2. SYNOPSIS

NAME OF COMPANY Genzyme Corporation 500 Kendall Street Cambridge, MA 02142 USA NAME OF FINISHED PRODUCT N/A NAME OF ACTIVE INGREDIENT Ad2/Hypoxia Inducible Factor (HIF)-1α/VP16	SUMMARY TABLE Referring to Part …… of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:

TITLE OF STUDY:

A Phase 2, Randomized, Double blind, Placebo-controlled, Parallel-group, Multicenter, Dose-Selection Study of Ad2/Hypoxia Inducible Factor (HIF)-1α/VP16 in Patients with Intermittent Claudication

INVESTIGATORS / STUDY CENTERS:

A total of 48 clinical study sites participated in this study (35 in the United States [US], 7 in the United Kingdom [UK], and 6 in Germany) of which 35 sites (27 in the US, 4 in the UK, and 4 in Germany) randomized at least 1 patient.

PUBLICATION (REFERENCE):

None

STUDIED PERIOD:

First patient in (date when the first patient was treated): 21 February 2005
Last patient out (date when the last patient completed study participation): 12 March 2010

PHASE OF DEVELOPMENT: 2

OBJECTIVES:

This Phase 2 therapeutic dose-selection study was conducted to:

1. Assess the safety and tolerability of 3 doses of Ad2/HIF-1α/VP16 compared to Placebo-control in the treatment of severe intermittent claudication (IC)
2. Assess the efficacy of 3 doses of Ad2/HIF-1α/VP16 compared to Placebo-control in the treatment of severe IC
3. Provide a basis for optimal dose selection for Phase 3 studies
4. Provide a basis for patient population, safety and efficacy parameters, and sample size determinations for Phase 3 studies

METHODOLOGY:

The original protocol was amended on 6 occasions and the following methodology description reflects those changes.

This was a prospective, randomized, double-blind, placebo-controlled, parallel group, multicenter, multinational Phase 2 therapeutic dose-selection study designed to investigate the safety and efficacy of 3 doses of adenovirus type 2/hypoxia inducible factor-1α/VP16 (Ad2/HIF-1α/VP16) to treat severe IC. A patient was considered enrolled after he/she had signed the informed consent form. After a patient had signed the informed consent, but prior to randomization, a patient was considered to be undergoing Screening. After a patient met the eligibility requirements, he/she was randomized to receive either 1 of 3 doses of Ad2/HIF-1α/VP16 or Placebo in a 1:1:1:1 ratio. Patient randomization was stratified based on diabetes mellitus status.

The 3 total doses of Ad2/HIF-1α/VP16 evaluated are $2x10^9$ viral particles (vp), $2x10^{10}$ vp, and $2x10^{11}$ vp. Seventy-five patients were to be enrolled into each of 4 treatment groups (including the placebo group) for a total

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of 300 patients. The study treatment for each patient consisted of a single dose administered intramuscularly (IM) to both lower limbs with 20 injections in each lower limb for a total of 40 injections.

The duration of each patient's participation in the study was 2 years (104 weeks), including a 1-year (52-week) Initial Treatment and Follow-up and a subsequent 1-year Extended Safety Follow-up. During the Initial Treatment and Follow-up, patients had scheduled visits at Day 1 and Weeks 1, 4, 12, 26, and 52.

The primary efficacy parameter for this study was the peak walking time (PWT), defined as the maximum time a patient walked before stopping due to claudication symptoms, using a standardized exercise treadmill test, which followed a modified Gardner protocol. The primary efficacy endpoint was the percent change from Baseline in PWT at 26 weeks following treatment administration. The unblinded analysis of the primary efficacy endpoint was conducted at the 6-month efficacy and safety analysis.

During the Extended Safety Follow-up, patients were scheduled for follow-up visits at Weeks 78 (18 months) and 104 (2 years). These visits focused on assessment of adverse events (AEs) including adverse vascular events (AVEs), as well as potential significant longer-term risks associated with Ad2/HIF-1α/VP16 and other potential late-occurring toxicities (e.g., de novo cancers, and hematological, neurological, and autoimmune disorders) that may be associated with the use of an investigational gene transfer product.

