baseline. The overall TG-lowering effect was transient and beyond week 12 the effect was gradually lost in all patients until baseline levels were reached by approximately 4–6 months. Muscle biopsies taken at 6 months (between weeks 25 and 27) indicated a certain proportion of patients with local transgene expression, intracellular staining for LPL and intracellular lipid accumulation (Fig. 4). This effect was not observed in the noninjected muscles nor accompanied with any marginal increase in systemic LPL levels and did not correlate with fasting plasma triglyceride response. However, additional data on markers of clinical efficacy were collected parallel to the study protocol (Fig. 5) and signs of clinical improvement, independent of plasma triglyceride, were noticed several months after triglyceride reverted to baseline (up to 2 years after LPL gene transfection). Taken together, these signs raised the possibility that postprandial lipoprotein lipase characteristics, particularly the size, lipid content and kinetics of buoyant chylomicrons, rather than plasma triglyceride concentration per se, might be a better surrogate marker to monitor the clinical expression of LPLD and the effect of alipogene tiparvovec administration. These observations led to the conception and execution of two additional studies specifically designed to evaluate the effect of alipogene tiparvovec on postprandial chylomicron metabolism, kinetics and clearance (CT-AMT-011-02) and on pancreatitis and abdominal pain (CT-AMT-011-03).

The CT-AMT-011-02 study was conducted to document the postprandial effect of alipogene tiparvovec and collect additional efficacy and safety data at a dose $1 \times 10^{12}$ gc/kg in combination with immunosuppressive treatment [21,36]. Efficacy endpoints at 12 and 52 weeks included the assessment of fasting and postprandial triglyceride and lipid–lipoprotein metabolism, chylomicron clearance and kinetics, muscle expression of the LPLS447X transgene, secondary symptoms to LPL deficiency and occurrence of pancreatitis or other LPLD comorbidities. Five eligible Canadian adults with LPLD and a prior history of acute pancreatitis were included in the study. Two weeks before and 14 weeks after administration, chylomicron metabolism and plasma palmitate and glycerol appearance rates were determined following ingestion of a low-fat meal containing $3^{\text{H}}$-palmitate, combined with (continuous) intravenous infusion of [U-$^{13}$C]-palmitate and [1,1,2,3,3-$^{2}$H]-glycerol. Postprandial chylomicron clearance analyses were repeated after 52 weeks in three consenting participants. Results at 14 weeks have been published [36] and show that following the administration of alipogene tiparvovec, the triglyceride content of the chylomicron fraction and the chylomicron-triglyceride/total plasma triglyceride ratio were reduced in all

<table>
<thead>
<tr>
<th>Study</th>
<th>Dose (gc/kg)</th>
<th>Patients</th>
<th>Main Objectives</th>
<th>Duration</th>
<th>Status</th>
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</thead>
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<tr>
<td>PREPARATION-01</td>
<td>None</td>
<td>18</td>
<td>Effect of the diet Occurrence of events</td>
<td>78 Weeks</td>
<td>Completed</td>
</tr>
<tr>
<td>AMT-010-01</td>
<td>$1 \times 10^{11}$ $3 \times 10^{11}$</td>
<td>4</td>
<td>Safety Effect on fasting TG at 12 weeks</td>
<td>5 Years</td>
<td>Active phase completed, follow-up ongoing</td>
</tr>
<tr>
<td>PREPARATION-02</td>
<td>None</td>
<td>22</td>
<td>Effect of the diet Occurrence of events</td>
<td>83 weeks</td>
<td>Completed</td>
</tr>
<tr>
<td>AMT-011-01</td>
<td>$3 \times 10^{11}$ $1 \times 10^{12}$</td>
<td>6</td>
<td>Safety Effect on TG at 12 weeks</td>
<td>5 years+</td>
<td>Active phase completed, follow-up ongoing</td>
</tr>
<tr>
<td>AMT-011-02</td>
<td>$1 \times 10^{12}$</td>
<td>5</td>
<td>Effect on CM clearance at 14–52 weeks</td>
<td>1 Year</td>
<td>Completed</td>
</tr>
<tr>
<td>AMT-011-03</td>
<td>None</td>
<td>22</td>
<td>Pancreatitis and abdominal pain incidence and characteristics</td>
<td>Retrospective and prospective</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

CM, chylomicrons; LPL, lipoprotein lipase; TG, triglyceridaemia.
throughout the postprandial period. The postprandial peak chylomicron $^3$H level and chylomicron $^3$H AUC were greatly reduced (by 79 and 93%, 6 and 24 h after the test meal, respectively). Intramuscular administration of alipogene tiparvovec resulted in a significant improvement of postprandial chylomicron metabolism in LPLD patients, without inducing large postprandial NEFA spillover.

