

Long-Term Retrospective Analysis of Gene Therapy with Alipogene Tiparvovec and Its Effect on Lipoprotein Lipase Deficiency-Induced Pancreatitis

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Alipogene tiparvovec (Glybera) is a gene therapy product approved in Europe under the “exceptional circumstances” pathway as a treatment for lipoprotein lipase deficiency (LPLD), a rare genetic disease resulting in chylomicronemia and a concomitantly increased risk of acute and recurrent pancreatitis, with potentially lethal outcome. This retrospective study analyzed the frequency and severity of pancreatitis in 19 patients with LPLD up to 6 years after a single treatment with alipogene tiparvovec. An independent adjudication board of three pancreas experts, blinded to patient identification and to pre- or post-gene therapy period, performed a retrospective review of data extracted from the patients’ medical records and categorized LPLD-related acute abdominal pain events requiring hospital visits and/or hospitalizations based on the adapted 2012 Atlanta diagnostic criteria for pancreatitis. Both entire disease time period data and data from an equal time period before and after gene therapy were analyzed. Events with available medical record information meeting the Atlanta diagnostic criteria were categorized as definite pancreatitis; events treated as pancreatitis but with variable levels of laboratory and imaging data were categorized as probable pancreatitis or acute abdominal pain events. A reduction of approximately 50% was observed in all three categories of the adjudicated post-gene therapy events. Notably, no severe pancreatitis and only one intensive care unit admission was observed in the post-alipogene tiparvovec period. However, important inter- and intraindividual variations in the pre- and post-gene therapy incidence of events were observed. There was no relationship between the posttreatment incidence of events and the number of LPL gene copies injected, the administration of immunosuppressive regimen or the percent triglyceride decrease achieved at 12 weeks (primary end point in the prospective clinical studies). Although a causal relationship cannot be established and despite the limited number of individuals evaluated, results from this long-term analysis suggest that alipogene tiparvovec was associated with a lower frequency and severity of pancreatitis events, and a consequent overall reduction in health care resource use up to 6 years posttreatment.

INTRODUCTION

LIPOPROTEIN LIPASE DEFICIENCY (LPLD) is a debilitating genetic disease for which there is currently no effective drug therapy available. The prevalence of LPLD is approximately 1–2 per million.^{1,2} Individuals with LPLD display marked chylomicronemia and severe hypertriglyceridemia.¹

LPL is the key enzyme responsible for the hydrolysis of triglycerides (TGs) in TG-rich lipopro-

teins.^{3,4} In patients with LPLD, the *LPL* gene defect leads to impaired LPL function; the rate of lipolysis of TG-rich particles is much lower than in normal subjects, causing extreme accumulation of chylomicron (CM) particles, clinically observed as chylomicronemia.

Major clinical signs of LPLD consist of plasma lactescence, lipemia retinalis, hepatosplenomegaly, and eruptive xanthomas.^{1,5} Feared complications

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include recurrent acute pancreatitis and possible development of pancreatic insufficiency and insulin-dependent diabetes.⁶ If infection or necrotizing pancreatitis develops, the risk of a fatal outcome may reach 5–15% of cases.⁷ Management of patients currently consists of severe restrictions of dietary fat intake to less than 20 g/day or less than 15% of caloric intake and the use of medium-chain triglycerides (MCTs).¹ Compliance with this strict dietary regimen is difficult and, even if it is good, such measures are usually not sufficient to reduce chylomicronemia. At present, no available drug is able to effectively modulate the course of the illness, so these patients remain at high risk of morbidity and mortality. Enzyme replacement therapy is not expected to be effective, due to the short intravascular half-life of the LPL protein (approximately 15 min). The disease is, however, amenable to gene therapy because it is a monogenic disease.⁸

Adeno-associated virus (AAV) is a nonpathogenic virus that has been safely administered to humans in numerous clinical trials.^{9–11} After single intramuscular administration of recombinant AAV vector particles¹² long-term transgene protein expression has been reported in preclinical studies.^{13–17} Given the established safety profile and persistent expression, an AAV vector was chosen in the clinical development of alipogene tiparvovec (Glybera) to deliver the therapeutic gene. The *LPL*^{S447X} variant, a gain-of-function allele associated with lower plasma TG levels, was selected as the candidate for gene therapy of patients with LPLD.^{18,19} Alipogene tiparvovec includes the human *LPL* gene variant *LPL*^{S447X} carried by an AAV vector, AAV1, in solution for intramuscular injection designed to target muscle cells.^{18,19}