As part of the conduct of this study, Genzyme implemented a safety evaluation plan that provided an ongoing review of AEs and assessed potential safety issues associated with the use of Ad2/HIF-1α/VP16. In addition, an independent Data Monitoring Committee (DMC) provided an ongoing, expert, independent review of safety data that provided an ongoing risk management during the conduct of the study. The DMC could recommend suspension of enrollment in the study to evaluate a safety issue that arose during the conduct of the study. Throughout the study, the DMC received notification of all serious adverse events (SAEs), including amputations and cancers, on an expedited basis, as described in the DMC Charter.

The DMC conducted the first interim assessment of patient safety on unblinded safety data when the first 60 patients had been treated and followed for at least 4 weeks. Further randomization and treatment was suspended during this interim safety review until authorization to proceed was received from the DMC. This first review of data from 60 patients also included review of data from potential adenoviral vector circulation and viral shedding data (throat, urine, and semen). According to the protocol, the DMC was to decide if also the samples from subsequent patients needed to be analyzed, and the DMC concluded that this was not necessary. Thereafter, DMC reviews of blinded patient safety data was to occur after each additional cohort of 60 patients (i.e., 120, 180, and 240 patients) had been enrolled and treated and had completed the Week 4 follow-up. However, in the event that enrollment was slower than anticipated, the time between DMC reviews of patient safety data would not exceed 6 months. Suspending enrollment for these reviews was only required if specified by the DMC. Following each DMC review, the DMC recommendations were provided to relevant regulatory authorities if required. At any time, the independent DMC had the option to request that study data be unblinded.

NUMBER OF PATIENTS (PLANNED AND ANALYZED):
Planned: 300 (75 per treatment arm)
Screened: 749
Randomized: 289 (74, $2x10^9$ vp [low dose]; 74, $2x10^{10}$ vp [middle dose]; 65, $2x10^{11}$ vp [high dose]; and 76, placebo)
Completed treatment: 238 (62, low dose; 58, middle dose; 54, high dose; and 64, placebo)
Discontinued treatment: 51 (12, low dose; 16, middle dose; 11, high dose; and 12, placebo)

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION/EXCLUSION:

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INCLUSION:

Patients fulfilling the following criteria were eligible for inclusion in the study:

1. Males and females 40 to 80 years of age, inclusive.
2. Clinical diagnosis of peripheral arterial disease (PAD), secondary to atherosclerosis, in both lower limbs, confirmed by objective evidence:
   - An ankle-brachial index (ABI) of ≤0.90 at rest in at least 1 lower limb. (Note: The index limb must be ≤0.90 at rest.)
   - The ABI after exercise must be reduced by ≥20% from the ABI at rest in the index leg (the most symptomatic leg during the treadmill testing). The post exercise ABI will also be performed on the other leg if the resting ABI >0.90. A patient may be eligible for the study with a resting ABI in the non-index limb >0.90 if:
     - the post exercise ABI in the non-index limb is also reduced by greater than or equal to 20% or
     - a medically significant stenosis (defined as ≥50%) of a femoropopliteal or infrapopliteal artery is present, as documented via an imaging study (such as magnetic resonance, conventional angiography, duplex ultrasound, or computed tomography)
   - If the ABI cannot be measured in either leg (due to noncompressable arteries), then a toe-brachial index (TBI) of ≤0.70 may be used in its place to confirm PAD.
3. Symptoms of severe IC in at least 1 lower limb persisting for ≥6 months.
4. Patients with a PWT of 1 to 12 minutes (inclusive) using the standardized exercise treadmill test at each of the 2 consecutive treadmill tests performed at least a week apart during the Screening period.
   - During Screening, patients must demonstrate consistency of PWTs between 2 standardized exercise treadmill tests (Walk 1 and Walk 2) performed at least 1 week apart.
   - Consistency of the PWT between the 2 visits is achieved if the difference between PWT at Walk 1 and Walk 2 is ≤25% of the higher of the 2 PWTs ([higher PWT - lower PWT]/higher PWT)
   - If the difference between PWT at Walk 1 and Walk 2 is >25% of the higher of the 2 PWTs, a third treadmill test (Walk 3) may be performed at the discretion of the Principal Investigator between 7 and 14 days following Walk 2. The variability in PWT warranting the performance of Walk 3 must be secondary to circumstances that may contribute to the observed variation (e.g., prior exertion, inconsistent timing, ingestion of a meal within 4 hours, etc.). To qualify for the study, the difference between PWT of either Walk 1 or Walk 2 as compared with Walk 3 must be ≤25% of the higher of the 2 PWTs, ([higher PWT- lower PWT]/higher PWT). The decision as to whether Walk 1 or Walk 2 would be used for comparison with Walk 3 was to be made prospectively and reviewed with the Sponsor.
   - An acceptable mean PWT must be achieved within 4 weeks of treatment administration.
5. Patients have been considered for other potential treatment options including exercise rehabilitation, smoking cessation, and pharmacological therapy prior to enrollment.
6. Claudication severity, concomitant medications for the treatment of coronary artery disease, PAD, and IC, smoking status and exercise habits should be clinically stable for 3 months prior to Enrollment.
7. Patients who are committed to following the protocol requirements as evidenced by written informed