Clinical outcomes were not a primary objective in the CT-AMT-011-01, CT-AMT-011-02 or CT-AMT-011-02 studies. However, up to 2 years after the intramuscular administration of AAV-LPLS447X, several participants self-reported signs of clinical benefits such as a capacity to eat more or to eat food they were unable to eat before or increased energy levels. One of the participants to the CT-AMT-011-02 became pregnant approximately 1 year post-alipogene tiparvovec administration. The pregnancy went very well and she delivered a healthy baby, despite the fact that pregnancy is an important trigger of pancreatitis [19] and can be life-threatening in LPLD.

Although based on a small number of patients and episodes and relatively short period of observation, it has also been noticed in the three intervention studies that overall pancreatitis incidence, or the intensity, duration or peak of acute abdominal pain episodes tend to be lower, up to 2 years after injection. On the basis of these observations, patients involved in the CT-AMT-011-01 and CT-AMT-011-02 studies, as well as untreated LPLD patients from Preparation-02, were invited to consent to participate in a case note review study (CT-AMT-011-03) designed to retrospectively and prospectively assess and confirm data about the incidence and severity of acute abdominal pain episodes in LPLD. From the available total population of 27 patients, 22 agreed to participate in CT-AMT-011-03. The aim of CT-AMT-011-03 was to generate unbiased and blinded assessment of the incidence rates of hospital presentations and admissions before and after alipogene tiparvovec treatment. The available data of all acute abdominal

![FIGURE 4. Influx of neutral lipid after intramuscular alipogene tiparvovec administration.](image)

![FIGURE 5. Surrogate markers of AAV-LPLS447X efficacy.](image)
pains events leading to hospital presentation and admission were retrieved and adjudicated by three independent experts. These experts were blinded to whether or not the patient had been treated with alipogene tiparvovec, and to whether or not the event occurred before or after treatment. Definite pancreatitis attacks were diagnosed according to the Revised Atlanta Diagnostic Criteria. Event’s severity for pancreatitis was measured using the Harmless Acute Pancreatitis Scale (HAPS). In total, 512 pain events have been classified to date as definite pancreatitis, probable pancreatitis, abdominal pain or other. The evaluated clinical markers before and up to 2 years after alipogene tiparvovec treatment showed a reduction of the corrected time incidence of pancreatitis episodes, the definite and probable pancreatitis episodes, the severity of the episodes, the number and duration of hospitalizations, both in general hospital unit and in ICU and the complexity hospital setup requirements.

CLINICAL SAFETY

Alipogene tiparvovec was well tolerated by the majority of the 27 LPLD patients having participated in the three interventional studies [33,35,36]. After the administration of AAV1-LPLS447X (AMT-010 or alipogene tiparvovec), the most frequent adverse reactions consisted of intramuscular injection-associated local reactions that developed immediately after and directly related to the administration procedure such as myalgia, pain in the legs and oedema. Bruising was commonly seen a few days after administration. These reactions were mild to moderate and showed no dose relationship. In all interventional studies, multiple injections (40–60 injection sites) were administered during a single procedure under spinal anaesthesia and most of the adverse reactions, swelling, bruising or transient fever, were self-limiting within a few days after the treatment. There are obvious risks associated with both spinal anaesthesia and a 3-month course of immunosuppression; but this did not translate in higher occurrence of SAEs in these studies.

On muscle biopsies, there were variable local responses observed in injected muscle tissue compared with noninjected muscle. Muscle biopsy assessments suggest a relationship between the extent of the local injection site response and the level of local LPL activity (e.g., LPL protein and activity, accumulation of intracellular lipids) rather than with observed anti-AAV1 immune responses. No apoptosis, excess fibrosis or necrosis were observed in injected muscle tissue, and there were no gross abnormalities. Overall muscle structure and function was preserved. Anti-LPLS447X antibodies were not observed in any patient. Anti-AAV1 antibodies, however, were detected in approximately half of the patients before alipogene tiparvovec administration, and all patients, whether exhibiting pre-existing antibodies or not, showed a treatment-emergent increase in anti-AAV1 antibodies after administration, persisting at high titre through the post-treatment period. Treatment-emergent anti-AAV antibody responses were not affected by immunosuppression or the termination of the immunosuppressant regimen.