Intramuscular administration of alipogene tiparvovec in 14 patients with LPLD (study CT-AMT-011-01) was well tolerated without emerging safety concerns up to 5 years of follow-up.^{20,21} Half of the patients demonstrated a $\geq 40\%$ reduction in total fasting plasma TG between 3 and 12 weeks after gene therapy. Total fasting plasma TG showed subsequent significant variability and tended to return to baseline values, although sustained *LPL*^{S447X} expression and changes in TG-rich lipoprotein characteristics were noted, independently of the effect of alipogene tiparvovec on total fasting plasma TG.²⁰ A later trial with alipogene tiparvovec in five patients with LPLD (CT-AMT-011-02) showed that the postprandial chylomicron (ppCM) kinetics significantly improved after 14 weeks of treatment.²² Signs of ppCM improvement of TG-rich lipoprotein kinetics in-

duced by alipogene tiparvovec were sustained at the 52-week assessment and were independent of pretreatment TG values.²³

To gain further insight into the long-term clinical efficacy of alipogene tiparvovec, retrospective medical record review studies (CT-AMT-011-03 and CT-AMT-011-05) were conducted to assess the frequency and severity of disease-related acute abdominal pain events reported from subjects with LPLD who were previously treated during the clinical development program for alipogene tiparvovec. Detailed information about acute abdominal pain events requiring hospital visits and/or admissions was collected from patients' medical records. Analyses were carried out for the entire disease time period (e.g., the period from first hospital event and the entire post-gene therapy follow-up) and for an equal pre- and post-gene therapy time period.

MATERIALS AND METHODS

Patients

Twenty-seven patients with LPLD have received alipogene tiparvovec or its predecessor AMT-010 in three clinical studies. All clinical studies were approved by the relevant ethics committees or institutional review boards and were performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Signed informed consent forms were obtained from all participants. Figure 1 describes the clinical development program.^{20,22}

None of the patients who received the alipogene tiparvovec predecessor (8 of the 27 patients who participated in the first study, CT-AMT-010-01) were included in the CT-AMT-11-03 and CT-AMT-11-05 analyses because hospital medical records pertaining to the historical period before gene therapy were mostly not available, and the gene therapy product was manufactured differently.²⁴ The CT-AMT-010-01 study used AMT-010, which comprised the same *LPL*^{S447X} gene construct and AAV vector as alipogene tiparvovec.⁴ However, AMT-010 was manufactured using a mammalian cell production system, whereas alipogene tiparvovec is produced in an insect cell-based system using baculovirus technology.⁴ Therefore, the analysis reported here includes 19 patients with LPLD treated with alipogene tiparvovec solely in two of three prospective clinical studies.

A subgroup of 12 patients with LPLD with pretreatment occurrence of severe or multiple definitive or probable pancreatitis episodes is described separately, as they constitute the "on label" patient population for the approved indication and dose of

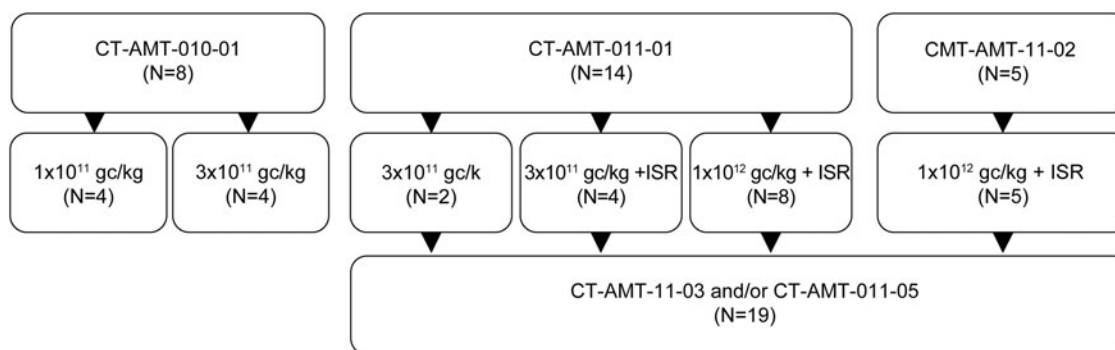


Figure 1. Overview of the clinical development program. Study CT-AMT-011-05 included patients who consented to that particular study, as well as those who consented only to the predecessor study, CT-AMT-011-03, as the databases for both studies were merged. ISR, immunosuppression (cyclosporine and mycophenylate mofetil) and added methylprednisolone for CT-AMT-011-02, as described in References 20 and 22; gc, genome copies.

alipogene tiparvec in the European Union (EU). Earlier results (3-year median posttreatment follow-up) from this group of patients, who participated in CT-AMT-011-03, had been assessed by the European Medicines Agency (EMA) during the regulatory review process.²⁵

Gene therapy and immunosuppressive treatment

In the CT-AMT-011-01 trial, six patients received alipogene tiparvec intramuscularly at a total dose of 3×10^{11} genome copies (gc)/kg and eight patients at a total dose of 1×10^{12} gc/kg.²⁰ Alipogene tiparvec was combined with an immunosuppressive regimen consisting of 12 weeks of cyclosporine A (3 mg/kg/day) and mycophenolate mofetil (2 g/day) postdosing in all but two patients.