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consent.

EXCLUSION:

Patients who met any of the following exclusion criteria were not eligible for participation in this study:

1. Patients with either current or any history of critical limb ischemia (that is, patients classified as Rutherford Category 4 [ischemic rest pain], Rutherford Category 5 [non-healing ischemic ulcers and minor tissue loss], or Rutherford Category 6 [non-healing ischemic ulcers and major tissue loss]).
2. Patients in whom arterial insufficiency in the lower extremity was the result of acute limb ischemia or an immunological or inflammatory non-atherosclerotic disorder (e.g., thromboangiitis obliterans [Buerger's Disease]) and systemic sclerosis [both limited and diffuse forms]).
3. A PAD-specific surgical revascularization procedure within 6 months of Enrollment or a PAD-specific percutaneous procedure within 3 months of Enrollment, or patients likely to have required a PAD-specific revascularization procedure within 6 months after Enrollment.
4. Patients with aortoiliac disease that limits inflow in either leg:
   - Patients with concomitant aortoiliac disease (i.e., patients with a significant component of inflow disease in the distal aorta, common or external iliac, or proximal common femoral artery) as assessed by an imaging modality (e.g., segmental limb pressures and waveform analysis, duplex ultrasound scanning, magnetic resonance angiography, or radio-contrast arteriogram) performed within 1 year prior to Enrollment. If subject had undergone a bypass after the imaging study, then documentation of graft patency was required within 6 months prior to Enrollment.
   - If it was suspected at Screening that a patient has aortoiliac disease based on vascular examination, an imaging modality (e.g., segmental limb pressures and waveform analysis, duplex ultrasound scanning, magnetic resonance angiography, or radio-contrast arteriogram) was performed to rule it out if there was not one available within the times specified above. If there was no suspicion of aortoiliac disease in the Principal Investigator's judgment, an imaging test at Screening was not required for study purposes.
5. Patients in whom walking impairment due to pain in the index leg was the result of these non-atherosclerotic co-morbid conditions: venous claudication, chronic compartment syndrome, peripheral nerve pain (e.g., severe peripheral neuropathy), pseudoclaudication caused by spinal cord compression, or acute limb ischemia which, in the Principal Investigator's judgment were severe enough to confound the assessment of the patient's IC.
6. Conditions other than IC of significant severity that could confound PWT on the standardized exercise treadmill test causing premature or inconsistent termination of exercise (e.g., angina pectoris, heart failure [New York Heart Association {NYHA} Classes III and IV], respiratory disease [e.g., chronic obstructive pulmonary disease], orthopedic disease, neurological disorders, rheumatologic disorders [e.g., severe degenerative joint diseases], dyspnea, fatigue, prior lower limb amputation, including amputations proximal to the metatarsal or phalangeal joints).
7. Presence or history of cancer within 5 years of Enrollment or were not current with recommended screening guidelines for colorectal, lung, prostate, breast, cervical, and uterine cancers, as described in full in the clinical study protocol, with the exception of low grade and fully resolved non-melanoma skin malignancy.
8. Patients with a well-defined clinical or genetic disorder predisposing to malignancy were excluded

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(e.g., von Hippel-Lindau, familial polyposis coli, BRCA1, BRCA2, etc.).

