

Protocol Cover Page

Protocol Title: A Phase 1/2 Dose Escalation Study of Subretinally Injected ATSN-101
Administered in Patients with Leber Congenital Amaurosis Caused by Biallelic Mutations in
GUCY2D

Protocol Version: 8.0

Protocol Date: 13SEP2023

NCT Number: NCT03920007

CLINICAL STUDY PROTOCOL AMENDMENT 08

COMPOUND: ATSN-101 (PREVIOUSLY REFERRED TO AS SAR439483)

A Phase 1/2 Dose Escalation Study of Subretinally Injected
ATSN-101 Administered in Patients with Leber Congenital Amaurosis
Caused by Biallelic Mutations in *GUCY2D*

STUDY NUMBER: ATSN-101-1 (PREVIOUSLY DFI14738)

Version Number: 8.0

EudraCT: Not applicable

IND Number(s): [REDACTED]

Date: 13 Sep 2023

WHO universal trial number: [REDACTED]

NAMES AND ADDRESSES OF

PRIMARY INVESTIGATOR

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Address: [REDACTED]
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1. SIGNATURE PAGE

RESPONSIBLE MEDICAL OFFICER SIGNATURE(S)

STUDY TITLE: A Phase 1/2 Dose Escalation Study of Subretinally Injected ATSN-101 Administered in Patients with Leber Congenital Amaurosis Caused by Biallelic Mutations in GUCY2D

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

INVESTIGATOR OR SPONSOR’S RESPONSIBLE MEDICAL OFFICER:

Approval Signature and Date is captured electronically [REDACTED]

NA

[REDACTED] Chief Medical Officer

(Date)

Atsena Therapeutics

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

CLINICAL STUDY SUMMARY

COMPOUND: ATSN-101 (previously referred to as SAR439483)		STUDY No.: ATSN-101-1 (previously DFI14738)
TITLE	A Phase 1/2 Dose Escalation Study of Subretinally Injected ATSN-101 Administered in Patients with Leber Congenital Amaurosis Caused by Biallelic Mutations in <i>GUCY2D</i>	
INVESTIGATOR/ STUDY LOCATION	[REDACTED], University of Pennsylvania [REDACTED], Oregon Health and Science University (OHSU)	
PHASE OF DEVELOPMENT	1/2	
STUDY OBJECTIVE(S)	<p>Primary Objective: To evaluate the safety and tolerability of ascending doses of ATSN-101 administered as a unilateral subretinal injection in subjects with Leber Congenital Amaurosis (LCA) caused by autosomal recessive <i>GUCY2D</i> mutations (<i>GUCY2D</i>-LCA).</p> <p>Secondary Objective: To evaluate the efficacy of ascending doses of ATSN-101 administered as a unilateral subretinal injection in subjects with <i>GUCY2D</i>-LCA.</p>	
STUDY DESIGN	<p>This is a Phase 1/2, multi-center, open-label, unilateral (one eye) dose escalation study of subretinally injected ATSN-101. The study is separated into two parts including a dose escalation phase (Part A) and a dose expansion phase (Part B). The dose escalation phase will evaluate three ascending dose levels of ATSN-101 in three successive cohorts (Cohorts 1-3). Approximately nine subjects ≥18 years of age (three subjects per cohort) are planned. Up to 15 subjects may be included based on the need for additional cohorts at intermediate doses or to replicate dose levels.</p> <p>For Cohort 1 of the dose escalation phase, there will be an interval of at least 14 days between dosing of each of the three subjects that will be observed in order to assess safety. For the remaining dose escalation phase cohorts (Cohorts 2 and 3), there will be an interval of at least 14 days between dosing the first and second subject and an interval of at least seven days between dosing the second and third subject that will be observed in order to assess safety.</p> <p>The dose expansion phase (Part B) will include two cohorts, both treated at the maximum tolerated dose (MTD) or maximum administered dose (MAD) determined from Part A. These two cohorts will enable further characterization of the risk-benefit profile of ATSN-101 in the adult (Cohort 4) and pediatric (Cohort 5) populations.</p> <p>Cohort 4 will include approximately three subjects ≥18 years of age and Cohort 5 will include approximately three subjects ≥6 years of age and <18 years of age. For Cohort 4, there will be an interval of at least seven days between dosing each subject that will be observed to assess safety. For Cohort 5, there will be an interval of at least 14 days between dosing each subject that will be observed to assess safety.</p>	

	<p>After completion of the 52-week Main Study Period, subjects in all five cohorts will continue in the Extension Study Period. The 208-week Extension Study Period will further evaluate the safety and efficacy of ATSN-101.</p> <p>Data Monitoring Committee (DMC):</p> <p>At least 14 days of safety data will be reviewed by an independent DMC for each cohort prior to dose escalation.</p> <p>The DMC will also review at least 14 days of safety data from the final dose escalation cohort in Part A prior to enrolling Part B.</p> <p>New cohorts will be initiated only after agreement by the Sponsor and DMC that the safety and tolerability demonstrated in prior cohorts support further dosing.</p> <p>The DMC will meet at least once more after the completion of the Main Study Period of the last subject to monitor safety.</p>
<p>STUDY POPULATION</p> <p>Main Selection Criteria:</p>	<p>Inclusion Criteria:</p> <p>I 01. Male or female patient with clinical diagnosis of Leber congenital amaurosis caused by biallelic mutations in the <i>GUCY2D</i> (retinal guanylate cyclase) gene with all of the following:</p> <ul style="list-style-type: none"> a) Documented mutations in both alleles of the <i>GUCY2D</i> gene per testing by a CLIA-approved laboratory b) For Cohorts 1 through 3, best corrected visual acuity (BCVA) of 20/200 or worse in the eye to be injected; subsequent cohorts may include BCVA of 20/80 or worse in the eye to be injected c) Photoreceptor (outer nuclear) layer structure identifiable on an optical coherence tomography (OCT) scan across the central retina with evidence of residual foveal structure <p>I 02. Age ≥ 18 years for Cohorts 1 through 4, and age ≥ 6 years and < 18 years for Cohort 5</p> <p>I 03. Women of childbearing potential must follow the contraception requirements as described in Appendix A</p> <p>I 04. Male patients must follow the contraception requirements as described in Appendix A</p> <p>I 05. Signed and dated written informed consent (or assent in the case of minors from their legal guardian/representative), in accordance with the local regulations</p> <p>I 06. Patients must agree to not donate blood, organs, tissues, cells, or sperm for at least three months following ATSN-101 administration.</p> <p>Exclusion Criteria:</p> <p>E 01. Complicating systemic diseases (such as medical conditions causing immunosuppression) that would preclude the gene transfer, ocular surgery, or planned study procedures</p> <p>E 02. History of HIV infection</p> <p>E 03. Pre-existing eye conditions in the study eye that would preclude the planned surgery or interfere with the assessment and interpretation of study endpoints: for example, glaucoma or optic neuropathy that has resulted in significant visual loss, corneal or lenticular abnormalities or opacities that would preclude view of the fundus or performance of the</p>

	<p>outcome measures, uveitis, retinopathy, and maculopathy that in the opinion of the Investigator are causing significant visual loss.</p> <p>E 04. Presence of significant ocular abnormalities in the study eye that in the opinion of the Investigator would preclude the planned surgery, effective safety follow-up, or interfere with the interpretation of study endpoints (e.g., glaucoma, corneal or significant lens abnormalities or opacities, pre-existing uveitis, intraocular infection, choroidal neovascularization)</p> <p>E 05. Any contraindication to the planned surgical procedure, such as contraindications to the use of anesthesia or allergy to medications planned in the peri-operative period</p> <p>E 06. Known allergy or hypersensitivity to any component of the IMP, diagnostic agents used during the study, or medications planned for use in the peri-operative period, particularly corticosteroids</p> <p>E 07. Women who are pregnant (defined as positive beta-HCG blood or urine test), lactating, or breastfeeding</p> <p>E 08. Any ocular procedure, either planned or performed, within six months of Day 1, which would interfere with the planned surgery or the interpretation of study endpoints in the opinion of the Investigator</p> <p>E 09. Laboratory test abnormalities or abnormalities in electrocardiogram that in the opinion of the Investigator would make the patient unsuitable for participation in the study</p> <p>E 10. Significant (as determined by the Investigator) intercurrent illness or infection during the 28 days prior to enrollment</p> <p>E 11. Current substance use disorder</p> <p>E 12. Use of any investigational agent administered within five times the elimination half-life of that investigational agent prior to ATSN-101 administration</p> <p>E 13. Enrollment in any other clinical treatment study, for any condition, including those relating to <i>GUCY2D</i>-LCA, throughout the duration of the ATSN-101 study participation</p> <p>E 14. Use of anticoagulation therapy within two weeks prior to surgery</p> <p>E 15. Use of systemic immunosuppressive medications</p> <p>E 16. Current, planned during the course of this study, or past (within five times the elimination half-life of that therapy prior to ATSN-101 administration) use of anti-viral therapy that would inactivate the investigational agent</p> <p>E 17. Received gene therapy within the last 15 years</p> <p>E 18. Any patient who, in the judgment of the Investigator, is likely to be noncompliant during the study, or unable to cooperate with the study procedures</p> <p>E 19. Any patient who is the Investigator or any sub-investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in conducting the study</p>
Total Expected Number of Subjects:	<p>Part A:</p> <p>Approximately nine subjects, but up to 15 based on need for additional cohorts at intermediate doses or to replicate dose levels.</p>

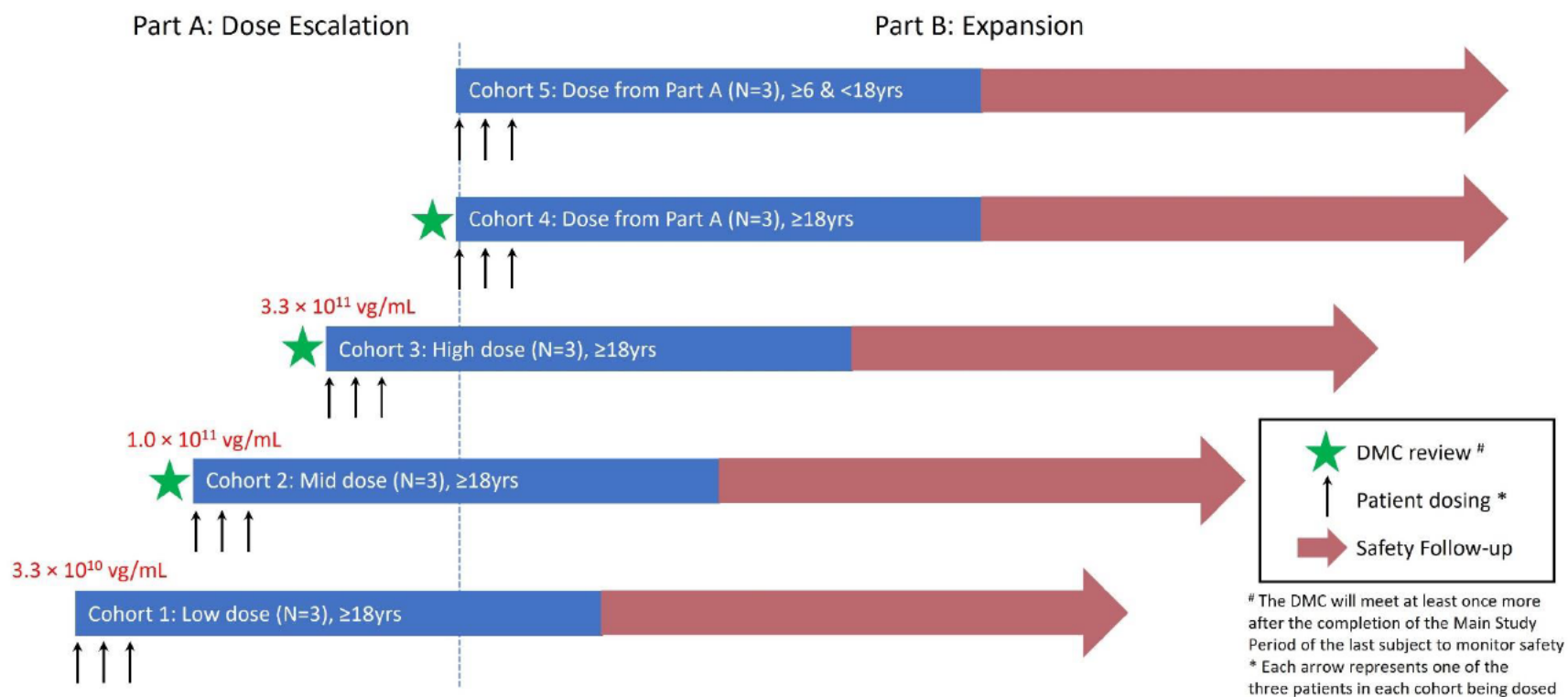
	Part B: <u>Cohort 4:</u> Approximately three subjects <u>Cohort 5:</u> Approximately three subjects																																						
Expected Number of Sites:	Two (multi-center)																																						
STUDY TREATMENT(s)																																							
Investigational Medicinal Product(s) Formulation:	AAV5-GUCY2D gene therapy (ATSN-101; previously referred to as SAR439483) ATSN-101 solution for injection: Solution for intraocular administration. Each single-use vial contains [REDACTED] [REDACTED] [REDACTED] [REDACTED] Packaged in a glass vial, stopper, crimp seal, to be stored upright at or below -60°C (-76°F).																																						
	ATSN-101 diluent solution: Diluent solution for parenteral use. Each single-use vial contains [REDACTED] [REDACTED] [REDACTED] [REDACTED] Packaged in a glass vial, stopper, crimp seal, to be stored upright at or below -60°C (-76°F).																																						
Route(s) Of Administration:	Subretinal injection in the central retina																																						
Dose Regimen:	Study drug will be administered as a single subretinal injection into the study eye. The study eye will be the eye that in the opinion of the Investigator and in consultation with the subject, is the worse seeing eye. In order to select the worse eye, the Investigator will review the subject's historical and Screening visual acuity and visual field data and also consider which eye is dominant and any preferences shown by the subject.																																						
	Planned Unilateral Doses by Cohort: <table><tr><th></th><th>Cohort^a</th><th>Age (years)</th><th>Number of subjects</th><th>Vector genomes per mL</th><th>Vector genomes per eye</th><th>Volume (µL)</th></tr><tr><td rowspan="3">A</td><td>1</td><td>≥18</td><td>3</td><td>3.3×10¹⁰</td><td>1.0×10¹⁰</td><td>300</td></tr><tr><td>2</td><td>≥18</td><td>3</td><td>1.0×10¹¹</td><td>3.0×10¹⁰</td><td>300</td></tr><tr><td>3</td><td>≥18</td><td>3</td><td>3.3×10¹¹</td><td>1.0×10¹¹</td><td>300</td></tr><tr><td rowspan="2">B</td><td>4</td><td>≥18</td><td>3</td><td>TBD^b</td><td>TBD^b</td><td>300</td></tr><tr><td>5</td><td>≥6 and <18</td><td>3</td><td>TBD^b</td><td>TBD^b</td><td>300</td></tr></table>		Cohort ^a	Age (years)	Number of subjects	Vector genomes per mL	Vector genomes per eye	Volume (µL)	A	1	≥18	3	3.3×10 ¹⁰	1.0×10 ¹⁰	300	2	≥18	3	1.0×10 ¹¹	3.0×10 ¹⁰	300	3	≥18	3	3.3×10 ¹¹	1.0×10 ¹¹	300	B	4	≥18	3	TBD ^b	TBD ^b	300	5	≥6 and <18	3	TBD ^b	TBD ^b
	Cohort ^a	Age (years)	Number of subjects	Vector genomes per mL	Vector genomes per eye	Volume (µL)																																	
A	1	≥18	3	3.3×10 ¹⁰	1.0×10 ¹⁰	300																																	
	2	≥18	3	1.0×10 ¹¹	3.0×10 ¹⁰	300																																	
	3	≥18	3	3.3×10 ¹¹	1.0×10 ¹¹	300																																	
B	4	≥18	3	TBD ^b	TBD ^b	300																																	
	5	≥6 and <18	3	TBD ^b	TBD ^b	300																																	
^a Additional cohorts may be included in part A of the study for intermediate dose levels, or to replicate doses as described above. ^b MTD or MAD																																							

Non-investigational medicinal product(s)	The following non-investigational medicinal products (NIMPs) comprise the recommended periprocedural corticosteroid and topical antibiotic medication regimen. These NIMPs will be provided by the site. This regimen may not be changed without prior discussion with the study Medical Monitor.
Formulation:	<ul style="list-style-type: none"> • Prednisone, tablet (or equivalent) • Triamcinalone acetonide, suspension, 40 mg/mL (or equivalent) • Prednisolone suspension, 1% (or equivalent) • Trimethoprim 1 mg and polymyxin B sulfate 10,000 units per 1 mL solution (or equivalent)
Dose regimen:	<ul style="list-style-type: none"> • Prednisone PO: 1 mg/kg/day PO prednisone (up to a maximum dose of 80 mg) administered in the morning (approximately 8AM) given on D-1, D1, D2, D3, D4, D5, and D6; followed by 0.5 mg/kg/day PO prednisone for seven days; followed by 0.25 mg/kg/day PO prednisone for four days; and followed by 0.125 mg/kg/day PO prednisone for three days (21 days in total). • Triamcinalone acetonide: 20 mg in 0.5 mL administered as a peri-ocular injection at the time of the procedure • Prednisolone 1% suspension: one drop QID D2 through D9 • Trimethoprim/polymyxin solution: one drop QID D2 through D9
PRIMARY ENDPOINT(S) AND MAIN SECONDARY ENDPOINT(S)	<p>Primary Endpoint: The incidence of adverse events (AEs, SAEs, and AESIs) over a 52-week period following a single subretinal dose of ATSN-101. Clinically significant changes from baseline in safety assessments will be noted.</p> <p>Secondary (Efficacy) Endpoints:</p> <ul style="list-style-type: none"> • Full-field stimulus testing • Best-corrected visual acuity <p>Exploratory Endpoints:</p> <ul style="list-style-type: none"> • Pupillometry • Oculomotor instability • Photoaversion • Low-luminance visual acuity (LLVA) • Optical coherence tomography (OCT) • Visual Function Questionnaire <ul style="list-style-type: none"> ○ National Eye Institute Visual Function Questionnaire (NEI VFQ-25) will be used for Cohorts 1 through 4; The Cardiff Visual Ability Questionnaire for Children (CVAQC) will be used for Cohort 5. • Patient's Experience Due to Changes in Vision (PECV) Questionnaire • Mobility testing <ul style="list-style-type: none"> ○ Mobility testing will be performed on subjects where testing is available.
ASSESSMENT SCHEDULE	See flow chart (Section 2.2)