In the subsequent trial (CT-AMT-011-02), all five patients received a total dose of 1×10^{12} gc/kg intramuscularly (Fig. 1).²² In this study, the immunosuppressive regimen was modified with cyclosporine A and mycophenolate mofetil administered 3 days before alipogene tiparvec injection and continued for 12 weeks postdosing; a single intravenous bolus of methylprednisolone (1 mg/kg) was given one half-hour before alipogene tiparvec administration.

Event adjudication

Acute abdominal pain events requiring hospital visits and/or hospitalizations underwent thorough review and assessment by an independent adjudication board in studies CT-AMT-011-03 and CT-AMT-011-05. The latter was blinded as to patient identification, whether the events occurred before or after gene therapy, the number of *LPL* gene copies injected, and the immunosuppressive regimen used. Each event was assessed by all three adjudication board members in the form of patient profiles with data extracted from medical records, in order to determine whether available information supported a diagnosis of pancreatitis according to the most recent revision of the Atlanta diagnostic criteria for pancreatitis (Table 1).²⁶ Events were evaluated and classified by the independent adjudication board according to the following definitions, which they devised on the basis of the Atlanta diagnostic criteria: (1) definite pancreatitis (required documentation of the event according to the 2012 Atlanta diagnostic criteria), (2) probable pancreatitis (typical pancreatitis abdominal pain with incomplete laboratory and/or imaging data in a subject with a previous documented definite pancreatitis event), (3) abdominal pain (typical pancreatitis abdominal pain with

Table 1. Criteria for diagnosis of acute pancreatitis

Clinical feature	Diagnosis of acute pancreatitis requires two of the following three features:
1	Abdominal pain consistent with acute pancreatitis (acute onset of a persistent, severe, epigastric pain often radiating to the back)
2	Serum lipase activity (or amylase activity) at least three times greater than the upper limit of normal
3	Characteristic findings of acute pancreatitis on contrast-enhanced computed tomography (CECT) and, less commonly, magnetic resonance imaging or transabdominal ultrasonography
	<ul style="list-style-type: none"> • If abdominal pain suggests strongly that acute pancreatitis is present, but the serum amylase and/or lipase activity is less than three times the upper limit of normal, as may be the case with delayed presentation, imaging will be required to confirm the diagnosis • If the diagnosis of acute pancreatitis is established by abdominal pain and by increases in the serum pancreatic enzyme activities, CECT is not usually required for diagnosis in the emergency room or on admission to the hospital

incomplete laboratory and/or imaging data), or (4) other (event that could not be placed in the preceding categories or abdominal pain events clearly due to causes other than pancreatitis). The severity of events was also classified by the adjudication board in accordance with the 2012 Atlanta diagnostic criteria for pancreatitis.²⁶ The agreement of the three experts was required to classify events into any given category and severity.

Statistics

Demographics including age and sex, as well as baseline TG levels, were descriptively provided. The following variables were analyzed: number, rate and duration of hospitalization, adjudication of events leading to hospitalization, and severity of events of pancreatitis. Variables were evaluated for an equal time pre- and posttreatment, ranging from up to 6 years pretreatment to up to 6 years posttreatment, and/or an entire disease time period, that is, the period since first event requiring hospital care and the whole post-gene therapy follow-up. Statistical analyses were performed with SAS version 9.3 or higher.

RESULTS

Patient characteristics

Baseline characteristics of patients with LPLD, including fasting TG pre-gene therapy levels, and 12 weeks, 1 year, 2 years, and 3–5 years post-alipogene tiparvovec treatment, are shown in Table 2. All patients had chylomicronemia and elevated TG pre-alipogene tiparvovec (>10 mM), shown as a median of five pre-gene therapy measurements before entry in the CT-AMT-011-01 and -02 trials. Median TG levels were 29.2 mM at baseline, whereas at 12 weeks to 3–5 years after alipogene tiparvovec treatment levels varied between 18.3 and 20.9 mM.

