STUDY PROTOCOL COVER PAGE

Official Title of the study/Protocol Title: AAV8-mediated Low Density Lipoprotein Receptor (LDLR) Gene Replacement in Subjects with Homozygous Familial Hypercholesterolemia (HoFH)

Protocol Number: FHGT002

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Document Date: 14 December 2018; version 10

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Protocol Number: FHGT002

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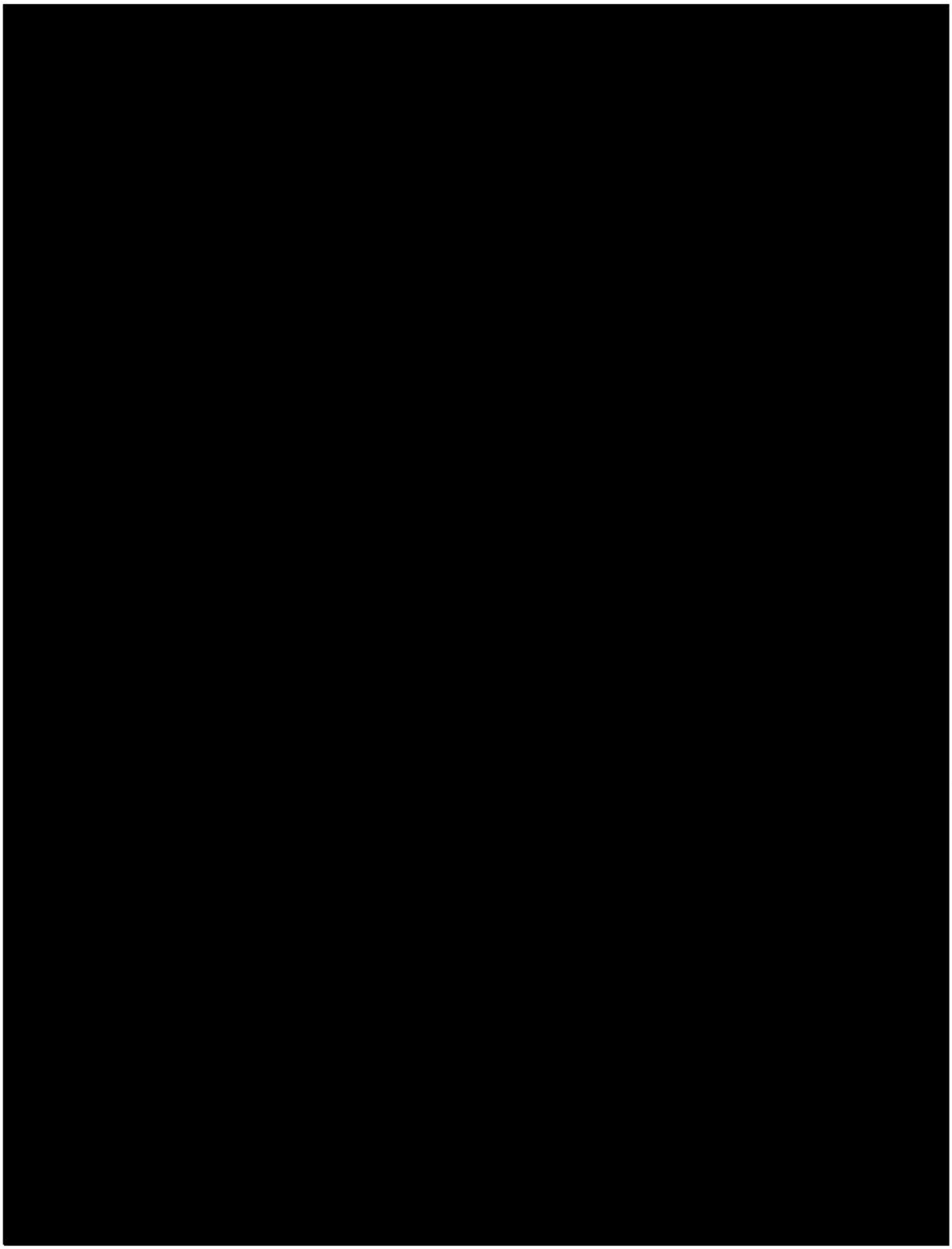
Investigational Product:	[redacted]
Indication:	Homozygous Familial Hypercholesterolemia (HoFH)
IND/EudraCT Number/Health Canada #:	IND 16710/ 2016-001446-25/ 199002
Regulatory Sponsor:	REGENXBIO Inc. 9600 Blackwell Road, Suite 210 Rockville, MD, USA 20850
Sponsor's Responsible Medical Monitor	[redacted]

This study is to be performed in compliance with the protocol, International Conference on Harmonization E6: Good Clinical Practice: Consolidated Guideline (ICH E6) and applicable regulatory requirements.



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LIST OF ABBREVIATIONS

AAV	Adenoassociated virus
ADR	Adverse drug reaction
AE	Adverse event
ALT	Alanine aminotransferase
Apo	Apolipoprotein
ApoB	Apolipoprotein B
ASCVD	Atherosclerotic Cardiovascular Disease
AST	Aspartate aminotransferase
CBC	Complete blood count
CPK	Creatine Phosphokinase
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common terminology criteria for adverse events
CTL	Cytotoxic T lymphocyte
CVD	Cardiovascular disease
DKO	Double knock-out or LDLR-/- Apobec-/-
DLT	Dose-limiting toxicity
DMEM	Dulbecco's minimal essential medium
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
EDC	Electronic data capture
ELISA	Enzyme-linked immunosorbent assay
EOS	End of study
FDA	Food and Drug Administration
FCR	Fractional catabolic rate
FH	Familial hypercholesterolemia
FSR	Fractional synthetic rate
FWA	Federalwide Assurance
GC	Genome copy
GCP	Good Clinical Practices
GGT	Gamma Glutamyl Transferase
GI	Gastrointestinal
GMP	Good Manufacturing Practices
HDL-C	High density lipoprotein cholesterol
hLDLR	Human low density lipoprotein receptor
HoFH	Homozygous familial hypercholesterolemia
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
INR	International Normalized Ratio

IRB	Institutional Review Board
ISC	Internal Safety Committee
ITR	Inverted terminal repeat
IV	Intravenous
Kg	Kilogram
L	Liter
LAHB	Double knock-out mouse with human APOB or LDRL-/- Apobec-/- hAPOB
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
LDLR	Low density lipoprotein receptor
LFTs	Liver function tests
Lp(a)	Lipoprotein a
LPL	Lipoprotein lipase
MED	Minimal effective dose
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
mL	Milliliter
mLDLR	Murine low density lipoprotein receptor
MPS VII	Mucopolysaccharidosis type VII
mRNA	Messenger ribonucleic acid
MRS	Magnetic resonance spectroscopy
MTD	Maximum tolerated dose
Nab	Neutralizing antibody
NCI	National Cancer Institute
NHLBI	National Heart, Lung and Blood Institute
NHP	Non-human primates
NOAEL	No observed adverse event level
Non-HDL-C	Non-HDL cholesterol
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCSK9	Proprotein convertase subtilisin/kexin type 9
PD	Pharmacodynamic
Penn	University of Pennsylvania
PHI	Personal health information
POC	Proof-of-concept
polyA	Polyadenylation
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
qPCR	Quantitative PCR
rAAV	Recombinant AAV
RNA	Ribonucleic acid
SAE	Serious adverse event

SAP	Statistical Analysis Plan
SD	Standard deviation
SDV	Source data verification
SDS PAGE	Sodium dodecyl sulphate polyacrilamide electrophoresis
SOC	System Organ Class
SRT	Safety review trigger
TBG	Thyroxine-binding globulin
TC	Total cholesterol
TG	Triglycerides
TSH	Thyroid stimulating hormone
UADR	Unexpected adverse drug reaction
ULN	Upper limit of normal
VLDL	Very low density lipoprotein
VLDL-C	Very low density lipoprotein cholesterol
Vg	Vector genomes
WBC	White blood cell
WHHL	Watanabe heritable hyperlipidemic

STUDY SUMMARY

Title	AAV8-mediated Low Density Lipoprotein Receptor Gene Replacement in Subjects with Homozygous Familial Hypercholesterolemia
Short Title	AAV8.TBG.hLDLR for FH
Protocol Number	FHGT002
Phase	Phase I/IIa
Study Design	This is a 104-week Phase I/IIa, multicenter, open-label, single arm, dose escalation study of AAV8.TBG.hLDLR in adults with a clinical presentation consistent with Homozygous Familial Hypercholesterolemia (HoFH) and carrying 2 mutations in the gene encoding for the LDL receptor (LDLR). Safety will be the primary focus, with a secondary focus on clinical response to AAV8.TBG.hLDLR. The primary safety endpoint is at Week 24. Approximately 12 subjects will be enrolled into one of 3 dose cohorts, 2.5×1012 GC/kg (Dose 1),7.5×1012 GC/kg (Dose 2), or 2.5×1013 GC/kg (Dose 3), and will receive a single dose of AAV8.TBG.hLDLR administered by IV infusion into a peripheral vein (or into an existing arteriovenous (AV) fistula). Following completion of the primary study period at Week 24, subjects will continue to be assessed (for safety and efficacy) for up to 104 weeks following treatment with AAV8.TBG.hLDLR. At the end of the study, all subjects will be invited to participate in a long-term follow-up study. In this trial, two groups will assess data accumulated from the trial, an external Data Safety Monitoring Board (DSMB) and the Sponsor's Internal Safety Committee (ISC). The primary role of the independent DSMB is to assess safety at periodic intervals and to provide recommendations on safety and dose escalation. The primary role of the ISC is to monitor safety on an ongoing basis. The initial Dose 1 cohort of AAV8.TBG.hLDLR will receive 2.5×10^{12} GC/kg. Dosing of subjects will be staggered by at least 4 weeks A formal review of all safety data will be performed by the DSMB after the 3rd subject is dosed and completes the 4 weeks post-dosing visit, primarily to determine if dose escalation can occur. However, the DSMB could recommend to expand the cohort, lower the dose, or stop the trial. If the recommendation is to dose escalate, the Dose 2 cohort will be initiated at a dose of 7.5×10^{12} GC/kg (Dose 2). Dosing of subjects will be staggered by at least 4 weeks. A formal review of all safety data will be performed by the DSMB after the 3rd subject is dosed and completes the 4 weeks post-dosing visit, primarily to determine if dose escalation can

occur. However, the DSMB could recommend to expand the cohort, lower the dose, or stop the trial. If expansion of Dose 2 cohort is recommended, up to 3 additional subjects will be enrolled and will receive prophylactic corticosteroids. Subject dosing will be staggered by at least 8 weeks. After enrollment of up to 3 subjects in the expanded Dose 2 cohort (or fewer subjects, upon agreement by the DSMB), a review of all the safety data will performed by the DSMB to determine if dose escalation can occur. After completion of the Dose 2 cohort, the DSMB will need to recommend dose escalation in order for the Dose 3 cohort to be initiated.

After initiation of the Dose 3 cohort (2.5×10^{13} GC/kg), the following will occur:

- Dosing of subjects will be staggered by at least 6 weeks. Up to 3 subjects will be enrolled into the cohort.

At any time, if there are safety concerns, the ISC may share safety concerns with the DSMB. The DSMB may recommend to stop the trial, dose additional subject(s) at the current dose, or proceed at a lower dose.

If at any time during dosing an event meets the criteria of a Stopping Rule, dosing of any new subjects will be suspended until a complete review of all safety data by the external DSMB and the ISC has been performed.

At any given DSMB meeting, whether called for by a Safety Review Trigger (SRT) or at the planned DSMB meeting at the conclusion of a dose cohort, the DSMB may recommend stopping the trial, dosing additional subjects at the current dose, proceeding to the next dose cohort, or proceeding at a lower dose. After the final subject has been dosed, a review of all safety data by the DSMB will be performed.

A summary of safety review triggers and expected actions is provided in Table 1.

Table 1: Safety Review Trigger Event and Actions

Safety Review Trigger Event	Safety Review Action
A Stopping Rule is met	An external DSMB will review all available safety data and provide a recommendation on whether to enroll additional subjects.
Any Grade 4 or 5 AE regardless of relationship to treatment	The chairs of the ISC and the external DSMB will review and decide whether to allow enrollment to continue or convene a full DSMB review of all available safety data which will then provide a recommendation on whether to enroll additional subjects.
Any Grade 3 AE considered treatment-related (by the Investigator)	

	<table><tr><td>Any Grade 3 AE considered unrelated to treatment (by the Investigator)</td><td rowspan="2">An ISC will review all available safety data. If safety concerns arise while a cohort is being enrolled, the committee may ask the DSMB to review and make a recommendation on whether to keep enrolling subjects in that cohort.</td></tr><tr><td>Any report by the Investigator of technical issues with the product administration that may warrant modifications to the procedure</td></tr></table> All subjects, including those that were deemed eligible based on their participation in a companion screening protocol, will be invited to undergo a screening visit (Visit 1) to confirm eligibility for this clinical trial. Subjects that agree to participate will be withdrawn from selected lipid-lowering drugs for at least 4 weeks prior to vector administration. An additional Visit 1a may be scheduled as a blood draw either at the study site or by a home healthcare nurse to perform a lipid panel up to 2 weeks prior to the dosing visit (Visit 3). All subjects will be admitted at the research inpatient unit the day of or the day before vector administration and eligibility and willingness to participate in the trial will be appropriately re-confirmed. Following AAV8.TBG.hLDLR administration, safety assessments and laboratory draws will occur for 24 hours post dosing, after which the subject will be discharged from the research unit. Subject will return to the Research Unit for a safety visit 48 hours post-dosing. Blood will be drawn weekly for safety testing from Week 2 to Week 12. Based on the experience accumulated with subjects dosed under earlier versions of this protocol, prophylactic corticosteroids will be administered to any future dosed subjects starting 1 day prior to dosing through the end of Week 13. After week 14, subjects will undergo study site visits at Weeks 18, 24, 36, and 52 for the active study and at Weeks 78 and 104 during the follow up period. Additional laboratory assessments will occur at least biweekly between Week 14 and Week 24. Both liver function tests (LFTs) and LDL-C collected locally either at local lab or by health care nurse will be recorded in the Clinical Database. Long-term follow-up will be conducted via a separate protocol. All visits and follow-up will be indexed to the date of the vector infusion. [redacted]
Study Duration	The study duration is 104 weeks, made up of an active study period through Week 52 and a follow-up period after Week 52 visit through Week 104 after which the subject will be invited to enter a long-term follow up study, as a separate study.

Study Center(s)	Approximately 13 sites in the US, Canada and Europe
Objectives	The primary objective will be to determine the safety of AAV8.TBG.hLDLR administration in this patient population. The key secondary objective is to assess the efficacy of LDL-C reduction achieved with AAV8.TBG.hLDLR administration.
Number of Subjects	Approximately12 subjects.
Diagnosis and Main Inclusion Criteria	The subject population will be male and female subjects with the following characteristics: 1. Age: 18 years or older; Carry 2 molecularly defined *LDLR* mutations and clinical presentation consistent with HoFH; No cardiovascular event or cardiovascular intervention within 12 weeks of enrollment; A baseline serum AAV8 neutralizing antibody titer ≤ 1:10.
Study Product, Dose, Route, Regimen	[redacted]
Reference Therapy	None
Statistical Methodology	All data will be presented in subject data listings. Categorical variables will be summarized using frequencies and percentages, and continuous variables will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). Graphical displays will be presented as appropriate. Safety and pharmacodynamic (PD) endpoints will be reported by dose group and may also be reported for all dose groups combined. No formal calculation was performed to determine sample size.

1. INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

In November 2018, the University of Pennsylvania notified all Regulatory Authorities that sponsorship of this study had been transferred to REGENXBIO Inc. This transfer was completed on November 12 2018, with updates of study documentation, including this protocol, reflecting REGENXBIO as the study sponsor.

1.1. Background

1.1.1. Genetics of Homozygous Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is a life-threatening disorder caused by mutations in genes that affect LDL receptor (LDLR) function (Goldstein et al. 2001). It is estimated that >90% of patients with molecularly confirmed FH carry mutations in the gene encoding for the LDLR (*LDLR*, *MIM 606945*). The remainder of the patients carry mutations on three additional genes: *APOB (MIM 107730)* encoding apolipoprotein (apo) B, *PCSK9 (MIM 607786)* encoding proprotein convertase subtilisin/kexin type 9 (PCSK9), and *LDLRAP1 (MIM 695747)* encoding LDLR adapter protein 1. The latter is the only gene mutation that is associated with a recessive trait. Homozygosis is usually conferred by the presence of mutations in the 2 alleles of the same gene; however, cases have been reported of patients with double heterozygosis (two heterozygous mutations, one each in two different genes). Based on prevalence rates of between 1 in 500 and 1 in 200 for heterozygous FH (Nordestgaard et al. 2013, Sjouke et al. 2014), it is estimated that between 7,000 and 43,000 people worldwide have homozygous FH (HoFH).