STATISTICAL CONSIDERATIONS	<p>Sample Size Determination: This is a Phase 1/2 dose escalation study. No formal sample size calculation has been performed.</p> <p>Analysis Population: All subjects who have signed informed consent will be included in the safety population. The activity/efficacy population will include all subjects in the safety population who have received subretinal administration of ATSN-101. For each efficacy and exploratory endpoint, all subjects in the activity/efficacy population who have both the baseline and at least one post-baseline examination for that specific endpoint measurement will be included in the analysis.</p> <p>Primary Analysis: The primary objective of safety and tolerability will be assessed descriptively by the incidence of adverse events (AEs) in subjects in the safety population at Week 52, the end of the Main Study Period. An additional safety analysis will be performed at Week 260, the end of the Extension Study Period. The number and percentage of subjects with AEs, SAEs, and AESIs will be summarized in each cohort and overall, and significant changes from baseline in safety assessments will be assessed. The type, frequency, seriousness, and relatedness of treatment-emergent adverse events (TEAEs) will be summarized and listed according to the Medical Dictionary for Regulatory Activities (MedDRA). Severity of TEAEs and laboratory abnormalities will be summarized according to the Common Terminology Criteria for Adverse Events (CTCAE; v5.0).</p> <p>Analysis of Secondary Endpoints: Secondary endpoints will be summarized with descriptive statistics at each visit, for each cohort and overall, with the main analysis occurring at Week 52. An additional analysis will be performed at Week 260.</p> <p>Analysis of Exploratory Endpoints: Exploratory endpoints will be summarized with descriptive statistics at each visit for each cohort and overall, with the main analysis occurring at Week 52. Additional analyses may be performed at Week 260.</p>
DURATION OF STUDY PERIOD (per subject)	<p>The Screening and Baseline period will be up to 56 days, the duration of the Main Study Period will be approximately 52 weeks, and the Extension Study Period will include an additional 208 weeks, for a total post-treatment study duration of approximately 260 weeks and a total study duration, including Screening and Baseline, of up to approximately 268 weeks.</p>
STUDY COMMITTEES	<p>Steering Committee: ☐ Yes ☒ No</p> <p>Data Monitoring Committee: ☒ Yes ☐ No</p> <p>Adjudication Committee: ☐ Yes ☒ No</p>

2. FLOW CHARTS

2.1. Graphical Study Design



2.2. Study Flow Chart

Phase	Screening	Baseline	Treatment	Main Study Period										Extension Study Period				
Day	D-56 to D-8 ^b	D-7 to D-1 ^b	D1	D2	D3	D4, D5, D6, D7	D8, D9, D10, D12, D14	D28 ±5d ^b	W8 ±7d ^b	W12 ±7d ^b	W24 ±2W ^b	W36 ±3W ^b	W52 ±3W ^{b,c}	W78 ±4W ^b	W104 ±4W ^b	W156 ±4W ^b	W208 ±4W ^b	End-of-Study W260 ±4W ^b
Informed consent / assent	X																	
Visit at clinical site ^a	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Anesthesiology consultation ^d		X ^e	X															
Visit at surgical site ^f		X ^g	X ^h															
Inclusion/exclusion criteria	X																	
Medical/surgical history	X																	
LCA genetic testing ⁱ	X																	
Demographic data	X																	
Prior/concomitant medications	<	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>
Confirm eligibility		X	X															
Study Treatment Administration																		
ATSN-101			X ^j															
Peri-procedural medications ^k		D-1	X	X	X	X	D8, D9											
Safety^l																		
Physical examination ^m	X																	
Height	X																	
Body weight	X																	

Phase	Screening	Baseline	Treatment	Main Study Period										Extension Study Period				
Day	D-56 to D-8 ^b	D-7 to D-1 ^b	D1	D2	D3	D4, D5, D6, D7	D8, D9, D10, D12, D14	D28 ±5d ^b	W8 ±7d ^b	W12 ±7d ^b	W24 ±2W ^b	W36 ±3W ^b	W52 ±3W ^{b,c}	W78 ±4W ^b	W104 ±4W ^b	W156 ±4W ^b	W208 ±4W ^b	End-of-Study W260 ±4W ^b
Archival blood sample		X																
HIV Serology	X																	
ECG	X																	
Vital signs (blood pressure, heart rate)	X	X	X	X	X	X	X	X	X	X	X	X	X					
Oral body temperature	X	X	X	X	X													
Hematology, biochemistry, urinalysis ⁿ	X	X				D7		X					X					
Pregnancy test (if applicable) ^o	X	X						X										
Plasma FSH (if applicable)	X																	
Humoral Immune response (anti-AAV5 and anti-RetGC1) ^p		X				D7		X		X	X		X					
Cellular Immune response (ELISpot) ^q		X						X		X			X					
Adverse event collection	<	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>
Ophthalmological Examinations																		
Best-corrected visual acuity (BCVA)	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Slit-lamp examination	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Ophthalmoscopy	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ATSN-101-1 Protocol
 Version 8.0, 13 Sep 2023

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Phase	Screening	Baseline	Treatment	Main Study Period										Extension Study Period				
Day	D-56 to D-8 ^b	D-7 to D-1 ^b	D1	D2	D3	D4, D5, D6, D7	D8, D9, D10, D12, D14	D28 ±5d ^b	W8 ±7d ^b	W12 ±7d ^b	W24 ±2W ^b	W36 ±3W ^b	W52 ±3W ^{b,c}	W78 ±4W ^b	W104 ±4W ^b	W156 ±4W ^b	W208 ±4W ^b	End-of-Study W260 ±4W ^b
Fundus photography (infrared) ^r	X	X		X	X			X	X	X	X	X	X	X	X	X	X	X
Fundus autofluorescence imaging		X						X						X	X	X	X	X
Intraocular Pressure (IOP) ^{r,s}	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Full-field stimulus testing ^{r,t}	X	X			X		X ^u	X	X	X	X	X	X	X	X	X	X	X
Photoaversion ^{r,t}		X						X	X	X	X	X	X					
Low-luminance visual acuity (LLVA) ^{r,t}		X						X	X	X	X	X	X					
Optical coherence tomography ^{r,t}	X	X						X	X	X	X	X	X	X	X	X	X	X
Pupillometry ^{r,t}	X	X						X	X	X	X	X	X					
Oculomotor instability ^{r,t}	X	X						X	X	X	X	X	X					
Additional Testing																		
Visual Function Questionnaire ^{v,w}		X						X		X	X		X	X	X	X	X	X
Patient's Experience Due to Changes in Vision (PECV) Questionnaire ^w								X		X	X		X	X	X	X	X	X
Mobility testing ^{x,t}		X						X		X	X		X	X	X	X	X	X

^a Additional clinic visits may be added throughout the study for safety and efficacy follow-up based on Investigator's judgment.

^b Visit may occur over multiple days to accommodate testing.

- c* The Screening and Baseline period will be up to 56 days, the Main Study Period will be approximately 52 weeks, and the Extension Study Period will include an additional 208 weeks, for a total post-treatment study duration of approximately 260 weeks and a total study duration, including Screening and Baseline, of up to approximately 268 weeks.
- d* May not be performed on all subjects based on the standard of care of the surgical site.
- e* If needed, will occur on D-3, D-2, or D-1.
- f* Additional visits may occur on Days 2 or 3 based on the judgement of the Surgeon.
- g* If needed on any day from D-7 to D-1 for anesthesiology consultation.
- h* The subject will be observed for approximately four hours at the surgical site post-operatively, with the final duration determined by the Surgeon and/or Investigator. If required based on clinical circumstances, the subject may be admitted for overnight observation in the hospital at the discretion of the Surgeon and/or Investigator.
- i* Gene mutation analysis results from the subject's medical record may be used provided that the analysis was conducted by a CLIA (Clinical Laboratory Improvement Amendments) certified laboratory, written results are provided to the site, and participants (subjects and/or the subject's parent[s]/legal guardian[s]) give consent to utilize the results. For subjects genotyped during the Screening period, results must be documented prior to the administration of ATSN-101.
- j* The subject will need to take nothing by mouth overnight or for approximately 12 hours prior to surgery.
- k* The recommended peri-procedural medication regimen is described in Section 5.2.2.3 and extends to Day 21.
- l* Refer to Section 10.2 for detailed safety investigations.
- m* Conducted at screening visit and as needed post-procedure based on subject symptoms.
- n* As described in Section 10.2.2.
- o* Beta-HCG plasma test at Screening, and urine pregnancy test at Baseline and D28.
- p* If two consecutive post-procedure values are decreasing, then subsequent draws are not required.
- q* Samples will be frozen and only run if there is evidence of ocular inflammation.
- r* Based on Investigator judgment, testing may be performed on additional days beyond what is indicated in this table if clinically indicated for safety follow-up or based on subject symptoms.
- s* Measured using applanation tonometry, Tonopen, or iCare tonometer.
- t* For Cohort 5 (pediatric), completion of these assessments is dependent on the cooperation level of the child.
- u* For D8, D9, D10, D12, and D14 full-field stimulus testing will be performed on one or more of the five days based on the Investigator's judgement.
- v* NEI VFQ-25 will be used for Cohorts 1-4, and CVAQC will be used for Cohort 5.
- w* To be administered (interviewer-administered) either by phone up to 3 days ($\pm$ 2 days) prior to the study visit day or, if preferable to the subject and/or Investigator, on the study visit day. These questionnaires should be performed prior to any other study-related activities, and prior to discussion of study participant's treatment or health status, or similar activities. The PECV should be performed first and the visual function questionnaire subsequently.
- x* Mobility testing will be performed on subjects where testing is available.

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4. LIST OF ABBREVIATIONS

AAV:	Adeno-Associated Virus
ADA:	Anti-Drug Antibody
ADL	Activities of Daily Living
AE:	Adverse Event
AESI:	Adverse Event of Special Interest
ALT:	Alanine Aminotransferase
BCVA:	Best-Corrected Visual Acuity
BD:	Biodistribution
BRVT:	Berkeley Rudimentary Vision Test
cGMP:	Cyclic Guanosine Monophosphate
CSR:	Clinical Study Report
CTCAE:	Common Terminology Criteria for Adverse Events
CVAQC:	Cardiff Visual Ability Questionnaire for Children
D:	Day
DMC:	Data Monitoring Committee
ECG:	Electrocardiogram
eCRF:	Electronic Case Report Form
eGFP:	Enhanced Green Fluorescent Protein
ELISpot:	Enzyme-Linked Immunosorbent Spot
ERG:	Electroretinogram
ETDRS:	Early Treatment Diabetic Retinopathy Study
FSH:	Follicle-Stimulating Hormone
FST:	Full-Field Stimulus Testing
GCP:	Good Clinical Practices
GLP:	Good Laboratory Practices
GMP:	Good Manufacturing Practices
GUCY2D:	Guanylate Cyclase 2D
HCG:	Human Chorionic Gonadotropin
hGRK1:	Human Rhodopsin Kinase
HIV:	Human Immunodeficiency Virus
HRT:	Hormonal Replacement Therapy
IEC:	Independent Ethics Committee
IMP:	Investigational Medicinal Product
IOP:	Intraocular Pressure
IRB:	Institutional Review Board
LCA:	Leber Congenital Amaurosis

LLVA:	Low-Luminance Visual Acuity
LRP:	Longitudinal Reflectivity Profile
MAD:	Maximum Administered Dose
MedDRA:	Medical Dictionary for Regulatory Activities
MTD:	Maximum Tolerated Dose
NEI VFQ:	National Eye Institute Visual Function Questionnaire
NIMP:	Non-Investigational Medicinal Product
NIR:	Near-Infrared
NLP:	No Light Perception
NOAEL:	No-Observed-Adverse-Effect Level
OCI:	Oculomotor Instability
OCT:	Optical Coherence Tomography
OE:	Ophthalmic Examination
PCSA:	Potentially Clinically Significant Abnormality
PECV:	Patient Experience Due to Changes in Vision Questionnaire
PLR:	Pupillary Light Reflex
PO:	Oral(ly)
PT:	Preferred Term
QID:	4 Times a Day
RetGC1:	Retinal Membrane Guanylyl Cyclase 1
RPE:	Retinal Pigment Epithelium
SAE:	Serious Adverse Event
SOC:	System Organ Class
TEAE:	Treatment-Emergent Adverse Event
VA:	Visual Acuity
vg:	Vector Genomes
WOCBP:	Woman of Childbearing Potential

5. INTRODUCTION AND RATIONALE

Leber congenital amaurosis (LCA) caused by mutations in guanylate cyclase 2D (*GUCY2D*) (*GUCY2D*-LCA) is a rare inherited disease of early childhood blindness that typically presents within the first year of life. It is an autosomal recessive disease caused by mutations in the gene *GUCY2D* encoding retinal guanylate cyclase (retGC1) which modulates phototransduction in rods and cones. RetGC1 is expressed in both cones and rods as a 120 kDa membrane protein that is responsible for the re-synthesis of cyclic guanosine monophosphate (cGMP) required for the recovery of the dark adapted state of photoreceptors after phototransduction. Thus, deficiencies in retGC1 function create the biological equivalence of chronic light exposure in rod and cone photoreceptors, but without the severe degeneration. Despite the loss of photoreceptor function, it is important to note that *GUCY2D*-LCA patients present with a mainly intact retina morphologically and there are retained rods and cones in both their macula and peripheral retina into adulthood (1, 2). Therefore, there remains a window for therapeutic intervention years after initial vision loss.

Preclinical studies in multiple *GUCY2D*-LCA mouse models have established that using gene therapy to deliver the *GUCY2D* gene to the rods and cones of the eye using a recombinant adeno-associated virus (AAV) vector is feasible and efficacious (3, 4, 5, 6, 7, 8). Structural preservation and functional restoration of photoreceptors was achieved as demonstrated by histological examinations, electroretinography (ERG) and improvements in vision-elicited behavior. Furthermore, AAV-mediated gene replacement has been demonstrated as safe and effective for the treatment of other retinal diseases, most notably LCA caused by mutations in *RPE65* (9, 10, 11, 12, 13, 14, 15, 16), for which there is now a US Food and Drug Administration approved AAV2-based gene therapy (Luxturna™; Spark Therapeutics).

ATSN-101 (AAV5-*GUCY2D*; previously referred to as SAR439483) is a gene therapy product being developed to introduce the functional human retinal guanylate cyclase 1 gene (*GUCY2D*) to photoreceptors in the eye, thereby altering positively the vision in patients with *GUCY2D*-LCA.

AAV5-*GUCY2D* is a non-replicating AAV (Serotype 5) containing the human retinal guanylate cyclase 1 gene under the transcriptional control of the photoreceptor-specific human rhodopsin kinase promoter.

LCA Disease Overview

Leber congenital amaurosis is a clinically-defined group of inherited retinal diseases that cause severe loss of vision in infancy or childhood. The diagnosis tends to be made in early life due to a parent noting that a child does not respond as expected to visual cues and has unstable fixation (nystagmus). This can lead to medical visits to pediatricians or referrals directly to ophthalmic practitioners. Given no obvious abnormalities in the pre-retinal anatomy or other systemic or more central nervous system causes of the visual loss, an electroretinogram (ERG) is usually considered. An abnormal ERG that points to major photoreceptor dysfunction as the source of the retina-wide low vision secures the diagnosis of LCA. In addition to the nystagmus, there can be refractive errors, keratoconus, pigmentary and vascular abnormalities on ophthalmoscopic examination or a normal-appearing fundus with minimal attenuation of retinal vessels.

It is now known that LCA is not a single disorder, but rather is a group of molecularly heterogeneous retinal degenerative diseases, mainly inherited as autosomal recessive traits. There are at least 20 monogenic causes of LCA (17, 18). The molecular subtypes reveal different disease mechanisms causing LCA, such as abnormalities in retinal development or photoreceptor structure, or disruptions in the visual (retinoid) cycle or phototransduction. LCA, as discussed here, is the non-syndromic type, but there are also syndromic forms (17). Despite major scientific advances leading to greater understanding of mechanisms and the increasing availability of animal models of various forms of LCA, there are still only limited therapeutic options for these severe blinding diseases.

Diagnosis of GUCY2D-LCA and Current Standard of Care for These Patients

Once the clinical diagnosis of LCA is made, further diagnostic refinement occurs through genetic testing. The first LCA gene was located on 17p13 (19) and identified to be *GUCY2D*, which encodes RetGC1, an enzyme required for the recovery process in the phototransduction pathway (20).

Treatment of LCA in general and *GUCY2D*-LCA specifically has been palliative; the severe visual disturbance has been accepted as unable to be remedied. Appropriately, patients are directed to agencies that can help them cope with their visual loss by providing them with educational tools (whether Braille or computer technology, for example) or training them to be independent through the teaching of useful life skills. Clinical eye examinations tend to include some measure of visual acuity (as possible), slit lamp, and ophthalmoscopic examinations to detect any abnormalities that may threaten the integrity of the eye and lead to chronic pain or even loss of an eye (for example, keratoconus, cataract, increased intraocular pressure, and Coats disease [21]).

Prospect of Retinal Gene Therapy for GUCY2D-LCA Depends on Identification of Sufficient Photoreceptors in Their Retinas

Clinical reports of *GUCY2D*-LCA in recent years have indicated that most patients were hyperopic; best-corrected visual acuities ranged from 20/125 to NLP (no light perception); and nystagmus was present. ERGs were not detectable in most patients; some had evidence of a small amplitude rod signal. Ophthalmoscopic examinations were described as normal or with a 'salt and pepper' appearance, attenuated retinal vessels and possibly a maculopathy. This is valuable clinical information, but not the type that will allow the decision of whether there are sufficient photoreceptor cells to be treated in these eyes.

The advent of optical coherence tomography (OCT) in the clinic has allowed an *in vivo* microscopic approach to human retinal structure. Such information was previously unavailable except in rare post-mortem eye donors with well-characterized retinal diagnoses (22). Now, microscopic details of cross-sectional retinal images have been able to be obtained in different molecular subtypes of LCA and measurement of retinal laminae performed (reviewed in [23]). Four patterns of retinal microstructure have been identified to date among young patients with different LCA genotypes: 1) near normal photoreceptor layer structure across the central retina; 2) severely dysplastic retina; 3) well-formed foveal architecture but structure limited to a central island only; and 4) pronounced macular disease due to degeneration or foveal maldevelopment (23).

The retinal laminar microstructural pattern into which *GUCY2D*-LCA patients fall is the first pattern listed above: near normal photoreceptor layer structure across a wide expanse of central retina, but in *GUCY2D*-LCA there can be abnormalities in the foveal region (1, 2). Identification of preserved photoreceptor cells in a cohort of patients with *GUCY2D*-LCA makes this form of LCA amenable to a gene augmentation strategy in the retina and, although differing in mechanism and exact pattern, it is reminiscent of the findings that were critical to initiating the plan for a gene augmentation strategy in the *RPE65* form of LCA (24). Shared by the two forms of LCA is the dissociation of function and structure, meaning that vision is severely reduced but there is sufficiently retained retinal structure to warrant treating the defective retinal pathway.

Measuring the Outcome of a Clinical Study of Gene Therapy for *GUCY2D*-LCA

In this first clinical study of ATSN-101 for *GUCY2D*-LCA, safety is the primary objective and the routine eye examination will be the primary method for assessment. Preliminary efficacy information will be secondary in this first study. The low level of vision in patients with *GUCY2D*-LCA, however, precludes use of many of the time-honored visual outcome measures in clinical studies of eye disease (25). Best-corrected visual acuity will be possible in some of the subjects using a conventional Early Treatment Diabetic Retinopathy Study (ETDRS) chart; in situations where subjects have off-chart vision, alternative methods will be used, specifically the Berkeley Rudimentary Vision Test (BRVT) (26). Perimetry is not able to be performed because of the degree of visual loss and nystagmus preventing fixation. The ERG is not detectable to all stimuli in most of these subjects, giving a non-detectable baseline. Feasible efficacy outcomes studied in a recent cohort of 28 *GUCY2D*-LCA patients were the following: the full-field stimulus test (FST), OCT across the central retina, pupillometry, and the National Eye Institute Visual Function Questionnaire (NEI VFQ-25) (1) and these will be included in this study.

5.1. Introduction

ATSN-101 is a gene therapy approach to treat *GUCY2D*-LCA. The *GUCY2D* gene is delivered to the retinal photoreceptor cells by subretinal injection of AAV5 virus containing the wild type gene. Preclinical models demonstrate both transduction of photoreceptor cells and improvement of visual function.

