1. SCIENTIFIC ABSTRACT

Program Overview and Background

Genzyme Corporation (Genzyme) is investigating a gene transfer product, AAV2-sFLT01, which is a replication defective, recombinant adeno-associated virus (serotype 2) vector that carries the gene construct for a soluble chimeric protein composed of domain 2 of tic soluble human vascular endothelial growth factor (VEGF) receptor 1 (FLT-1, Flt-1, Fms-like tyrosine kinase 1: VEGFR1) and the Fc region of human IgG1. The gene construct is named sFLT01.

Recombinant adeno-associated virus (rAAV) vector mediated gene delivery has been safely used in nonclinical short-and long-term models. Additionally, the vector has been well tolerated in clinical trials for cystic fibrosis, hemophilia, Leber's Congenital Amaurosis and currently in Parkinson’s disease. Genzyme therefore believes that rAAV vectors offer an attractive method to achieve long term local delivery of proteins into the eye.

We have demonstrated that intravitreal administration ofAAV2-sFLT01 mediates transduction of retinal ganglion cells resulting in persistent expression of sFLT01 protein in the rodent eye for at least 1 year with no gross toxicities upon histological examination. There have been reports of intraocular delivery ofAAV2 based vectors in the dog model giving expression of therapeutic protein for over 6 years. In our nonclinical experiments, intravitreal delivery of AAV2-sFLT01 has resulted in inhibition of retinal neovascularization in the mouse retinopathy of prematurity (abbreviated as either ROP or oxygen induced retinopathy (OIR) model and inhibition of choroidal neovascularization (CNV) in the laser-induced CNV model. We have observed stable sFLT01 protein expression out to approximately 1 year in rodents and primates. The vector appears to transduce retinal ganglion and transitional epithelial cells of the pars plana in primates.

Evidence exists to link VEGF to angiogenesis and neovascularization. There is reason to believe that by blocking the biological action of VEGF through binding to sFLT01, the transgene product may modify pathological processes characterized by inappropriate neovascularization. Genzyme's objective is to develop AAV2-sFLT01 for use in neovascular age-related macular degeneration.

Age-related Macular Degeneration

Age-related macular degeneration (AMD) is the major cause of central vision loss in individuals 65 years of age and older in industrialized countries. It presents as part of a clinical spectrum ranging from the more common "dry" or atrophic macular changes to the less common, visually devastating neovascular ("wet") form of the disease. It is estimated that over 153,000 new cases of neovascular AMD develop each year in the United States. Given the bilateral nature of this disease in an increasing aged population and that 42 % of neovascular AMD patients develop it in the fellow eye within 5 years; this number is expected to rise significantly.

The neovascular form of the disease is characterized by a choroidal neovascularization which is marked by proliferation of vessels and of a number of cells including those of the retinal pigment epithelium. Additionally, there is a recruitment of inflammatory cells such as neutrophils and macrophages. In many aspects, the process resembles that of a tissue repair response, and the role of the individual factors in the pathogenesis and the course of the disease is poorly understood. It is however, wel1 established that the vascular aspects of the process leads to leakage and bleeding in the retinal or in the subretinal space, which is one of the key factors leading to vision loss. Ultimately, photoreceptor death and fibrous disciform scar formation result in a severe loss of central vision and the inability to read, write, recognize faces or drive. Many patients can no longer maintain gainful employment, carry out daily activities and consequently report a diminished quality of life.

Clinically, patients may present with distorted vision (metamorphopsia), spots missing from their vision (scotoma) and/or decreased visual acuity. Diagnosis is often apparent upon clinical examination and confirmed via intravenous fluorescein angiography and stereoscopic fundus photography. Optical coherence tomography (OCT), a non-invasive imaging technique, has become increasingly useful in the diagnosis and management of retinal diseases characterized by increased retinal thickness such as AMD.

There is a limited understanding of the pathogenetic events leading to neovascular AMD. Thus preventative therapies have demonstrated little effect and therapeutic strategies have focused primarily on treating the neovascular lesion.

Vascular Endothelial Growth Factor in AMD

Vascular endothelial growth factor (VEGF) plays a critical role in endothelial cell proliferation and vessel growth which are key components required for neovascularization in health and disease. There are several reports of nonclinical and clinical studies (Macugen® and Lucentis®) that demonstrate that antagonizing VEGF is a useful strategy for treating neovascular AMD. We have designed and constructed a soluble hybrid protein inhibitor of VEGF named sFLT01 based on the VEGF receptor flt-1.

Currently Available Therapies for Neovascular AMD)

There is an existing body or information regarding intraocular and systemic use of VEGF inhibitors in humans. This information indicates that drugs with this mechanism of action are efficacious for the intended use, and that the safety profile is acceptable.