9. Patients with baseline funduscopic evidence of active proliferative diabetic retinopathy, preproliferative diabetic retinopathy, or wet age-related macular degeneration (AMD)

   and/or

   Patients with a history of treatment for active proliferative diabetic retinopathy or wet AMD within 5 years of Enrollment.
10. Diabetes type 1 (juvenile onset).
11. Poorly controlled type 2 diabetes (i.e., HbA_{1C} >10%) at Screening.
12. Active hepatitis defined as clinically significant increase in liver enzymes (i.e., 3 times the upper limit of normal or other current infectious disease.
13. Patients who had symptoms of respiratory infection at time of Screening and/or randomization period and/or patients who had been on systemic or oral antibiotics for active infection within 2 weeks of study drug administration.
14. Patients who had clinically significant abnormal hematology (e.g., hematocrit <30%, white blood cell count >14,000), blood chemistry, renal, hepatic, or other laboratory parameters that could have been the result of an underlying malignancy or systemic infection (e.g., serum creatinine ≥2.5 mg/dL), as judged by the investigator.
15. Patients with the following co-morbidities who may not have been healthy enough to successfully complete all protocol requirements or in whom results may have been particularly difficult to assess:
    - Concurrent severe congestive heart failure (NYHA Classes III and IV)
    - Life-threatening ventricular arrhythmias, unstable angina (characterized by increasingly frequent episodes with modest exertion or at rest, worsening severity, and prolonged duration), and/or myocardial infarction within 4 weeks before Enrollment
    - Coronary artery bypass grafting or percutaneous coronary intervention within 3 months before Enrollment
    - A renal and/or carotid revascularization procedure within 1 month of Enrollment
    - Transient ischemic attack within 3 months before Enrollment
    - Deep vein thrombosis within 3 months before Enrollment
    - Severe chronic obstructive pulmonary disease (room air arterial PO_2 <60 mmHg or PCO_2 >50 mmHg, or abnormal pulmonary function tests (forced expiratory volume [FEV_1] <1.2 L/sec)
    - Thrombocytopenia (defined as platelet count ≤100,000/mm^3)
    - Undergoing hemodialysis
    - Patients with immunocompromised conditions, organ transplant recipients and/or need for immunosuppressive therapy
    - Neurological dementia (i.e., Alzheimer's Disease)
    - Hemorrhagic stroke
16. Patients who had a known allergy to the vehicle, placebo control, or any other medications or imaging agents required for participation in this study.

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17. Fertile women who were pregnant (as confirmed by a serum pregnancy test at the Screening visit and a urine pregnancy test at Day 0 prior to study drug administration), nursing, or using either no or an inadequate form of contraception.
18. Fertile men and women who were not willing to use barrier-type contraception for at least 90 days post treatment.
19. Patients who had a recent history of alcoholism or drug abuse, or severe emotional, behavioral or psychiatric problems, who may not have been able to adequately comply with the requirements of the study.
20. Patients who were receiving experimental medications or participating in another study using an experimental drug or experimental procedure within 30 days of Enrollment into this study.
21. Patients who previously enrolled in a prior angiogenic gene therapy clinical study, unless patient was a known placebo patient.

TEST PRODUCT, DOSE, AND MODE OF ADMINISTRATION; BATCH NUMBER:
Test product: Ad2/HIF-1α/VP16 in a formulation buffer (phosphate-buffered saline + 10% sucrose + 0.02% polysorbate 80 [Tween 80])
Dose: $2x10^9$ vp (low dose), $2x10^{10}$ vp (middle dose), $2x10^{11}$ vp (high dose)
Mode of Administration: IM (Twenty 100 µL injections to each lower limb for a combined total of 40 injections [4.0 mL total body volume]). Ad2/ HIF-1α/VP16 test article solution (2.0 mL) and diluent solution (3.0 mL) were packaged in separate 5-mL vials. After vials of the test article were diluted, 1-mL syringes were filled to the 0.1 mL (100 µL) mark, placed in bags containing 40 syringes each, and provided to the clinician.
Lot numbers:
[redacted]

DURATION OF TREATMENT:
Each patient received a single treatment and was followed for 2 years (52-week [1 year] Initial Treatment and Follow-up and subsequent 1-year Extended Safety Follow-up).

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION; BATCH NUMBER:
Reference product: Placebo solution (phosphate-buffered saline + 10% sucrose + 0.02% polysorbate 80 [Tween 80])
Dose: Volume matching test product
Mode of Administration: Identical to test product
Lot number: [redacted]

CRITERIA FOR EVALUATION:

EFFICACY:

The primary efficacy evaluation was PWT using a standardized exercise treadmill test. Other efficacy parameters evaluated but not reported in this abbreviated clinical study report included claudication onset time using a

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standardized exercise treadmill test, ABI and two Quality of Life questionnaires (a global instrument-Short Form-36 (SF-36) and a disease-specific instrument, the Walking Impairment Questionnaire.