5.1.1. Toxicology

In order to assess the safety of AAV5-*GUCY2D* as a therapeutic approach to treating *GUCY2D*-LCA, two cynomolgus monkey Good Laboratory Practices (GLP) toxicology studies were conducted. The first study evaluated five groups: vehicle control and four dose levels of AAV5-*GUCY2D* by subretinal injection at a volume of 150 μ L with concentrations ranging from 1.0×10^{12} to 4.9×10^{13} vector genomes (vg)/mL. Ointment containing dexamethasone 0.1% together with broad spectrum antibiotics (neo-poly-dex) was applied to the eye twice daily on Days -1 through 3 of the study. Animals were observed for nine months following administration of AAV5-*GUCY2D*. In this nine-month study, the no-observed-adverse-effect level (NOAEL) was not established. In all groups receiving AAV5-*GUCY2D*, there were one or more animals per group with adverse findings, as assessed by either ophthalmic examination (OE), ERG, and/or OCT. These adverse findings were present at study termination, at which time microscopic analysis established histochemical correlates to the OE/ERG/OCT adverse findings, including retinal damage with disruption of the retinal pigment epithelium (RPE) and

evidence of persistent ocular inflammation. The OE/ERG/OCT adverse findings present at nine months were identifiable as early as the three-month time point and generally persisted without improvement/recovery for the duration of the nine-month study.

Based on the results of the nine-month study, a three-month monkey toxicology study was conducted with eight groups to evaluate lower dose levels of AAV5-*GUCY2D* administered by subretinal injection. Injections at a volume of 150 μ L with three concentrations of AAV5-*GUCY2D* were evaluated: 1.0×10^{11} , 3.3×10^{11} , and 1.0×10^{12} vg/mL, with the highest dose corresponding to the lowest dose administered in the nine-month monkey study. Considering the inflammation observed in the nine-month study, two regimens of oral corticosteroid treatment (both combined with a single 2 mg subconjunctival dexamethasone injection on Day 1) were utilized in the three-month study: a short duration regimen consisting of oral prednisolone 1-2 mg/kg one day prior to injection through Day 3, and a long duration regimen consisting of oral prednisolone 1-2 mg/kg given three days prior to injection, daily through Week 5, and then every other day during Week 6. In addition, all animals received the same topical corticosteroid/antibiotic ointment regimen that was used in the nine-month study, as described above.

In the three-month monkey toxicology study, no difference was observed between the corticosteroid treatment regimens. The NOAEL was determined to be the highest dose level evaluated: 150 μ L of AAV5-*GUCY2D* administered at a concentration of 1.0×10^{12} vg/mL. In all AAV5-*GUCY2D*-dosed groups, non-adverse observations included minimal/mild ophthalmic inflammation. Specifically, acute anterior/posterior segment inflammation was observed early, but declined rapidly over time and by the end of the three-month period was at a level approximately equivalent to that of the vehicle control group. Grey-white subretinal/choroidal foci, representing a focal inflammatory response (as confirmed by microscopy), appeared after two months, persisted through the end of the study, and were located within the boundaries of the injection site.

Microscopic examination revealed non-adverse observations of increased RPE hypertrophy/hyperpigmentation and minimal/slight RPE cell loss, considered to be procedure-related phenomena. RPE cell loss was observed in all AAV5-*GUCY2D*-dosed groups at an incidence slightly higher than that observed in the vehicle control group. Minimal/slight mononuclear cell choroidal inflammation was also observed, corresponding to the grey-white foci observed via OE. At the highest concentration (1.0×10^{12} vg/mL), minimal/slight photoreceptor disorganization and loss of the photoreceptor outer segment (only), plus slightly decreased pigmentation of the RPE, and minimal vitreous exudate (observed in only one animal) were present. Multi-focal and full-field ERGs indicated no changes at any dose level, indicating that the microscopic changes observed, even those only at the highest dose level, were without apparent functional consequences, within the limits of testing as conducted in this species and in this study. These results at 1.0×10^{12} vg/mL in this three-month study with the addition of oral and peri-ocular injected corticosteroid treatment are a noted contrast to that of the nine-month study without such treatment in which OE, ERG, and OCT adverse findings were observed at both the three-month and nine-month time points. Based on the lack of functional effects (as measured by ERG) in the three-month study and generally minimal/slight observations by all other measures, the NOAEL was determined to be 1.0×10^{12} vg/mL administered in a 150 μ L injection volume.

5.1.2. Biodistribution

The biodistribution (BD) of AAV5-*GUCY2D* to non-target tissues and biological fluids (blood) was evaluated in a rat BD study and limited BD endpoints were also included in the nine-month monkey GLP toxicology study.

In the rat BD study, Long-Evans rats were administered 2 μ L of either vehicle or AAV5-*GUCY2D* at 1.0×10^{12} or 1.0×10^{13} vg/mL subretinally in the right eye. Of note, the low dose evaluated in this study corresponds to the NOAEL that was identified in the three-month monkey toxicology study. The vector lot of material that was administered in the rats was the same vector lot administered in the GLP toxicology studies. Samples were collected at Day 4, Day 15, Day 29, and Day 92 to evaluate for the vector presence, persistence, and clearance profile. The testing strategy was defined so that if a tissue was positive for vector genomes, then an adjacent piece of tissue was analyzed for transgene expression. Furthermore, our testing strategy was outlined so that sequential analysis was performed until two consecutive timepoints were negative for vector genomes.

As expected, the highest level of vector DNA was detected in the injected eye, with lower vector DNA levels detected in the optic nerve and brain. In general, vector DNA in tissue and fluids was greater in the high dose animals (1.0×10^{13} vg/mL) as compared to the low dose animals (1.0×10^{12} vg/mL) and the vector DNA levels were greater during earlier timepoints, indicating that the detection of AAV5-*GUCY2D* vector was dose-dependent and progressively decreased over time. Vector DNA was only detectable in the blood at Day 4 in the high dose (1.0×10^{13} vg/mL) group. Significantly lower, transient levels of vector DNA were detected in all/the majority of peripheral tissues evaluated and by Day 92 the majority of tissues were negative for vector genomes. Of note, in the low dose group (1.0×10^{12} vg/mL), ovaries and testes were positive for vector genomes in 3/5 and 1/5 animals, respectively, at Day 4 and negative for vector genomes at Days 15 and 29 (not tested at Day 92). In the high dose group (1.0×10^{13} vg/mL), ovaries and testes were positive for vector genomes in 5/5 and 2/5 animals, respectively, at Day 4. While a few animals were positive for vector genomes at Days 15 and 29, all animals were negative for vector genomes in ovaries and testes at Day 92. It is also noteworthy that most of the animals (all but one animal in the low dose group) had observations of vitreous and/or subretinal hemorrhage noted in the eye immediately following the subretinal procedure, and this may have contributed to distribution of the vector systemically. Importantly, no non-target tissues were positive for *GUCY2D* transgene expression, consistent with the use of a photoreceptor specific promoter (hGRK1) in our vector.

In the nine-month monkey GLP toxicology study, blood was collected throughout the study and tissues were collected at study termination for limited biodistribution analysis. AAV5-*GUCY2D* vector DNA was detected in a dose dependent manner in blood samples. The highest positive samples were found at Week 2 and levels progressively decreased over time to Week 13, at which time only one sample was found to be positive. All blood samples collected subsequently were negative for vector DNA. The highest levels of vector DNA detected were in the optic nerve (as expected) while the lowest levels detected were in the spleen and liver. Only one liver sample from the high dose group (Group 5; 4.9×10^{13} vg/mL) expressed *GUCY2D* mRNA transgene out of 101 liver and spleen samples analyzed. This single positive sample was from the high dose group which is 49X higher than the NOAEL and over 1400X higher than the proposed first-in-human dose. Moreover, no systemic toxicity (including histopathology) was observed.

These results suggest that low levels of vector DNA can be detected systemically following a subretinal administration and should take into consideration the high doses evaluated and the technical challenges of a subretinal administration in rodents. Importantly, no non-target tissues were positive for *GUCY2D* transgene expression in the rats and monkey, consistent with the use of a photoreceptor specific promoter (human rhodopsin kinase [hGRK1] promoter).

5.2. Rationale

5.2.1. Study Rationale

This is a Phase 1/2, first-in-human, dose escalation study. The duration of the Main Study Period will be approximately 52 weeks, after which all subjects will continue in the Extension Study Period for an additional approximately 208 weeks of follow-up. This study is designed to assess whether ATSN-101 is safe, well tolerated, and efficacious in subjects with *GUCY2D*-LCA. Before administering a new investigational medicinal product (IMP) such as ATSN-101 to subjects in a clinical efficacy study, it is necessary as a first step in clinical development to assess its safety and tolerability in a closely monitored setting. Evaluation of a safe and effective dose of ATSN-101 in this study will enable optimal dose identification and further characterization of the safety profile in the target population in a pivotal study.

5.2.2. Design Rationale

5.2.2.1. Rationale for Delivery Procedure

The eye is a suitable target organ for gene therapy for the following reasons:

- The eye is the affected sensory organ in *GUCY2D*-LCA (and other inherited retinal degenerative diseases), and access to the diseased retinal cells (the photoreceptors) for targeted delivery of the gene therapy is possible via microsurgical separation of the neural retina from the retinal pigment epithelium.
- The treatment approach is to correct the underlying genetic abnormality in the affected cells. Administration of ATSN-101 directly in the eye and into the subretinal space provides a means of delivering the gene therapy to the targeted photoreceptor cells in order to restore retinal function. The blood-ocular barrier does not permit the gene therapy product to access the target cells if administered systemically. Subretinal administration of gene therapy has been demonstrated to be effective for the treatment of other retinal diseases, including *RPE65*-LCA (for example: 9, 10, 11, 12, 13, 14, 15, 16). AAV therapy is now approved by the FDA for the treatment of *RPE65*-LCA (Luxturna™; Spark Therapeutics).
- The eye is readily accessible to safety monitoring using multiple methodologies developed and advanced in ophthalmic practice over many decades.

5.2.2.2. Rationale for Unilateral Administration

In this study, the contralateral non-administered eye will act as the primary control (i.e., intra subject control). The more severely affected of the two eyes will be injected. The study eye will be the eye that, in the opinion of the Investigator and in consultation with the subject, is the worse seeing eye. In order to select the worse eye, the Investigator will review the subject's historical and Screening visual acuity and visual field data and also consider which eye is dominant and any preferences shown by the subject. This is done with the intention of minimizing risk to the less affected eye and thereby reducing risk to the subject's remaining vision.

5.2.2.3. Rationale for Peri-Procedural Medication Regimen

The primary rationale for the corticosteroid regimen proposed for this clinical study, which utilizes three different routes of administration, comes from the GLP cynomolgus monkey toxicology data generated for ATSN-101. In the nine-month GLP toxicology study (TSA1563), retinal damage with evidence of persistent ocular inflammation was observed following subretinal injection of ATSN-101 at dose levels ranging from 1.0×10^{12} to 4.9×10^{13} vg/mL in an injection volume of 150 μ L. This study only included a topical corticosteroid (dexamethasone ointment). The NOAEL was not established in that study. Based on these results, a second three-month monkey GLP toxicology study (TSA1578) was conducted that utilized a lower range of doses, 1.0×10^{11} to 1.0×10^{12} vg/mL (thus including the lowest dose level from the nine-month study), and also included the addition of corticosteroids administered via two additional routes, systemic and peri-ocular injection. Specifically, a subconjunctival injection of dexamethasone and oral prednisolone (either a long or short course) were added to the topical dexamethasone in order to provide enhanced protection against ocular inflammation. In this second three-month study (TSA1578) the NOAEL was determined to be the highest dose level evaluated: 150 μ L of ATSN-101 administered at a concentration of 1.0×10^{12} vg/mL (a dose tested in the nine-month study that caused persistent ocular inflammation when given with only a topical corticosteroid). No differences were observed between the long or short course prednisolone regimens. These results indicated that the use of corticosteroids by three routes (oral, peri-ocular injected, and topical) should effectively mitigate the risk of developing ocular inflammation following administration of ATSN-101 and provide strong support for the use of a similar regimen in this clinical study.

For this clinical study, the use of corticosteroids administered via three different routes is proposed to mirror the regimen used in the three-month toxicology study (TSA1578) and to avoid the type of ocular inflammation observed in the nine-month toxicology study (TSA1563). When determining the optimal corticosteroid regimen, the published literature from other subretinal gene therapy clinical studies using AAV serotypes was also taken into careful consideration. For example, among the original first-in-human *RPE65*-LCA gene therapy studies, all used peri-procedural corticosteroids as a preventive measure against ocular inflammation, though the composition of the regimens and duration of treatment varied widely. One clinical study used a three-part regimen: oral corticosteroids for one week before the injection and four weeks after (tapering), a subconjunctival corticosteroid at surgery completion, and then topical steroids for four weeks after surgery (12). Another study used a retrobulbar injection of corticosteroid at the time of surgery and an oral corticosteroid starting three days before injection and continuing for a week after the procedure (13). The third study used a

subconjunctival corticosteroid at surgery and a topical corticosteroid for approximately three weeks after surgery (9, 15). This literature review indicated that administration of corticosteroids via three routes has precedence in the ocular gene therapy field, though no published study has used a regimen that is identical to the one proposed for ATSN-101-1. To reduce the risk of inflammation resulting from surgery or from the vector/transgene, all subjects in this study will be given a 21-day course of oral prednisone, based off the 17-day protocol established in the LCA2 Phase 3 AAV gene therapy clinical study (13) with an additional four-day taper, for a total oral prednisone course of 21 days.

The specific corticosteroids proposed for use in this clinical study and the duration of treatment with each were selected based on both the regimen used in the three-month GLP toxicology study, the clinical experience of the Investigators and Surgeons for this study, as well as context within the field of previous and current ocular AAV subretinal gene therapy studies as described above.

In addition to corticosteroids, subjects in this clinical study will use a broad-spectrum topical antibiotic post-procedure to minimize the risk of post-operative infection. The peri-procedural corticosteroid and topical antibiotic regimen is a recommendation, however, and may be changed based on the subject's clinical condition and at the discretion of the Surgeon and/or Investigator (e.g., for signs of post procedure ocular inflammation, infection, or adverse reactions to any of these medications).

5.2.2.4. Rationale for Study Design

The selected design for this study is open-label, ascending single doses performed at two centers. The primary objective of the study is safety, which is evaluated by comparing findings in the treated versus the non-treated eye. The non-treated eye will serve as the primary control for the safety assessments. The study design, with minimal study sites, allows the ability to minimize variability in administration procedures and provides optimal control of subject enrollment. To ensure the best conditions of safety, there will be a minimum of 14 days between dosing the first and second subjects in all cohorts of Part A and Cohort 5 of Part B. In addition, for Cohorts 1 and 5, there will also be a minimum of 14 days between dosing the second and third subjects. In the nine-month GLP monkey toxicology study, ocular inflammation was rapid in onset during the first week following the procedure (as measured eight days later) and declined, though still present, by the end of the second week. As such, we anticipate that 14 days will be a sufficient observation period. There will be a minimum of seven days between dosing the second and third subjects in Cohorts 2 and 3. For Cohort 4, which is an expansion cohort of adults receiving the maximum tolerated dose (MTD)/maximum administered dose (MAD) defined in Part A, there will be an interval of at least seven days between dosing each subject.

An independent Data Monitoring Committee (DMC) will be convened to monitor safety and will meet regularly either at face-to-face meetings or by teleconference. The DMC will review the available safety data for each individual subject, and make recommendations regarding dose escalation, study continuation, and study conduct.

The DMC will review at least 14 days of safety data from the final dose escalation cohort in Part A prior to enrolling Part B.

The DMC will meet at least once more after the completion of the Main Study Period of the last subject to monitor safety.

Cohorts 1 through 4 will be conducted in adults. Before enrolling pediatric subjects (Cohort 5), a preliminary summary of safety and biological activity data of Part A along with a rationale for the dose to be used in Cohort 5 will be provided to the DMC for review. Cohort 1-3 will be limited to subjects with severe vision loss (best-corrected visual acuity [BCVA] of 20/200 or worse). Subjects with less severe vision loss (BCVA of 20/80 or worse) will be enrolled in subsequent cohorts.

After completion of the 52-week Main Study Period, subjects in all five cohorts will continue in the Extension Study Period. The 208-week Extension Study Period will further evaluate the safety and efficacy of ATSN-101.

5.2.2.5. Rationale for Population

For this first-in-human study, the population to be included is adult and pediatric subjects having the clinical diagnosis of LCA with the molecular cause being mutations in the *GUCY2D* gene (*GUCY2D*-LCA). As ATSN-101 is a gene therapy requiring an invasive ocular procedure for administration, administration would result in excessive risk and no benefit for healthy subjects. However, AAV-mediated gene replacement has proven safe and effective for the treatment of other retinal diseases in adults and children, including *RPE65*-LCA (for example: 9, 10, 11, 12, 13, 14, 15, 16); AAV therapy is now approved by the FDA for the treatment of *RPE65*-LCA in adults and children (Luxturna™; Spark Therapeutics). Currently, there is no treatment option for adult or pediatric patients with *GUCY2D*-LCA, so participation in a first-in-human clinical study would not result in a lost treatment opportunity.

Women will be included in this first-in-human study to reflect the disease population. To prevent potential harm to an unborn child, pregnant women will be excluded, and pregnancy testing will be performed twice before administration. Double contraceptive measures will be required for both women and men to prevent pregnancy and exposure of women of child-bearing potential for three months after treatment. Additional pregnancy testing will be performed on Day 28 of the study. Subjects will be asked to report pregnancies while participating in this study. Lactating women will be excluded, whether or not they are currently breast feeding.

The first three cohorts in this study (Cohorts 1-3) will enroll subjects with more severe vision loss (BCVA of 20/200 or worse). However, all subjects enrolled in this study must have an intact photoreceptor layer (as determined by OCT) to be included in the study, and therefore even these severely affected subjects have the potential to respond to and benefit from receiving this treatment. The preclinical data gathered in mouse models of *GUCY2D*-LCA indicate that even the lowest dose administered in this study (Cohort 1, 3.3×10^{10} vg/mL) has the potential to demonstrate clinical efficacy and improvement in vision. Subsequent cohorts (Cohorts 4 and 5) may enroll subjects with less severe vision loss (BCVA of 20/80 or worse).

GUCY2D mutations result in a severe, early onset form of LCA that is typically diagnosed during infancy or early childhood. Considering the increased awareness of LCA among pediatricians and the wide availability of molecular testing, there is now a population of pediatric patients potentially available for the proposed study treatment. There are no established treatments available for pediatric patients to treat the severe vision loss associated with

GUCY2D-LCA. ATSN-101, at the doses that will be used in this clinical study, demonstrated a meaningful impact on ERG parameters in a mouse model of *GUCY2D*-LCA.

Pediatric patients will not be included in the dose escalation phase (Part A) and will only be included in the dose expansion phase (Part B). Enrollment of pediatric subjects will only occur once the safety and tolerability of ATSN-101 at the MAD/MTD are considered satisfactory based on review of at least 14 days of safety data from the final dose escalation cohort in Part A. In addition, short-term efficacy data will be available for the MAD/MTD at the time pediatric subjects will be enrolled. The MAD/MTD is the dose that is anticipated to have the highest chance of showing clinical efficacy based on clinical evaluation of Cohorts 1-3. As such, pediatric subjects will be enrolled at a point in the study when an optimal and safe dose has been identified, thereby maximizing the benefit to risk balance.

5.2.3. Risk Assessment

Potential risks associated with ATSN-101 can be informed by other AAV-mediated gene replacement therapies as well as other ocular gene therapies with a similar delivery procedure.