Since December 2004, two anti-VEGF molecules [the aptamer pegaptanib sodium (Macugen®) and the humanized monoclonal antibody fragment ranibizumab (Lucentis®)] have been approved for treatment of neovascular AMD. Pegaptanib binds specifically $VEGF_{165}$ while ranibizumab binds all VEGF-A isoforms. Both of these drugs have been reported to be well tolerated in recommended doses. In contrast to pegaptanib, which maintains visual acuity in 70 % of patients treated, ranibizumab both maintains vision in almost all (95 %) patients and improves (> 3 line) visual acuity in 34-40 % of patients. These drugs represent milestone achievements in the treatment of AMD.

Additionally, Avastin®, a VEGF inhibitor with a similar mode of action is also approved and clinically used systemically for the treatment or some metastatic cancers. The drug has been demonstrated to increase the progression-free survival and overall survival in some patient populations, and its side effects are well characterized.

Laser thermal photocoagulation and photodynamic therapy (PDT) using verteporfin (Visudyne®) are two therapies that do not involve the inhibition of VEGF.

Laser thermal photocoagulation treatment involves the destruction of well defined choroidal neovascular membranes greater than 200 microns from the center of the foveal avascular zone (extrafoveal) (Fletcher, 2007. *Vaughan & Asbury's General Ophthalmology*). The procedure uses high energy burns over and around the entire

membrane, also resulting in the destruction of the overlying retina. Patients may experience visual loss due to the expanding laser scar and the rate of reoccurrence of the neovascular membrane is high.

Verteporfin, approved by the Food and Drug Administration (FDA) for the treatment of' CNV in 2000, is a porphyrin derivative used in PDT as a photosensitizing agent. Following intravenous administration, verteporfin is activated by a non-thermal light source (689 nm wavelength laser) at the site of choroidal neovascularization. The activated verteporfin damages neovascular endothelium, resulting in vascular occlusion. In contrast to laser thermal photocoagulation, PDT causes less tissue and retinal damage since the CNV complex is specifically targeted (Clinical Pharmacology, 2008, [Verteporfin Monograph]). The procedure is repeated every 3 months as needed.

Rationale for AAV2-sFLTO1Phase 1 Study

While some currently used therapies may slow the progression of vision loss or in some cases improve vision, none of these treatments prevent neovascularization from recurring, and each has to be re-administered to prevent the disease from worsening. Each of the currently available VEGF inhibitor therapies requires continuous repeat intravitreal injections at 4-week (ranibizumab) or 6-week (pegaptanib) schedules. The administration of repeat treatments can incur additional risk and is inconvenient for both patient and treating physician.

Thus there is significant need for a long acting therapeutic. A gene transfer product with a long duration of action could fulfill this need, and could have a profound impact on the treatment of this disease.

The aim of the proposed study is to evaluate the safety and tolerability of a single uniocular injection of AAV2-sFLT01, a long acting (at least 12 months) gene transfer product. This study will also evaluate the biological activity of AAV2-sFLT01. Finally; this study will assess potential doses of AAV2-sFLT01 for inclusion in a Phase 2 study.

1.1 Proposed Phase 1Study for AMD

The proposed Phase I study is an open label, dose escalating, safety and tolerability study of a single administration via intravitreal injection of an adeno-associated virus vector containing a chimeric vascular endothelial growth factor (VEGF) receptor DNA expression cassette (AAV2-sFLT01,test article) in patients with neovascular (wet) AMD. Four closes will be evaluated: 2 x 10^8 vector genomes (vg), 2 x 10^9 vg, 6 x 10^9 vg and 2 x 10^9 vg. The starting close is 10-fold below the NOAEL established in the non-human primate toxicology study. This study consists of a screening period (up to 14 days), an enrollment and test article administration period (1 day) a safety observation period (8 weeks) and a follow-up period (44 weeks) for each patient. There are 2 dosing phases in this study, a Dose-Escalation Phase for identifying dose-limiting toxicity (DLT) followed by a Maximum Tolerated Dose (MTD)-Evaluation Phase for further evaluation of safety and biological activity at and below the MTD.

Patient Population

All patients considered for participation in the study will meet screening criteria; patients enrolled in the Dose-Escalation Phase will have subfoveal disciform scarring, and best-corrected visual acuity (BCVA) of 20/60 or worse in the study eye, while those patients enrolled in the MTD-Evaluation Phase, will not have subfoveal disciform scarring and will have BCVA or20160 or worse in the study eye.

Written informed consent will be obtained from all study patients prior to their participation in any study related procedures. Screening will determine patient eligibility for the study according to written inclusion and exclusion criteria which include both general medical and AMD-specific criteria.

Study Design

It is anticipated that up to 34 patients may be enrolled in this study. In the Dose-Escalation Phase, 3 patients will he enrolled into each of the 4 dosing cohorts. Within each cohort, there will be a 2-day waiting period between the dosing of patients to ensure that there are no immediate adverse reactions to the treatment. Once 3 patients in a cohort receive their dose they must complete the first 8 week safety observation period. If a patient drops out of the study prior to the completion of the first 8 weeks, they will be

replaced. A total of 3 patients must complete the first 8 week safety observation period for a cohort to be considered complete. If certain adverse events (AEs) are observed after the initial 3 patients have been treated in each closing cohort, an additional 3 patients may be enrolled in that dosing cohort to further evaluate the DLT. Once DLT is identified an additional 5 patients will be enrolled at the MTD, and an additional 5 patients will be enrolled at 1 dose below the MTD for further evaluation of safety and biological activity.