The median follow-up post-gene therapy period for the 19 treated subjects was 5.6 years (range, 1.5–6.2 years). Included with the 19 treated subjects are the 12 “on label” patients; the median follow-up of those 12 subjects was 5.8 years (range, 1.5–6.2 years). On an equal pre- versus posttreatment time period basis, the CT-AMT-011-05 study doubled the assessment of events to 91.6 patient years for each (pre- and post-) time period, versus 42.5 patient years in the earlier CT-AMT-011-03 study.

Events adjudication during the entire disease time period

Per-patient displays for definitive pancreatitis, probable pancreatitis, and abdominal pain events

Table 2. Characteristics of patients who received alipogene tiparvovec in trials CT-AMT-11-01 (n = 14) and CT-AMT-11-02 (n = 5)

Trials CT-AMT-11-01 and CT-AMT-11-02 ^a	
Age (yr)	
n	19
Mean	44.3
Min	20
Max	62
Sex	
F	11
M	8
Baseline triglycerides (mM)	
n	19
Median	29.2
Min	13.0
Max	65.5
Triglycerides (mM), 12 wk posttreatment	
n	19
Median	19.7
Min	3.8
Max	51.5
Triglycerides (mM), 1 yr posttreatment	
n	19
Median	18.3
Min	6.7
Max	57.4
Triglycerides (mM), 2 yr posttreatment	
n	13 ^b
Median	20.9
Min	12.2
Max	46.1
Triglycerides (mM), 3–5 yr posttreatment	
n	12 ^b
Median	20.5
Min	9.8
Max	38.4

^aThe CT-AMT-11-02 study completed at 1 year.

^bLong-term triglyceride data (after 1 year) are from study CT-AMT-11-01 only.

for the entire disease time period and equal pre-versus post-gene therapy period are provided in Table 3. Most patients had multiple events, but there was wide interpatient variability (median, 7.0 events; range, 1–92) and inpatient variability in the interval between episodes (Table 3).

Overall, 380 events were evaluated by the adjudication board for the 19 patients (Table 3). Eighty-six events were sufficiently documented in medical records to meet the 2012 Atlanta diagnostic criteria and were therefore classified as definite pancreatitis; 11 of these events occurred after gene therapy. Thirty-nine events were categorized as probable pancreatitis; 6 of these occurred after gene therapy. Finally, 161 events were categorized as abdominal pain; 7 of these events occurred after gene therapy (Table 3). Of note, 94 events were categorized as “other.” Excluding the “other” category, 17 events were classified as severe

Table 3. Definite pancreatitis, probable pancreatitis, and abdominal pain events in patients treated with alipogene tiparvec on entire disease time period and on equal pre- to posttreatment period

Subject no.	Consented to CT-AMT-011-03	Consented to CT-AMT-011-05	Dose	Age (yr)/sex	ISR (Y/M)	Lifetime events, pre-alipogene tiparvec treatment			Study CT-AMT-011-05 (equal pre- and post-gene therapy time periods)						Duration of post-alipogene tiparvec follow-up (yr)	
						Definite pancreatitis	Probable pancreatitis	Abdominal pain	Definite pancreatitis		Probable pancreatitis		Abdominal pain			
									Pre	Post	Pre	Post	Pre	Post		
18 ^a	Y	Y	3 × 10 ¹¹	50/M	N	3	0	1	0	0	0	0	0	0	0	6.2
17 ^a	Y	Y	3 × 10 ¹¹	51/M	N	2	0	2	0	0	0	0	0	0	0	6.2
16 ^a	Y	Y	3 × 10 ¹¹	56/F	Y	1	1	4	0	0	0	0	0	0	0	6.0
13 ^a	Y	Y	1 × 10 ¹²	29/M	Y	2	0	1	0	0	0	0	0	0	1	5.8
12 ^a	Y	Y	1 × 10 ¹²	62/F	Y	7	1	2	4	1	1	0	0	0	0	5.9
11 ^a	Y	Y	1 × 10 ¹²	48/F	Y	3	0	1	2	0	0	0	0	1	0	5.9
14 ^a	Y	Y	3 × 10 ¹¹	51/F	Y	8	8	18	0	1	0	0	2	0	0	6.0
9 ^a	Y	N	1 × 10 ¹²	50/F	Y	6	7	13	0	0	0	0	0	0	0	2.2
6 ^a	Y	Y	1 × 10 ¹²	39/F	Y	6	1	5	4	3	0	0	5	0	0	5.4
5 ^a	Y	Y	1 × 10 ¹²	57/M	Y	10	4	72	3	1	0	1	8	4	0	4.5
4 ^a	Y	Y	1 × 10 ¹²	45/F	Y	16	8	27	3	0	4	5	2	1	0	4.1
2 ^a	Y	N	1 × 10 ¹²	46/M	Y	6	2	2	0	1	0	0	0	0	0	1.5
Total: 12 "on label" patient						70	32	148	16	7	5	6	18	6	59.7 patient-years	
19	Y	Y	3 × 10 ¹¹	60/F	Y	1	0	3	0	0	0	0	2	0	0	6.0
15	Y	N	3 × 10 ¹¹	42/M	Y	0	0	1	0	0	0	0	0	0	0	3.1
10	N	Y	1 × 10 ¹²	40/F	Y	1	0	1	1	3	0	0	1	1	0	5.8
8	N	Y	1 × 10 ¹²	37/M	Y	1	1	0	1	0	1	0	0	0	0	5.6
7	Y	Y	1 × 10 ¹²	28/F	Y	0	0	0	0	1	0	0	0	0	0	5.6
3	Y	Y	1 × 10 ¹²	33/F	Y	1	0	0	1	0	0	0	0	0	0	4.1
1	Y	N	1 × 10 ¹²	20/M	Y	1	0	1	0	0	0	0	0	0	0	1.6
Total of all 19 patients						75	33	154	19	11	6	6	21	7	91.6 patient-years	