5.2.3.1. Risks Associated with the Surgical Procedure Involving Subretinal Injection

Vitreotomy and subretinal injection(s) have been performed in previous gene therapy clinical studies in both adult and pediatric patients (for example: 9, 10, 11, 12, 13, 14, 15, 16, 27). Based on the published results of these studies, the feasibility of this method of gene transfer has been demonstrated and safety considerations have been elucidated. The potential risks associated with subretinal gene therapy are discussed below. The risks associated with the procedure, and management of the complications of subretinal surgery, are similar in pediatric and adult patients. The human eye has reached approximately its adult size by six years of age (28), and so the same dosing strategy can be used for adult and pediatric populations in this study. The procedure will be performed by a surgeon with expertise in both adult and pediatric surgery.

Specifically associated with subretinal injections, there is a risk of surgical trauma to the retina causing a degree of retinal cell loss (13, 15). There is also a risk of cataract development that could require additional surgery. There is a risk of retinal tears or retinal detachment and there is the possibility that these could lead to worse vision. Exogenous intraocular infection (endophthalmitis) is rare but can occur. The most common pathogens can be treated, but poor vision (in patients with far better vision than those entering this study) can result from infection despite successful antibiotic treatment. Intraocular hemorrhage is rare in patients without bleeding diathesis. Vitreous hemorrhage is benign and usually resolves spontaneously. Choroidal or subretinal hemorrhage is usually self-limited and could affect vision (or potential vision) significantly. Large choroidal or subretinal hemorrhages can be surgically drained, if necessary. There may be corneal abrasion during the course of surgery. This usually resolves spontaneously but may require pressure patching or a bandage contact lens. Persistent corneal abrasion can result in mild corneal clouding. Temporary increases in intraocular pressure can occur following ocular/retinal surgery and are treatable with topical or systemic medications to lower eye pressure. Some of the increases in intraocular pressure after subretinal surgery have been attributable to the topical steroids used to treat inflammation or prevent inflammation – i.e., steroid-induced ocular hypertension. The latter can be treated by decreasing or cessation of the topical medicine and/or adding an anti-glaucoma topical agent. Additional adverse

events (AEs) associated with the procedure can include ocular hyperemia, ocular pain. Risk of local anesthesia is low. Risks associated with general anesthesia include nausea, disorientation, low blood pressure, prolonged unconsciousness or drowsiness, pain, allergic reaction, vein irritation, irregular or fast heartbeat, pneumonia, problems with breathing, stroke, or death. There is a small risk of penetration of the eye or optic nerve from periocular injection and also the possibility of hemorrhage, which can be decompressed if necessary. General and local anesthesia do not pose an additional risk to pediatric patients.

5.2.3.2. Inflammatory Reaction and Immune Response

In clinical studies of other subretinal gene therapy products involving both adult and pediatric subjects, transient mild ocular inflammation has been observed: for example, in 2 of 20 subjects treated with AAV2-hRPE65v2 (voretigene neparvovec) (16). Based on non-clinical toxicology data generated for AAV-*GUCY2D*, the doses that will be used in this clinical study were associated with procedure-related minimal/mild ocular inflammation when peri-procedural corticosteroids were administered. Peri-procedural corticosteroids will be used in the current study to minimize the risk of ocular inflammation. Due to the local administration of ATSN-101, a systemic inflammatory response is unlikely. This is consistent with what has been observed for *RPE65*-LCA in clinical studies, with no clinically significant systemic inflammatory immune response reported (9, 10, 11, 12, 13, 14, 15, 16). Anti-RetGC1, anti-AAV5, and enzyme-linked immunosorbent spot (ELISpot) will be used in this study to monitor humoral and cellular immune response.

There is currently no evidence to suggest that the immune response to vector particles or a gene product in children and adolescents will differ from the adult population. Notably, in studies utilizing AAV vectors subretinal injection was well tolerated in children (16). An increased incidence of adverse immunological reactions in children following subretinal delivery of an ocular gene therapy is therefore considered unlikely. To ensure effective monitoring in children and adolescents, anti-RetGC1, anti-AAV5, and ELISpot will be collected to monitor humoral and cellular immune response.

5.2.3.3. Risks Associated with Study Assessments

Risks associated with visual function tests are similar for adult and pediatric patients and include risks from eye drops. Rarely, a patient may experience redness, discomfort, or an allergic reaction to the eye drops used to dilate the pupil or anesthetize the cornea. Angle closure glaucoma may be precipitated by the dilating drops in a susceptible individual. If a patient has hypertension or cardiac disease, dilating drops can, in rare circumstances, temporarily exacerbate these conditions. Meticulous care with light calibration before each test, a knowledge of the literature on the topic and understanding of the specific disease being studied has prevented harm to the thousands of patients who have been examined with these tests by the investigators over the past 20 years. Phlebotomy has the risks of discomfort, psychological stress leading to syncopal reactions, and hematomas at the site of venipuncture.

5.2.3.4. Risks Associated with Peri-Procedural Medications

The risks associated with the recommended peri-procedural medications, which include oral, topical, and peri-ocularly injected corticosteroids and a topical antibiotic, are similar between adult and pediatric patients. Some of the risks associated with corticosteroids include weight

gain, skin thinning, elevated IOP (particularly with topical and peri-ocular injected corticosteroids), cataract, hypertension, gastritis, peptic ulcer disease, osteoporosis, avascular necrosis, myopathy, euphoria, depression, insomnia, mania/psychosis, hyperglycemia, hypothalamic-pituitary-adrenal axis insufficiency, leukocytosis, and increased risk of infection. In children, systemic corticosteroids can cause impaired growth, however these effects are observed with long-term treatment. In general, the risks associated with corticosteroids increase as a function of dose and time of exposure. Subjects will be exposed to high-dose systemic corticosteroids and topical corticosteroids for a brief period of time (21 days for oral, eight days for topical). Subjects will be seen daily by the Investigator for the first two weeks postoperatively. Subjects will also have IOP monitored daily during the time period when they are receiving topical corticosteroids. Of note, the dose and duration of the corticosteroids may be adjusted after discussion with the study Medical Monitor.

The topical antibiotic trimethoprim/polymyxin can be associated with circumocular rash, burning/stinging sensation of the eyes, eyelid edema, eye pruritis, and eye redness. The topical antibiotic regimen can be adjusted as needed based on the clinical circumstances at the discretion of the Investigator and/or Surgeon (e.g., adverse reactions to the medication).

5.2.4. Specific Parameters Rationale

5.2.4.1. Assessment of Primary Endpoints – Safety and Tolerability

The primary safety endpoint in this study will be the incidence of AEs (AEs, serious adverse events [SAEs], and adverse events of special interest [AESIs]) over a 52-week period following a single subretinal dose of ATSN-101.

Clinically significant changes from baseline in safety assessments (including ocular examination, clinical safety laboratories, vital signs, and immunological tests) will be noted.

Ocular examination: Routine eye examinations will be performed before and after vector administration, and the Surgeon will be alert to intra-operative complications. The clinical ocular examinations for toxicity will include slit lamp examination, applanation tonometry or tonopen, and gonioscopy (as needed in cases of increased intraocular pressure to examine for anterior chamber abnormalities). Direct and indirect ophthalmoscopy and biomicroscopy will be the mainstay of the retinal examinations. Fundus photography (with infrared illumination and autofluorescence) will also be taken to document fundus appearance and optical coherence tomography will be used to obtain cross-sectional images of the retina; both techniques are described further below. Visual acuity will be evaluated using the BCVA score, assessed using ETDRS standardized protocol. The BRVT (26) will be used for subjects with off-chart vision.

Systemic examination: Physical examination will be performed by a medical practitioner at the screening visit; further examinations post-operatively will be performed as needed pending subject symptoms. Hematology, biochemistry, and urinalysis will be performed as described in Section 10.2.2 at the Baseline visit (serving also as a pre-operative evaluation) and on post-treatment evaluations.

Measurements of antibodies to the AAV capsid and RetGC1 will be performed prior to and after vector administration as markers of the humoral immune response. Samples will also be taken for measurement of the cellular immune response by ELISpot, though these will be frozen and

only run if there is evidence of ocular inflammation. At each contact with the subject, information will be obtained on AEs by specific questioning.

Severity of AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE; v5.0), and AEs will be classified by system organ class (SOC)/preferred term (PT) according to the last available version of the Medical Dictionary for Regulatory Activities (MedDRA).

5.2.4.2. Assessment of Secondary (Efficacy) Endpoints

BCVA: Visual acuity will be evaluated using the BCVA score, assessed using ETDRS standardized protocol. The BRVT (26) will be used for subjects with off-chart vision.

Full-field stimulus testing (FST): Candidates for this gene transfer safety study will have severe retina-wide dysfunction. Traditional tests of vision, such as perimetry, cannot be used in most of these subjects because of nystagmus and the lack of stable fixation. Also, the dynamic range of traditional instruments is not sufficient for testing the severe loss of vision. Therefore, a full-field stimulus threshold test (FST) that has been developed for LCA and is based on a commercially-available unmodified Colordome desktop ganzfeld and Espion console (Diagnosys LLC, Littleton, MA, or equivalent) will be used. Full-field nature of the stimulus made the test mostly independent of fixation and achromatic stimuli were detected by all subjects under dark-adapted conditions including those with severe retinopathies; variability of the measure was similar to more traditional dark-adapted psychophysics (29). Thresholds will be measured with blue (peak 465 nm) and red (peak 637 nm) stimuli in the dark-adapted and light-adapted (steady white 2.7 log₁₀ Td background) states using published methods (2, 29, 30). Intervisit variability has been established in LCA patients and the methods have been used in a previous clinical study of gene therapy for *RPE65*-LCA (15) and specifically in patients with *GUCY2D*-LCA (1).

5.2.4.3. Assessment of Exploratory Endpoints

Pupillometry (PLR): The direct transient PLR, an objective measure of visual function, will be recorded in the subjects using a commercially-available instrument (Roland Consult, Brandenburg a.d. Havel, Germany, or equivalent) and methods specifically developed for patients with LCA (1, 10, 15, 34). PLRs will be recorded to full-field achromatic and chromatic stimuli of short (100 ms) and long (4.5 s) duration with increasing intensities (up to 3.6 log cd.m²), presented monocularly in the dark-adapted (>1 hour) state. The difference between the thresholds estimated from two colors is used to determine photoreceptor mediation.

Oculomotor instability (OCI): Patients with severe childhood-onset retinopathies, such as *GUCY2D*-LCA, have a wide spectrum of oculomotor abnormalities. There is limited or no voluntary control of eye position. Measurements of oculomotor instability before and after administration of therapy would be an important assay of change. Conventional measures of ocular movement depending on the electro-oculogram and therefore the corneal-retinal standing potential (electro-nystagmography) would not be useful because of the retinal dysfunction and impaired signal from this method in LCA. Therefore, a method that has been developed to quantify OCI will be employed (35). Video imaging of the eye will be performed with a confocal scanning laser ophthalmoscope (Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany, or equivalent) using near-infrared (815 nm) reflectance mode (30° lens; 25% laser

power setting; 55% detector sensitivity; automatic normalization off; high speed recording mode). The focus will be held constant at +20D for all subjects. Subjects will be instructed to try to “look straight ahead” in primary gaze. The camera head will be moved along the z-axis to bring the iris into focus (without changing the focus knob), and then the camera moved laterally such that both eyelids are visible and centered in the frame, and the medial (nasal) canthus is just visible at the edge of the frame. In a dark room (light from the computer monitor blocked from reaching the subject’s eye), two sets of 30 s long video recordings will be performed per eye. One set will be recorded with the internal fixation turned off and the second set will be recorded with the internal fixation target turned on.

Photoaversion: A photoaversion threshold is determined by testing a series of increasing light intensities using the full-field ganzfeld ColorDome (Diagnosys LLC) without dilation or dark adaptation.

Low-luminance visual acuity (LLVA): LLVA is measured by placing a 2.0-log-unit neutral density filter over the front of each eye and having the subject read the normally illuminated ETDRS chart. Initially, letters are read at a distance of 4 meters from the chart. If <20 letters are read at 4 meters, testing at 1 meter should be performed.

Optical coherence tomography (OCT): A spectral-domain OCT system (RTVue-100, Optovue Inc., Fremont, CA, or equivalent) will be used to acquire the cross-sectional imaging data. Horizontal and vertical scans (9 mm in length, 1019 longitudinal reflectivity profiles [LRPs] each) will be taken through the fovea and in the rod-dominant region of the bleb (typically 10-12 degrees from the fovea at a location specified to represent the bleb) (1, 31, 32, 33).

Visual Function Questionnaire: For Cohorts 1 through 4, the NEI VFQ-25 will be administered and scored as previously described (36). This questionnaire has been used to assess vision-related quality of life and visual function in patients with *GUCY2D*-LCA (1). For Cohort 5, the 25-item Cardiff Visual Ability Questionnaire for Children (CVAQC), which was developed and validated for use in children and young people with visual impairment, will be used as previously described (37). The NEI VFQ-25 and CVAQC will be administered in an interview format.

Patient’s Experience Due to Changes in Vision Questionnaire (PECV): Subject experience data will be captured using an internally developed measure, the PECV questionnaire. Subjects will be asked about changes in vision they have had since receiving treatment with ATSN-101.

Mobility testing: Mobility testing assesses the ability of the subject to navigate standardized obstacle courses at varying light levels, and has been used in prior gene therapy studies for *RPE65*-LCA to assess the impact of the therapeutic intervention on functional vision (13, 15, 16).

5.2.5. Dose Levels

5.2.5.1. Choice of Dose Levels Rationale

The starting dose of ATSN-101 for this study was selected based on a consideration of the pre-clinical efficacy and GLP toxicology data that was generated for AAV5-*GUCY2D*. There were two key measures of efficacy for this gene therapy approach; the impact on visual function (based on ERG) in mouse models of *GUCY2D*-LCA and transduction efficiency in the monkey

retina. The mouse model data indicated that there is a meaningful impact on ERG parameters following subretinal administration of AAV5-*GUCY2D* at doses ranging from 3.3×10^{10} to 1.0×10^{12} vg/mL in a 1 μ L injection volume. Transduction efficiency was measured using subretinal administration of an AAV5-eGFP (enhanced green fluorescent protein) vector followed by immunohistochemistry evaluation of eGFP expression. These results indicated that doses ranging from 3.3×10^{10} to 1.0×10^{12} vg/mL in a 120 μ L injection volume achieved measurable levels of transduction efficiency in the monkey retina. The GLP toxicology data from the three-month monkey study established a NOAEL for AAV5-*GUCY2D* at a dose of 1.0×10^{12} vg/mL in a 150 μ L injection volume when peri-procedural corticosteroids are utilized. Based on these collective data, the starting dose for this study, which will utilize peri-procedural corticosteroids, is 3.3×10^{10} vg/mL, a concentration that is 1.5 log units below the NOAEL established by the three-month toxicology study, in an injection volume of 300 μ L, a volume used frequently in subretinal gene therapy studies of *RPE65*-LCA (15, 16, 38). The GLP toxicology data provide confidence that this should be a safe starting dose, and the efficacy data suggest that there may potentially be an improvement in vision at this dose.

Following completion of the nonclinical pharmacology and GLP toxicology studies, the Good Manufacturing Practices (GMP) material produced for this study was found to be qualitatively more potent than the GLP material used for the pharmacology and GLP toxicology studies based on an *in vitro* assay. In order to further investigate the significance of this *in vitro* result, an additional *in vivo* bridging study was conducted to compare the impact of the GLP and GMP batches of ATSN-101 on the ERG response and retinal thickness in a mouse model of *GUCY2D*-LCA. In this study, the lower of the two doses tested was chosen to correspond to the low dose level for the clinical study (3.3×10^{10} vg/mL). Both batches of ATSN-101 at this dose level caused an improvement in ERG response. The improvement in response caused by these two batches were comparable at the low dose. These data indicate that at a dose of 3.3×10^{10} vg/mL there is not a significant potency difference between the GLP and GMP batches, and they provide further support that 3.3×10^{10} vg/mL has the potential to cause a clinically significant improvement in vision.

The middle dose for the clinical study, 1.0×10^{11} vg/mL, represents a half-log increase above the starting dose. This dose is 10-fold below the NOAEL. It falls within the range of doses that showed a meaningful impact on ERG parameters in the mouse model of *GUCY2D*-LCA and also achieved measurable levels of transduction efficiency in the monkey retina. In the bridging study cited above, a second dose of the GMP and GLP lots of material (1.5×10^{11} vg/mL) comparable to the proposed middle dose for this clinical study (1.0×10^{11} vg/mL) was also tested. Those results indicated that for both the GMP and GLP batches, there is a significant improvement in the ERG response following treatment with this dose of ATSN-101. However, there was a small and statistically significantly larger ERG response following treatment with the GMP material compared with the GLP material. Of note, the magnitude of the ERG response observed with the GMP lot of material was comparable to the ERG response that had been observed in a prior experiment with the GLP material, and so the difference observed between the GMP and GLP batches in ERG response within this bridging study falls within the range of inter-experiment variability. In addition, there was no difference in mean retinal outer nuclear layer thickness in mice treated with either the GLP or GMP material. Given that the middle dose represents a 10-fold safety margin below the NOAEL, the collective pre-clinical data support this as a safe dose.

The highest dose for this study was selected based on careful evaluation of the data from the GLP monkey toxicology studies as well as the non-clinical bridging study. Based on the findings of the three-month monkey GLP toxicology study, the NOAEL was considered to be 1.0×10^{12} vg/mL in a 150 μ L injection volume when AAV5-*GUCY2D* is co-administered with peri-procedural corticosteroids. Further, data from the nonclinical bridging study indicated a small increase in ERG response at the middle dose proposed for the study as described above. Therefore, to account for a potential small increase in potency, the highest dose for this study was selected conservatively at 3.3×10^{11} vg/mL, which is three-fold lower than the NOAEL established by the three-month toxicology study, administered in an injection volume of 300 μ L. This dose also demonstrated robust evidence of efficacy in a mouse model of *GUCY2D*-LCA, as well as robust photoreceptor transduction in the monkey transduction efficiency studies, and so it has the potential to result in significantly improved vision in patients.

5.2.5.2. Dose Escalation Rationale

The dose escalation steps for Part A are provided in Table 1. The dose escalation steps will be half log dose increases, as smaller dose increases would challenge the ability to differentiate between doses in the analytical assay used to determine titer. This dose escalation scheme is typical for AAV gene therapy vectors.

5.2.5.3. Choice of Doses

The following single doses (Table 1) are planned to be administered in an ascending manner. These doses are subject to change based on the method of dose escalation process described in Section 5.2.6.1.

Table 1 Doses for Part A

Cohort	Concentration (vg/mL)	Volume (μ L)	Vector genomes delivered per eye (vg/eye)	Increment factor to NOAEL ^a
1	3.3×10^{10}	300	1.0×10^{10}	30
2	1.0×10^{11}	300	3.0×10^{10}	10
3	3.3×10^{11}	300	1.0×10^{11}	3

^a Calculation of increment factor to NOAEL is based on concentration

Cohorts 4 and 5 (Part B) will be treated at the MTD/MAD established in Part A. Before enrolling pediatric subjects (Cohort 5), a preliminary summary of safety and biological activity data of Part A along with a rationale for the dose to be used in Cohort 5 will be provided to the DMC for review. This information will also be submitted to the Regulatory Authorities and other review committees as required by local site requirements.

5.2.6. Study Conduct and Method of Dose Escalation

5.2.6.1. Method of Dose Escalation

As the first priority is safety, the dose escalation in Part A will progress from the first dose to highest dose based on a continued favorable safety profile. The dose escalation will be paused as

soon as consequences regarding subject protection or significant safety signal are identified that change the benefit risk balance based on the judgement of the Sponsor and Investigator.