Adverse events and SAEs are described with more details in the publications corresponding to each study.

Pooled shedding data illustrate that alipogene tiparvovec was present transiently in serum, urine, saliva and, at extremely low levels, in semen. The product is gradually eliminated from the various body fluids, including semen, and no vector DNA is detected in the serum beyond 12 weeks. No effect of the immunosuppressant regimen was observed when comparing the shedding profiles between patients with or without immunosuppressant treatment.

CONCLUSION

LPLD is a very rare disease and gene therapy techniques are relatively new and complex to develop, to apply and to evaluate. It is indeed very difficult to generate evidence when dealing with diseases affecting small numbers of patients [38]. Furthermore, medications based on transgene transfer present several scientific, clinical, social and ethical challenges for researchers, clinicians, regulatory authorities and the society as a whole [39–42,43]. In the light of the findings of the clinical studies described above, however, and taking into consideration the perspective of the patients and that of the clinical teams having been involved, the overall benefit–risk ratio of alipogene tiparvovec appears positive to date, particularly for the patients presenting the highest risk of complications. The clinical development of AAV1-LPLS447X gene therapy significantly contributes to our understanding of the clinical expression of LPLD and severe hypertriglyceridaemia. The effect of alipogene tiparvovec on chylomicron metabolism, which appears to be independent of its effect on fasting plasma triglyceride concentration, was unexpected and represents a paradigm shift.

Acknowledgements

This study is dedicated to all patients affected by LPLD or severe hypertriglyceridaemia. The authors would like to thank the participants in the clinical studies and their...
families; Diane Brisson, PhD, and the staff from the ECOG-ENET-21 Clinical Research Center, Department of Medicine, Université de Montréal, Chicoutimi Hospital; staff from the Academic Medical Center (AMC), The Netherlands; André Carpenter’s team, Sherbrooke University Hospital, Canada; Dr Claude Gagné and the staff of the clinique de lipidologie de Québec inc. as well as staff from Amsterdam Molecular Therapeutics (now UniQue B.V.). J.M. was a Université de Montréal postdoctoral fellow and received support from the Canadian Institutes for Health Research (CIHR) during the studies and D.G. was holder of the Canada Research Chair in preventive genetics and community genomics (www.chairs.gc.ca), which is also supported by a CIHR team grant (# CTP-82941).

Conflicts of interest

D.G. has no financial interest in Amsterdam Molecular Therapeutics (AMT now uniQue B.V.) and has currently only a consultancy with AMT. He received funds to participate in the conception and conduct of clinical studies with aligpogene tiparvovec. J.M. declares no conflict of interest. J.K. was one of the co-founders of AMT. He has currently only a consultancy with AMT and owns stock options of AMT.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 388).


15. This review highlights the contribution of genetic variants to hyperlipidaemia compared to other complex traits. It emphasizes the importance of the contribution of genetic factors to hyperlipidaemia, particularly in those with extreme scores such as familial chylomicronaemia.


24. In this study, the molecular basis of type 1 and type V hypertriglyceridaemia was investigated in 86 patients and 327 controls. The coding regions of LPL, APOC2, APOA5, GPIHPB1 and LMF1 were sequenced. Mutations in LPL, GPIHPB1, APOC2 and APOA5 were associated with severe hypertriglyceridaemia.


Gene therapy for lipoprotein lipase deficiency Gaudet et al. 2012
36. Carpentier A, Frisch F, Labbe SM, et al. Gene therapy with alipogene tiparvovec results in enhanced postprandial clearance of chylomicrons in LPLD patients. J Clin Endocrinol Metab 2012; 97:1635–1644. This study presents the results of the first phase of a study assessing the effect of AAV1-LPLS447X on markers of postprandial metabolism at baseline, 14 and 52 weeks after administration. Intramuscular administration of alipogene tiparvovec in five LPLD patients resulted in a significant improvement of chylomicron clearance 14 weeks after injection in all patients, without inducing large postprandial NEFA spillover.


43. Kay MA. State-of-the-art gene-based therapies: the road ahead. Nat Rev Genet 2011; 12:316–328. This review provides insights into the accomplishments made with gene-based therapies and the major barriers that need to be overcome before they are more widely implemented.