F, female; ISR, immunosuppression; M, male; N, no; Y, yes.

^a"On label" patient.

by the adjudication board; none of these 17 severe events occurred after alipogene tiparvec treatment (data not shown).

In the "on-label" 12-patient population, 77 events of definite pancreatitis were categorized as such by the adjudication board; 7 of these events occurred after gene therapy (Table 3). Thirty-eight events were categorized as probable pancreatitis; 6 of them occurred after gene therapy. Finally, 154 events were categorized as abdominal pain; 6 of these occurred after gene therapy.

Per-patient results on an equal pretreatment versus posttreatment time period

The adjudicated events for the equal pre- versus post-gene therapy time period, as well as the entire disease time period, in individual patients are shown in Table 3. On an equal time period comparison, the total posttreatment numbers of definitive pancreatitis, probable pancreatitis, and abdominal pain events are consistently lower than at reciprocal time intervals, in both the 19-patient population (posttherapy, 24 vs. pretherapy, 46 events) and the 12-patient "on label" group (post-

therapy, 19 vs. pretherapy, 39 events). In the 19-patient group, post-gene therapy reduction was observed in two of the three categories of event: a 42% reduction in definite pancreatitis, no reduction in probable pancreatitis, and a 67% reduction in abdominal pain events. The same analysis in the 12-patient "on label" subgroup revealed a 56% reduction in definite pancreatitis, a 20% increase in probable pancreatitis, and a 67% reduction in abdominal pain events.

Annualized hospitalization rates and days in hospital

During the entire disease time period of the 19 patients, a total of 105 hospitalizations (18 of them in an intensive-care unit [ICU]) occurred before gene therapy, whereas 16 hospitalizations occurred after treatment with alipogene tiparvec, and only 1 of them was in an ICU (data not shown).

Because all of the severe events requiring ICU hospitalization were in the definite pancreatitis category, annualized hospitalization rates and days in hospital in Table 4 for that event category are shown separately, from probable pancreatitis

Table 4. Annualized hospitalization rates and days in-hospital per patient per year during an equal period of pre- and posttreatment follow-up

Nineteen Treated Patients								
Definite pancreatitis								
Patients	Pretreatment				Posttreatment			
	Events	Rate (events/yr)	Total days	Days/yr	Events	Rate (events/yr)	Total days	Days/yr
19	19	0.21	143	1.56	10	0.11	75	0.82
Probable pancreatitis and abdominal pain events								
19	18	0.20	116	1.27	11	0.12	107	1.17
Twelve "On Label" Patients								
Definite pancreatitis								
12	16	0.27	120	2.01	7	0.12	62	1.04
Probable pancreatitis and abdominal pain events								
12	17	0.28	113	1.89	10	0.17	105	1.76