The study will be immediately suspended according to the criteria outlined below (Section 5.2.6.2). Subjects will be informed about the study suspension, the reason for the suspension, and any necessary instructions. Once it is confirmed by the Sponsor and Investigator, based on the recommendations of the DMC, that it is safe for the study to continue, subjects will receive all available information allowing them to confirm their decision to continue or not in the study.

A decision to proceed from dose “n” to the next higher “n+1” dose during Part A will be made jointly by the Sponsor and the Investigator based on the recommendations of the DMC review of at least two weeks (14 days) of post-administration data for three subjects in dose level cohort “n”.

The relevant data for this decision should include, at a minimum, ophthalmological examinations (BCVA, slit-lamp, indirect ophthalmoscopy) and AEs.

For Part A, depending on the safety and tolerability profile and the available biological activity observed in the dose level cohort “n,” one of the following decisions will be taken for cohort “n+1”:

- Dose escalation may continue as scheduled.
- The same dose may be administered in additional subjects.
- A higher intermediate dose between the current dose (“n”) and the next planned dose (“n+1”) may be administered to the next cohort.
- A lower intermediate dose between the current dose (“n”) and the previous dose (“n-1”) may be administered to the next cohort, including the possibility of administering a lower dose than the starting dose.
- Part A may be stopped.
- Part B may be initiated before all three cohorts of Part A are completed based on safety, tolerability, and efficacy observed, after review by the DMC.

5.2.6.2. Guidance for Stopping Rules

In addition to close assessment of SAEs and the occurrence/grading of other AEs by the Sponsor and the Investigator, after exploring potential confounding factors, the following criteria would lead to the decision to suspend dose escalation or to stop IMP administration to additional subjects. In this event, the DMC will review the AE and may recommend to stop further IMP administration, continue IMP administration with modifications to the protocol, or continue IMP administration without modifications to the protocol. The decision to stop further IMP administration, continue IMP administration with modifications to the protocol, or continue IMP administration without modifications to the protocol will be made by the Sponsor based on the recommendations of the DMC. Stopping criteria will also be included in the DMC charter.

Criteria Definition

1. Any severe (Grade 3 or higher, as defined by the CTCAE) or persistent (28 days) ocular inflammation without signs of resolution despite treatment
2. Any other significant ocular toxicity (e.g., evidence of direct toxicity, such as retinal degeneration associated with the region of subretinal injection)
3. Any other systemic toxicities (e.g., acute allergic reaction) Grade 3 or higher, as defined by the CTCAE, possibly related to gene therapy
4. Any safety issue that has been identified that adversely changes the benefit/risk balance to study subjects
5. Any serious event that leads to hospitalization of a study subject and could be considered related to the study treatment and/or study procedures

The Sponsor may decide to stop gene therapy administration based on other safety signals not described in the above criteria.

In all cases when the decision to stop the study is taken by the Sponsor, the appropriate Institutional Review Board (IRB)/Ethics Committee and Regulatory Authorities will be informed according to applicable regulatory requirements.

6. STUDY OBJECTIVES

Primary Objective

To evaluate the safety and tolerability of ascending doses of ATSN-101 administered as a unilateral subretinal injection in subjects with Leber Congenital Amaurosis (LCA) caused by autosomal recessive *GUCY2D* mutations (*GUCY2D*-LCA).

Secondary Objective

To evaluate the efficacy of ascending doses of ATSN-101 administered as a unilateral subretinal injection in subjects with *GUCY2D*-LCA.

7. STUDY DESIGN

7.1. Description of the Study

This is a Phase 1/2, multi-center, open-label, unilateral (one eye) dose escalation study of subretinally injected ATSN-101. The study is separated into two parts including a dose escalation phase (Part A) and a dose expansion phase (Part B).

The dose escalation phase will evaluate three ascending dose levels of ATSN-101 in three successive cohorts (Cohorts 1-3). Approximately nine subjects ≥ 18 years of age (three subjects per cohort) are planned. Up to 15 subjects may be included based on the need for additional cohorts at intermediate doses or to replicate dose levels.

For Cohort 1 of the dose escalation phase, there will be an interval of at least 14 days between dosing of each of the three subjects that will be observed in order to assess safety. For the remaining dose escalation phase cohorts (Cohorts 2 and 3), there will be an interval of at least 14 days between dosing the first and second subject and an interval of at least seven days between dosing the second and third subject that will be observed in order to assess safety.

The dose expansion phase (Part B) will include two cohorts, both treated at the MTD or MAD determined from Part A. These two cohorts will enable further characterization of the risk-benefit profile of ATSN-101 in the adult (Cohort 4) and pediatric (Cohort 5) populations.

Cohort 4 will include approximately three subjects ≥ 18 years of age and Cohort 5 will include approximately three subjects ≥ 6 years of age and < 18 years of age. For Cohort 4, there will be an interval of at least seven days between dosing each subject that will be observed to assess safety. For Cohort 5, there will be an interval of at least 14 days between dosing each subject that will be observed to assess safety.

After completion of the 52-week Main Study Period, subjects in all five cohorts will continue in the Extension Study Period. The 208-week Extension Study Period will further evaluate the safety and efficacy of ATSN-101.

Data Monitoring Committee (DMC):

At least 14 days of safety data will be reviewed by an independent DMC for each cohort prior to dose escalation.

The DMC will also review at least 14 days of safety data from the final dose escalation cohort in Part A prior to enrolling Part B.

New cohorts will be initiated only after agreement by the Sponsor and DMC that the safety and tolerability demonstrated in prior cohorts support further dosing.

Before enrolling pediatric subjects (Cohort 5), a preliminary summary of safety and biological activity data of Part A along with a rationale for the dose to be used in Cohort 5 will be provided to the DMC for review. This information will also be submitted to the Regulatory Authorities and other review committees as required by local site requirements.

The DMC will meet at least once more after the completion of the Main Study Period of the last subject to monitor safety.

Graphical study design/flow charts – please refer to flow charts in Section 2.

7.2. Duration of Study Participation

7.2.1. Duration of Study Participation for Each Subject

- Screening duration: Up to 49 days
- Baseline duration: Seven days
- Main Study Period (from IMP administration to Week 52): Approximately 52 weeks including one treatment day
- Extension Study Period (Week 52 to Week 260): Approximately 208 weeks
- End of study: Approximately 260 weeks after IMP administration
- Total duration from Screening per subject: Up to approximately 268 weeks

7.2.2. Determination of End of Clinical Study (All Subjects)

The end of the clinical study is defined as the day the last subject completes his/her last study visit.

7.3. Interim Analysis

Interim analyses may be performed in case of unexpected safety concerns and/or for internal decision making. The outcome of interim analyses may lead to early termination of the study, continuation without any changes, or continuation with changes.

8. SELECTION OF SUBJECTS

8.1. Number of Subjects Planned

Part A:

Approximately nine subjects, but up to 15 based on need for additional cohorts at intermediate doses or to replicate dose levels.

Part B:

Cohort 4: Approximately three subjects

Cohort 5: Approximately three subjects

8.2. Inclusion Criteria

- I 01. Male or female patient with clinical diagnosis of Leber congenital amaurosis caused by biallelic mutations in the *GUCY2D* (retinal guanylate cyclase) gene with all of the following:
 - a) Documented mutations in both alleles of the *GUCY2D* gene per testing by a CLIA-approved laboratory
 - b) For Cohort 1 through 3, best corrected visual acuity (BCVA) of 20/200 or worse in the eye to be injected; subsequent cohorts may include BCVA of 20/80 or worse in the eye to be injected
 - c) Photoreceptor (outer nuclear) layer structure identifiable on an optical coherence tomography (OCT) scan across the central retina with evidence of residual foveal structure
- I 02. Age ≥ 18 years for Cohorts 1 through 4, and age ≥ 6 years and < 18 years for Cohort 5
- I 03. Women of childbearing potential must follow the contraception requirements as described in [Appendix A](#)
- I 04. Male patients must follow the contraception requirements as described in [Appendix A](#)
- I 05. Signed and dated written informed consent (or assent in the case of minors from their legal guardian/representative), in accordance with the local regulations
- I 06. Patients must agree to not donate blood, organs, tissues, cells, or sperm for at least three months following ATSN-101 administration

8.3. Exclusion Criteria

Patients who have met all the inclusion criteria listed in Section 8.2 will be screened for the following exclusion criteria:

- E 01. Complicating systemic diseases (such as medical conditions causing immunosuppression) that would preclude the gene transfer, ocular surgery, or planned study procedures
- E 02. History of HIV infection

- E 03. Pre-existing eye conditions in the study eye that would preclude the planned surgery or interfere with the assessment and interpretation of study endpoints: for example, glaucoma or optic neuropathy that has resulted in significant visual loss, corneal or lenticular abnormalities or opacities that would preclude view of the fundus or performance of the outcome measures, uveitis, retinopathy, and maculopathy that in the opinion of the Investigator are causing significant visual loss
- E 04. Presence of significant ocular abnormalities in the study eye that in the opinion of the Investigator would preclude the planned surgery, effective safety follow-up, or interfere with the interpretation of study endpoints (e.g., glaucoma, corneal or significant lens abnormalities or opacities, pre-existing uveitis, intraocular infection, choroidal neovascularization)
- E 05. Any contraindication to the planned surgical procedure, such as contraindications to the use of anesthesia or allergy to medications planned in the peri-operative period
- E 06. Known allergy or hypersensitivity to any component of the IMP, diagnostic agents used during the study, or medications planned for use in the peri-operative period, particularly corticosteroids
- E 07. Women who are pregnant (defined as positive beta-HCG blood or urine test), lactating, or breastfeeding
- E 08. Any ocular procedure, either planned or performed, within six months of Day 1, which would interfere with the planned surgery or the interpretation of study endpoints in the opinion of the Investigator
- E 09. Laboratory test abnormalities or abnormalities in electrocardiogram that in the opinion of the PI would make the patient unsuitable for participation in the study
- E 10. Significant (as determined by the Investigator) intercurrent illness or infection during the 28 days prior to enrollment
- E 11. Current substance use disorder
- E 12. Use of any investigational agent administered within five times the elimination half-life of that investigational agent prior to ATSN-101 administration
- E 13. Enrollment in any other clinical treatment study, for any condition, including those relating to *GUCY2D*-LCA, throughout the duration of the ATSN-101 study participation
- E 14. Use of anticoagulation therapy within two weeks prior to surgery
- E 15. Use of systemic immunosuppressive medications
- E 16. Current, planned during the course of this study, or past (within five times the elimination half-life of that therapy prior to ATSN-101 administration) use of anti-viral therapy that would inactivate the investigational agent
- E 17. Received gene therapy within the last 15 years
- E 18. Any patient who, in the judgment of the Investigator, is likely to be noncompliant during the study, or unable to cooperate with the study procedures

-
- E 19. Any patient who is the Investigator or any sub-investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in conducting the study

9. TREATMENTS

9.1. Investigational Medicinal Product(s)

A complete description of the IMP and its proper handling will be provided in a Pharmacy Manual available at the clinical site.

9.1.1. AAV5-*GUCY2D* Gene Therapy (ATSN-101; Previously Referred to as SAR439483)

- **ATSN-101 Solution for Injection:**

- Solution for intraocular administration
- Each single-use vial contains [REDACTED]
[REDACTED]
[REDACTED].
- The drug product is packaged in a glass vial, stopper, crimp seal, and to be stored upright at or below -60°C (-76°F).
- Dose of ATSN-101 per administration will be dependent on dose cohort. For planned dose levels, see [Table 2](#).
- Duration of treatment: one single dose

- **ATSN-101 Diluent Solution:**

- Diluent solution for parenteral use
- Each single-use vial contains [REDACTED]
[REDACTED]
[REDACTED].
- The diluent is packaged in a glass vial, stopper, crimp seal, and to be stored upright at or below -60°C (-76°F).
- Duration of treatment: one single dose

- **Dose of Drug Per Administration:**

The IMP will be administered as a single subretinal injection into the study eye at the doses indicated in [Table 2](#). The study eye will be the eye that in the opinion of the Investigator and in consultation with the subject, is the worse seeing eye. In order to select the worse eye, the Investigator will review the subject's historical and Screening visual acuity and visual field data and also consider which eye is dominant and any preferences shown by the subject.

- **Method of Administration:**

The IMP will be administered via subretinal injection (location: macular area) by a trained vitreoretinal surgeon with pediatric experience. The surgical protocol and

post-operative care will be detailed in a separate manual. The subject will be observed for approximately four hours at the surgical site post-operatively, with the final duration determined by the Surgeon and/or Investigator. If required based on clinical circumstances, the subject may be admitted for overnight observation in the hospital at the discretion of the Surgeon and/or Investigator.

Table 2 Planned Unilateral Doses by Cohort

	Cohort ^a	Age (years)	Number of subjects	Vector genomes per mL	Vector genomes per eye	Volume (μL)
A	1	≥18	3	3.3×10 ¹⁰	1.0×10 ¹⁰	300
	2	≥18	3	1.0×10 ¹¹	3.0×10 ¹⁰	300
	3	≥18	3	3.3×10 ¹¹	1.0×10 ¹¹	300
B	4	≥18	3	TBD ^b	TBD ^b	300
	5	≥6 and <18	3	TBD ^b	TBD ^b	300

^a Additional cohorts may be included in Part A for intermediate dose levels, or to replicate doses as described above.

^b MTD or MAD

9.2. Non-investigational Medicinal Products

The following non-investigational medicinal products (NIMPs) comprise the recommended peri-procedural corticosteroid and topical antibiotic medication regimen. These NIMPs will be provided by the site. This regimen may not be changed without prior discussion with the study Medical Monitor.

9.2.1. Prednisone

- Prednisone tablet (or equivalent)
- **Dose of Drug Per Administration:** 1 mg/kg/day oral (PO) prednisone (up to a maximum dose of 80 mg) administered in the morning (approximately 8AM) given on Day (D) -1, D1, D2, D3, D4, D5, and D6; followed by 0.5 mg/kg/day PO prednisone for seven days; followed by 0.25 mg/kg/day PO prednisone for four days; and followed by 0.125 mg/kg/day PO prednisone for three days (21 days in total).

9.2.2. Triamcinalone acetonide

- Triamcinalone acetonide, suspension, 40 mg/mL (or equivalent)
- **Dose of Drug Per Administration:** Triamcinalone acetonide 20 mg in 0.5 mL administered as a peri-ocular injection at the time of the procedure.

9.2.3. Prednisolone

- 1% prednisolone suspension (or equivalent)
- **Dose of Drug Per Administration:** one drop 4 times a day (QID) given D2 through D9.

9.2.4. Trimethoprim and Polymyxin B

- Trimethoprim 1 mg and polymyxin B sulfate 10,000 units per 1 mL solution (or equivalent)
- **Dose of Drug Per Administration:** one drop QID given D2 through D9.

9.3. Method of Assigning Subjects to Treatment Group

Before including a subject on Day 1, the Investigator or designee should confirm that the subject meets all of the inclusion criteria and none of the exclusion criteria.

9.4. Packaging and Labeling

The IMP described in Section 9.1 is contained in single use vials that will be provided to the study center by the Sponsor or designee. The IMP is packaged and labeled according to GMP and local regulatory specifications and requirements. Refer to the pharmacy manual for additional details.

9.5. Storage Conditions and Shelf Life

ATSN-101 is a frozen liquid formulation, stored at $\leq -60^{\circ}\text{C}$, in aliquots of 0.75 mL. Investigators or other authorized persons (e.g., pharmacists) are responsible for storing IMP provided by the Sponsor in a secure and safe place in accordance with local regulations, labeling specifications, policies, and procedures.

Control of storage conditions for IMP, especially control of temperature (e.g., frozen storage) and information on in-use stability and instructions for handling the Atsena Therapeutics, Inc. IMP should be managed according to the rules provided by the Sponsor.

9.6. Responsibilities

The Investigator, the clinical site pharmacist, or other personnel allowed to store and dispense IMP/NIMP will be responsible for ensuring that the IMP/NIMP used in the clinical study is securely maintained as specified by the Sponsor and in accordance with the applicable regulatory requirements.

All IMP/NIMP shall be dispensed in accordance with the Investigator's prescription, and it is the Investigator's responsibility to ensure that an accurate record of IMP/NIMP issued and returned is maintained.

Any quality issue noticed with the receipt or use of an IMP provided by the Sponsor (deficiency in condition, packaging, appearance, pertaining documentation, labeling, expiration date, etc.) should be promptly notified to the Sponsor, who will initiate a complaint procedure.

A potential defect in the quality of the IMP provided by the Sponsor may be subject to initiation of a recall procedure by the Sponsor. In this case, the Investigator will be responsible for promptly addressing any request made by the Sponsor, in order to recall the IMP and eliminate potential hazards.

Under no circumstances will the Investigator supply the IMP provided by the Sponsor to a third party, allow the IMP provided by the Sponsor to be used other than as directed by this clinical study protocol, or dispose of the IMP provided by the Sponsor in any other manner.

9.7. Concomitant Medication

Refer to the exclusion criteria (Section 8.3) for information on which medications are not allowed during this study. If a specific treatment is required for any reason, an accurate record must be entered in the electronic case report form (eCRF), including the name of the medication (international nonproprietary name), daily dosage, and duration of use. This information will be collected from the informed consent to the last study visit and includes information regarding NIMP use.

9.8. Treatment Accountability and Compliance

- Compliance of IMP:
 - The IMP will be administered as a single subretinal injection into the study eye and an appropriate record will be made in the source data by the Investigator or delegate.
 - The Investigator records the dosing information on the appropriate page(s) of the case report form.
- Compliance of NIMP:
 - Compliance to NIMP dosing regimen will be documented in the source data by the Investigator or delegate and entered in the eCRF.
- Accountability of NIMP/IMP:
 - An accurate accountability record of products received, dispensed, discarded, and returned or destroyed by the pharmacy at the Investigator site must be maintained on the Product accountability and Inventory forms at site level by site staff.
 - The Site Monitor will perform documentation checks during monitoring visits. A final reconciliation will be performed on all accountability documentation at the end of the study by the Site Monitor together with the site personnel responsible for product management.

9.9. Return and/or Destruction of Treatments

Whenever possible, all partially used, used, or unused IMP/NIMP will be destroyed on-site according to the standard practices and capabilities of the site after reconciliation verification by the monitor.

A detailed treatment log of the destroyed IMP/NIMP will be established with the Investigator (or the pharmacist) and countersigned by the Investigator and the monitoring team.

The Investigator will not destroy any IMP/NIMP unless the Sponsor provides written authorization. When destruction at site cannot be performed, all IMP will be retrieved by the Sponsor or designee for destruction.

10. ASSESSMENT OF INVESTIGATIONAL MEDICINAL PRODUCT

10.1. Activity or Pharmacodynamics

10.1.1. Assessment Methods

Best-Corrected Visual Acuity

The visual acuity (VA) will be evaluated using the BCVA score, assessed using the ETDRS standardized protocol. The BRVT will also be applied for subjects with off-chart BCVA.