Characterization of mutant *LDLR* alleles has revealed a variety of mutations including deletions, insertions, missense mutations, and nonsense mutations (Goldstein et al. 2001). More than 1700 *LDLR* mutations have been reported. This genotypic heterogeneity leads to variable consequences in the biochemical function of the receptor which are classified in four general groups. Class 1 mutations are associated with no detectable protein and are often caused by gene deletions. Class 2 mutations lead to abnormalities in intracellular processing of the protein. Class 3 mutations specifically affect binding of the ligand LDL, and Class 4 mutations encode receptor proteins that do not cluster in coated pits. Based on residual LDLR activity assessed using patients' cultured fibroblasts, mutations are also classified as receptor negative (<2% residual activity of the LDLR) or receptor-defective (2 to 25% residual activity). Patients that are receptor-defective have, on average, lower LDL-C levels and a less malignant cardiovascular course compared to those that are receptor negative. This was evident among a cohort of patients that was followed over several years at the University of Pennsylvania (Kolansky et al. 2008), in which receptor-defective patients had lower cholesterol levels, a delayed appearance of xanthomas and clinically evident cardiovascular disease, and a better response to standard lipid-lowering therapy.

As a consequence of impaired LDL receptor function, untreated total plasma cholesterol levels in patients with HoFH are typically greater than 500 mg/dl, resulting in premature and aggressive atherosclerosis often leading to cardiovascular disease (CVD) before age 20 and death before age

30 (Cuchel et al. 2014, Goldstein et al. 2001). Early initiation of aggressive treatment for these patients is therefore essential (Kolansky et al. 2008). Unfortunately, the available options are severely limited. Statins are considered the first line for pharmacological treatment. Even at maximal doses, only a 10 to 25% reduction in LDL-C plasma levels is observed in most patients (Marais et al. 2008, Raal et al. 2000). The addition of the cholesterol absorption inhibitor, ezetimibe, to statin therapy may result in a further 10 to 20% reduction in LDL-C levels (Gagne et al. 2002). Use of other cholesterol lowering medications, including bile acid sequestrants, niacin, fibrates, and probucol have been used successfully in the pre-statin era and can be considered to achieve further LDL-C reduction in HoFH; however, their use is limited by tolerability and drug availability. This approach has been shown to reduce CVD and all-cause mortality (Raal et al. 2011). Despite the implementation of an aggressive multi-drug therapy approach, the LDL-C levels of HoFH patients remain elevated and their mean life expectancy remains approximately 32 years (Raal et al. 2011). Several non-pharmacological options have also been tested over the years. Surgical interventions, such as portacaval shunting (Bilheimer 1989, Forman et al. 1982) and ileal bypass (Deckelbaum et al. 1977), have resulted only in partial and transient LDL-C lowering and are now considered nonviable approaches. Orthotopic liver transplantation has been demonstrated to substantially reduce LDL-C levels in HoFH patients (Ibrahim et al. 2012, Kucukkartallar et al. 2011), but disadvantages and risks limit the use of this approach, including the high risk of post-transplantation surgical complications and mortality, the scarcity of donors, and the need for life-long treatment with immunosuppressive therapy (Malatack 2011, Starzl et al. 1984). The current standard of care in HoFH includes lipoprotein apheresis, a physical method of purging the plasma of LDL-C which can transiently reduce LDL-C by more than 50% (Thompson 2003, Vella et al. 2001). Rapid re-accumulation of LDL-C in plasma after treatment sessions (Eder and Rader. 1996) necessitates weekly or biweekly apheresis. Although this procedure may delay the onset of atherosclerosis (Thompson et al. 1995, Vella et al. 2001), it is laborious, expensive, and not readily available. Furthermore, although it is a procedure that is generally well tolerated, the fact that it requires frequent repetition and intravenous access can be challenging for many HoFH patients.

Recently, three new drugs have been approved by the FDA as add-on therapy specifically for HoFH. Two of them, lomitapide and mipomersen, inhibit the assembly and secretion of apoB-containing lipoproteins, although they do so via different molecular mechanisms (Cuchel et al. 2007, Raal et al. 2010). This approach results in a significant reduction of LDL-C that reaches an average of ~50% with lomitapide (Cuchel et al. 2013) and ~25% with mipomersen (Raal et al. 2010). However, their use is associated with an array of adverse events that may affect tolerance and long-term adherence and include liver fat accumulation, the long term consequences of which have not yet been fully clarified.

The third is part of a novel class of lipid-lowering drugs, monoclonal antibodies against PCSK9 that have been shown to be effective in lowering LDL-C levels with an apparently favorable safety profile in patients with heterozygous FH (Raal et al. 2012, Raal et al. 2015, Stein et al. 2012). Treatment of HoFH with the PCSK9 inhibitor evolocumab 420 mg every 4 weeks for 12 weeks has been shown to provide about a 30% reduction in LDL-C as compared with placebo (Raal et al. 2015). Efficacy of PCSK9 inhibitors is, however, dependent on the residual LDLR activity, with no effect in patients with no residual LDLR activity (Raal et al. 2015, Stein et al. 2013). Although the addition of PCSK9 inhibitors may become standard of care for FH and may

provide an additional further reduction to lower cholesterol in a subset of HoFH patients, it will not dramatically impact the clinical management of this condition. Therefore, there is a tremendous unmet medical need for new medical therapies for this life-threatening orphan disease.

1.1.2. Liver Directed Gene Therapy for HoFH

The correction of the hypercholesterolemia observed in patients with HoFH who underwent liver transplant (Ibrahim et al. 2012, Kucukkartallar et al. 2011) underscores the importance of the liver in regulating levels of circulating lipid and lipoproteins. Thus, a gene therapy approach that focuses on delivering the correct transgene to the liver may represent a viable approach. A considerable number of proof-of principle studies using gene transfer of the LDLR have been performed in animal models of HoFH. The administration of a first-generation adenovirus encoding the human LDLR transiently reduced LDL cholesterol levels in chow-fed LDLR deficient mice (Ishibashi et al. 1993). Injection of a recombinant first-generation adenovirus encoding the LDLR into Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model homozygous for a natural mutation in the LDLR, transiently reduced LDL cholesterol levels (Kozarsky et al. 1994). A helper-dependent adenovirus expressing *LDLR* was shown to provide long-term reduction in cholesterol in LDLR KO mice (Nomura et al. 2004). An AAV8-based vector encoding *LDLR* also provided sustained correction of hypercholesterolemia (Lebherz et al. 2004). A pilot human study was previously conducted at the University of Pennsylvania to evaluate the effect of *ex vivo* gene therapy in patients with HoFH (Grossman et al. 1995). In that study, five patients underwent hepatic resection, and isolated hepatocytes were infected *ex vivo* with a recombinant retrovirus encoding the human *LDLR* gene. The genetically modified autologous hepatocytes were re-infused into the patients via a portal catheter placed at the first procedure. Three of the five patients had a transient 6 to 25% reduction in their LDL cholesterol levels. Liver biopsies performed 4 months after treatment revealed LDLR transgene expression by *in situ* hybridization in all five patients. Based on preclinical studies conducted more recently using an AAV8-based vector, an investigational agent has been developed to be tested in a Phase I/IIa study in patients with HoFH.

1.2. Investigational Agent

The investigational agent is an AAV8 vector expressing the transgene human low-density lipoprotein receptor, (hLDLR) under control of a liver-specific promoter (thyroxine-binding globulin, TBG) and is referred to in this document as AAV8.TBG.hLDLR. [redacted]

[redacted]

1.3. Preclinical Data

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

1.4. Rationale for Dose Choice and Escalation

This is a Phase I/IIa dose escalation study of AAV8.TBG.hLDLR in patients with HoFH. The study will characterize the safety and tolerability of this vector and identify doses for further clinical investigation. The rationale for implementing a Phase I/IIa dose-escalating clinical study is based on a robust biological rationale established through proof-of-concept animal studies and non-clinical pharmacology/toxicology data. As this is a first-in-human study using AAV8.TBG.hLDLR, a staggered dose escalation is utilized to evaluate safety prior to treating a subsequent subject.

[redacted]

As further discussed in Section 1.5, the doses proposed for this study are within the range of the intravenous doses in other ongoing AAV8-based gene therapy trials (Table 2). These include X-linked myotubular myopathy (up to 5×10^{14} GC/kg), Crigler-Najjar Syndrome (up to 1.5×10^{13} GC/kg) and late onset OTC deficiency (up to 1×10^{13} GC/kg).

Based on these data, this trial includes up to three single-dose cohorts, 2.5×10^{12} GC/kg, 7.5×10^{12} GC/kg, and 2.5×10^{13} GC/kg. These doses represent half-log, stepwise increases that could inform a dose-response and that represent a dose range that is supported by the non-clinical testing. The introduction of prophylactic corticosteroids in the clinical protocol is anticipated to improve the safety of product administration by attenuating or preventing immune mediated hepatocyte injury.

1.5. Clinical Data to Date

Information obtained from recent ongoing gene therapy trials of systemically administered AAV8 has also informed the design and safety monitoring plan of this trial. Importantly, monitoring for increases in transaminases as a marker of potential liver injury has been incorporated into this trial as an adverse event of special interest.

Accumulating data from clinical experience with AAV8 supports the safety of this vector across gene therapy trials. A list of some recent and ongoing AAV8 based gene therapy trials is presented in Table 2.

Table 2: AAV8-based gene therapies listed on ClinicalTrials.gov

ClinicalTrials.gov Identifier	Indication	Sponsor
NCT00979238	Hemophilia B	St. Jude Children's Research Hospital
NCT01620801	Hemophilia B	Spark Therapeutics
NCT01687608	Hemophilia B	Shire
NCT01899092	Chronic Hepatitis C	Benitec Biopharma
NCT02317887	X-linked Retinoschisis	NIH / National Eye Institute (NEI)
NCT02651675*	Homozygous familial hypercholesterolemia	REGENXBIO
NCT02991144	Late onset OTC deficiency	Ultragenyx Pharmaceutical Inc
NCT03001830	Hemophilia A	University College London (UCL)
NCT03066258	Age-related Macular Degeneration	REGENXBIO

ClinicalTrials.gov Identifier	Indication	Sponsor
NCT03173521	Mucopolysaccharidosis Type VI	Fondazione Telethon
NCT03199469	X-linked myotubular myopathy	Audentes Therapeutics
NCT03223194	Crigler-Najjar Syndrome	Audentes Therapeutics
NCT03370172	Hemophilia A	Shire
NCT03374202	HIV-1	NIH/ National Institute of Allergy and Infectious Diseases (NIAID)
NCT03374657	Retinitis Pigmentosa	Novartis Pharmaceuticals
NCT03517085	Glycogen Storage Disease Type Ia (GSDIa)	Ultragenyx Pharmaceutical Inc

*Current protocol

In a publication on the long-term safety and efficacy of factor IX gene therapy in hemophilia B using an AAV8 vector (Nathwani et al. 2014; NCT00979238), the majority of reported adverse events were mild in severity. The most common study related adverse reaction reported was an asymptomatic elevation in the alanine aminotransferase (ALT) level, which occurred approximately 7 to 10 weeks after vector infusion in 4 of 6 patients receiving 2×10^{12} vector genomes (vg)/kg. Only 2 of these 4 patients had elevation greater than the upper limit of normal range for ALT (45 IU/L); including an increase from 32 to 64 IU/L in one patient and from 13 to 202 IU/L in the second. All 4 patients received a tapering dose of prednisolone, which resulted in resolution of the elevated ALT over a range of 2 to 35 days. Of note, an asymptomatic increase in the serum ALT level was associated with a decline in factor IX levels in 3 out of 4 patients, suggesting a loss of transduced hepatocytes in these patients. No patient had a recurrent episode of an elevated ALT level and there have been no new late adverse events observed by the study investigators. With respect to efficacy for this trial, a single IV infusion of the vector resulted in an increase in plasma factor IX activity that ranged from less than 1% of normal at baseline to steady-state levels of 1% to 6% of normal in 10 patients. AAV8 mediated factor IX expression has remained relatively stable over a period of up to 4 years (Nathwani et al. 2014).

A recently published trial of an AAV5 based gene therapy for hemophilia A (Rangarajan et al. 2017) utilized a dose escalation scheme whereby escalation to subsequent dose cohorts (three cohorts in total) occurred after a single patient had received what was considered a safe dose along with having a factor VIII activity level less than 5 IU/dL at study week 3 post gene transfer. This allowed the third subject enrolled, as well as six additional subjects, to receive the highest dose (6×10^{13} vg/kg), resulting in sustained therapeutic factor VIII levels at least 1 year after gene transfer. This approach illustrates the use of individual safety and efficacy data to support the

benefit:risk of dose escalation while minimizing the number of subjects exposed to subtherapeutic doses.

Data has also been reported from a Phase I/II clinical trial assessing the safety and optimal dosing level of an investigational factor IX rAAV8-based gene therapy treatment for hemophilia B (NCT01687608) (Shire). Based on public information released by the sponsor, a total of seven patients in three sequentially-ascending dosing cohorts [2×10^{11} vg/kg (n=2), 1×10^{12} vg/kg (n=3) and 3×10^{12} vg/kg (n=2)] have been treated in the trial with evidence of a dose-related response. Two patients in the high dose cohort are reported by the sponsor to have experienced an immune response which led to decreased factor IX expression, with one patient resuming regular factor IX infusions (due to inadequate production of transgene product).

Additional subjects have been dosed with AAV8 based gene therapy trials for hemophilia B (NCT01620801), chronic HCV infection (NCT01899092), late onset OTC (NCT02991144) and age-related macular degeneration (NCT03066258). Accumulating data from ongoing trials continue to support a favorable benefit:risk for AAV based gene therapy for these conditions.

Notably, the doses proposed for this study are within the range of the intravenous doses being investigated in other ongoing AAV8-based gene therapy trials shown in Table 2. These include X-linked myotubular myopathy (up to 5×10^{14} GC/kg), Crigler-Najjar Syndrome (up to 1.5×10^{13} GC/kg) and late onset OTC deficiency (up to 1×10^{13} GC/kg). Data on the first 3 subjects enrolled into the myotubular myopathy gene therapy trial, all treated at an IV dose of 1×10^{14} GC/kg, has been made publicly available (Audentes. 2018). Although data is limited for this trial, reported possibly/probably treatment-related AEs include asymptomatic elevations in liver enzymes (n=1), elevated troponin (n=1) and exacerbation of pre-existing elevated bilirubin (n=1); all of these responded to treatment, including to corticosteroids. For the first 6 subjects enrolled into the OTC deficiency trial (2×10^{12} GC/kg (n=3) and 6×10^{12} GC/kg (n=3)), the only treatment-related adverse events reported in Cohort 1 were mild, clinically asymptomatic elevations in alanine aminotransferase (ALT) in two subjects (Ultragenyx 2018). The only treatment-related adverse events in Cohort 2 were the mild, clinically asymptomatic elevations in ALT in the first patient in Cohort 2.

As in this study, these trials also employ a dose escalation design (including half-log increases) in which the safety of each cohort is carefully reviewed before escalating to the next higher dose cohort. As in other trials, asymptomatic increases in transaminases have been observed in this trial in the first 3 subjects in Cohort 2 (7.5×10^{12} GC/kg) approximately 4-6 weeks post dosing. These transaminase elevations have responded to oral prednisone and resulted in normalization of transaminase levels. It is anticipated that the prophylactic administration of corticosteroids during the first 13 weeks of the trial may attenuate or possibly eliminate transaminase increases post dosing.

To summarize, the AAV8 vector containing an identical capsid as that used in the AAV8.TBG.hLDLR program are being or have been investigated as a gene therapy vector in clinical trials across a range of conditions and organ systems. Accumulating data has shown that recombinant, replication deficient AAV8 as a gene therapy vector is safe for investigational use in patients, as well as results in promising preliminary evidence of clinical benefit. A T-cell response to the capsid or the transgene product with subsequent hepatocyte injury may explain the asymptomatic elevations in transaminases, although the relationship between this and loss of

transgene product expression remains unclear. It is possible that immunosuppression with corticosteroids may mitigate this effect (Gernoux et al. 2017).

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Primary Objectives

- To determine the safety of AAV8.TBG.hLDLR administration in patients with homozygous familial hypercholesterolemia (HoFH) as assessed by the number of reported adverse events, changes noted on physical examinations, and clinical laboratory parameters assessed up to 24 weeks post vector administration.