and abdominal pain events during an equal period of pre- and posttreatment follow-up (up to 6 years). The annualized rate of hospitalizations for definite pancreatitis in the 19 patients was 0.21 events per patient per year; after gene therapy, the rate was 0.11 events per patient yearly, thus corresponding to a 48% lower rate in hospitalizations for this event category, and a 48% lower number of hospital days per year. Similarly, the annualized hospitalization rate for probable pancreatitis and abdominal pain was 0.20 events per patient per year (before) versus 0.12 events per patient per year after gene therapy, a 40% lower hospitalization rate for these two events, and 8% less hospital days per year than before treatment with alipogene tiparvovec. A similar magnitude of reduction in hospitalization rates and days in hospital was evident in the 12 "on label" patients. The annualized hospitalization rate for definite pancreatitis was 0.27 events per patient per year pre-gene therapy, which decreased to 0.12 events per patient per year posttreatment (a 56% lower rate of hospitalization), and a 48% reduction in total hospital days per year. The rate of probable pancreatitis and abdominal pain events in the 12 "on label" patients was reduced by 39% (0.28–0.17 events per patient per year) and total days in hospital per year by 7%.

Comparison of results between study CT-AMT-011-03 and study CT-AMT-011-05

As displayed in Fig. 2, the annualized rate of events per patient per year after treatment with alipogene tiparvovec observed at the time of Gly-

bera approval (study CT-AMT-011-03; median follow-up, 3 years) did not increase during the additional period of observation (study CT-AMT-011-05; median follow-up, 5.6 years).

The longer follow-up of study CT-AMT-011-05 allowed a more accurate calculation of annualized event rates (Fig. 2). On an equal time period comparison, the post-gene therapy annualized rate of all three events, namely, definite pancreatitis, probable pancreatitis, and abdominal pain events, was 0.26 and 0.32 in the 19 patients (Fig. 2A) and the 12-patient "on label" subgroup (Fig. 2B), respectively, versus 0.50 and 0.65 events per patient per year pretreatment in the 19 patients and the 12-patient "on label" subgroup, respectively, corresponding to a 48% decrease in the 19 patients and a 51% decrease in the 12-patient "on label" subgroup. This is the most conservative approach, as it includes all three event categories. If only definite and probable pancreatitis are analyzed in the same two patient populations, the annualized rates for the two event categories are 0.19 and 0.22 events per patient per year after gene therapy versus 0.27 and 0.35 events per patient per year pre-gene therapy, respectively, corresponding to a 30% decrease in the 19-patient population and a 37% decrease for the same events in the 12-patient "on label" subgroup (data not shown).

DISCUSSION

On the basis of a systematic, independent, and retrospective adjudication of data extracted from individual medical records of 19 patients treated with alipogene tiparvovec, a reduction of