An Independent Data Monitoring Committee (DMC) will provide an ongoing, expert, independent review of safety data to assure that the risks to study patients are minimized. This ongoing review will include expedited notification of any serious adverse events (SAEs) as well as all ocular AEs and SAEs. Patients will be enrolled and receive treatment in a staggered manner within a particular cohort. After the last patient in a cohort has completed 8 weeks safety observation, the DMC will review safety data per the DMC charter. Approval from the DMC is required before enrollment into the next cohort can proceed. The DMC has the option to halt or suspend enrollment or to recommend that enrollment within a particular dosing cohort be expanded up to 6 patients should the DMC require additional data to better evaluate any potentially significant safely signal, SAE possibly related to the AAV2-sFLT01 or other condition expressly included in the DMC charter and that may represent a MTD. Consequently, as many as thirty-four (34) patients may be enrolled in this study. Up to seven (7) investigational sites may participate in this study.

The primary objective of this study is to evaluate the safety and tolerability of a single uniocular intravitreal injection of AAV2-sFLT01 in patients with neovascular AMD. The secondary objective of this study is to assess the biological activity of a single uniocular intravitreal injection ofAAV2-sFLT01 in patients with neovascular AMD. In addition to the planned evaluations between dosing cohorts described above, Genzyme will implement a risk assessment plan as part of this study as outlined in the IB to provide an ongoing evaluation of the nature, frequency, severity and causality of specific AEs identified as potential risks associated with the use ofAAV2-sFLT01.

Based on currently available data (Appendix M-II-B-2-b-(2)), it is expected that sufficient expression of sFLT01 occurs at 1-2 months. Therefore; there will be a minimum follow-up of 8 weeks from administration of the study treatment before alternative treatments are considered. The investigator will be asked to consider treating,

with best available appropriate care, any patient who has experienced: at least a 2 line loss of distance visual acuity; persistent or worsening of intra- or subretinal fluid on OCT; and/or other concerning vitreal or retinal findings. When possible: the investigator will be asked to consult with the Genzyme medical monitor to discuss a particular case prior to initiating rescue treatment. However, a patient may be treated with best available care at any time according to the medical judgment of the investigator. Any case in which a patient is given additional care under these specific circumstances the investigator will be asked to promptly report the additional care to the Genzyme medical monitor. Ocular conditions in the study eye other than vitreal or retinal will be managed per standard of care, and will be reported to the Genzyme medical monitor via standard documentation by the patient case report form (CRF). In the event that a patient receives additional AMD therapy beyond the study treatment, that patient will continue in the trial for collection of safety data. However, if this additional treatment occurs prior to the initial 8 week follow-up this patient will be replaced for purposes of safety evaluation.

Assessments of Safety and Biological Activity

After treatment, patients will return for follow-up visits on Days 1, 3, 7, and Weeks 2, 4, 8, 12, 18, 26, 38, and 52 to complete study assessments including: an eye exam with distance BCVA with or without refraction, intraocular pressure (IOP) testing by Goldmann tonometry, biomicroscopy, dilated or undilated fundus examination including indirect ophthalmoscopy, stereoscopic fundus photography, FA and OCT; physical examination with vital signs; clinical chemistry laboratory testing and patient-reported AEs. Further assessment of retinal function and RPE integrity may be performed in a subset of patients at investigative sites with electroretinogram (ERG) and AF capabilities. Assessors of visual acuity will be masked to previous measurements and a central reading facility will be used for a masked independent assessment of FA and stereoscopic fundus photography. If a patient requires more frequent follow-up visits for the clinical management of AMD symptoms than is specified in the protocol, then he/she can be evaluated via an unscheduled visit. Applicable data from any unscheduled visits will be captured in the study database.

Analysis of vector distribution in the blood and testing for potential virus shedding in the nasopharynx, urine, and semen (consenting fertile male patients only) will occur early in

the course of the study and will continue until 2 consecutive negative samples are obtained at which point testing may be discontinued.

AAV-specific immunity will include evaluation of anticapsid T cells and serum antibodies to AAV2 capsid. In addition, an evaluation of circulating levels of sFLT01 protein and potential sFLT01 antibodies will be performed.

After a total of 52 weeks of follow-up, patients will be enrolled in an extended, follow-up program for up to 5 years. In collaboration with the FDA, an assessment of the accumulated data will be made at or prior to the 5-year time point to determine if modification or discontinuation is warranted. The long-term follow-up program will be an observational program reflecting the FDA's recommendations as outlined in the *Guidance for Industry-Gene Therapy Clinical Trials-Observing Subjects for Delayed Adverse Events.* In addition, Genzyme's program will include ocular safety and efficacy assessments. Participation in long-term follow up is strongly encouraged; however, patients can choose not to participate.

Please refer to Part 4 of this application for the complete clinical protocol.