2.2. Secondary Objectives

- To assess the LDL-C reduction achieved with AAV8.TBG.hLDLR administration as defined by percent change in LDL-C at 12 weeks (Cohort 1 only) or 18 weeks after vector administration (or 4 weeks after steroid termination or prior to changing lipid lowering therapies) compared to baseline.
- To assess changes in other lipid parameters at 12 weeks (Cohort 1 only) or 18 weeks after vector administration (or 4 weeks after steroid termination or prior to changing lipid lowering therapies) compared to baseline values, specifically percent change in total cholesterol (TC), non-high density lipoprotein cholesterol (non-HDL-C), HDL-C, fasting triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), lipoprotein(a) (Lp(a)), apolipoprotein B (apoB), and apolipoprotein A-I (apoA-I).
- To determine the safety of AAV8.TBG.hLDLR administration as assessed by the number of reported adverse events, changes noted on physical examinations and clinical laboratory parameters assessed at multiple time points up to 104 weeks post vector administration.
- To assess vector shedding in plasma and urine.

[redacted]

2.4. Safety Endpoints

Safety assessment is the primary objective of this Phase I/IIa study. Safety assessments will be performed before and at multiple time points after vector administration up to the 104 week post dosing visit, as detailed in Table 16.1 (see Section 16.1). The primary study period will be at Week 24. Safety assessment will include reported adverse events, changes noted on physical examinations, and laboratory parameters.

Laboratory Assessments

Biochemical Profile: sodium, potassium, chloride, carbon dioxide, glucose, blood urea nitrogen, lactate dehydrogenase (LDH), creatinine, creatine phosphokinase, calcium, total protein, albumin, AST, ALT, alkaline phosphatase, total bilirubin. Gamma glutamyl transferase (GGT) will be measured at screening and only as clinically indicated through the study.

Complete Blood Count (CBC): white blood cell (WBC) count, hemoglobin, hematocrit, platelet count, red cell distribution width, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

HbA1c

Coagulation: prothrombin time (PT), international normalized ratio (INR), partial thromboplastin time (PTT) at screening and baseline, and as needed through the study.

Urinalysis: urinary color, turbidity, pH, glucose, bilirubin, ketones, RBCs, protein, WBCs

- Vector concentration: AAV8 concentration in plasma and urine, measured as vector genomes by PCR.

Adverse Events of Special Interest

Based on the pre-specified laboratory and clinical assessments described above, the number of subjects who have the following will be presented:

- Liver injury
  - Common Terminology Criteria for Adverse Events (CTCAE) v4.0 Grade 2 or higher lab result for bilirubin (>1.5 × ULN) or liver enzymes (AST > 3 × ULN, ALT > 3 × ULN, AlkPhos > 2.5 × ULN).
- Hepatotoxicity (i.e., meet criteria for “Hy’s law”)
  - ALT or AST ≥ 3 × ULN and total bilirubin ≥ 2 × ULN and no other reason can be found to explain the changes observed. This event would meet the study Stopping Rules (Section 9.3).

Additionally, ALT or AST elevations that may trigger initiation or adjustment of corticosteroid therapy will be flagged and reported.

2.5. Efficacy Endpoints

The secondary endpoints are based on a detailed assessment of the percent change in lipid parameters at 12 weeks (Cohort 1 only) or 18 weeks after vector administration (or 4 weeks after

steroid termination or prior to changing lipid lowering therapies), following administration of AAV8.TBG.hLDLR compared to baseline. This will include:

- Percent changes in LDL-C
- Percent changes in Total Cholesterol, VLDL-C, HDL-C, non-HDL-C, TG, apoA-I, apoB, and Lp(a)

Efficacy will primarily be defined using LDL-C measured by beta quantification [redacted] The baseline LDL-C value will be determined using qualified LDL-C drawn prior to administration of AAV8.TBG.hLDLR. Qualified LDL-C draws will be those taken after sufficient washout of selected lipid lowering medications has occurred and pre-apheresis in subjects receiving apheresis (i.e., the day of apheresis). Baseline LDL-C value will be calculated as the average of up to 2 of the most recent qualified fasting LDL-C levels obtained during stable lipid lowering treatment before administration of AAV8.TBG.hLDLR. The 2 most recent LDL-C values should be no more than approximately 4 weeks apart. Baseline LDL-C values based on both beta-quantification and direct methods will be based on the same visit dates. Similar methods to define the baseline value will be applied to other lipid parameters. Lipid lowering treatment must be stable as per inclusion/exclusion criteria by the time of the first laboratory assessment.

[redacted]

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- [redacted]
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- [redacted]
- [redacted]
- [redacted]

3. STUDY DESIGN

3.1. General Design

This is a 104-week Phase I/IIa, multicenter, open-label, single arm, dose escalation study of AAV8.TBG.hLDLR in adults with a clinical presentation consistent with HoFH and carrying 2 mutations in the LDLR gene. Safety will be the primary focus, with a secondary focus on clinical response to AAV8.TBG.hLDLR. The primary safety endpoint is at Week 24. Approximately 12 subjects will be enrolled into one of 3 possible dose cohorts, 2.5×10^{12} GC/kg (Dose 1), 7.5×10^{12} GC/kg (Dose 2), or 2.5×10^{13} GC/kg (Dose 3), and will receive a single dose of AAV8.TBG.hLDLR administered by IV infusion into a peripheral vein (or into an existing AV fistula). Following completion of the primary study period at Week 24, subjects will continue to be assessed (for safety and efficacy) for up to 104 weeks following treatment with AAV8.TBG.hLDLR. At the end of the study, all subjects will be invited to participate in a long-term follow-up study.

In this trial, two groups will assess data accumulated from the trial, an external DSMB and the Sponsor's ISC. The primary role of the independent DSMB is to assess safety at periodic intervals and to provide recommendations on safety and dose escalation. The primary role of the ISC is to monitor safety on an ongoing basis.

The initial Dose 1 cohort of AAV8.TBG.hLDLR will receive 2.5×10^{12} GC/kg. Dosing of subjects will be staggered by at least 4 weeks. A formal review of all safety data will be performed by the DSMB after the 3rd subject is dosed and completes the 4 weeks post-dosing visit, primarily to determine if dose escalation can occur, however the DSMB may recommend to expand the cohort, lower the dose, or stop the trial.

If the recommendation is to dose escalate, the Dose 2 cohort will be initiated at a dose of 7.5×10^{12} GC/kg (Dose 2). Dosing of subjects will be staggered by at least 4 weeks. A formal review of all safety data will be performed by the DSMB after the 3rd subject is dosed and completes the 4 weeks post-dosing visit, primarily to determine if dose escalation can occur. However, the DSMB could recommend to expand the cohort, lower the dose, or stop the trial. If expansion of the Dose 2 cohort is recommended, up to 3 additional subjects will be enrolled and receive prophylactic corticosteroids as described in this protocol (Section 6.5). Subject dosing will be staggered by at least 6 weeks. After enrollment of the 3rd subject in the expanded Dose 2 cohort (or fewer subjects, upon agreement by the DSMB), a review of all safety data will be performed by the DSMB to determine if dose escalation can occur. After completion of the Dose 2 cohort, the DSMB will need to recommend dose escalation in order for the Dose 3 cohort to be initiated.

After initiation of the Dose 3 cohort (2.5×10^{13} GC/kg), dosing of subjects will be staggered by at least 6 weeks. Up to 3 subjects will be enrolled into the cohort.

At any time, if there are safety concerns, the ISC may share safety concerns with the DSMB. The DSMB may recommend to stop the trial, dose additional subject(s) at the current dose, or proceed at a lower dose.

If at any time during dosing an event meets the criteria of a Stopping Rule (Section 9.3), dosing of any new subjects will be suspended until a complete review of all safety data by the external DSMB and the ISC has been performed.

At any given DSMB meeting, whether called for by a Safety Review Trigger (SRT) or at the planned DSMB meeting at the conclusion of a dose cohort, the DSMB may recommend stopping the trial, dosing additional subjects at the current dose, proceeding to the next dose cohort, or proceeding at a lower dose. After the final subject has been dosed, a review of all safety data by the DSMB will be performed.

A summary of safety review triggers and expected actions is provided in Table 3.

Table 3: Safety Review Trigger Event and Actions

Safety Review Trigger Event	Safety Review Action
A Stopping Rule is met	An external DSMB will review all available safety data and provide a recommendation on whether to enroll additional subjects.
Any Grade 4 or 5 AE regardless of relationship to treatment	The chairs of the ISC and the external DSMB will review and decide whether to allow enrollment to continue or convene a full DSMB review of all available safety data which will then provide a recommendation on whether to enroll additional subjects.
Any Grade 3 AE considered treatment-related (by the Investigator)	
Any Grade 3 AE considered unrelated to treatment (by the Investigator)	An ISC will review all available safety data. If safety concerns arise while a cohort is being enrolled, the committee may ask the DSMB to review and make a recommendation on whether to keep enrolling subjects in that cohort.
Any report by the Investigator of technical issues with the product administration that may warrant modifications to the procedure	

All subjects, including those that were deemed eligible based on their participation in a companion screening protocol, will be invited to undergo a screening visit (Visit 1) to confirm eligibility for this clinical trial. Subjects that agree to participate will be withdrawn from selected lipid-lowering drugs for at least 4 weeks prior to vector administration. An additional Visit 1a may be scheduled as a blood draw either at the study site or by a home healthcare nurse to perform a lipid panel up to 2 weeks prior to the dosing visit (Visit 3).

All subjects will be admitted at the research inpatient unit the day of or the day before vector administration and eligibility and willingness to participate in the trial will be appropriately re-confirmed. Following AAV8.TBG.hLDLR administration, safety assessments and laboratory draws will occur for 24 hours post dosing as per Table 16.1 (see Section 16.1), after which the subject will be discharged from the research unit. The subjects will be asked to come to the research until for a blood draw 48 hours post administration. Blood will be drawn weekly for safety testing from Week 2 to Week 14. Based on the experience accumulated with subjects dosed under earlier versions, prophylactic corticosteroids will be administered to subjects from 1 day prior to dosing through the end of Week 13.

After week 14, subjects will undergo study site visits at Weeks 18, 24, 36, 52, 78 and 104. Additional laboratory assessments will occur at least biweekly between Week 12 and Week 24, and a single laboratory draw at Week 30. Subjects will be also asked to allow the collection of any LFTs or LDL-C collected locally after Week 13 for other reasons than the participation in this clinical trial, and the data recorded in the clinical database. Long-term follow-up will be conducted via a separate protocol. All visits and follow-up will be indexed to the date of the vector infusion.

[redacted]

4. SUBJECT SELECTION AND WITHDRAWAL

4.1. Inclusion Criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Male or female ≥ 18 years of age.
2. Untreated and/or treated LDL-C levels and clinical presentation consistent with the diagnosis of homozygous FH
3. Molecularly defined LDLR mutations at both *LDLR* alleles.
4. Concurrent allowed lipid lowering medication must be stable for ≥ 4 weeks before the baseline visit and must remain stable until 18 weeks after vector administration (or 4 weeks post steroid termination). These include but are not limited to: statins, ezetimibe, bile acid sequestrants, PCSK9 inhibitors, and LDL and/or plasma apheresis. Subjects on other lipid-lowering medications are eligible for the study but must wash out of these medications for the pre-specified time period.
5. Females of childbearing potential must have a negative pregnancy test at screening and baseline visits and be willing to have additional pregnancy tests during the study.
6. Sexually active subjects (both female and male) must be willing to use a medically accepted method of contraception from screening visit until 6 months after vector administration
7. A baseline serum AAV8 NAb titer ≤ 1:10.
8. Subjects must be able to comprehend and be willing to provide a signed institutional review board/ethics committee (IRB/EC) approved Informed Consent Form (ICF).
9. Subjects must be willing to comply with all study-related procedures and be available for the duration of the study.

4.2. Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

1. Unwilling to wash out of the following lipid lowering therapies for the pre-specified time period:
    a. niacin > 250 mg/day: within 6 weeks of baseline
    b. fibrates: within 4 weeks of baseline
    c. lomitapide: within 8 weeks of baseline
    d. mipomersen: within 24 weeks of baseline
2. Heart failure defined by the NYHA classification as functional Class III with history of hospitalization(s) within 12 weeks of the baseline visit or functional Class IV.
3. History within 12 weeks of the baseline visit of a myocardial infarction (MI), unstable angina leading to hospitalization, coronary artery bypass graft surgery (CABG), percutaneous coronary intervention (PCI), uncontrolled cardiac arrhythmia, carotid surgery

or stenting, stroke, transient ischemic attack, carotid revascularization, endovascular procedure or surgical intervention.

4. Uncontrolled hypertension defined as: systolic blood pressure > 180 mmHg, diastolic blood pressure > 95 mmHg.
5. Uncontrolled diabetes defined as HbA1c > 8.5% or an average fasting glucose ≥ 160 mg/dl.
6. Known hypersensitivity to prednisone
7. History of cirrhosis or chronic liver disease based on documented histological evaluation or non-invasive imaging or testing.
8. Documented diagnosis of any of the following liver diseases:
    a. Nonalcoholic steatohepatitis (biopsy-proven)
    b. Alcoholic liver disease
    c. Autoimmune hepatitis
    d. Liver cancer
    e. Primary biliary cirrhosis
    f. Primary sclerosing cholangitis
    g. Wilson's disease
    h. Hemochromatosis
    i. α_1 anti-trypsin deficiency
9. Abnormal liver function tests (LFTs) at screening (AST or ALT > 2 × upper limit of normal (ULN) and/or Total Bilirubin of > 1.5 × ULN unless patient has unconjugated hyperbilirubinemia due to Gilbert's syndrome).
10. Hepatitis B as defined by positive for HepB SAg, or Hep B Core Ab, and/or viral DNA
11. Chronic active Hepatitis C as defined by positive for HCV Ab and viral RNA.
12. History of chronic alcohol abuse within 52 weeks of the screening visit.
13. Certain prohibited medications known to be potentially hepatotoxic, especially those that can induce microvesicular or macrovesicular steatosis. These include but are not limited to: Accutane (isotretinoin), amiodarone, HAART medications, heavy acetaminophen use (2 g/day more than 3 times a week), isoniazid, methotrexate, tetracyclines, tamoxifen, or valproate.
14. Active tuberculosis, systemic fungal disease, or other chronic infection.
15. History of immunodeficiency diseases, including a positive HIV test result.
16. Chronic renal insufficiency defined as estimated GFR < 30 mL/min/1.73m^2.
17. History of cancer within the past 5 years, except for adequately treated basal cell skin cancer, squamous cell skin cancer, or in situ cervical cancer.
18. Previous organ transplantation.
19. Administration of an investigational drug within 12 weeks or 5 half-lives of the drug (whichever is longer) prior to the screening visit and until 52 weeks after receiving

AAV8.TBG.hLDLR. Subjects are not prohibited from receiving investigational drugs after 52 weeks.

20. Any major surgical procedure occurring less than 3 months prior to the screening visit, or any planned future surgical procedure within 3 months of baseline.
21. Serious or unstable medical or psychological conditions that, in the opinion of the investigator, would compromise the subject's safety or successful participation in the study.
22. Any other medical condition or finding that would make it not in the subject's best interest to participate in the study
23. Study staff member or any direct family member.

4.3. Subject Recruitment and Screening

Subjects will be recruited from study sites. Patients may be contacted through a companion screening protocol entitled "Screening Protocol for a Gene Therapy Trial in Subjects with Homozygous Familial Hypercholesterolemia." Methods to identify patients used in this and in the companion screening protocol include IRB/EC approved recruitment letters, referrals, and non-profit research foundations. Institutionally approved consent forms will be provided to patients. Consent of subjects that are not fluent in English will be obtained using appropriately translated informed consent forms (ICFs) and appropriate interpreters. Those who express an interest during the screening process will have the opportunity to discuss the study in detail again and confirm their consent to participate before vector administration.

4.3.1. Screening Protocol

Subjects with a diagnosis of HoFH that may have participated in a separate companion Screening Protocol will be contacted to participate in this clinical trial. Screening Protocol procedures include genetic analysis to confirm the presence of two mutations in the *LDLR* gene, lipid profile, NAb titer, and the collection of a detailed medical history as well as a detailed description of the procedures and risks of participating in the gene therapy trial. Subjects who preliminarily qualified based on the results from the Screening Protocol and that expressed interest in participating in the gene therapy trial will be invited to participate in this gene therapy clinical trial. Participation in the companion Screening Protocol is not obligatory. Eligibility for this clinical trial will be determined based on data collected during the screening visit (Visit 1) of the protocol described herein.