Full-Field Stimulus Testing (FST)

Subjects will be dilated and dark-adapted (>45 min) first and then will be positioned in front of the FST stimulator. Test sessions will be performed in each eye while the contralateral eye is “triple” patched with a combination of adhesive eye patch (such as Opticlude, Nexcare, 3M Health Care, St. Paul, MN, or equivalent), black opaque photographic tape (Scotch, 3M, St. Paul, MN, or equivalent), and black eye patch with elastic band (such as Wilson Ophthalmic, Palatine, IL, or equivalent). Pre-testing with the white stimulus is used for explanation of the test and practice at performance. All fixation lights are turned off and infrared video monitoring is used throughout the session to confirm open eyelids and attentive subjects. Each uniocular test session will consist of repeated measurements of psychophysical threshold to a full-field stimulus using white, red and blue flashes of 200 ms duration. Five thresholds will be collected for each color. A short pause will be given between threshold determinations to avoid fatigue. A light-adapted (steady white 2.7 log₁₀ Td background) FST session with the same stimuli will follow the dark-adapted one. Repeat testing will be performed at similar times of the day. Details of this method of evaluating visual function in individuals with severe retinal degenerations and specifically in patients with *GUCY2D*-LCA have been published (1, 29).

Pupillometry

The direct PLR will be recorded with the pupillometer (Roland Consult, Brandenburg a.d. Havel, Germany, or equivalent) as published (39) using methods appropriate for LCA patients (2, 10, 15, 34). PLRs will be recorded to full-field achromatic and chromatic stimuli of short (100 ms) and long (4.5 s) duration with increasing intensities (up to 3.6 log cd.m²), presented monocularly in the dark-adapted (>1 hour) state.

Oculomotor Instability

Video imaging of the eye will be performed with a confocal scanning laser ophthalmoscope (such as Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany, or equivalent) using near-infrared (815 nm) reflectance mode (30° lens; 25% laser power setting; 55% detector sensitivity; automatic normalization off; high speed recording mode). The focus will be held constant at +20D for all subjects. The subject will be instructed to “look straight ahead” in primary gaze. The camera head will be moved along the z-axis to bring the iris into focus (without changing the focus knob), and then the camera moved laterally such that both eyelids are visible and centered in the frame, and the medial (nasal) canthus is just visible at the edge of the frame. In a dark room (light from the computer monitor blocked from reaching the subject’s eye), two sets of 30 s long video recordings will be performed per eye. One set will be recorded with the internal fixation turned off and the second set will be recorded with the internal fixation target turned on.

Photoaversion

A photoaversion threshold is determined by testing a series of increasing light intensities using the full-field ganzfeld ColorDome (Diagnosys LLC) without dilation or dark adaptation.

Low-luminance Visual Acuity

LLVA is measured by placing a 2.0-log-unit neutral density filter over the front of each eye and having the subject read the normally illuminated ETDRS chart.

Initially, letters are read at a distance of 4 meters from the chart. If <20 letters are read at 4 meters, testing at 1 meter should be performed.

Optical Coherence Tomography

A spectral-domain OCT system (RTVue-100, Optovue Inc., Fremont, CA, or equivalent) will be used to acquire the cross-sectional imaging data.

Horizontal and vertical scans (9 mm in length, 1019 LRP each) will be taken through the fovea and in the rod-dominant region of the bleb (typically 10-12 degrees from the fovea at a location specified to represent the bleb) (1, 32, 33, 34).

Fundus Imaging

En face imaging will be performed with a confocal scanning laser ophthalmoscope (such as Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany, or equivalent) to obtain near-infrared (NIR) reflectance, and NIR excited reduced-illumination autofluorescence. All images will be acquired with the high-speed mode wherein either 30°×30° square or 55° diameter circular fields are sampled onto 768×768 pixels.

Visual Function Questionnaire

The NEI VFQ-25 and CVAQC will be administered and scored as previously described (36, 37). To be administered (interviewer-administered) either by phone up to 3 days (± 2 days) prior to the study visit day or, if preferable to the subject and/or Investigator, on the study visit day. This questionnaire should be performed prior to any other study-related activities, and prior to discussion of the subject's treatment or health status, or similar activities. The PECV should be performed first and the visual function questionnaire subsequently.

Patient Experience Due to Changes in Vision Questionnaire (PECV)

Subject experience data will be captured using an internally developed measure, the PECV questionnaire. Subjects will be asked about changes in vision they have had since receiving treatment with ATSN-101. To be administered (interviewer-administered) either by phone up to 3 days (± 2 days) prior to the study visit day or, if preferable to the subject and/or Investigator, on the study visit day. This questionnaire should be performed prior to any other study-related activities, and prior to discussion of study participant's treatment or health status, or similar activities. The PECV should be performed first and the visual function questionnaire subsequently.

Mobility Testing

A mobility course system will be used in the study utilizing pre-validated levels. Several different mobility course layouts will be provided for testing. The different layouts are designed to vary in difficulty, with harder courses featuring low contrast pathways and hard to see obstacles, and easier courses featuring high contrast pathways and easy to see obstacles.

10.1.2. Assessment Schedule

The assessment timing can be found in the study flow chart (Section 2.2).

10.2. Safety

10.2.1. Baseline Demographic Characteristics

Baseline demographic characteristics will consist of:

1. Age (years)
2. Height (cm)
3. Body mass index
4. Race
5. Gender

Subject race or ethnicity will be collected in this study, because analysis of results according to race/ethnicity/demographics are required by several health authorities.

10.2.2. Safety Assessment at Screening, Baseline, and During the Study

The tolerability investigations at Screening, Baseline, and during the study will consist of:

1. Adverse events, spontaneously reported by the subject or observed by the Investigator, will be monitored

2. Physical examination (includes at a minimum: heart and respiratory auscultation; peripheral arterial pulse; peripheral lymph nodes and abdomen examination)
3. Body weight (kg)
4. Oral body temperature
5. Electrocardiogram
6. Vital signs (heart rate, systolic and diastolic blood pressure measured after 10 minutes in supine resting position)
7. Laboratory tests:
 - Hematology: red blood cell count, hematocrit, hemoglobin, white blood cell count with differential count (neutrophils, eosinophils, basophils, monocytes, and lymphocytes), platelets, international normalized ratio, and activated partial thromboplastin time
 - Biochemistry:
 - Plasma/serum electrolytes: sodium, potassium, chloride, calcium,
 - Liver function: aspartate aminotransferase, alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase, total and conjugated bilirubin,
 - Renal function: urea, creatinine,
 - Metabolism: glucose, albumin, total proteins, total cholesterol, triglycerides,
 - Potential muscle toxicity: creatine phosphokinase
8. Archival blood sample: a 3.5 mL blood sample will be collected into a dry, gold topped tube, decanted at room temperature no more than 30 minutes following collection, then centrifuged at approximately 1300-2000 g for 10 minutes at 15-25°C; the serum will then be transferred into one labelled cryovial, which will be immediately capped and frozen in an upright position at $\leq -60^{\circ}\text{C}$. This sample will be used if any unexpected safety issue occurs to ensure that a pre-administration baseline value is available for previously non-assessed parameters (e.g., serology). If this sample is not used, the Investigator will destroy it after the Sponsor's approval.
9. Serology tests: anti-human immunodeficiency virus (HIV) 1 and anti-HIV2 antibodies
10. Urinalysis will include measurement of glucose, protein, erythrocytes, and leukocytes
11. If woman of childbearing potential, beta-HCG plasma test at Screening and urine pregnancy test at Baseline and Day 28
12. If postmenopausal female, plasma follicle-stimulating hormone test
13. Immune response to ATSN-101:
 - a) Anti-drug antibody (ADA) will be measured via detection of anti-AAV5 and anti-RetGC1 antibodies. If two consecutive post-procedure values are decreasing, then subsequent draws are not required.
 - b) Samples will be collected and frozen for the cellular immune response to be measured by ELISpot. Samples will be stored and will only be run if clinical evidence of ocular inflammation is observed.
14. Ocular examinations: BCVA, slit lamp, ophthalmoscopy, fundus photography:
 - a) The examinations will include evaluation of BCVA, the condition of the lid/lashes, conjunctiva, cornea, anterior chamber, iris, and (through a dilated pupil) lens, vitreous, fundus, and optic nerve. Graded observations will be recorded using a five-point scale. Pupils have to be dilated by application of a mydriatic agent

- (i.e., tropicamide or equivalent). Fundus photography (with infrared illumination and autofluorescence) will also be taken to document fundus appearance.
- b) Ocular findings that are deemed clinically significant will be reported as such on the eCRF page. Significant findings made after the screening visit that meet the definition of an AE must be recorded in the AE eCRF page.
15. Optical coherence tomography:
- a) OCT will be used to obtain cross sectional images of the retina. Horizontal and vertical scans will be taken through the fovea.
16. Intraocular Pressure:
- a) IOP will be measured using slit-lamp applanation tonometry, Tonopen, or iCare tonometer. The measurement along with the date and time of day the measurement was made will be recorded in the eCRF.

10.2.3. Sample Handling Procedure

Instructions for Humoral and Cellular Immune response sample preparation and shipping are summarized in Table 3 and Table 4, respectively. Further details will be provided to the study site in the Laboratory Manual.

Table 3 Summary of handling procedures for humoral immune response (anti-AAV5 and anti-RetGC1)

Blood Sample Volume	3.5 mL
Anticoagulant tube type	Blood will be collected into a 3.5 mL vacutainer tube containing clot activator (serum separator tube)
Handling procedures	Instructions included in laboratory manual
Serum aliquot split	2 aliquots (0.5 mL in aliquots 1 and 2)
Serum storage conditions	Polypropylene tubes with screw caps (e.g., Nunc 1.8 mL cryovial tubes). Frozen promptly at $\leq -60^{\circ}\text{C}$.
Serum shipment conditions	Dry ice

Table 4 Summary of handling procedures for cellular immune response (ELISpot)

Blood Sample Volume	24 mL
Anticoagulant tube type	Blood will be collected into three 8 mL BD Vacutainer® CPT™ tubes containing sodium heparin
Handling procedures	Instructions included in laboratory manual
PBMC aliquot split	1 mL aliquots each containing 50×10^6 cells/mL/tube
PBMC storage conditions	Nunc 1.8 mL cryovial tubes. Frozen promptly at -80°C .
PBMC shipment conditions	Dry ice

10.3. Sampled Blood Volume

Table 5 Approximate sampled blood volume

Type	Approximate Volume Per Sample (mL)	Sample Number	Total Volume (mL)
HIV Serology	3.5	1	3.5
Hematology	5	9	45
Biochemistry	10.5	9	94.5
Plasma FSH (if applicable)	3.5	1	3.5
Pregnancy test (if applicable)	3.5	1	3.5
ELISpot	24	9	216
Anti-AAV5/Anti-RetGC1	3.5	11	38.5
LCA genetic testing (if applicable)	5	1	5
Archival sample	3.5	1	3.5
Total for Males ^a			406
Total for Females ^a			413

^a The total approximate blood volumes are the same for adult and pediatric subjects.

10.4. Future Use of Samples

Not all of the samples collected during this study may be required for the tests planned in this clinical study. For subjects who have consented to it, any unused specimens collected during this study will be stored for up to 15 years. The samples may be used for other research purposes related to ophthalmology than those defined in the present protocol.

These other research analyses will help to understand either disease subtypes or therapeutic response, or to develop and/or validate a bioassay method, or to identify new therapeutic targets or biomarkers.

These samples will remain labeled with the same identifier as the one used during the study (i.e., subject ID). They will be transferred to an Atsena Therapeutics, Inc. site (or a subcontractor site), which can be located outside of the country where the study is conducted. The Sponsor has included safeguards for protecting subject confidentiality and personal data (see Section 17.3 and Section 17.5).

11. SUBJECT SAFETY

The Investigator is the primary person responsible for making all clinically relevant decisions on safety issues.

If judged necessary, the opinion of a Specialist (with pediatric expertise, when appropriate, for subjects in Cohort 5) should be sought in a timely manner (e.g., acute renal failure, convulsions, skin rashes, angioedema, cardiac arrest, electrocardiographic changes).

11.1. Adverse Event Monitoring

All events will be managed and reported in compliance with all applicable regulations and included in the final clinical study report (CSR).

11.2. Definitions of Adverse Events

11.2.1. Adverse Event

An AE is any untoward medical occurrence in a subject that develops or worsens in severity during the conduct of a clinical study of a pharmaceutical product and does not necessarily have a causal relationship to the investigational product. This can, therefore, be any unfavorable and unintended physical sign, symptom, or laboratory parameter that develops or worsens in severity during the course of the study, whether or not considered related to the investigational product.

All AEs must be described in the appropriate section of the eCRF and their seriousness and putative relationship (causality) to the study medication and/or protocol procedures noted. Adverse events are recorded following signature of the informed consent; this is to ensure that any AEs associated with the screening procedures are captured.

11.3. Severity of an Adverse Event

Intensity of AEs will be graded according to CTCAE (v5.0) and AEs will be classified by SOC/PT according to the last available version of the MedDRA dictionary. The Investigator will be required to assess the intensity of the AE/biologic experience using the CTCAE general guideline:

- **Grade 1:** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2:** Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL). Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
- **Grade 3:** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. Self-care ADL refer to bathing, dressing, and undressing, feeding self, using the toilet, taking medications and not bedridden.
- **Grade 4:** Life-threatening consequences; urgent intervention indicated.
- **Grade 5:** Death related to AE.

11.3.1. Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death, or,
- Is life-threatening, or,

Note: The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- Requires inpatient hospitalization or prolongation of existing hospitalization, or,
- Results in persistent or significant disability/incapacity, or,
- Is a congenital anomaly/birth defect, or,
- Is a medically important event:
 - Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the definition above.

11.3.2. Adverse Event of Special Interest

An AESI is an AE (serious or non-serious) of scientific and medical concern specific to the Sponsor’s product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them. Adverse events of special interest may be added or removed during a study by protocol amendment. Reporting guidelines for AESIs are provided in Section 11.4.3.

The following AEs are considered AESIs. This list includes all AESIs defined by Atsena Therapeutics, Inc. for any program, although these events have not been observed with serious adverse reaction.

Acute hypersensitivity/anaphylaxis.

Pregnancy of a female subject occurring at any time after treatment with IMP/NIMP.

Pregnancy occurring in a female partner of a male subject if within three months of her male partner treatment with IMP/NIMP.

The pregnancy will be qualified as an SAE only if it fulfills one of the seriousness criteria (see Section 11.3.1).

Follow-up of the pregnancy in a female subject or in a female partner of a male subject is mandatory until the outcome has been determined.

Symptomatic Overdose (Serious or Non-Serious) with IMP:

An overdose (accidental or intentional) with the IMP is an event suspected by the Investigator or spontaneously notified by the subject and defined as at least 30% more than the intended dose.

Of note, asymptomatic overdose has to be reported as a standard AE.

Increase in ALT ≥ 2 times the upper limit of normal.

The Following Adverse Events Are Defined as Project Specific AESIs:

Ocular infection, particularly any opportunistic infection,

Immunological reactions (e.g., new incidence or exacerbation of rheumatologic or other autoimmune disorder).

Ocular AESIs:

AEs that cause a decrease in VA of ≥ 15 ETDRS letters or $\geq +0.3$ LogMAR (compared with baseline or the VA assessment at the latest visit) lasting >1 week or two successive visits,

AEs that require surgical intervention (e.g., conventional surgery, vitreous tap or biopsy with intravitreal injection of anti-infectives, or laser or retinal cryopexy with gas) to prevent permanent loss of sight,

AEs associated with severe intraocular inflammation (i.e., >2 step increase in anterior chamber cells and flare or a >2 step increase in vitreous haze),

AEs that, in the opinion of the Investigator, may require medical intervention to prevent permanent loss of sight.

11.4. Obligation of the Investigator Regarding Safety Reporting

11.4.1. General Guidelines for Reporting Adverse Events

All AEs, regardless of seriousness or relationship to IMP/NIMP, spanning from the signature of the informed consent form until the end of the study as defined by the protocol for that subject, are to be recorded on the corresponding page(s) or screen(s) of the eCRF.

Whenever possible, diagnosis or single syndrome should be reported instead of symptoms. The Investigator should specify the date of onset, intensity (see definitions in Section 11.2), action taken with respect to IMP/NIMP, corrective treatment/therapy given, additional investigations performed (e.g., in the case of dermatologic lesions photographs are required), outcome, and Investigator's opinion as to whether there is a reasonable possibility that the AE was caused by the IMP/NIMP.

In order to ensure the safety of the subject, the Investigator should take appropriate measures to follow all AEs until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized, or until death. This may imply that observations will continue beyond the last planned visit per protocol, and that additional investigations may be requested by the monitoring team.

If the study is prematurely discontinued, subjects are to be assessed using the procedure planned for the end-of-study visit (Week 260) as defined by the protocol.

Laboratory and vital signs, or electrocardiogram (ECG) abnormalities are to be recorded as AEs in the eCRF only if:

- Symptomatic, and/or,
- Requiring either corrective treatment or consultation, and/or,
- Leading to IMP/NIMP discontinuation or modification of dosing, and/or,
- Fulfilling a seriousness criterion, and/or,
- Defined as an AESI.

11.4.2. Guidelines for Reporting Serious Adverse Events

In the case of occurrence of an SAE, the Investigator or any designees must immediately:

- ENTER, in the eCRF (within 24 hours), the information related to the SAE in the appropriate screens of the eCRF; the system will automatically send a notification to the Sponsor after approval of the Investigator within the eCRF.
- SEND (Atsenatx@medcomminc.com) a photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the subject's identity is protected and the subject's identifiers in the clinical study are properly mentioned on any copy of a source document provided to the Sponsor. For laboratory results, include the laboratory normal ranges.
- All further data updates should be recorded in the eCRF as appropriate, and further documentation as well as additional information (for laboratory data, concomitant medications, subject status, etc.) should be sent (Atsenatx@medcomminc.com) to the monitoring team within 24 hours of knowledge of the follow-up information. In addition, every effort should be made to provide further documentation of any SAE that is fatal or life-threatening within a week (seven days) of the initial notification.

Any SAE brought to the attention of the Investigator at any time after the end of the study for the subject and considered by him/her to be caused by the IMP with a reasonable possibility, should be reported to the Sponsor.

11.4.3. Guidelines for Reporting Adverse Events of Special Interest

The need for specific monitoring, documentation, and management of AESIs are described in this section.

For each defined AESI, consider carefully the need to collect additional specific information that would impact the study, such as:

- Preexisting related condition or lifestyle of interest for the AE (e.g., habits, cardiovascular risk factor).
- Expected list of associated signs and symptoms.
- Corrective actions (e.g., treatment discontinuation, concomitant treatment).
- Diagnostic actions (e.g., test(s) or procedure(s) results).

- Additional descriptive factors.
- Sequelae.

For AESI, the Sponsor is to be informed immediately (i.e., within 24 hours) even if a seriousness criterion is not met, using the corresponding pages of the case report form (to be sent) or screens in the eCRF.

Refer to Section 11.3.2 for the definition of AESI.

11.4.4. Guidelines for Management of Specific Laboratory Abnormalities

Once the subject is included in the clinical study, the following laboratory abnormalities must be monitored, documented, and managed according to standard of care.

- Neutropenia
- Thrombocytopenia
- Increase of ALT
- Acute renal impairment
- Increase in creatine phosphokinase suspected to be on non-cardiac origin and not related to an intensive physical activity

11.5. Obligations of the Sponsor

During the course of the study, the Sponsor will report in an expedited manner:

- All SAEs that are both unexpected and at least reasonably related to the IMP (suspected unexpected serious adverse reaction), to the Health Authorities, IRBs/Independent Ethics Committees (IECs) as appropriate and to the Investigators.
- The AESI deemed medically important by Sponsor will be reported to the Health Authorities.

Any AE not listed as an expected event in the Investigator's Brochure or in this protocol will be considered as unexpected.

Adverse events that are considered as expected events will be specified in the reference safety information (Investigator's Brochure).

The Sponsor will report all safety observations made during the conduct of the study in the CSR.