SAFETY:

The safety data collected for this Phase 2 therapeutic dose-selection study include AEs, physical examination findings, vital signs, and ECGs, clinical laboratory parameters (hematology, liver function tests, renal function tests, blood chemistry, ESRs, urinalysis), selected cancer screening tests, Ad2 antibody and neutralizing antibody titers, eye exams, and SAEs that are adverse vascular events.

STATISTICAL METHODS:

EFFICACY:
The primary efficacy endpoint was the change from Baseline to Week 26 (6 months) in PWT using a standardized exercise treadmill test for the Full Analysis Set. The primary efficacy endpoint was to be compared between the 3 Ad2/HIF-1α/VP16 treatment groups and the Placebo group using the stratified Wilcoxon-Mann-Whitney (WMW) test, where the strata were defined by the presence/absence of diabetes mellitus at Baseline. The WMW test was applied separately to the 3 comparisons between each treatment group and the Placebo group. Missing data was imputed using last observation carried forward.

SAFETY:
Safety analysis of treatment-emergent adverse events (TEAEs) and SAEs (but not AVEs) collected cumulatively up to 24 months post-treatment was performed for the Safety Set.

While all reported AEs were displayed in by-patient data listings, only treatment emergent AEs were included in the summary tables. Treatment-emergent AEs, SAEs, and AVEs were summarized by treatment group and by System Organ Class, Preferred Term, relationship to study treatment, and maximum severity. Frequencies and percentages were presented in all summary tables for AEs, SAEs, and AVEs. Should a patient have more than 1 occurrence of an AE for a specific Preferred Term or System Organ Class, the patient was counted only once for that Preferred Term or System Organ Class of the closest relation to study treatment or the maximum severity.

Other safety assessments were summarized by treatment group and overall for the Safety Set. Categorical variables were presented with frequencies and percentages. Descriptive statistics (number of non-missing values, sample mean, median, standard deviation, minimum, and maximum) were displayed for continuous variables. No inferential statistical tests were performed.

SUMMARY – CONCLUSIONS

EFFICACY:

The primary efficacy endpoint was the percent change from Baseline in PWT at Week 26 following study drug treatment. The percent change from Baseline was 30.44%, 30.80%, 23.20%, and 34.70% for the placebo, low, middle and high dose groups, respectively. There were no statistically significant differences observed between each of the three doses of Ad2/HIF-1α/VP16 administered and placebo treatment at Week 26 indicating a lack of efficacy of the treatment in this patient group.

SAFETY:

Overall, no major adverse consequences and no new safety concerns of Ad2/HIF-1α/VP16 treatment were

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identified during the 2 year follow-up period.

- While most of the patients had TEAEs which were considered moderate or severe in intensity, there were no important differences in distribution of mild, moderate, and severe TEAEs among the 4 treatment groups. No severe TEAE was reported in more than 4 patients in a treatment group and most were single occurrences.
- Most of the TEAEs considered definitely related to study drug were injection-associated reactions, with injection site hematoma the most common definitely related TEAE. Overall, injection site reactions considered definitely related to study drug occurred more frequently among patients in the Ad2/HIF-1α/VP16-treatment groups than in the placebo group.
- None of the SAEs reported during the study were considered probably or definitely related to Ad2/HIF-1α/VP16.
- Deaths occurred at a similar frequency in all Ad2/HIF-1α/VP16 treatment and placebo groups. A total of 20 patients died during the study: 5 patients each in the low dose and middle dose groups, 4 patients in the high dose group, and 6 patients in the placebo group. The causes of death were consistent with the natural history of patients with PAD. Two patients had fatal TEAEs that were considered by the investigators to be possibly related to study drug: 1 patient in the middle dose group with non-small cell lung cancer and 1 patient in the high dose group with metastatic small cell lung cancer. Eleven of the 20 patients died from cardiovascular-related TEAE. No TEAE that resulted in death was considered to be probably or definitely related to study drug.
- No conclusions can be drawn regarding treatment related causality of malignancy.

CONCLUSION: [redacted]