4.4. Informed Consent

Written informed consent will be obtained from each potential study subject prior to the conduct of any study procedures. Both the protocol and consent forms will be discussed with the potential study subject during documented informed consent sessions. Copies of the consent forms will be provided in advance for the patient's perusal. The subject will have ample time to ask questions and to discuss the study with their family and personal care physicians before and after the screening visit.

During the informed consent sessions, particular emphasis will be given to the following:

- The experimental nature of the study and the relative lack of experience with AAV8 vectors in humans.
- The potential for no benefit.
- The need to discontinue other selected lipid lowering medications for at least 4 weeks to establish baseline plasma cholesterol levels, and for 18 weeks (or 4 weeks post steroid termination if steroids have not been discontinued by at least 4 weeks at the Week 18 visit or prior to changing lipid lowering therapies) after vector infusion to assess efficacy. Lipoprotein and/or plasma apheresis will be allowed to continue as per standard of care if available and necessary for safety of the patient as determined by the caring physician.
- The observation of asymptomatic and transient increase transaminases in subjects enrolled in the previous cohort, risk of liver injury and treatment with corticosteroids.
- The toxicity and efficacy of AAV8 vectors in preclinical animal studies.
- The outcome of the previous studies that used AAV-based vector, including complications.
- The theoretical potential for germline transmission and malignancy.
- The potential for development of anti-human NAbs (e.g., inhibitors to LDLR).
- The nature and duration of follow-up including the need for long-term follow-up.
- The alternatives to gene transfer.
- That the subject is free to refuse to enter the study or to withdraw from it at any time.
- That the subject will be provided any new information during the course of the study that might affect their continued participation in the study.

Subjects who live within driving distance of the study site will be consented during an in-person visit. Subjects who live a long distance from the study site may be consented remotely at the discretion of the investigator. In this case, subjects will be provided with two copies of the consent form so that they have one copy to keep and one copy to mail back to the study site. They will be asked to set up a remote appointment (via phone or video-conference) with the research team after receiving and reading the consent form. During the in-person or remote consent session, the potential study participant and the research team will have an opportunity to review the study in detail. The subject will also have the opportunity to have all questions answered. These conversations will be documented in the subject's file.

A representative(s) of the potential subject's choosing will be encouraged to attend the consenting session with the subject. At the end of the consent session, subjects will be asked to sign the consent form. Subjects consented remotely will be asked to return the signed consent form using pre-paid envelopes. Subjects are considered enrolled when they have signed the informed consent document. No study procedures will be performed in subjects consented remotely until a signed copy of the consent form is received by the study site. Each subject enrolled in the study will receive the study agent, the dose of which will be dependent on the cohort to which he/she is admitted.

4.5. Early Withdrawal of Subjects

Any subject who withdraws from the study after signing the final consent document, but before the administration of the vector, will be replaced. This clinical trial involves a single administration of vector and therefore "withdrawal" after the single dose of vector applies only to loss of follow-up, not to ongoing administration of study agent. Every effort will be made to continue to follow-up subjects for study duration (104 weeks) after enrollment and vector administration.

4.6. Withdrawal of Informed Consent for Data and Biological Samples

Biological Samples Obtained for the Main Study

Study data are protected by the use of an identification number, which is a number specific to the subject. The investigator is in control of the information that is needed to connect a study sample to a subject. A subject's consent to the use of data does not have a specific expiration date, but the subject may withdraw consent at any time by notifying the investigator. If consent is withdrawn, any samples collected prior to that time may still be given to and used by the sponsor but no new data or samples will be collected unless specifically required to monitor safety of the subject.

Samples Obtained for Future Research

Research samples, including serum, plasma, DNA, RNA, and cells will be collected and stored for follow-up exploration of study findings, efficacy measures and/or adverse events (e.g., measurement of immune response, antibodies against LDLR, measurement of cytokine and chemokine levels or other inflammatory biomarkers) in this or subsequent studies of AAV8.TBG.hLDLR. Any unused sample collected throughout the study for routine lab analysis may also be stored for future research.

Samples will be labeled with the same identification number used for other study-related information and will not be labeled with personal identifiers such as the subject's name. If the subject withdraws consent, this link will allow the sponsor to locate the subject's sample and destroy it. The coding of samples and results is to ensure that these research results are kept confidential by keeping the subject's identity and these results separate.

This additional research may not start immediately and may start at any time during the storage period. The subject's sample(s) will be stored with similar samples from other subjects at a secure central laboratory for up to 10 years for use in research as noted above.

If consent is withdrawn after a sample has been taken but before the subject's sample is sent to the central laboratory, the investigator will arrange to have it destroyed. If consent is withdrawn after the subject's research sample(s) have been stored, the sponsor and the investigator will ensure that these sample(s) are destroyed unless the sample identification number has been removed and the subject can no longer be linked to any sample(s). However, if the subject's samples have already been used for research, the sponsor is not required to destroy results of this research. In this case only the remaining sample(s) will be destroyed.

4.7. Confirmation of Eligibility

Eligibility is initially determined by the site primary Principal Investigator. The study Medical Monitor will confirm eligibility of all subjects who will be dosed, regardless of the local site at which they are enrolled. To confirm eligibility, sites must provide the Medical Monitor with key medical records which provide supporting documentation of eligibility criteria. These documents should be provided shortly after eligibility is determined by the local site and before the subject travels to the University of Pennsylvania or other selected sites for dosing.

5. STUDY PROCEDURES

Refer to the "Schedule of Events" table in the Appendix (see Section 16.1) for a list and frequency of study procedures and laboratory parameters that will be performed as part of this clinical trial.

5.1. Clinical Laboratory Testing

Samples for laboratory testing will be collected at time points according to the "Schedule of Events" and analyzed by a central laboratory (Central Lab) during the study. Laboratory samples may be drawn at a site local to the subject's residence or by a home nursing service and shipped to the Central Lab. If local labs are drawn, including lipid panel, results should be entered into the Clinical Database. Detailed instructions for blood sample collection and processing will be described in a separate laboratory manual. The investigator must document his or her review of each laboratory report by signing or initialing and dating each report. Laboratory values that fall outside a clinically accepted reference range or values that differ significantly from previous values must be evaluated. When multiple laboratory values are out of range but not clinically significant (NCS), "all labs NCS" or a general comment may be written on the laboratory page. However, all clinically significant (CS) laboratory values must be individually marked with "CS."

5.2. Safety Assessments

Safety will be evaluated by the incidence, severity, and relationship to study drug of adverse events (AEs), and changes from baseline in laboratory test results, physical examination and vital sign measurements at any time after the subject has received study drug.

Laboratory assessments include:

- Biochemical panel: sodium, potassium, chloride, carbon dioxide, glucose, blood urea nitrogen, lactate dehydrogenase, creatinine, creatine phosphokinase, calcium, total protein, albumin, AST, ALT, alkaline phosphatase, total bilirubin, and GGT (GGT will be measured at screening and only as clinically indicated through the study).
- CBC: WBC, hemoglobin, hematocrit, platelet count, red cell distribution width, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration
- HbA1c

- Coagulation: PT, INR, PTT (at screening and baseline, and through the study as deemed necessary)
- Urinalysis: urine color, turbidity, pH, glucose, bilirubin, ketones, RBCs, protein, WBCs
- Vector concentration: AAV8 concentration in plasma and urine, measured as vector genomes by PCR.

Physical Examinations include:

The complete physical examination will include evaluation of the following organ or body systems:

- Skin
- Head, eyes, ears, nose, and throat
- Thyroid
- Respiratory system
- Cardiovascular system
- Abdomen (liver and spleen)
- Lymph nodes
- CNS
- Musculoskeletal

The targeted physical examination will include evaluation of the following organ or body systems:

- Respiratory system
- Cardiovascular system
- Abdomen (liver and spleen)
- Any other system prompted by complaints/symptoms

5.2.1. Adverse event driven safety evaluation

Additional ad hoc laboratory testing may be performed if a subject is experiencing an adverse event that is preliminarily deemed at least possibly related to the study vector. If an inflammatory response is suspected, additional testing may include (but may not be limited to) inflammatory markers, such as high-sensitivity C-reactive protein (hsCRP), ferritin, fibrinogen and cytokines panel.

5.3. Pharmacodynamic/ Efficacy Assessments

Given the difficulty to directly assess the presence of the gene product, the membrane-bound LDLR, efficacy will be primarily assessed by changes in plasma lipid and lipoprotein profile. In the context of this trial, we assume that LDL-C is the main indirect measure of LDLR expression. The following efficacy laboratory tests will be evaluated under fasting conditions as described in the "Schedule of Events":

- LDL-C
- Lipid panel: total cholesterol, VLDL-C, non-HDL-C, HDL-C, TG, Lp(a)
- Apolipoproteins: apoB and apoA-I

Lipid lowering efficacy will be assessed as percent changes from baseline at 12 (Cohort 1 only), 18 (or 4 week post steroid termination or prior to changing lipid lowering therapies), 24, 36, 52, 78 and 104 weeks post vector administration. Baseline LDL-C value will be calculated as the average of up to 2 of the most recent qualified fasting LDL-C levels obtained during stable lipid lowering treatment before administration of AAV8.TBG.hLDLR. The 2 most recent LDL-C values should be no more than approximately 4 weeks apart. The percent change from baseline in LDL-C at 12 weeks (Cohort 1 only) or 18 weeks post vector administration (or 4 weeks after steroid termination or prior to changing lipid lowering therapies) will be considered the primary measure of gene transfer activity.

[redacted]

5.3.1. Efficacy Assessment in Subjects Receiving Apheresis

For subjects receiving apheresis, efficacy will be evaluated on the basis of pre-apheresis lipid levels.

Apheresis treatment causes a sharp drop in LDL-C, followed by a rebound phase and therefore in subjects undergoing apheresis, it is important that lipid parameters are evaluated at a time that is as close as possible to and before the scheduled apheresis treatment. **Once established, this time point for lipid assessment blood draws should always be maintained, relative to the previous apheresis visit, so that lipid assessments are always performed at the same point on the LDL rebound curve**. During the run-in phase a stable frequency regimen (i.e., apheresis every X number of days) will be established and needs to be maintained on as regular a schedule as possible during the efficacy phase. Frequency of apheresis is usually personalized to meet the patients' needs and the centers' capacity, and therefore we have included a window for each visit to allow for flexibility when patients can be seen. If the visit schedule is inconsistent with a subject's apheresis schedule, then the investigator should contact the Contract Research Organization (CRO) and Sponsor. If a subject is unable to come for an apheresis treatment per his/her usual regimen, apheresis should be rescheduled as soon as possible. If apheresis was missed at a time a study visit was scheduled, then blood for lipid parameters should be drawn before apheresis treatment and as close as possible to the apheresis regimen established during

the run-in period or as revised if during the safety phase. However, fasting lipids must be drawn just prior to the apheresis treatment and apheresis must occur ± 1 day from the regimen established during the run-in period.

Subjects will be asked to allow the collection of pre-apheresis LDL levels collected as part of standard of care to be captured in the clinical database.

5.3.2. Efficacy Assessment in Subjects Receiving Prophylaxis Steroid Treatment

Steroid treatment has the potential to affect lipid metabolism and, consequently, to affect efficacy assessments, thus limiting the possibility to assess efficacy during such treatment. In subjects who undergo steroid treatment, the primary measure of gene transfer activity will be the percent change from baseline in LDL-C assessed 4 weeks after steroid treatment termination. This assessment may coincide with week 18 post vector administration in subjects that receive steroid prophylaxis as per Section 6.5, but may vary if steroid treatment needs to be personalized. Secondary measures of efficacy are changes from baseline of lipid and lipoprotein parameters at 18 (or 4 week post steroid termination if steroids have not been discontinued by at least 4 weeks at the Week 18 visit or prior to changing lipid lowering therapies), 24, 36, 52, 78 and 104 weeks post vector administration.

5.4. Assessment of Pharmacokinetics and Immune Response to AAV8

The following tests will be evaluated as described in the "Schedule of Events":

- Immune response monitoring:
  - AAV8 NAb; sample used for testing the AAV8 NAb titer and for assay validation
  - T-cell responses to AAV8 vector and hLDLR. An additional PBMC sample may be collected at the discretion of the Sponsor Medical Monitor to assess immune response based on safety events.
- Vector concentration: AAV8 concentrations in plasma and urine
- Human Leukocyte Antigen Typing (HLA type): HLA-A, HLA-B, HLA-C for Class I and HLA-DRB1/DRB345, DQB1 and DPB1 for Class II

5.5. Future Use Samples

Future use samples, including serum, DNA, and RNA will be collected and stored for follow-up exploration of study findings, efficacy measures and/or adverse events (e.g., measurement of immune response, antibodies against LDLR, measurement of cytokine and chemokine levels or other inflammatory biomarkers) in this or subsequent studies of AAV8.TBG.hLDLR. Future use for these samples may include, but will not be limited to, research and development in the cardiovascular indication, assay validation, or any other area that supports the furthering of science in these areas. Research samples will be coded to maintain patient confidentiality and may be stored for up to 10 years for use in research as noted above.

5.6. Xanthoma Assessment

Physical exams will include identification, examination, and description of any xanthomas. Documentation of xanthoma location and type will be determined by investigator (i.e., cutaneous, palpebral (eye), tuberous, and/or tendinous). Where possible, metric rulers or calipers will be used to document size of xanthomas (largest and smallest extents) during physical exam. All efforts should be made to take photographs using a standard digital camera of xanthomas that are the most extensive and readily identifiable, with placement of a tape ruler (metric with millimeters) next to the lesion(s).

5.7. Specific Study Procedures by Visit

Subjects will be asked to fast (except water) for a minimum of 12 hours prior to all visits unless otherwise noted. A detailed study time-table and list of all procedures to be performed at each visit is included in the "Schedule of Events" table located in the Appendix (see Section 16.1).

5.7.1. Screening Protocol

Potential subjects may participate in a separate companion Screening Protocol to obtain results for genotyping, lipids and NAb titer, as well as medical records. Subjects who are interested in participating in the gene therapy trial and who preliminarily qualify based on the results from the Screening Protocol will be invited to participate in the gene therapy clinical trial. Participation to the Screening protocol is not obligatory. Final eligibility of subjects that participated in the screening protocol, including AAV8 NAb titer as well as all other eligibility criteria, except for the genetic testing, will be reassessed during the study screening visit (Visit 1) procedures.

5.7.2. Visit 1: Consent, Screening and Washout (Weeks -26 to Day -1)

Potential subjects will be contacted and asked to schedule a visit at the Study Site.

The following procedures will be performed during this visit:

- Subject to review and sign the IRB-approved ICF (see Section 4.4 "Informed Consent" for detailed description of the consenting procedures).
- Review medical history including current medications, tobacco, drug, and alcohol consumption. [redacted]
- Collect blood and urine samples for laboratory testing as described in the "Schedule of Events" table (Section 16.1).
- Collect a blood sample to confirm molecular diagnosis (only for subjects that did not participate to the Screening Protocol)
- Concomitant medications will be recorded.

- Have procedures performed as described in the "Schedule of Events" table (Section 16.1), including physical examination, vitals, 12-lead ECG, and xanthoma assessment (can be assessed at Visit 1 or 3).
- Assessment of AEs

These procedures can be performed over several days/weeks. Subjects that are known to carry *LDLR* gene mutations at both alleles and that are potentially eligible based on their participation in the companion Screening Protocol and/or their medical history may, in agreement with the caring physician, start washout from selected lipid lowering medications before other screening procedures are completed (see "Subject Selection and Withdrawal" section for washout requirements). Subjects with genetic diagnosis determined by a reputable laboratory (medical history) could start washout before receiving the genetic results of Visit 1.

The baseline LDL-C value will be determined using qualified LDL-C drawn prior to administration of AAV8.TBG.hLDLR. Qualified LDL-C draws will be those taken after sufficient washout of selected lipid lowering medications has occurred and pre-apheresis in subjects receiving apheresis (i.e., the day of apheresis). Baseline LDL-C value will be calculated as the average of up to 2 of the most recent qualified fasting LDL-C levels obtained during stable lipid lowering treatment before administration of AAV8.TBG.hLDLR. The 2 most recent LDL-C values should be no more than approximately 4 weeks apart.

5.7.3. Visit 1a: Laboratory Retesting

Subjects will undergo Visit 1a up to 2 weeks prior to the dosing visit (Visit 3) if any of the following conditions apply:

- **The interval between the Visit 1 blood draw date for NAbs and Visit 3 is or will be greater than 26 weeks:** Blood for NAbs must be redrawn to determine eligibility. Retesting of NAb titer should be scheduled to allow for sufficient time for NAb titer analysis prior to dosing.
- **The interval between the Visit 1 blood draw date for LDL-C and Visit 3 is or will be greater than approximately 4 weeks:** Visit 1a will be scheduled at the end of the wash-out period. Blood for a complete lipid panel including LDL-C by beta-quantification will be performed at Visit 1a. The baseline LDL-C value can be calculated by averaging the LDL-C levels obtained under fasting conditions at Visit 1a and Visit 3 (prior to AAV8.TBG.hLDLR administration). The blood drawn for lipid panel for subjects undergoing apheresis must be done prior to the apheresis treatment as described in Section 5.3.1.