12. HANDLING OF SUBJECT WITHDRAWAL

12.1. Withdrawal Criteria

Once ATSN-101 has been administered into the eye, it cannot be removed, therefore subjects are unable to withdraw from the study treatment. However, an individual may withdraw consent for any study-related procedures at any time throughout the study, and this will not affect the standard of care they receive. Regardless of the reasons for withdrawal below, the Sponsor commits to follow the subject until their anticipated end-of-study visit at Week 260.

Individual subjects may be withdrawn from the study under the following circumstances:

- The subject requests early discontinuation.
- Investigator's request (e.g., if the Investigator considers that the subject's health is compromised by remaining in the study or the subject is not sufficiently cooperative).
- The subject is lost to follow-up (all attempts will be made to locate subjects lost to follow-up).

Subjects requesting withdrawal from the study should be informed that withdrawal of consent for follow-up will jeopardize the public health value of the study. Subjects who withdraw should be explicitly asked about the contribution of possible AEs to their decision to withdraw consent, and any AE information elicited should be documented. Such information about the reason of subject's withdrawal will be filled in in the appropriate eCRF page.

In case of withdrawal, preferably the subject should withdraw consent in writing and, if the subject or the subject's representative refuses or is physically unavailable, the site should document and sign the reason for the subject's failure to withdraw consent in writing. The informed consent for the study should note that although a subject is free to leave the study, the Investigators hope the subject will remain for follow up status evaluations.

If a subject withdraws/is withdrawn from the study prematurely, the subject will be requested to return to the site to complete the end-of-study (Week 260) procedures, if possible.

12.2. Replacement of Subjects

Subjects withdrawing from follow-up monitoring before the primary endpoint has been established at 52 weeks after administration of ATSN-101 may be replaced in order to have three evaluable subjects per cohort. More than three subjects may be enrolled per cohort to accommodate replacement. Decisions regarding replacement will be made by the Sponsor. However, in the case of a discontinuation related to an AE, the replacement will be discussed between the Investigator and the Sponsor.

12.3. Withdrawal Follow-Up Procedure

All study withdrawals should be recorded by the Investigator on the appropriate case report form pages or screens for eCRF when considered as confirmed.

If possible, subjects are to be assessed using the procedures planned for the end-of-study visit (Week 260).

For any subject who fails to return to the site, the Investigator should make every effort to contact the subject (e.g., contact the subject's family or private physician, review available registries or health care database), and to determine his/her health status, including at least his/her vital status. Attempts to contact the subject must be documented in the subject's records (e.g., times and dates of attempted telephone contact, receipt for sending a registered letter).

Subjects withdrawn from the study must not be re-included in the study. Their Subject ID must not be reused.

13. STUDY PROCEDURES

13.1. Visit Schedule

13.1.1. Screening Procedures

Screening procedures will be carried out within 56 days prior to Day 1. The patient will receive information on the study objective(s) and procedures from the Investigator. The patient will have to sign the informed consent prior to any action related to the study. Each screened patient will receive an incremental identification number corresponding to his/her order of screening in the study.

The Screening visit will include the investigations listed in the Study Flow Chart (Section 2.2) and detailed in Section 10.

Rechecking of any parameters at Screening can be performed at the Investigator's discretion.

Patients who meet all the inclusion criteria and none of the exclusion criteria will be eligible for participation in the study. Reasons for screen failure will be recorded in the case report form.

Re-screening will be allowed once.

13.1.2. Baseline Procedures

The Baseline visit will be carried out during the days prior to IMP administration (Day -7 to -1) and will include the investigations listed in the Study Flow Chart (Section 2.2) and detailed in Section 10. Eligibility will be confirmed at the Baseline visit prior to any other study procedures.

Rechecking of any Baseline parameter is at the Investigator's discretion. Results of a rechecked value should be known before the inclusion. In the case of BCVA and FST, the baseline can be the average of values obtained at Baseline, noting in addition the variability of these measures. The baseline for a given parameter is reported in the case report form and is defined as the last assessment for this parameter before first administration of IMP, with the exception of BCVA and FST, where, as noted, the average of multiple baseline measurements can be used.

Eligibility will be confirmed again prior to any study procedures on Day 1.

13.1.3. Description by Type of Visit

Post-Procedure Period

Following the subretinal injection procedure, subjects should immediately contact the Investigator or one of the clinical unit managers in the event of any unexplained symptom or any unexpected effect or event occurring during the study. For this reason, subjects will be informed that they can contact the clinical unit by telephone 24 hours a day. Subjects must give the Investigator a telephone number where they can be contacted in an emergency. Surgical complications, ocular inflammation, increased intra-ocular pressure, and any other post-procedural AEs will be managed according to the standard of care at the clinical site, with any needed consultation with the surgical site.

Main Study Period

For study procedures and assessments, refer to Section 2.2 for schedule and to Section 10 for details.

Extension Study Period

For study procedures and assessments, refer to Section 2.2 for schedule and to Section 10 for details.

End-of-Study Visit

The end-of-study visit will be carried out approximately 260 weeks after administration of ATSN-101.

For study procedures and assessments, refer to Section 2.2 for schedule and to Section 10 for details.

13.1.4. Study Restriction(s)

Study restrictions will be further specified in the Surgical Manual.

13.2. Definition of Source Data

All evaluations that are reported in the case report form must be supported by appropriately identified source documentation.

The results of certain examinations or evaluations recorded in the case report form may be considered to be source data:

- History
- Vital signs
- Height and weight
- Body temperature
- Physical exam
- Concomitant medications
- ECG
- Laboratory results
- BCVA
- Photoaversion
- LLVA
- Visual function questionnaire (NEI VFQ-25 or CVAQC)
- Slit lamp exam
- Intraocular pressure

- Ophthalmoscopy

14. STATISTICAL CONSIDERATIONS

An overview of the statistical approach for data analysis is provided in this section. A statistical analysis plan, describing in detail the statistical analysis methods, will be issued prior to database lock for the primary analysis of Week 52 data.

14.1. Determination of Sample Size

This is a Phase 1/2 dose escalation study. No formal sample size calculation has been performed.

Cohorts of three subjects will be screened and treated at the same dose level during the dose escalation phase in three dose levels (3.3×10^{10} vg/mL, 1.0×10^{11} vg/mL, and 3.3×10^{11} vg/mL). The actual number of dose levels examined and the emerging ATSN-101 related toxicities will determine the total sample size during the dose escalation phase.

It is anticipated that approximately nine subjects, but up to 15 based on need for additional cohorts at intermediate doses or to replicate dose levels, will be required to establish the MTD of ATSN-101. Approximately three subjects may be enrolled in Cohort 4, and approximately three subjects may be enrolled in Cohort 5. The goal is to have a total of approximately 15 evaluable subjects treated.

14.2. Subject Description

14.2.1. Disposition of Subjects

The total number of subjects in each of the following populations will be summarized using counts and percentages by cohorts and overall:

- Screened population
- Safety population
- Activity/efficacy population

Reasons for discontinuation will be presented within the safety population.

All withdrawals from the study, taking place on or after IMP/NIMP administration, will be fully documented in the body of the CSR.

14.2.2. Protocol Deviations

During review of the database, compliance with the protocol will be examined with regard to inclusion and exclusion criteria, treatment compliance, prohibited therapies, and timing and availability of planned assessments. Protocol deviations will be identified by the study team before database lock and listed in the Data Review and Surveillance Report, including missing data and study drug discontinuations, and classified as major or minor deviations.

14.3. Analysis Populations

The number of subjects included in each study population (screened population, safety population, activity/efficacy population) will be provided. All exclusions from any analysis populations will be fully documented in the CSR.

14.3.1. Safety Population

All subjects who have signed informed consent will be included in the safety population.

14.3.2. Activity/Efficacy Population

The activity/efficacy population will include all subjects in the safety population who have received subretinal administration of ATSN-101. For each efficacy and exploratory endpoint, all subjects in the activity/efficacy population who have both the baseline and at least one post-baseline examination for that specific endpoint measurement will be included in the analysis.

14.3.3. Statistical Method

Unless otherwise specified, analyses will be descriptive and performed based on the safety population.

The baseline for a given parameter is defined as the last assessment for this parameter before first administration of IMP.

Continuous variables will be summarized using number of available data, mean, standard deviation, median, minimum, and maximum for each cohort and overall. Categorical and ordinal data will be summarized using number and percentage of subjects in each cohort and overall.

14.4. Demographic and Baseline Characteristics

14.4.1. Subject Demographic Characteristics, Medical History, and Diagnoses

Subject demographic and baseline characteristics, including age, height, weight, BMI, gender, race, and medical history, collected at Screening (Day -56 to Day -8) and/or Baseline (Day -7 to Day -1) visits will be summarized for the safety population by cohort and overall.

14.5. Extent of Study Treatment Exposure and Compliance

The extent of IMP exposure (ATSN-101) and compliance will be assessed and summarized by cohort and overall within the activity/efficacy population.

14.6. Prior/Concomitant Medication/Therapy

Medications will be coded according to the World Health Organization Drug Dictionary (WHO Drug Dictionary, last available version before database lock). Medications that were stopped before IMP administration and concomitant medications with the IMP will be listed separately by subject.

14.7. Analysis of Safety Data

The primary analyses will be based on the incidence of AEs in subjects in the safety population at Week 52. An additional safety analysis will be performed at Week 260, the end of the Extension Study Period. The number and percentage of subjects with AEs, SAEs, and AESIs will be summarized in each cohort and overall. Significant changes from baseline in other safety assessments will be assessed.

For all safety data, the observation period will be divided into two segments:

- The **pre-treatment period** is defined as the time between informed consent signature and the first IMP administration.
- The **treatment-emergent adverse event (TEAE) period** is defined as the time from the first IMP administration through Week 260 or discontinuation of the study, whichever occurs earlier.

14.7.1. Adverse Events

The number and percentage of subjects with TEAEs will be summarized.

An overall summary will include the number and percentage of subjects with:

- A fatal AE (death)
- At least one serious AE
- At least one severe AE
- At least one related AE
- No AEs

An additional table will show the number and percentage of subjects with TEAEs broken down by SOC and PT. Related AEs will be summarized separately.

The type, frequency, seriousness, and relatedness of TEAEs will be summarized and listed according to MedDRA. Severity of TEAEs and laboratory abnormalities will be summarized according to the CTCAE (v5.0).

14.7.1.1. Definitions

Adverse events will be coded to a “Preferred Term (PT)” and “High Level Group Term (HLGT)”, “High Level Term (HLT)” and primary “System Organ Class (SOC)” using the Medical Dictionary for Regulatory Activities (MedDRA, version currently in use by the sponsor at the time of database lock). Their severity will be graded according CTCAE (v5.0).

They will be classified into predefined standard categories according to chronological criteria:

- **Pre-treatment AEs:** AEs that occurred or worsened during the pretreatment period.
- **Treatment-emergent AEs:** AEs that occurred or worsened during the TEAE period.

All AEs reported in the study will be listed, sorted by subject, onset date, and time.

14.7.1.2. Treatment-Emergent Adverse Events

The following TEAE summaries will be provided by treatment for the safety population:

- Overview of TEAEs: number and percentage of subjects with any TEAE, any serious TEAE, any TEAE leading to death (if any occurred), and any TEAE leading to permanent treatment discontinuation.
- Summary of TEAEs by primary SOC and PT:
 - Number and percentage of subjects with at least one TEAE,
 - Number of occurrences of TEAEs.

Subjects presenting TEAEs will be listed by primary SOC and PT.

14.7.1.3. Deaths, Serious, and Other Significant Adverse Events

Any deaths, serious, and other significant AEs will be listed.

14.7.1.4. Adverse Events Leading to Treatment Discontinuation

Any AEs leading to permanent treatment discontinuation will be listed.

14.7.1.5. Adverse Events of Special Interest

Number (%) of subjects experiencing treatment-emergent AESIs will be presented by AESI category and PT, sorted by decreasing incidence of PT within each AESI category.

14.7.2. Ophthalmological Safety Endpoints

Changes in safety endpoints will be summarized at each visit; in addition, where appropriate, individual profile plots and mean plot of changes in the variable against time will be presented. The primary end points will be considered the Week 52 visit. Additional analysis will be performed at Week 260.

14.7.3. Clinical Laboratory Evaluations

Baseline Definition

The values to be used as the baselines will be the Day -7 to -1 pre-dose assessment values. If any of the scheduled baseline tests are repeated for any subject, the last rechecked values will be considered as baselines, provided they were done before IMP administration.

Abnormalities Analyses

For parameters with laboratory ranges and/or abnormality criteria (potentially clinically significant abnormalities [PCSAs]), analysis will be performed using all post-baseline assessments done during the TEAE period, including all unplanned and rechecked values. Counts of subjects with PCSAs according to baseline status will be presented by cohort. The same type of summary tables will be provided for out-of-normal laboratory range values.

PCSA Listings

A listing of individual data from subjects with post-baseline PCSAs will be provided; values will be flagged when outside the laboratory limits and/or when reaching the PCSA criteria.

14.7.4. Vital Signs

Vital signs will be summarized and listed by cohort and visit.

Heart rate and systolic and diastolic blood pressures will be analyzed as raw parameter value and change from baseline.

Baseline Definition

The values to be used as baselines will be the Day 1 pre-dose assessment values.

Abnormalities Analyses

For all parameters, analysis will be performed using all post-baseline assessments done during the TEAE period, including all unplanned and rechecked values. Counts of subjects with PCSAs will be presented by treatment group, regardless of the baseline status.

Descriptive Statistics and Plots

For heart rate and blood pressures, raw data and changes from baseline will be summarized in descriptive statistics (including mean, median, standard deviation, minimum, and maximum) by cohort and scheduled time of measurement.

Additionally, graphs over time will be presented plotting mean by treatment group.

PCSA Listings

A listing of individual data from subjects with post-baseline PCSAs will be provided; values will be flagged when reaching the PCSA criteria.

14.8. Analysis of Efficacy Data

Efficacy summaries will include descriptive statistics of the observed value and/or change or percentage change from Baseline to Month 12 (or last study assessment). These descriptive statistics will be summarized overall and by cohort and treatment group. The main analysis will occur at Week 52, and an additional analysis will be performed at Week 260.

14.9. Interim Analysis

Interim analyses will be performed in preparation for DMC as well as after Week 52 of the last subject. Interim analyses may also be performed in case of unexpected safety concerns and/or for internal decision making.

15. ETHICAL AND REGULATORY CONSIDERATIONS

15.1. Ethical and Regulatory Standards

This clinical study will be conducted by the Sponsor, the Investigator, delegated Investigator staff and Sub-investigator, in accordance with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the World Medical Assemblies, and the ICH guidelines for Good Clinical Practice (GCP), all applicable laws, rules and regulations.

The study will not begin, and supplies will not be shipped to the site until all required Health Authority (Competent Regulatory Authority) and IRB/IEC/IBC approvals are received.

This clinical study will be recorded in a free, publicly accessible, internet-based registry, no later than 21 days after the first subject enrollment, in compliance with applicable regulatory requirements and with Atsena Therapeutics, Inc. public disclosure commitments.

15.2. Informed Consent

The Investigator (according to applicable regulatory requirements), or a person designated by the Investigator and under the Investigator's responsibility, should fully inform the subject of all pertinent aspects of the clinical study including the written information giving approval/favorable opinion by the ethics committee (IRB/IEC). All subjects should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a subject's participation in the clinical study, the written informed consent form should be signed, name filled in, and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written informed consent form will be provided to the subject.

The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject's consent. Any revised written informed consent form, and written information should receive the IRB/IEC's approval/favorable opinion in advance of use. The subject or the subject's legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the study. The communication of this information should be documented. In case of study suspension due to safety concerns, study subjects will be informed of this study suspension and the reason for it. Once it is confirmed that it is safe for the study to continue, study subjects will be asked to confirm their agreement to continue the study.

15.3. Institutional Review Board/Independent Ethics Committee (IRB/IEC) and Other Required Committees

As required by local regulation, the Investigator or the Sponsor must submit this clinical study protocol to the Health Authorities (Competent Regulatory Authority) and the appropriate IRB/IEC and other required committees and is required to forward to the respective other party a copy of the written and dated approval/favorable opinion signed by the chairman with committee composition.

The clinical study (study number, clinical study protocol title and version number), the documents reviewed (clinical study protocol, informed consent form, Investigator's Brochure with any addenda, Investigator's curriculum vitae, etc.) and the date of the review should be clearly stated on the written IRB/IEC approval/favorable opinion.

IMP will not be released at the study site and the Investigator will not start the study before the written and dated approval/favorable opinions are received by the Investigator and the Sponsor.

During the clinical study, any amendment or modification to the clinical study protocol will be submitted to the Health Authorities (Competent Regulatory Authority), as required by local regulation, in addition to the IRB/IEC and other required committees before implementation, unless the change is necessary to eliminate an immediate hazard to the subjects, in which case the Health Authorities (Competent Regulatory Authority) and the IRB/IEC and other required committees should be informed as soon as possible. They will also be informed of any event likely to affect the safety of subjects or the continued conduct of the clinical study, in particular any change in safety. All updates to the Investigator's Brochure will be sent to the IRB/IEC, Health Authorities (Competent Regulatory Authority), and other required committees as required by local regulation.

A progress report is sent to the IRB/IEC and other required committees annually or as required and a summary of the study's outcome at the end of the clinical study, according to local requirements.

16. STUDY MONITORING

16.1. Responsibilities of the Investigator(s)

The Investigator is required to ensure compliance with all procedures required by the clinical study protocol and with all study procedures provided by the Sponsor (including security rules). The Investigator agrees to provide reliable data and all information requested by the clinical study protocol (with the help of the case report form, discrepancy resolution form, or other appropriate instruments) in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents to Sponsor representatives.

If any circuit includes transfer of data, particular attention should be paid to the confidentiality of the subject's data to be transferred.

The Investigator may appoint such other individuals as he/she may deem appropriate as Sub-investigators to assist in the conduct of the clinical study in accordance with the clinical study protocol. All Sub-investigators shall be appointed and listed in a timely manner. The Sub-investigators will be supervised by and work under the responsibility of the Investigator.

The Investigator will provide them with a copy of the clinical study protocol and all necessary information.

16.2. Responsibilities of the Sponsor

The Sponsor of this clinical study is responsible to regulatory authorities for taking all reasonable steps to ensure the proper conduct of the clinical study as regards ethics, clinical study protocol compliance, and integrity and validity of the data recorded on the case report forms. Thus, the main duty of the monitoring team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical, and regulatory quality in all aspects of the clinical study.

At regular intervals during the clinical study, the site will be contacted, through monitoring visits, letters, or telephone calls, by a representative of the monitoring team to review study progress, Investigator and subject compliance with clinical study protocol requirements, and any emergent problems. These monitoring visits will include but not be limited to review of the following aspects: subject informed consent, subject recruitment and follow-up, SAE documentation and reporting, AESI documentation and reporting, AE documentation, IMP allocation, subject compliance with the IMP regimen, IMP accountability, concomitant therapy use, and quality of data.

16.3. Source Document Requirements

According to the ICH GCP, the monitoring team must check the case report form entries against the source documents, except for the pre-identified source data directly recorded in the case report form. The informed consent form will include a statement by which the subject allows the Sponsor's duly authorized personnel, the IRB/IEC, and the regulatory authorities to have direct access to original medical records which support the data on the case report forms (e.g., subject's medical file, appointment books, original laboratory records). These personnel, bound by professional secrecy, must maintain confidentiality of all personal identity or personal medical information (according to confidentiality and personal data protection rules).