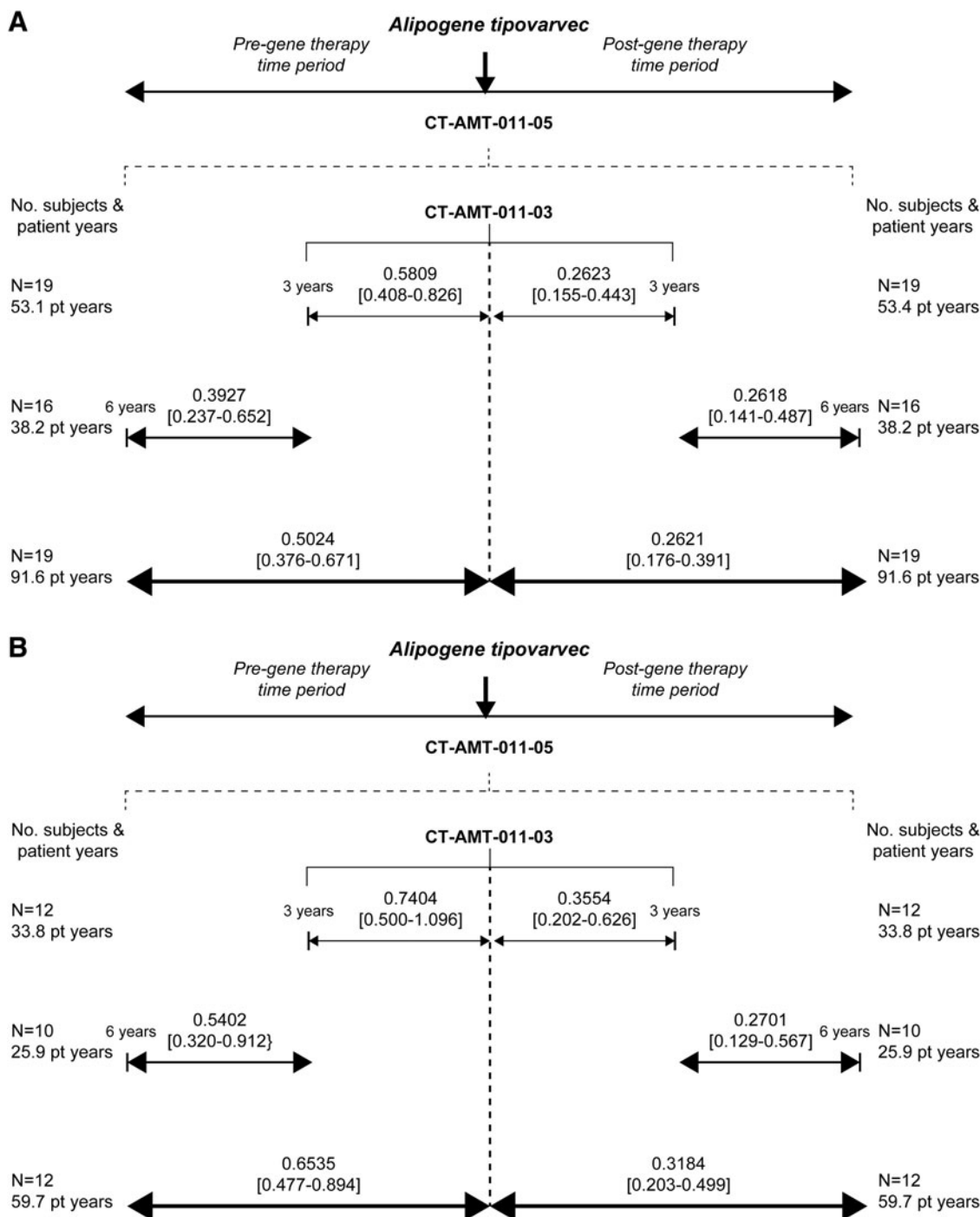


Figure 2. Annualized rate of definite pancreatitis, probable pancreatitis, and abdominal pain events [95% confidence intervals] for selected time intervals in study CT-AMT-011-03 (median follow-up, 3 years) and study CT-AMT-011-05 (median follow-up, 5.6 years for the 19 patients; 5.8 years for the 12 “on label” patients). **(A)** The 19-patient population; **(B)** the 12 “on label” patients.

approximately 50% in the annualized rate of the three categories of LPLD-related acute abdominal events requiring hospital care and/or hospitalization was observed. A decrease in the number of hospitalization days per year after gene therapy was also observed, being larger in the definite pancreatitis category than in the probable pan-

creatitis and abdominal pain event categories combined. The inclusion of abdominal pain events that require hospital attention and/or hospitalization in the outcomes of this study is justified, considering these events have an impact on health care costs and significantly affect the quality of life of patients with LPLD.

Results from CT-AMT-011-03 and CT-AMT-011-05 studies provide significant new information on the persistence of the effect of AAV1-based gene therapy in muscle tissue. Although new events occurred during the longer follow-up period, analysis of the annualized rate of events occurring at later time intervals, 3–6 years posttreatment, was comparable to the earlier time interval, roughly corresponding to 0–3 years of follow-up. More importantly, on an equal time period comparison, the post-alipogene tiparvec annualized rate of LPLD-related abdominal events in the pooled patient population remained markedly lower than the annualized rate of events at equivalent pretreatment time periods. These results are consistent with reports of persistence up to 10 years after intramuscular administration of an AAV8-based factor IX gene therapy product in patients with hemophilia B.²⁷

In a per-patient analysis it is also evident that gene therapy with alipogene tiparvec does not completely eliminate the risk of pancreatitis and that important interindividual differences exist. In addition, in the pre- versus posttreatment equal time period analysis, not all 19 patients were clearly informative for efficacy. For example, eight patients did not have any definitive or probable pancreatitis events in either time period. Notably, when these eight patients are excluded from the analysis (subjects 1, 9, 13, and 15–19), a decrease in the number of definitive or probable pancreatitis events was still evident, corresponding to a 38% posttreatment reduction for the remaining 6 “on label” patients and a 28% reduction in the remaining 11 patients in the total patient group. In general, patients with two or more post-gene therapy events tended to be those subjects with a higher number of events pre-gene therapy during the entire disease time period. This pattern of events may be the result of a negative impact of other genes affecting TG lipolysis²⁸ and/or a chronic lower level of compliance with severely fat-restricted diets.