Blood sampling for this visit may be conducted either at the study site or by a home healthcare nurse.

If the interval between Visit 1 and Visit 3 is less than 26 weeks, repetition of standard clinical laboratory assessment may be conducted at the discretion of the investigator. In this case, the assessment will be recorded as "unscheduled visit".

5.7.4. Visit 2

This visit only refers to a visit that is performed for the optional kinetic study.

5.7.5. Visit 3: Vector Administration (Day -1, Day 1 and Day 2)

This visit will take place at the University of Pennsylvania or at other selected dosing sites. For subjects that need to travel to the dosing site, a communication plan to share relevant medical information will be implemented between the 2 sites before the subject travels. Subjects will be admitted the day of or the day before vector administration and eligibility confirmed before dosing.

Subjects enrolled under this protocol will begin prophylactic corticosteroids and receive the first dose of oral prednisone (40mg daily) on Day -1. Prior to discharge, subjects will be provided with a sufficient supply of prednisone to last for at least 8 weeks. See Section 6.5.

The following will be performed prior to AAV8.TBG.hLDLR administration:

- Subject to review the IRB-approved ICF (see Section 4.4 "Informed Consent" for detailed description of the consenting procedures) and re-confirm willingness to participate in the clinical trial
- Review eligibility prior to vector administration
- Review medical history including current medications and alcohol consumption
- Conduct complete physical exam as described in Section 5.2
- Xanthoma assessment
- 12 lead-ECG
- Measure vital signs as listed in the Schedule of Events
- Collect blood and urine samples for laboratory testing as described in the "Schedule of Events".
- Dipstick urine pregnancy test for women
- Assessment of AEs
- Initiate oral corticosteroids

Subjects will receive AAV8.TBG.hLDLR via a peripheral vein by IV administration. The volume and infusion rate varying according to the dose given and the weight of the subject at time of admission. Vital signs will be monitored frequently during the subject's stay in the research unit.

The following will be performed 24 hours after AAV8.TBG.hLDLR administration:

- Conduct targeted physical exam as described in Section 5.2
- 12 lead-ECG
- Measure vital signs as listed in the Schedule of Events
- Collect blood samples for laboratory testing as described in the "Schedule of Events"

The subject can be discharged from the inpatient unit after 24 hours of observation post-vector infusion or may remain admitted for a longer duration if deemed necessary by the investigator.

5.7.6. Visit 4

Subjects will have a visit 48 hours after AAV8.TBG.hLDLR administration. The following will be performed:

- Review medical history including current medications and alcohol consumption
- Conduct physical exam as described in Section 5.2
- Measure vital signs as listed in the Schedule of Events
- Collect blood and urine samples for laboratory testing as described in the "Schedule of Events" table (Section 16.1)
- Assessment of AEs

5.7.7. Visit 6

Subjects will have a visit on Day 7 (±2 days) after AAV8.TBG.hLDLR administration to assess vital signs, AEs, and medical history & medications including subject's prophylactic corticosteroid compliance. This visit can be conducted at a study site or at the subject's home by a home healthcare nurse visit.

Note: Visit 5 was removed from Protocol version 10 and later.

5.7.8. Visit 7, 9, 13: Safety Visits

A full safety visit will occur on days 14, 28, and 56 (±3 days)). These study visits will be performed at a study site. The following procedures will be performed during these visits:

- Review medical history including current medications and alcohol consumption
- Confirm subject's prophylactic corticosteroid compliance
- Conduct physical exam as described in Section 5.2 (complete at Visit 9, targeted at Visits 7 and 13)
- Measure vital signs as listed in the Schedule of Events
- 12 lead-ECG (Visits 9 and 13)
- Collect blood and urine samples for laboratory testing as described in the "Schedule of Events" table (Section 16.1)
- Dipstick urine pregnancy test for women (Visit 9 only)
- Assessment of AEs

5.7.9. Visit 8, 10, 11, 12, 14, 15, 16, 17: Laboratory assessments

Subjects will have labs drawn for liver function tests, as well as LDL-C, ApoB, and other laboratory assessment as listed in the "Schedule of Events" table on days 21, 35, 42, 49, 63, 70,

77 and 84 (±3 days). Blood sampling for these visits may be conducted by a home healthcare nurse or local laboratory to be sent sample to central laboratory. Other safety laboratories will only be drawn if needed and at the discretion of the investigator and will be entered into the clinical database.

Subjects will be contacted by the site on Day 63, 70, 77, and 84 (Visit 14, 15, 16, and 17) to review and confirm the subject's prophylactic corticosteroid regimen and dose.

5.7.10. Visit 17.1: 14 Weeks Post Vector Administration

Subjects will be asked to return to the study site for an outpatient visit. The following procedures will be performed during this visit:

- Review medical history including current medications and alcohol consumption
- Confirm subject's prophylactic corticosteroid compliance
- Conduct complete physical exam as described in Section 5.2
- Measure vital signs as listed in the Schedule of Events
- 12 lead-ECG
- Collect blood and urine samples for laboratory testing as described in the "Schedule of Events" table (Section 16.1)
- Assessment of AEs

5.7.11. Visits 17.2, 17.4, 17.5: Bi-Weekly Lab Assessments and Telephone Contact

After Visit 17.1, subjects will have blood drawn for liver function testing and lipid panel, including ApoB every two weeks to Week 24 (Visit 18). These visits can be assessed at the local study site or by a home nurse visit with samples sent to the Central Laboratory. Investigators may draw additional blood for LFTs or LDL-C at their discretion. Subject will be asked to allow sharing any laboratory samples collected outside of the protocol specified schedule of events during the study to be recorded in the Clinical Database.

5.7.12. Visit 17.3

At Week 18 (or 4 week post steroid termination if steroids have not been discontinued by at least 4 weeks at the Week 18 visit or prior to changing lipid lowering therapies), subjects will be asked to return to the study site for an outpatient visit. The following procedures will be performed during the visit:

- Review medical history including current medications and alcohol consumption
- Conduct complete physical exam as described in Section 5.2
- Xanthoma assessment
- Measure vital signs as listed in the Schedule of Events
- 12 lead-ECG

- Collect blood and urine samples for laboratory testing as described in the "Schedule of Events" table (Section 16.1)
- Dipstick urine pregnancy test for women
- Assessment of AEs

Subjects will be permitted to receive lipid lowering therapies as per standard of care after the Week 18 visit (or 4 week post steroid termination). [redacted]

5.7.13. Visits 18,19, 20

Subjects will return to the study site for a 24, 36, and 52 week visit post vector administration. The following procedures will be performed during these visits:

- Review medical history including current medications and alcohol consumption
- Conduct complete physical exam (Week 24 and 52 only) or targeted exam (Week 36 only) as described in Section 5.2
- Measure vital signs as listed in the Schedule of Events
- 12 lead-ECG
- Collect blood and urine samples for laboratory testing as described in the Schedule of Events
- Dipstick urine pregnancy test for women
- Assessment of AEs
- Xanthoma assessment (only at the 52 week visit)

Week 52 is the last visit of the active study period. Subjects will not be prohibited from receiving other investigational therapies after Week 52, should they wish to do so.

5.7.14. Visit 18.1

Subjects enrolled under Protocol version 10 and following will have a week 30 visit that will be a laboratory visit only. Subjects will have a blood drawn at for liver function testing and lipid panel, including ApoB on Week 30 (±2 weeks) (Visit 18.1). This visit can occur at the local study site, by a home nurse visit or other testing laboratory, with samples sent to the Central Laboratory.

5.7.15. Visit 21: 78 weeks (± 2 weeks) Post Vector Administration

This visit can be assessed at the local study site, by a home nurse visit or with a local laboratory (results sent to central laboratory). Subjects may be contacted by telephone by the study site.

- Review medical history including current medications and alcohol consumption
- Assessment of AEs
- Fasting standard lipid panel (may be performed locally)

5.7.16. Visit 22: 104 weeks (± 2 weeks) Post Vector Administration / Early Termination Visit

Subjects will return to the study site for a 104 week visit post vector administration. This is the last visit of the follow up study period. The following procedures will be performed:

- Review medical history including current medications and alcohol consumption
- Conduct complete physical exam
- 12 lead-ECG
- Measure vital signs as listed in the Schedule of Events
- Collect blood and urine samples for laboratory testing as described in the Schedule of Events
- Assessment of AEs

Subjects will be asked to participate in a separate long-term follow-up protocol after the Week 104 visit (Visit 22). The primary objective of this study will be safety and will also include assessment of LDL-C.

6. STUDY DRUG

6.1. Description

The investigational agent is an AAV8 vector expressing the transgene for the human low density lipoprotein receptor (hLDLR) under control of a liver-specific promoter (thyroxine-binding globulin, TBG) and is referred to in this document as AAV8.TBG.hLDLR.

6.2. Treatment Regimen

Subjects will receive a single dose of AAV8.TBG.hLDLR administered via a peripheral vein by infusion.

6.2.1. Method for Assigning Subjects to Treatment Groups

There is no randomization in this Phase I/IIa, open-label, single ascending dose study. Subjects will be assigned to dose cohorts as defined in the overall study design.

6.3. Preparation of Vector for Injection

The vector will be prepared for administration at the study site. Full details regarding product preparation and administration are found in the study Investigational Product Manual. Upon thaw of the frozen product, the product should be a clear colorless liquid with no evidence of solids. The vials should be swabbed extensively with 70% sterilizing agent before withdrawing contents in a laminar flow biosafety cabinet. After drug preparation, the syringe containing the product should be stored at room temperature. Vector administration within 0-4 hours after preparation is recommended. Should there be any delay period before administration, the syringe containing the product could be stored at room temperature for up to 8 hours.

6.4. Prior and Concomitant Therapy

Subjects will be withdrawn from selected lipid lowering medications as specified in the "Subjects Selection and Withdrawal" section for at least one month prior to vector administration. Subjects will be permitted to resume lipid lowering therapies 18 weeks (or 4 weeks post steroid termination) after vector administration. Use of other medications not excluded in the entry criteria will be permitted at the discretion of the investigator. Any medication, including over the counter (OTC) medications, used during the course of the study will be documented.

6.5. Corticosteroid Prophylaxis

Subjects dosed under Protocol version 10 and later will receive daily prophylactic corticosteroids beginning on Study Day -1 prior to dosing. The starting dose is prednisone 40 mg once daily with a taper beginning at Week 9 and continuing through the end of Week 13. The first dose should be given on Day -1 at least 8 hours before scheduled dosing with study vector.

This intervention is based on the premise that elevated transaminases post-AAV based gene therapy are the result of an immune response that results in a cytotoxic (e.g., T-cell mediated) destruction of hepatocytes that have been transfected with the AAV vector. This may be of added importance at higher vector doses given the potential for dose-dependent toxicity. Therefore, prophylactic immunosuppression theoretically could halt the loss of hepatocytes expressing the transgenic LDL receptor. However published data to date have not conclusively demonstrated the effectiveness of this approach (Nathwani 2014, Rangarajan 2017, George 2017). Regardless, corticosteroids are relatively safe and justified as an attempt to preserve transgene expression. Use of corticosteroids such as prednisone may cause acne, dizziness, facial flushing, general body discomfort, headache, increased appetite, weight gain, increased sweating, nausea, nervousness, and sleeplessness (Prednisone Prescribing Information 2016). Additional information will be outlined in the Investigator's Brochure.

The daily prednisone dose of each study week is presented in Table 4.

Table 4: Prednisone Dose by Study Week

Week(s)	Day -1 to Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Week 14
Daily Prednisone dose (mg/day)*	40	30	20	15	10	5	0

* If an alternative to prednisone is considered, a corticosteroid dose equivalent must be provided and discussed with the Medical Monitor.

Any adverse event that is thought to be at least possibly related to the steroid prophylaxis will be treated as per standard of care (see Section 10.1.3).

Based on observed transaminase levels during routine monitoring, the dose of corticosteroids may be modified or the taper extended after a discussion between the PI, the study hepatologist and the Sponsor Medical Monitor. Corticosteroids may be reintroduced if transaminase elevations are observed after the taper is completed, as described in Section 9.1.15. During the corticosteroid taper, the use of alternative corticosteroids or doses, such as oral hydrocortisone, is up to the discretion of the investigator if adrenal insufficiency is suspected. After completion of corticosteroid taper, biweekly monitoring of LFTs are performed to Week 24. The primary efficacy measure (LDL-C) will be assessed 4 weeks after tapering ends.

6.6. Packaging

The vector is stored in vials in a cryobox. The vials are labeled with the vector name, lot number, date of manufacture and storage condition. In addition, the label contains statements that by law, limit use to investigational use. When required, investigational drug product will be transferred to the dosing site. The investigational drug product vials will be placed in a cryobox and bulk shipped on dry ice in a qualified shipping container to maintain temperatures at ≤-60°C. The investigational drug product should be handled at the Institution as a biohazardous material under biosafety level 1 containment.

6.7. Receipt of Drug Supplies

The vector will be transferred from storage to the investigational pharmacy. Upon receipt of the drug product, an inventory will be performed, and accountability records will be filled out according to pharmacy procedures. The responsible person that received the shipment will count the number of vials and verify that the shipment contains all the items noted in the shipment inventory.

6.8. Storage

The vector is stored at ≤-60°C until required vector preparation for administration as detailed in the IP manual. After drug preparation, the syringe containing the product should be stored at room temperature for up to 8 hours.

6.9. Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug provided, drug used, and drug remaining. This reconciliation will be logged on a drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return of unused study drug. Drug destroyed on site will be performed according to institutional policy and with prior written permission of the sponsor. On-site drug destruction will be documented and filed in the study files.

6.10. Reporting Product Complaints

Any defects with the investigational product must be reported *within 24 hours* to the Sponsor's Product Complaint Department by the site with further notification to the site monitor. All defects will be communicated to the Product Compliant Department and investigated further.

The Product Compliant Department contact information for reporting product complaints:

productcomplaints@regenxbio.com.

7. STATISTICAL PLAN

This section describes the planned statistical analyses in general terms. A complete description of the methodology will be specified in a statistical analysis plan (SAP). Any changes in the statistical methods described in this protocol that occur prior to database lock will be documented in the SAP and will not require a protocol amendment.

7.1. General

All data will be presented in subject data listings. Categorical variables will be summarized using frequencies and percentages, and continuous variables will be summarized using descriptive statistics (number of non-missing observations, mean, standard deviation, median, minimum, and maximum). Graphical displays will be presented as appropriate.

After all subjects have completed the primary study period (i.e., through Week 24), analyses will be performed for all safety, PD, and efficacy endpoints for primary period.

After all subjects have completed the end of study (EOS) period (through Week 104), analyses will be performed for all safety, PD, and efficacy endpoints.

Safety, PD, and efficacy endpoints will be reported by dose cohort and may also be reported for all dose cohorts combined.

7.2. Sample Size Determination

The sample size chosen for this study is not based on statistical considerations, as HoFH is a very rare disease. Approximately 12 subjects are planned to be treated.

7.3. Statistical Methods

7.3.1. Disposition

The number of subjects enrolled, the number of subjects treated, the number of subjects who complete the study, and the number of subjects who withdraw from the study, along with the reasons for their withdrawal, will be summarized by dose cohort and by the study overall. In addition, the total number of subjects screened and the number of screen failures, along with the reason for screen failure, will be summarized.

7.3.2. Demographics and Baseline Characteristics

Demographic and baseline characteristics will be summarized descriptively by dose cohort, as well as for the study overall.

Past and current medical history will be summarized by treatment group using the system organ class (SOC) as coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary.

Deviations from inclusion/exclusion criteria as well as subject's status will only be listed.

7.3.3. Efficacy Analyses

Observed values and changes from baseline over time (as applicable) will be summarized descriptively by dose cohort and by the study overall for the PD measures and efficacy endpoints (defined in the SAP).

7.3.4. Safety Analysis

7.3.4.1. Adverse Events

AEs occurring prior to a treatment dose will be listed and presented separately from those that occur during or after receiving a treatment dose. The incidences of treatment-emergent adverse events (TEAEs), treatment-related TEAEs, and TEAEs by severity (intensity) will be provided. All AEs will be coded using MedDRA and will be summarized by SOC and Preferred Term.

7.3.4.2. Clinical Laboratory Tests

Observed values and changes from baseline (as applicable) through Week 104 will be summarized descriptively by dose cohort and by the study overall for each biochemical profile, CBC, coagulation, and urinalysis parameter. In addition, the incidence of subjects who meet pre-defined criteria for clinically significant abnormal values (or abnormal change) may also be summarized by dose cohort and by the study overall.

7.3.4.3. Vital Signs

Observed values and changes from baseline (as applicable) through Week 104 will be summarized descriptively by dose cohort and by the study overall. In addition, the incidence of subjects who meet pre-defined criteria for clinically significant abnormal values (or abnormal change) may also be summarized by dose cohort and by the study overall.

7.4. Subject Population(s) for Analysis

All subjects who receive a treatment dose will be included in all analyses.

7.5. Interim Analysis

No interim analysis is planned.

[redacted]

[redacted]

[redacted]

9. SAFETY AND ADVERSE EVENTS

9.1. Definitions

9.1.1. Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness, or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

9.1.2. Adverse (Drug) Reaction

All noxious and unintended responses to a medicinal product related to any dose should be considered an ***adverse drug reaction*** (ADR). "Responses" to a medicinal product means that a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

9.1.3. Unexpected Adverse Drug Reaction

An ***Unexpected Adverse Drug Reaction*** (UADR) is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information. For AAV8.TBG.hLDLR the reference safety information is included in the Investigator's Brochure.

9.1.4. Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** (SAE) is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a

seizure that did not result in inpatient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

Adverse events that do not meet any of the criteria for serious adverse events should be regarded as *non-serious adverse events*.

9.1.5. Adverse Event Reporting Period

All AEs will be recorded from the time the subject signs the ICF to the end of the study (Week 104). Long-term follow-up will be conducted via a separate protocol (starting after 104 weeks post vector administration) for up to 5 years' cumulative duration in this study plus the follow-up study.

9.1.6. Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

9.1.7. General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. During the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event, or any preexisting physical exam findings that worsen, must also be recorded and documented as adverse events.

9.1.8. Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

9.1.9. Pregnancy

Sexually active subjects (both females of childbearing potential and males) must be willing to use a highly effective method of contraception from screening visit until 6 months (26 weeks) after vector administration.

Male subjects engaged in sexual relationship with a female of childbearing potential will be required to use condoms from screening visit until 6 months after vector administration, and must also encourage their partner to use a medically accepted method of contraception from screening visit until 6 months (26 weeks) after vector administration.

For the purpose of this study, highly effective methods of contraception include the following:

1. Combined hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
2. Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
3. Intrauterine device (IUD)
4. Intrauterine hormone-releasing system (IUS)
5. Bilateral tubal occlusion
6. Vasectomized partner
7. Sexual abstinence

Females of childbearing potential must have a negative pregnancy test at screening and baseline visits. Pregnancy tests will be repeated at regular intervals throughout the study. Additionally, females of childbearing potential must be willing to have additional pregnancy tests during the study when steroid treatment is initiated (prior to initiation) and if a menstrual cycle is delayed by more than 1 week.

If the subject or partner of a subject participating in the study becomes pregnant within 6 months from vector administration, the investigator should report the pregnancy to Medpace Clinical Safety within 24 hours of being notified. Medpace Clinical Safety will then forward the Exposure In Utero form to the investigator for completion.

The subject or partner should be followed by the investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the investigator should notify Medpace Clinical Safety. At the completion of the pregnancy, the investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting an SAE.

9.1.10. Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if the following conditions are met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality.
- The abnormality suggests a disease and/or organ toxicity.
- The abnormality is of a degree that requires active management; e.g., change of dose, discontinuation of drug, more frequent follow-up assessments, further diagnostic investigation, etc.

9.1.11. Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.1.12. Severity of Adverse Events

Wherever possible, the severity of all AEs will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE, v4.0). The majority of AEs can be graded using the CTCAE criteria; however, if an AE cannot be graded using the CTCAE criteria, it should be graded according to the following definitions:

- **Mild (Grade 1):** Awareness of signs or symptoms, but easily tolerated and are of minor irritant type causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.
- **Moderate (Grade 2):** Events introduce a low level of inconvenience or concern to the participant and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
- **Severe (Grade 3):** Events interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.
- **Life-threatening (Grade 4):** Events that place the subject at immediate risk of death or are disabling requiring urgent intervention.
- **Death (Grade 5):** Events that result in death.

9.1.13. Relationship to Study Drug

The investigator will assess the potential relationship of the AE to study drug using the following definitions:

- **Not Related:** This category applies to an AE that is clearly not related to the investigational product/procedure, beyond a reasonable doubt. That is, another cause of the event is most plausible; and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the exposure to study drug and/or causal relationship is considered biologically implausible.
- **Possibly Related:** This category applies to an AE that follows a reasonable temporal sequence from administration of the study drug and that follows a known or expected response pattern to the suspected study drug, but that could readily have been produced by a number of other factors.
- **Probably Related:** This category applies to an AE that follows a reasonable temporal sequence from administration of the study drug and that follows a known or expected response pattern to the suspected study drug and that could not be reasonably explained by the subject's concurrent disease or other drugs or chemicals.
- **Definitely Related:** This category applies to an AE where there is clear evidence that the event is related to the use of study drug.

An event that has been rated "possibly related," "probably related" or "definitely related" will be classified as "related" for regulatory reporting requirements. An event that has been rated "not related" will be classified as "unrelated" for regulatory reporting requirements.

9.1.14. Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

9.1.15. Adverse Events of Special Interest

Liver function tests abnormalities identified either by the Central Lab or by local labs as part of regular medical care have specific reporting requirements.

The following adverse events of special interest will require expedited reporting to the sponsor within 1 working day, regardless of whether the adverse event meets the definition of a SAE:

- Common Terminology Criteria for Adverse Events (CTCAE) v4.0 Grade 2 or higher lab result for bilirubin (> 1.5 × ULN) or liver enzymes (AST > 3 × ULN, ALT > 3 × ULN, AlkPhos > 2.5 × ULN).

- Hepatotoxicity (i.e., meet criteria for "Hy's law")
  - ALT or AST ≥ 3 × ULN and total bilirubin ≥ 2 × ULN and no other reason can be found to explain the changes observed. This event would meet the study Stopping Rules (Section 9.3)

Use of corticosteroids for presumed T-cell mediated immune transaminitis

All subjects dosed under Protocol version 10 and later will receive a 13 week course of daily prednisone beginning on the day prior to dosing (Day -1) and ending with a taper (see Section 6.5). An increase in ALT or AST (e.g., a doubling from baseline levels or ≥ 2 × ULN) may trigger a discussion between the PI and the Sponsor's Medical Monitor to decide on whether to administer or modify the dose of corticosteroids to prevent a potential loss of transgene expression. More frequent monitoring of ALT or AST may be considered initially (e.g., every 48-72 hours) to determine the subject's response to corticosteroids. The initiation and duration of the corticosteroid taper should be dictated by the clinical status of the subject, time course of the transaminase elevations, and per the site's accepted standards of care. Transaminases should be monitored more frequently in these subjects until levels consistently decline and/or approach normalization.

9.2. Reporting of Serious Adverse Events – Procedures for Investigators

All SAEs occurring from the time of consent must be reported to Medpace Clinical Safety **within 24 hours** of the knowledge of the occurrence. SAEs should be collected through 104 weeks post vector administration.

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, Medpace Safety personnel will be notified electronically and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to Medpace Safety at medpace-safetynotification@medpace.com or call the Medpace SAE hotline (phone number listed below), and fax the completed paper SAE form to Medpace (fax number listed below) within 24 hours of awareness. When the EDC system becomes available, the SAE information must be entered within 24 hours of the system becoming available.

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Follow-Up Reports

The investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment) or the subject dies.

Within 24 hours of receipt of follow-up information, the investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (e.g., subject discharge summary or autopsy reports) to Medpace Clinical Safety via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

9.2.1. Investigator Reporting

Each investigator must report to their local regulatory committees as required by their institution.

9.2.2. Regulatory Sponsor Reporting

The sponsor of the IND/CTA is required to report certain study events in an expedited fashion to the FDA and other regulatory agencies. These written notifications of adverse events are referred to as safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

The sponsor will report all relevant information about suspected unexpected serious adverse reactions that are fatal or life-threatening as soon as possible to the regulatory authorities, and in any case no later than seven days after knowledge by the sponsor of such a case, and that relevant follow-up information will subsequently be communicated within an additional eight days.

All other suspected unexpected serious adverse reactions will be reported to the regulatory authorities as soon as possible but within a maximum of 15 days of first knowledge by the sponsor.

9.3. Safety Review and Stopping Rules

A summary of safety review triggers and expected actions is provided in Table 5.

Table 5: Safety Review Trigger Events and Actions

Safety Review Trigger Event	Safety Review Action
A Stopping Rule is met	An external DSMB will review all available safety data and provide a recommendation on whether to enroll additional subjects.
Any Grade 4 or 5 AE regardless of relationship to treatment	The chairs of the ISC and the external DSMB will review and decide whether to allow enrollment to continue or convene a full DSMB review of all available safety data which will then provide a recommendation on whether to enroll additional subjects.
Any Grade 3 AE considered treatment-related (by the Investigator)	
Any Grade 3 AE considered unrelated to treatment (by the Investigator)	An ISC will review all available safety data. If safety concerns arise while a cohort is being enrolled, the committee may ask the DSMB to review and make a recommendation on whether to keep enrolling subjects in that cohort.
Any report by the Investigator of technical issues with the product administration that may warrant modifications to the procedure	

If any of the following events occur, a Stopping Rule will be considered to have been met and dosing of any new subjects will be suspended until a complete review of all safety data has been performed by the Sponsor and the DSMB:

- A Grade 4 or 5 AE that the Investigator considers to be related to the investigational product
- ALT or AST $\geq 3 \times$ ULN and total bilirubin $\geq 2 \times$ ULN and no other reason can be found to explain the changes observed

9.3.1. Medical Monitoring

It is the responsibility of the Investigators to oversee the safety of the subjects at the site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. The Medical Monitor, a key member of the Sponsor's team, will be responsible for review and evaluation of information (non-clinical and clinical) and/or documentation relevant to the safety of the investigational product and the eligibility and safety of the study subjects. The Medical Monitor's responsibilities will include review of deviations and exceptions as well as overseeing monitoring activities.

9.3.2. Independent Data and Safety Monitoring Board

An independent DSMB will assure the safety of participants in this trial as well as the validity and integrity of the data generated. A primary role of the independent DSMB is to assess safety at periodic intervals and to provide recommendations on safety and dose escalation. The membership of the DSMB as well as the responsibilities and procedures used to carry out these responsibilities are described separately in the DSMB charter.

9.3.3. Internal Safety Committee (ISC)

The Internal Safety Committee will be comprised of REGENXBIO employees and designees and be charged with Sponsor oversight for the safe conduct of the study.

10. RISK / BENEFIT ASSESSMENT

10.1. Risks

The clinical safety and efficacy of AAV serotypes carrying a therapeutic transgene is supported by an approved gene therapy, GLYBERA® (alipogene tiparvovec; Goldstein et al. 2001) for lipoprotein lipase deficiency, as well as by clinical trials for inherited forms of blindness (MacLaren et al, 2014; Maguire et al. 2008), hemophilia B (Nathwani et al 2014) and many other diseases (Luo et al. 2015). In addition, LUXTURNA™ (voretigene neparvovec), an AAV2 vector gene therapy for the treatment of patients with vision loss due to confirmed biallelic RPE65-mediated inherited retinal disease, marks the first approved AAV gene therapy in the US (Luxturna Prescribing Information, 2017) and Europe. AAV8 has been studied in various human diseases as described previously and has a growing track record of safety supporting ongoing clinical trials. However, clinical data addressing long-term risks of AAV-mediated gene transfer are limited, and the long-term risks remain unknown.

The Phase I/IIa study is a first-in-human study to evaluate the safety and collect preliminary data on efficacy of an AAV8-based vector for gene transfer in patients with HoFH. Because this is a new experimental treatment, its risks are largely unknown. Evaluation of potential risks comes from preclinical studies and clinical studies that assessed the safety of a similar vector for gene transfer in a different disease.

10.1.1. Risks Associated with Gene Transfer

Potential risks associated with the administration of AAV8.TBG.hLDLR (the gene and gene transfer vector) include:

Humoral Immune response to the vector (AAV8):

Existing data suggest that the administration of AAV results in a serotype-specific production of NAbs and that once these antibodies are present in circulations, re-administration of the same vector is ineffective. Thus, participation in this trial may preclude future administration of an AAV8-based gene transfer vector, which could be a problem if a sub-therapeutic dose is administered. For this reason the lower dose used in the proposed study has been selected to be safe and to have realistic potential to result in a clinically relevant LDL lowering. Subjects that at screening have a NAb titer against AAV8 greater than 1:10 will be excluded from the study.

Humoral Immune response to the transgene (LDLR):

There is the theoretical possibility of the production of antibodies against the transgene product LDLR. This has not been observed in preclinical studies conducted up to now. Samples will be collected through the study for the assessment of antibodies against LDLR.

Vector-induced hepatitis and hepatotoxicity:

Based on observations in the current study, as well as preclinical and clinical studies with a similar vector, hepatotoxicity is the most likely treatment-related toxicity. Immune mediated transaminitis in some patients is an anticipated consequence of AAV vector administration. While not seen in preclinical studies, elevation in other LFTs, such as alkaline phosphatase and bilirubin, are also possible. Preclinical data in mice and NHPs suggest a low likelihood of these events; however a transient delayed increase in transaminases was observed in previous gene therapy trials using similar vector (Nathwani et al. 2014, Baxalta 2015). The possible mechanisms of this event are discussed in the Introduction. Because of this anticipated possible toxicity, LFTs will be carefully monitored at regular intervals. The Medical Monitor will review all LFTs and will consult as needed with the study site, the Sponsor's Internal Safety Committee or other expert consultants (e.g., hepatologist) to discuss any concerns. As suggested by the experience of previous and ongoing trials with AAV vectors, increases of transaminases may be a sign of T-cell mediated immune transaminitis that is some cases can be associated with loss of transgene activity. To limit the immune response, a course of corticosteroids is initiated in those clinical trials.

All subjects dosed under Protocol version 10 and later will receive a 13 week course of daily prednisone beginning one day prior to dosing and ending with a taper through Week 13 (see Section 6.5). An increase in ALT or AST (e.g., a doubling from baseline levels or ≥ 2 × ULN) may trigger a discussion between the PI and the Sponsor's Medical Monitor to decide on whether to administer or modify the dose of corticosteroids to prevent a potential loss of transgene expression.

Risk of integration into the host chromosome

Recombinant AAV vector genomes display inefficient integration into the host chromosome and predominantly persist in episomal form (McCarty et al. 2004). However, there is uncertainty regarding the long-term risk of integration. The most extensive experience is with AAV2 expressing different genes than used in this study. There is one report of an increased incidence of hepatocellular carcinomas in mucopolysaccharidosis type VII (MPS VII) mice (a C57BL/6 strain) treated neonatally with IV recombinant AAV containing a β-glucuronidase gene and a chicken β-actin promoter (Donsante et al. 2001). However, the number of affected animals was very small and there was no difference in numbers of GCs of vector per diploid genome in tumor tissue versus non-tumor tissue, suggesting that the tumors were not related to insertional mutagenesis. In a larger and more controlled study that examined the risk of insertional mutagenesis in several strains of mice and including specific experimental measures to increase the likelihood of tumor promotion, an increase in tumors over mock-infected controls was only observed in C3H/HeJ mice, which are much more tumor-prone than C57BL/6 mice (Rosas et al. 2012). Similarly, an imbalance of liver tumors was observed in male mice of the B6C3F1 hybrid background that had been treated with the LacZ gene. As before, there was no increase in vector DNA in tumor tissue relative to non-tumor tissues (Bell et al. 2005). Several other studies in adult mice, monkeys, and humans that received AAV vectors have not reported cancer findings at a higher rate than controls (Bell et al. 2005, Gil-Farina et al. 2016, Li et al. 2011, Nathwani et al. 2011). In view of the relatively high background incidence of hepatocellular neoplasms in mice, albeit varying by strain, and the lack of evidence of neoplasms in other tissues, the weight of evidence suggests that

recombinant AAV vectors are not carcinogenic (Bell et al. 2006). However, the theoretical concern regarding insertional mutagenesis cannot be completely eliminated, although most AAVs are episomal and the frequency of integration by recombinant AAV vectors is significantly lower than that observed for retroviral vectors. In summary, the risk of tumorigenesis in humans due to insertional mutagenesis is unknown but considered low at this time.

As the true risk of this serious complication is unknown at this time, long-term safety monitoring is warranted. Subjects will be followed for up to 104 weeks after vector administration in the study and then invited to participate in a longer-term safety follow-up study for up to 5 years' cumulative duration since the vector administration in the parent (previous) study.