In retrospectively analyzing the rate of pancreatitis in patients with LPLD according to the 2012 Atlanta diagnostic criteria,²⁶ one can expect to underestimate the true incidence of definite pancreatitis events for a number of reasons. First, the Atlanta diagnostic criteria are designed to guide clinicians in confirming acute pancreatitis, as they present at clinic,²⁶ not retrospectively. Second, in patients with LPLD, abdominal CT scans for diagnosis are predominantly used for the first event, but less frequently for subsequent events.^{6,29} A CT scan will typically be performed only if the patient deteriorates or in case of diag-

nostic uncertainty.²⁹ Third, even a prospective study would be seriously hampered when only the Atlanta diagnostic criteria for acute pancreatitis are used, as these criteria were not designed with the particularities of LPLD pancreatitis in mind (i.e., the masking effect of hyperlipidemia on elevated amylase concentrations).^{30,31} It is also well recognized that patients with LPLD experiencing an acute pancreatic event often present with lactescent serum, but nonsignificantly elevated serum and urinary amylase.^{32–36} This has also been occasionally confirmed in hemorrhagic pancreatitis documented at laparotomy.^{37–39} Normal (or almost normal) amylase levels can be observed in the presence of marked hypertriglyceridemia, most probably as a consequence of interference of the lipids with the assay,⁴⁰ or of an inhibitor of the amylase assay in the plasma and urine.^{30,31}

Given the favorable safety profile of the compound up to 6 years after treatment,^{41–44} results from this study suggest long-term clinical benefits of alipogene tiparvec with regard to the frequency and severity of posttreatment pancreatitis, as well as health care resources use. However, this study has limitations. There are large inter- and intraindividual differences in the frequency and interval of events. Underestimation of the number of events in the pretreatment period is possible, due to less complete collection of data in medical records pre-gene therapy. Each patient served as his/her own historical control, but this study was not designed to establish a causal relationship between long-term clinical outcomes and the exposure to alipogene tiparvec, or to control for dietary fat intake. Historical controls are vulnerable to biases such as regression to the mean and the positive effect of participating in a clinical trial. Last, the reduction in pancreatitis rate and severity observed up to 6 years after alipogene tiparvec administration in this study does not correlate with baseline total fasting plasma TG levels, posttreatment TG levels (primary end point in AMT-011-01 and AMT-011-02 studies), number of injected *LPL* genome copies (drug dose), immunosuppressive regimen, or systemic posttreatment plasma LPL activity. However, signs of LPL activity were observed at the site of injection up to 1 year, several months after plasma TG values reverted to baseline. It has also been established that alipogene tiparvec profoundly modified the postprandial TG-rich lipoprotein characteristics²⁰ and chylomicron kinetics²² among participants in these studies, independently of its long-term effect on total fasting plasma TG levels. Signs of clinical benefits beyond 2 years have also been observed,

including during pregnancy.⁴¹ The alternative, a clinical study with an untreated control arm, would require a sample size of >300 patients to confirm the reduction in pancreatitis rate of approximately 50% observed in this report. Given the rarity of the disease, enrolling this number of patients is not feasible.

Additional evidence of efficacy may come from an even longer period of follow-up of patients treated with alipogene tiparvec in a registry and an increased number of treated patients.

The precise mechanisms for the pathogenesis of hypertriglyceridemia-induced acute pancreatitis have not been fully established. At present, several theories are discussed for the pathogenesis of LPLD-associated acute pancreatitis, among which are pancreatitis induced by the proinflammatory effects of hypertriglyceridemia or pancreatitis resulting from chylomicron accumulation (chylomicron toxicity). Although animal models favor the latter, the two theories are not mutually exclusive.^{45,46} Preliminary data obtained in patients treated with alipogene tiparvec suggest that several other mechanisms are likely to be involved. In this respect, it is interesting to note that alipogene tiparvec improved postprandial chylomicron metabolism,²² an effect that was sustained (>4 years) in one-half of the treated patients, and long-term improvement of chylomicron kinetics was the only studied covariate associated with lower incidence and severity of pancreatitis (data not shown). Although no proper analysis has yet been performed, this finding is consistent with the hypothesis that postprandial improvement of chylomicron metabolism could contribute to the reduction in pancreatitis incidence and severity after gene therapy in patients with LPLD.

Taking into consideration the small number of treated patients and the other limitations described previously, results of this study suggest that gene therapy with alipogene tiparvec tends to be associated with a marked reduction in the number and severity of acute abdominal events and LPLD burden in several patients, as expressed by annualized hospitalization rates, days in hos-

pital per year, and number of ICU admissions. Pre- and posttreatment gene expression profiling and functional analyses are ongoing to further document long-term effect of alipogene tiparvec on the occurrence of acute abdominal pain and pancreatitis. The evaluation of the long-term efficacy of alipogene tiparvec will, however, require a larger number of treated patients and longer period of observation.

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