Risk of transfer to the offspring

A theoretical risk for AAV8.TBG.hLDLR therapy is the transfer of the AAV vector through semen to the offspring of the treated subject. In human studies with IV administered AAV2, the vector has been found to be detectable in semen for several weeks, but data were consistent with the notion that the vector is present in the seminal fluid without transducing motile sperm (Manno et al. 2006). This result was corroborated by studies evaluating the risk of germline transmission of AAV2 and AAV8 in an animal model (Favaro 2009). In this study, sexually active subjects (both females of childbearing potential and males) must be willing to use a highly effective method of contraception from screening visit until 6 months after vector administration.

10.1.2. Risks Associated with Withdrawal of Lipid-lowering Treatment

Subjects will be allowed to continue conventional lipid-lowering treatment with statin, ezetimibe, bile acid sequestrants, PCSK9 inhibitors, and LDL or plasma apheresis, but will be asked to withdraw from other lipid lowering drugs at least one month to 24 weeks depending on the medication and for 18 weeks (or 4 weeks post steroid termination) following the administration of the vector. This will facilitate the assessment of efficacy (a secondary objective) by monitoring of LDL-C levels. Withdrawal from selected concomitant medications is a common requirement in clinical trials with investigational lipid-lowering treatments, including those in HoFH (Raal et al. 2000, Raal et al. 2010, Cuchel et al. 2007, Raal et al. 2015). Historical clinical data suggest that withdrawal from lipid-lowering drugs for a length of time similar to the one proposed in the clinical protocol, does not pose any acute risk to subjects. Although it is probable that during this period LDL-C levels will increase, there is no known acute risk associated with hypercholesterolemia and no acute events were reported in the cited studies during the wash out period. The protocol does not contain any upper limit for LDL-C to trigger a lipid-lowering intervention; however, lipid levels will be monitored frequently until additional medication can be re-initiated.

10.1.3. Risks of Corticosteroids

In order to prevent a potential loss of transgene expression, subjects dosed under Protocol version 10 and later will receive a 13 week course of daily prednisone beginning on study Day -1 and ending with a taper (see Section 6.5). Corticosteroids may be administered to any subject in the trial with an increase in ALT or AST. Short-term use of corticosteroids such as prednisone may cause acne, dizziness, facial flushing, general body discomfort, headache, stomach ache, nausea,

increased appetite, weight gain, increased sweating, nausea, nervousness, increased blood sugar, and sleeplessness (Prednisone Prescribing Information 2016).

Any adverse event that is thought to be at least possibly related to the steroid prophylaxis will be treated as per standard of care.

Patients that for their pre-existing conditions may be at higher risk of onset of steroid associated adverse events will receive ad-hoc monitoring. Eligible patients with a diagnosis of heart failure will be asked to monitor their weight daily while on prophylaxis steroid treatment, keep a daily chart, and instructed to promptly notify the study team if a weight gain of > 2 pounds is noted within 1 -2 days. It is responsibility of the study team to ensure appropriate communication with the patients' cardiologist to provide additional treatment as per standard of care. Eligible patients with a diagnosis of diabetes will have their fasting glucose levels monitored at time of the weekly lab draw during the length of steroid treatment. Urgent consult for initiation or intensification of insulin therapy will be required for fasting glucose levels ≥200 mg/dl. It is responsibility of the study team to ensure appropriate communication with the physician that follow the subjects' diabetes to provide additional treatment as per standard of care if the steroid treatment is associated with worsening of diabetes.

10.1.4. Phlebotomy Risks

Risks and discomfort associated with venipuncture and presence of an IV catheter are minor and include swelling, bruising/bleeding at the site, fainting, and, more rarely, the risk of infection at the needle puncture site and the possibility of a clogged catheter that requires replacement. These risks are associated with blood draw during laboratory testing, or with infusion of the AAV8.TBG.hLDLR or the deuterated leucine. These risks are modest and minimized by use of good clinical techniques. [redacted]

10.2. Potential Study Benefits

Patients with HoFH are minimally responsive to available conventional drug therapy and have limited treatment options. Thus, if efficacy and safety are confirmed, the primary benefit of the proposed study is the potential of AAV8.TBG.hLDLR gene transfer to significantly lower LDL-C levels and improve response to lipid lowering drugs, such as PCSK9 inhibitors. It may also allow a reduction in the frequency of or requirement for apheresis. Furthermore, the lower LDL-C levels could impact favorably atherosclerosis progression in HoFH patients.

10.3. Benefit:Risk Assessment

AAV8.TBG.hLDLR is an investigational biologic that is being developed for the treatment of HoFH, a life-threatening disease characterized by absence of a functional LDLR, severe hypercholesterolemia and development of severe atherosclerotic cardiovascular disease (ASCVD) in childhood or adolescence. Current therapy is inadequate to prevent the development of serious cardiovascular disease. LDLR expression in the liver plays a crucial role in the control of circulating LDL cholesterol levels. Liver-directed stable gene delivery and expression of the LDLR would allow an improved response to existing and novel therapies that would result in a substantial improvement of the disease management, also in absence of a complete normalization

of the hypercholesterolemia. Thus benefit:risk assessment based on all available information appear to be acceptable with the potential for a clinically significant LDL lowering accompanied by relatively low probability of serious toxicity. Furthermore, HoFH could be a model for the development of liver-directed gene therapy for the treatment of other conditions. Refer also to Section 1.5 for review of the clinical data available from other AAV based gene therapy programs.

10.4. Alternatives to Participation

The alternative to participation is not to participate.

11. DATA HANDLING AND RECORD KEEPING

11.1. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

11.2. Source Document and Case Report Forms

It is the Investigator's responsibility to prepare and maintain adequate and accurate case histories that record all observations and other data related to the study for each subject. Case histories include the CRFs, and supporting data (i.e. Source Documents) such as signed and dated ICFs, medical records, laboratory reports, etc. Case histories for each subject will document that informed consent was obtained prior to participation in the study.

A validated Electronic Data Capture (EDC) system will be used for entry of the data into electronic CRFs designed. All data entered in to the CRF must be verifiable; therefore, CRFs will be routinely monitored for accuracy, completeness, and clarity and will be cross-checked for consistency with source documents.

11.3. Data Quality Assurance

During routine monitoring visits, the Monitor will perform source data verification (SDV) against CRFs to ensure data accuracy, completeness, and clarity including laboratory reports and other

subject records with the stipulation that subject confidentiality will be strictly maintained in accordance with local and federal regulations, including HIPAA requirements. Instances of missing or uninterpretable data will be resolved in coordination with the Investigator.

In addition to routine monitoring of the data by the Monitor, the electronic data capture (EDC) system will include internal quality controls by employing Skip Logics and Edit Checks. Skip Logics are programs in the database that restricts entry to particular data fields based on previous entered data thus restricting the entry of irrelevant data. Edit Checks are a set of programmed instructions in the database to identify and flag discrepancies in the entered data.

11.4. Records Retention

All study-related documents must be retained for at least 2 years after the last approval of a marketing application or until at least 2 years have elapsed since the formal discontinuation of the clinical development of the IP. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. All study-related documents must be stored in a secure limited access facility.

12. STUDY MONITORING, AUDITING, AND INSPECTING

12.1. Study Monitoring Plan

It is the responsibility of the Investigator to ensure that the study is conducted in accordance with the protocol, Declaration of Helsinki, ICH E6 GCP, and applicable regulatory requirements, and that valid data are entered into the eCRFs.

To achieve this objective, the monitor's duties are to aid the Investigator and, at the same time, the Sponsor in the maintenance of complete, legible, well organized, and easily retrievable data. Before the enrollment of any subject in this study, the Sponsor or their designee will review with Investigator and site personnel the following documents: protocol, IB, eCRFs and procedures for their completion, informed consent process, and the procedure for reporting SAEs.

The Investigator will permit the Sponsor or their designee to monitor the study as frequently as deemed necessary to determine that data recording and protocol adherence are satisfactory. During the monitoring visits, information recorded on the eCRFs will be verified against source documents and requests for clarification or correction may be made. After the eCRFs data is entered by the site, the CRA will review the data for safety information, completeness, accuracy, and logical consistency. Computer programs that identify data inconsistencies may be used to help monitor the clinical study. If necessary, requests for clarification or corrections will be sent to Investigators. The Investigator and his/her staff will be expected to cooperate with the monitor and provide any missing information, whenever possible. Description of monitoring and reporting activities are provided in Data and Safety Monitoring Plan.

12.2. Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies and quality assurance groups of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data

etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities' quality assurance offices.

13. ETHICAL CONSIDERATIONS

This study is to be conducted according to applicable ICH GCP and FDA regulations and local and Institutional research policies and procedures. This protocol and any amendments will be submitted to a properly constituted independent IRB/EC, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB/EC concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members or Federalwide Assurance (FWA) number and their affiliate to the sponsor.

All subjects for this study will be provided a consent form, describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed and dated by the subject, and the investigator-designated research professional obtaining the consent.

14. STUDY FINANCES

14.1. Funding Source

Funding sources for this trial are the National Heart, Lung, and Blood Institute (NHLBI).

14.2. Conflict of Interest

All Investigators will be responsible for following the applicable policies related to review and management of conflicts of interests at their respective institutions.

15. PUBLICATION PLAN

Reporting and publication planning shall follow applicable guidelines of the International Committee of Medical Journal Editors. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor or as otherwise permitted in accordance with the clinical trial agreement for this study. Any investigator involved with this study is obligated to provide the study sponsor with complete test results and all data derived from the study.

Study information from this protocol will be posted on www.clinicaltrials.gov within 21 days of enrollment of the first participant.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical trial report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a Sponsor site or other mutually agreeable location.

The Sponsor will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate. The results summary will be posted to www.clinicaltrials.gov no later than 12 months after the last subject's last visit or sooner, if required by legal agreement, local law, or regulation. A manuscript will be processed for publication in the scientific literature if the results provide important scientific or medical knowledge and will be submitted to a peer-reviewed journal for publication.

16. APPENDICES

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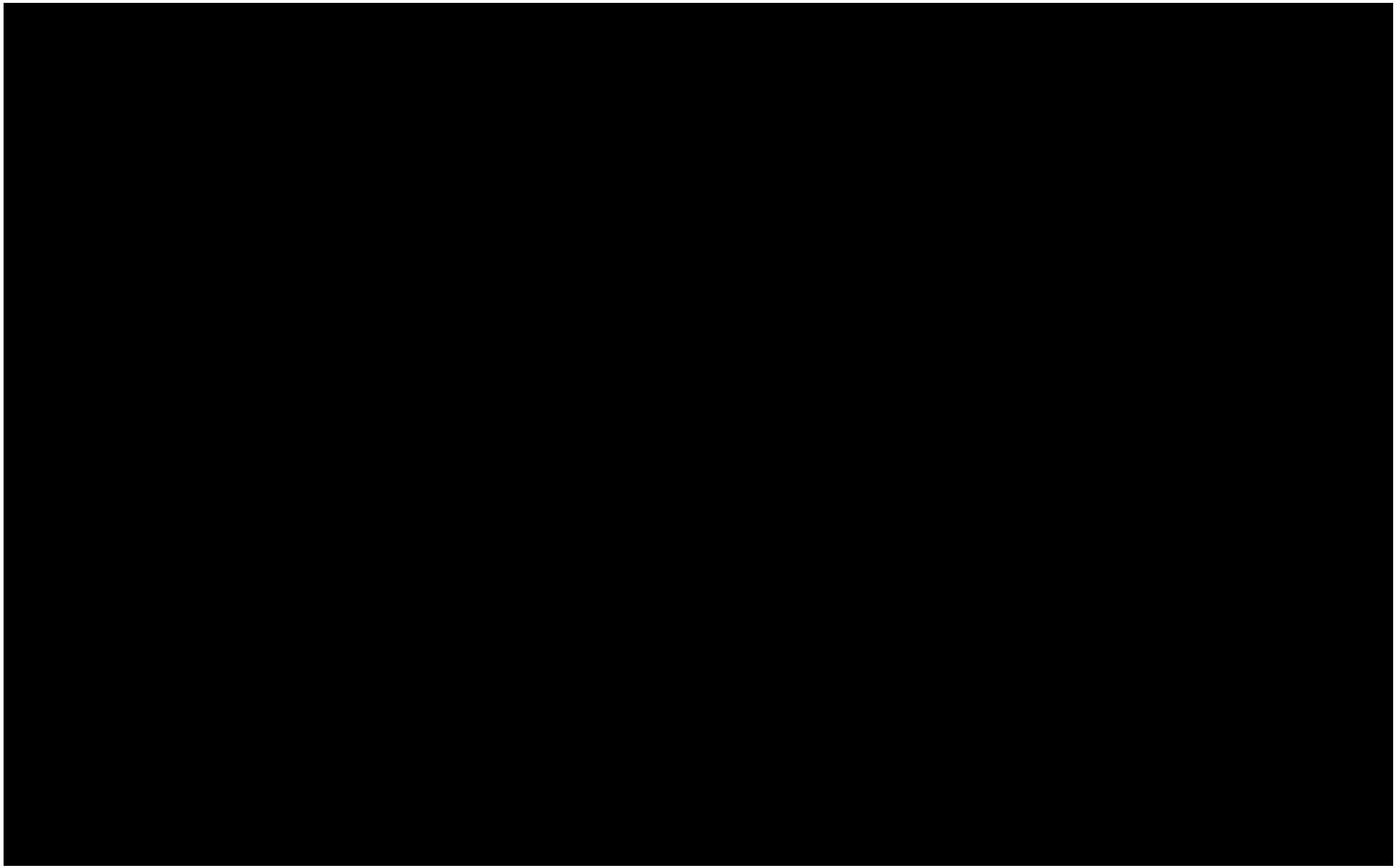

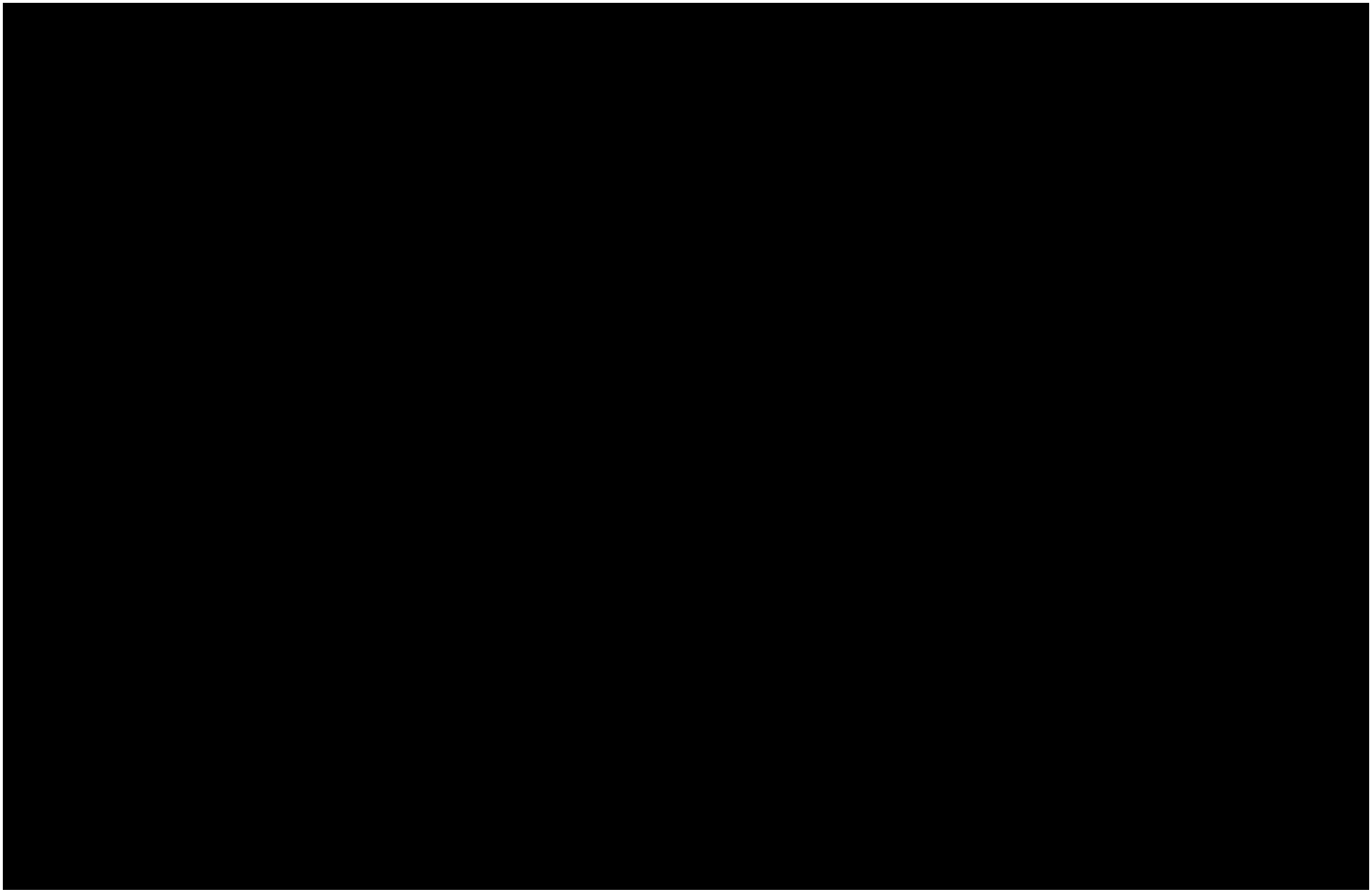

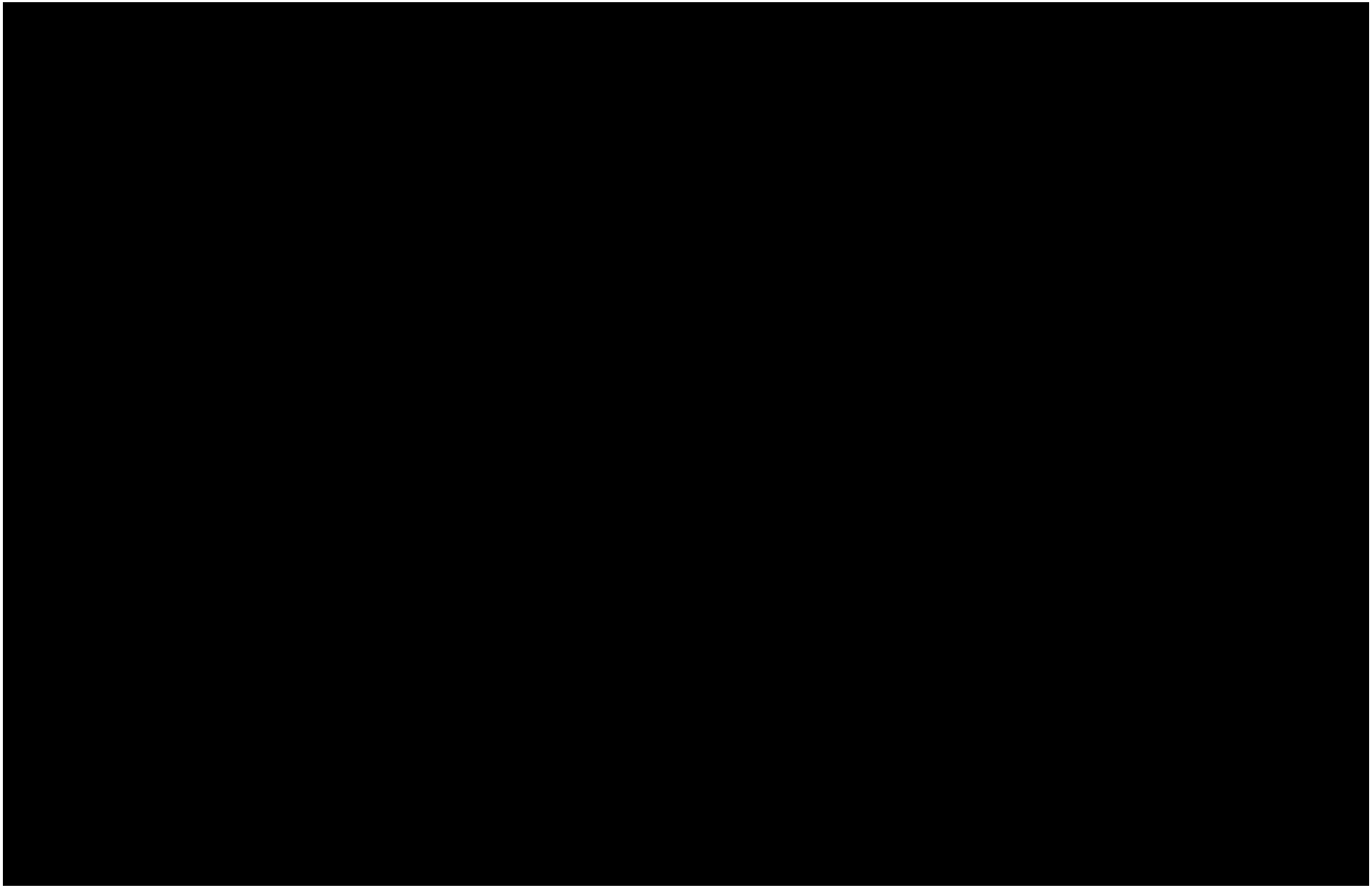

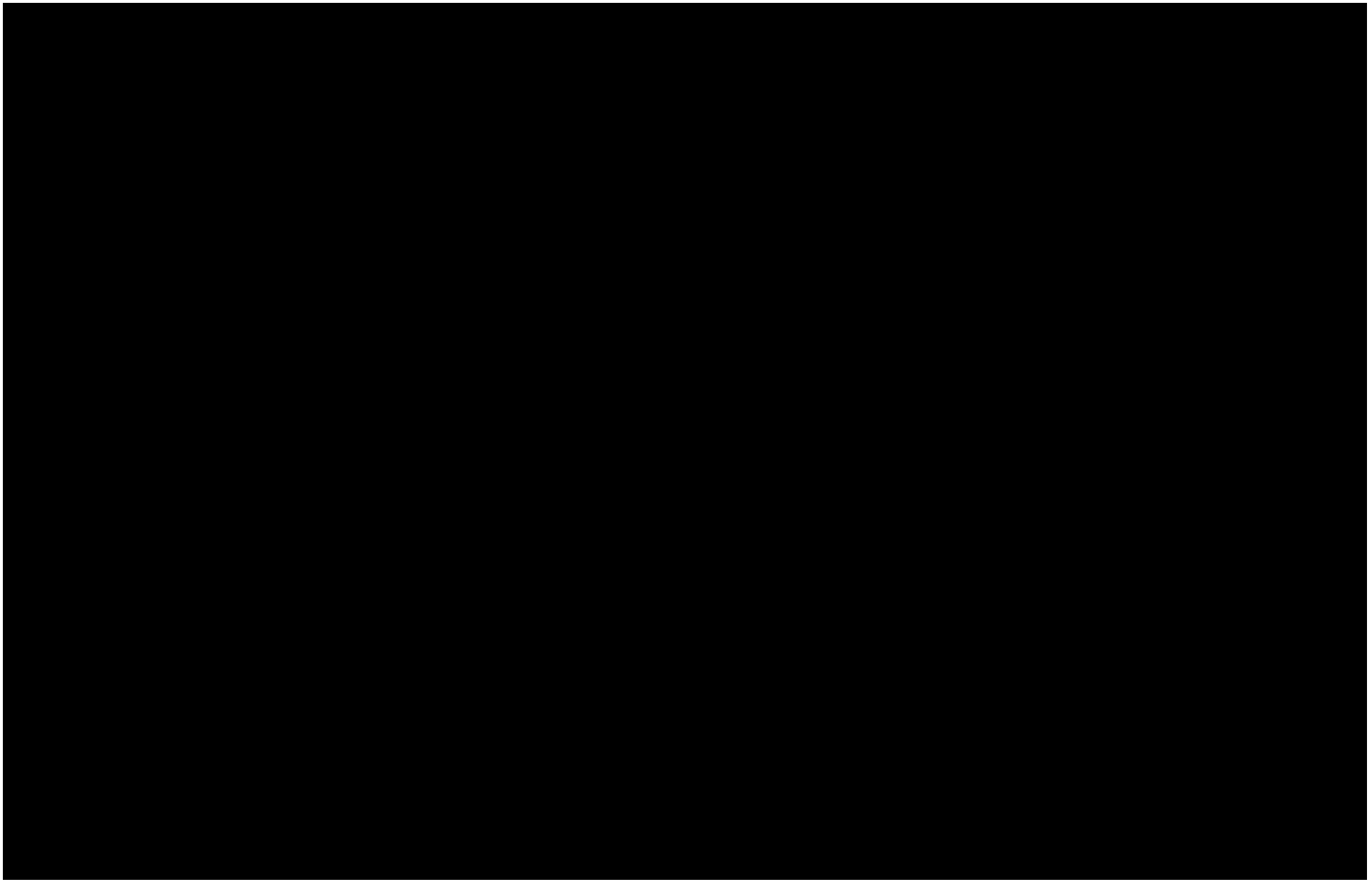

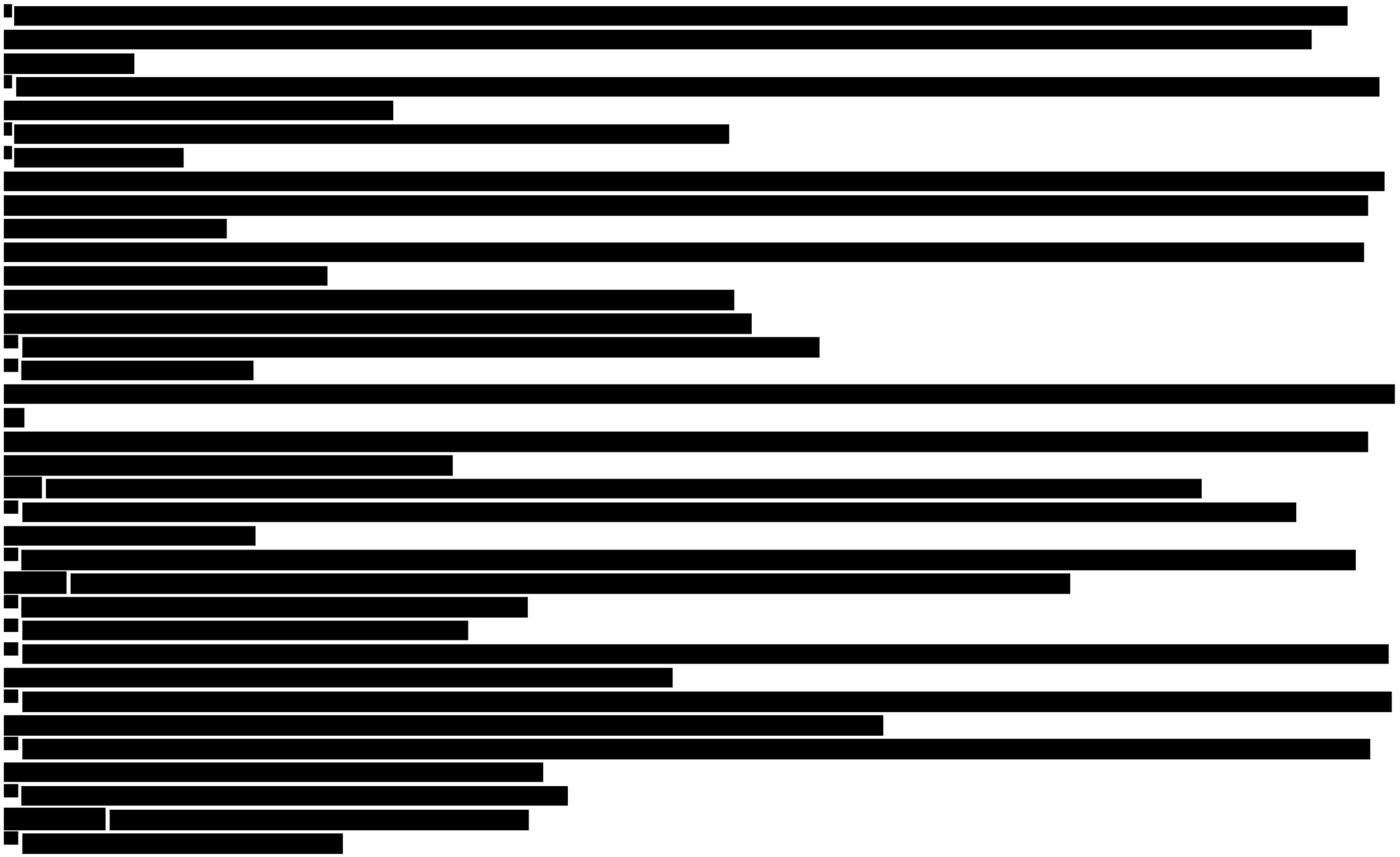

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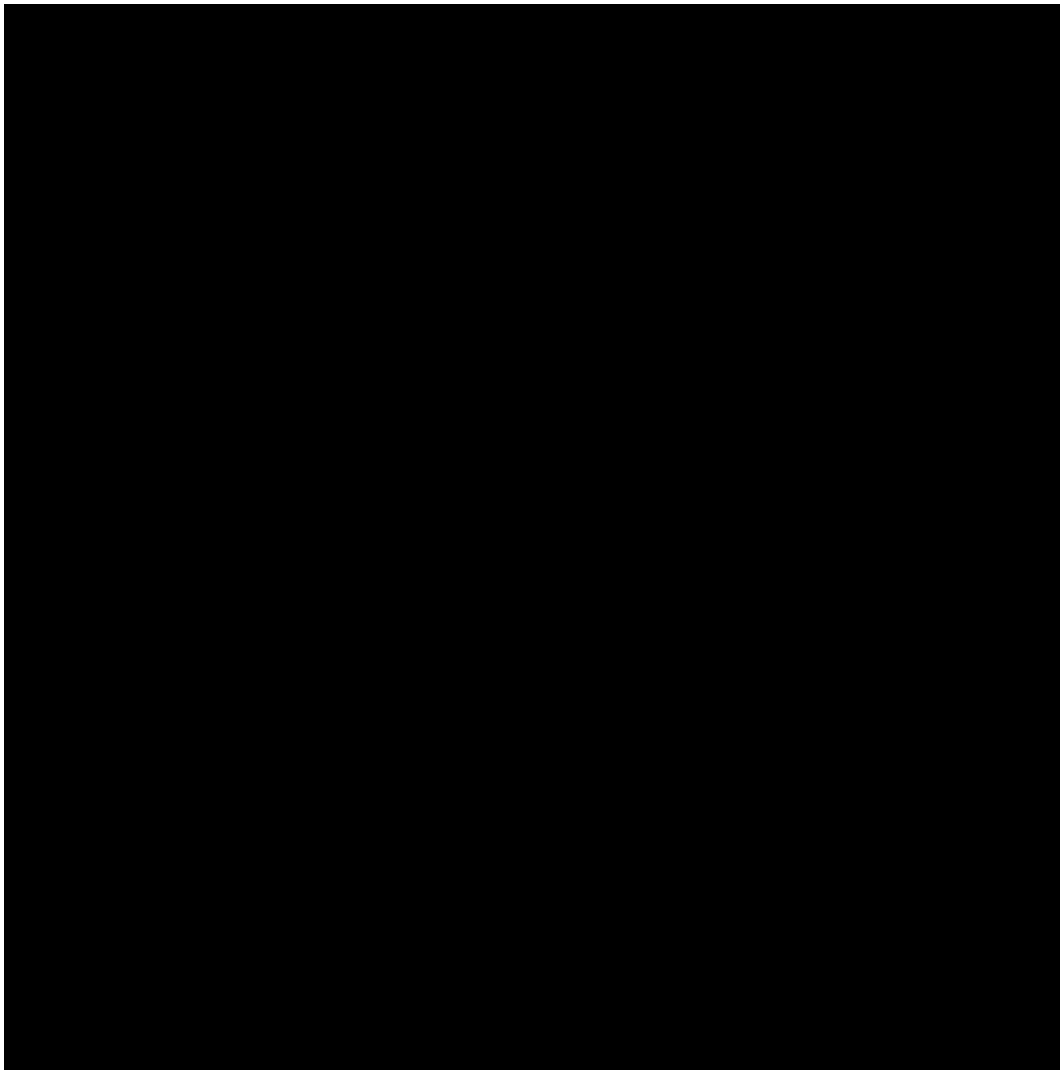

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18. SIGNATURE PAGE

Protocol Title: AAV8-mediated Low-Density Lipoprotein Receptor Gene Replacement in Subjects with Homozygous Familial Hypercholesterolemia

Protocol Number: FHGT002

I have read Protocol FHGT002. I agree to conduct the study as detailed in this protocol and in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP), and all applicable regulatory requirements and guidelines.

_____ _____

Investigator Signature Date

Printed Name: _____

Sponsor Signature:

As the Sponsor representative, I confirm that REGENXBIO will comply with all Sponsor obligations as detailed in applicable guidelines and regulations. I will ensure that the Investigator is informed of all relevant information in a timely manner that becomes available during the conduct of this study.

[redacted]