16.4. Use and Completion of Case Report Forms and Additional Requests

It is the responsibility of the Investigator to maintain adequate and accurate case report forms (according to the technology used) designed by the Sponsor to record (according to Sponsor instructions) all observations and other data pertinent to the clinical investigation in a timely manner. All case report forms should be completed in their entirety in a neat, legible manner to ensure accurate interpretation of data.

Should a correction be made, the corrected information will be entered in the eCRF overwriting the initial information. An audit trail allows identification of the modification.

Data are available within the system to the Sponsor as soon as they are entered in the eCRF.

The computerized handling of the data by the Sponsor may generate additional requests (discrepancy resolution forms) to which the Investigator is obliged to respond by confirming or modifying the data questioned. The requests with their responses will be managed through the eCRF.

16.5. Use of Computerized Systems

The complete list of computerized systems used for the study is provided in a separate document which is maintained in the Sponsor and Investigator study files.

17. ADDITIONAL REQUIREMENTS

17.1. Curriculum Vitae

A current copy of the curriculum vitae describing the experience, qualification and training of each Investigator and Sub-investigator will be signed, dated, and provided to the Sponsor prior to the beginning of the clinical study.

17.2. Record Retention in Study Site(s)

The Investigator must maintain confidential all study documentation and take measures to prevent accidental or premature destruction of these documents.

The Investigator should retain the study documents at least 15 years after the completion or discontinuation of the clinical study.

However, applicable regulatory requirements will be taken into account in the event that a longer period is required.

The Investigator must notify the Sponsor prior to destroying any study essential documents following the clinical study completion or discontinuation.

If the Investigator's personal situation is such that archiving can no longer be ensured by him/her, the Investigator shall inform the Sponsor and the relevant records shall be transferred to a mutually agreed upon designee.

17.3. Confidentiality

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf), or produced during the clinical study, including, but not limited to, the clinical study protocol, personal data in relation to the subjects, the case report forms, the Investigator's Brochure, and the results obtained during the course of the clinical study, is confidential, prior to the publication of results. The Investigator and any person under his/her authority agree to undertake to keep confidential and not to disclose the information to any third party without the prior written approval of the Sponsor.

However, the submission of this clinical study protocol and other necessary documentation to the IRB/IEC is expressly permitted, the IRB/IEC members having the same obligation of confidentiality.

The Sub-investigators shall be bound by the same obligation as the Investigator. The Investigator shall inform the Sub-investigators of the confidential nature of the clinical study.

The Investigator and the Sub-investigators shall use the information solely for the purposes of the clinical study, to the exclusion of any use for their own or for a third party's account.

17.4. Property Rights

All information, documents, and IMP provided by the Sponsor or its designee are and remain the sole property of the Sponsor.

The Investigator shall not and shall cause the delegated Investigator staff /Sub-investigator not to mention any information or the Product in any application for a patent or for any other intellectual property rights.

All the results, data, documents, and inventions, which arise directly or indirectly from the clinical study in any form, shall be the immediate and exclusive property of the Sponsor.

The Sponsor may use or exploit all the results at its own discretion, without any limitation to its property right (territory, field, continuance). The Sponsor shall be under no obligation to patent, develop, market or otherwise use the results of the clinical study.

As the case may be, the Investigator and/or the sub-investigators shall provide all assistance required by the Sponsor, at the Sponsor's expense, for obtaining and defending any patent, including signature of legal documents.

17.5. Data Protection

- The subject's personal data, which are included in the Sponsor database, shall be treated in compliance with all applicable laws and regulations.
- When archiving or processing personal data pertaining to the Investigator and/or to the subject, the Sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.
- The Sponsor also collects specific data regarding Investigator as well as personal data from any person involved in the study which may be included in the Sponsor's databases, shall be treated by both the Sponsor and the Investigator in compliance with all applicable laws and regulations.

17.6. Insurance Compensation

The Sponsor certifies that it has taken out a liability insurance policy covering all clinical studies under its sponsorship. This insurance policy is in accordance with local laws and requirements. The insurance of the Sponsor does not relieve the Investigator and the collaborators from any obligation to maintain their own liability insurance policy. An insurance certificate will be provided to the IEC/IRB or regulatory authorities in countries requiring this document.

17.7. Sponsor Audits and Inspections by Regulatory Agencies

For the purpose of ensuring compliance with the clinical study protocol, Good Clinical Practice and applicable regulatory requirements, the Investigator should permit auditing by or on the behalf of the Sponsor and inspection by regulatory authorities.

The Investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that these personnel are bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The Investigator will make every effort to help with the performance of the audits and inspections, giving access to all necessary facilities, data, and documents.

As soon as the Investigator is notified of a planned inspection by the authorities, he will inform the Sponsor and authorize the Sponsor to participate in this inspection.

The confidentiality of the data verified and the protection of the subjects should be respected during these inspections.

Any result and information arising from the inspections by the regulatory authorities will be immediately communicated by the Investigator to the Sponsor.

The Investigator shall take appropriate measures required by the Sponsor to take corrective actions for all problems found during the audit or inspections.

17.8. Premature Discontinuation of the Study or Premature Close-Out of a Site

17.8.1. By the Sponsor

The Sponsor has the right to terminate the participation of either an individual site or the study at any time, for any reason, including but not limited to the following:

- The information on the product leads to doubt as to the benefit/risk ratio.
- Subject enrollment is unsatisfactory.
- The Investigator has received from the Sponsor all IMP, means, and information necessary to perform the clinical study and has not included any subject after a reasonable period of time mutually agreed upon.
- Non-compliance of the Investigator or Sub-investigator, delegated staff with any provision of the clinical study protocol, and breach of the applicable laws and regulations or breach of the ICH GCP.

In any case, the Sponsor will notify the Investigator of its decision by written notice.

17.8.2. By the Investigator

The Investigator may terminate his/her participation upon thirty (30) days' prior written notice if the study site or the Investigator for any reason becomes unable to perform or complete the clinical study.

In the event of premature discontinuation of the study or premature close-out of a site, for any reason whatsoever, the appropriate IRB/IEC and regulatory authorities should be informed according to applicable regulatory requirements.

17.9. Clinical Study Results

The Sponsor will be responsible for preparing a CSR and to provide a summary of the study results to the Investigator.

17.10. Publications and Communications

The Investigator undertakes not to make any publication or release pertaining to the study and/or results of the study prior to the Sponsor's written consent, being understood that the Sponsor will not unreasonably withhold its approval.

The Investigator shall provide the Sponsor with a copy of any presentation or publication for review and comment at least 30 days in advance of any presentation or submission for publication. In addition, if requested by the Sponsor, any presentation or submission for publication shall be delayed for a limited time, not to exceed 90 days, to allow for filing of a patent application or such other justified measures as the Sponsor deems appropriate to establish and preserve its proprietary rights.

The Investigator shall not use the name(s) of the Sponsor and/or of its employees in advertising or promotional material or publication without the prior written consent of the Sponsor. The Sponsor shall not use the name(s) of the Investigator and/or the collaborators in advertising or promotional material or publication without having received his/her and/or their prior written consent(s).

The Sponsor has the right at any time to publish the results of the study.

18. CLINICAL STUDY PROTOCOL AMENDMENTS

All appendices attached hereto and referred to herein are made part of this clinical study protocol.

The Investigator should not implement any deviation from, or changes of the clinical study protocol without agreement by the Sponsor and prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to clinical study subjects, or when the change(s) involves only logistical or administrative aspects of the study. Any change agreed upon will be recorded in writing, the written amendment will be signed by the Investigator and by the Sponsor, and the signed amendment will be filed with this clinical study protocol.

Any amendment to the clinical study protocol requires written approval/favorable opinion by the IRB/IEC prior to its implementation, unless there are overriding safety reasons.

In some instances, an amendment may require a change to the informed consent form. The Investigator must receive an IRB/IEC written approval/favorable opinion concerning the revised informed consent form prior to implementation of the change and subject signature should be re-collected if necessary.

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20. APPENDICES

[Appendix A Contraceptive Guidance and Collection of Pregnancy Information](#)

[Appendix B Protocol Amendment History](#)

[Appendix C Principal Investigator's Agreement](#)

APPENDIX A. CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

DEFINITIONS

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

Women in the Following Categories Are Not Considered WOCBP

1. Premenarchal.
2. Premenopausal female with one of the following procedures occurring at least three months prior to ATSN-101 administration:
 - Documented hysterectomy,
 - Documented bilateral salpingectomy,
 - Documented bilateral oophorectomy.
3. Postmenopausal:
 - A postmenopausal state is defined as no menses for two years without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of two years of amenorrhea, a single FSH measurement is insufficient.
 - Females on HRT and whose menopausal status is in doubt will be required to use the required non-hormonal highly effective contraception methods required by this study if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

CONTRACEPTION GUIDANCE

Male Subjects

- Male subjects with female partners of childbearing potential are eligible to participate if they agree to ONE of the following from the inclusion up to three months after IMP/NIMP administration:
 - Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
 - Agree to use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in [Table 6](#) when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.

- Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration from the inclusion up to three months after IMP/NIMP administration.
- Refrain from donating sperm from the inclusion up to three months after IMP/NIMP administration.

Female Subjects

WOCBP, as defined above, must use from inclusion up to three months after IMP/NIMP administration a double contraception method. The accepted double contraception methods include the use of one of the highly effective contraceptive methods consistently and correctly as described in Table 6 in addition to one of the following contraceptive options: (1) condom; (2) diaphragm or cervical/vault cap; (3) spermicide.

Table 6 Highly effective contraceptive methods

<p>Highly Effective Contraceptive Methods That Are User Dependent^a</p> <p><i>Failure rate of <1% per year when used consistently and correctly</i></p> <ul style="list-style-type: none"> • Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation: <ul style="list-style-type: none"> - Oral, - Intravaginal, - Transdermal. • Progestogen-only hormone contraception associated with inhibition of ovulation: <ul style="list-style-type: none"> - Oral, - Injectable.
<p>Highly Effective Methods That Are User Independent^a</p> <ul style="list-style-type: none"> • Implantable progestogen-only hormonal contraception associated with inhibition of ovulation: <ul style="list-style-type: none"> - Intrauterine device (IUD), - Intrauterine hormone-releasing system (IUS). • Bilateral tubal occlusion. • Vasectomized partner. <p><i>(Vasectomized partner is a highly effective contraceptive method provided that the partner is the sole sexual partner of the woman of childbearing potential and the absence of sperm has been confirmed. If not and less than one year after vasectomy, additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.)</i></p> <ul style="list-style-type: none"> • Sexual abstinence. <p><i>Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.</i></p>

NOTES:

- a* Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

COLLECTION OF PREGNANCY INFORMATION

Male Subjects with Partners Who Become Pregnant

- The Investigator will attempt to collect pregnancy information on any male subject's female partner who becomes pregnant while the male subject is in this study. This applies only to male subjects who receive study treatment.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy if it meets AESI criteria.
- The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor.
- Generally, the follow-up will be no longer than six to eight weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

Female Subjects Who Become Pregnant

- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study.
- Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy after treatment with IMP/NIMP.
- The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate, and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than six to eight weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- Any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.
- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any post-study pregnancy-related SAE considered reasonably related to the study treatment by the Investigator will be reported to the Sponsor. While the Investigator is not obligated to actively seek this information in former study subjects, he or she may learn of an SAE through spontaneous reporting.

APPENDIX B. PROTOCOL AMENDMENT HISTORY

PROTOCOL AMENDMENT- SUMMARY OF CHANGES

Amended Protocol 08, 13 Sep 2023

OVERALL RATIONALE FOR THIS AMENDMENT

The rationale for this protocol amendment is to: (1) update Global Study Manager information, (2) change compound name from SAR439483 to ATSN-101, (3) change study name from DFI14738 to ATSN-101-1, (4) clarify that a single additional safety analysis will be performed after the Main Study Period at W260, (5) remove vital sign, laboratory, immunogenicity, photoaversion, LLVA, pupillometry, and OCI testing after W52, (6) add FAF to Extension Study Period visits, (7) update planned data analysis and minimize redundancy with the SAP.

Substantive changes are documented in the Summary of Changes table below.

Section	Description of Change	Brief Rationale
Names and Addresses (page 2)	Updated Global Study Manager name and contact information	Mallory Bissett will replace Mike DeSordi as Global Study Manager
Clinical Study Summary and 14.7 Analysis of Safety Data	Removed that additional safety analyses will be performed at W78, W104, W156, and W208	After the Main Study Period, a single additional safety analysis will be performed at W260 (End of Study)
2.2 Study Flow Chart	Removed vital signs (blood pressure, heart rate), hematology, biochemistry, urinalysis, humoral immune response (anti-AAV5 and anti-RetGC1), cellular immune response (ELISpot), photoaversion, LLVA, pupillometry, and OCI testing after W52 Added FAF to W78, W104, W156, W208, and W260/EOS	Decrease testing burden and focus on high-yield parameters following completion of Main Study Period Facilitate long-term follow-up of ocular safety, including any RPE changes
10.1 Activity or Pharmacodynamics, 14.4.2 Baseline Characteristics, and 14.8 Analysis of Efficacy Data	Detailed description of data analysis was removed	Details regarding data analysis are described in a separate SAP

Amended Protocol 07, 22 March 2023

OVERALL RATIONALE FOR THIS AMENDMENT

The rationale for this protocol amendment is to: (1) update PI information; (2) update language regarding DMC review.

Substantive changes are documented in the Summary of Changes table below.

Section	Description of Change	Brief Rationale
Names and Addresses (page 2)	Updated PI name and email address; Updated Sponsor's address and phone numbers	[REDACTED] will replace [REDACTED] as PI; the Sponsor has a new office location
Signature Page	Updated Sponsor's address	The Sponsor has a new office location
Clinical Study Summary, Graphical Study Design, 5.2.2.4 Rationale for Study Design, 7.1 Description of the Study	Replaced "The DMC will meet at least once per year throughout the duration of the study to monitor safety" with "The DMC will meet at least once more after the completion of the Main Study Period of the last subject to monitor safety"	Updated to more accurately reflect the current plan for DMC monitoring according to the DMC Charter

Amended Protocol 06, 17 December 2021

OVERALL RATIONALE FOR THIS AMENDMENT

The rationale for this protocol amendment is to: (1) add photoaversion as an exploratory efficacy endpoint; (2) add low-luminance visual acuity as an exploratory efficacy endpoint; (3) remove the ability for NIMP dose regimen to be changed based on Surgeon/Investigator discretion and, instead, require discussion with the Medical Monitor before any changes are made; (4) require mobility testing where available; (5) increase the window for baseline relative to surgery (Day 1); (6) add OCT assessment at Baseline; (7) remove mobility testing at Screening; (8) remove some details on the planned statistical analyses; (9) update the definition of pre-treatment AEs and treatment-emergent AEs; and (10) update language regarding interim analyses.

Substantive changes are documented in the Summary of Changes table below.

Section	Description of Change	Brief Rationale
Synopsis, 2.2 Study Flow Chart, 5.2.4.3 Assessment of Exploratory Endpoints, 10.1.1.3 Exploratory Parameters, 10.1.2 Assessment Methods, 13.2 Definition of Source Data, 14.4.2 Baseline Characteristics, 14.8.5 Photoaversion	Added photoaversion as an exploratory endpoint	Photoaversion was added to further assess a subject's changes to discomfort and/or avoidance of bright lights as a result of the treatment.
Synopsis, 2.2 Study Flow Chart, 5.2.4.3 Assessment of Exploratory Endpoints, 10.1.1.3 Exploratory Parameters, 10.1.2 Assessment Methods, 13.2 Definition of Source Data,	Added low-luminance visual acuity as an exploratory endpoint	Low-luminance visual acuity was added to further assess a subject's changes to visual function in a low light setting as a result of the treatment.

14.4.2 Baseline Characteristics, 14.8.6 Low-luminance Visual Acuity		
Synopsis, 5.2.3.4 Risks Associated with Peri-Procedural Medications, 9.2 Non-investigational Medicinal Products	Removed the ability for NIMP dose regimen to be changed based on Surgeon/Investigator discretion and, instead, amended to require discussion with the Medical Monitor before any changes are made	Reinforces the recommended starting dose of systemic corticosteroid (1 mg/kg/day) and close contact between Medical Monitor and PI for any changes.
Synopsis, 2.2 Study Flow Chart, 10.1.1.3 Exploratory Parameters, 10.1.2 Assessment Methods	Amended language to require mobility testing where available	This testing is not optional and must be performed at investigational sites in which the testing is available.
2.2 Study Flow Chart, 7.2.1 Duration of Study Participation for Each Subject, 13.1.2 Baseline Procedures, 14.4.1 Subject Demographic Characteristics, Medical History, and Diagnoses, 14.7.3 Clinical Laboratory Evaluations	Increased the window for baseline assessments relative to surgery (Day 1)	Allow more flexibility in scheduling the surgical procedure.
2.2 Study Flow Chart	Added OCT assessment at Baseline	A baseline assessment, which was inadvertently omitted in previous versions of the protocol, is needed to serve as the comparison for all post-treatment OCT assessments.
2.2 Study Flow Chart, 14.8.10 Mobility Testing	Removed mobility testing at Screening	Removed to alleviate subject burden at the Screening visit.
10.1.1.2 Secondary (Efficacy) Parameters, 10.1.1.3 Exploratory Parameters, 14.7.4 Vital Signs, 14.8 Analysis of Efficacy Data	Removed some details on the planned statistical analyses	Removed details that may be addressed in the statistical analysis plan.
14.7.1.1 Definitions	Updated the definition of pre-treatment AEs and treatment-emergent AEs	Removed “or became serious”, as there is a separate definition for serious AEs.
14.9 Interim Analysis	Updated language regarding interim analyses	Updated language to align with the statistical analysis plan.

Amended Protocol 05, 17 September 2021

OVERALL RATIONALE FOR THE AMENDMENT

The rationale for this protocol amendment is to include details that describe an extended follow-up period. The Extension Study Period is being lengthened to 48 months, resulting in a total post-treatment follow-up of 260 weeks. The amendment to include the additional Extension Study Period is being instituted to collect additional data on the persistence of effect. Moreover, additional amendments included reflect that the study will now be multi-center.

Substantive changes are documented in the Summary of Changes table below. Additional administrative changes were made throughout the protocol for style, grammar, and formatting. Not all of these administrative changes are tracked in the redlined version of the protocol amendment.

Section	Description of Change	Brief Rationale
Names and Addresses	Name of Sponsor Medical Officer changed	Change in staffing
Study Location	Amended to reflect the inclusion of an additional site	Study will now be multi-center
Study Design	Amended to reflect the Extension Study Period out to Week 260	Extension Period to collect additional data on the persistence of effect
1.2 Study Flow Chart	Amended to reflect Extension Study Period inclusive of annual ophthalmological assessments	Amended to reflect required assessments during the Extension Study Period
4.2.2.3 Rationale for Peri-Procedural Medication Regimen	Amended to add verbiage, "To reduce the risk of inflammation resulting from surgery or from the vector/transgene, all subjects in this study will be given a 21-day course of oral prednisone, based off the 17-day protocol established in the LCA2 Phase 3 AAV gene therapy clinical study (13) with an additional four-day taper, for a total oral prednisone course of 21 days."	In light of the 17-day steroid regimen course used in the unilateral eye treatment studies for the now FDA-approved retinal gene therapy voretigene neparvovec and in review of other publicly available current day ocular AAV gene therapy protocols, it was decided to extend the oral prednisone coverage for subjects from four days in the current protocol to a 21-day regimen with a slower taper. The risk of oral steroids for a short 21-day course is quite low in comparison to the benefit of reducing the risk of development of ocular inflammation post treatment.
4.2.4.3 Assessment of Exploratory Endpoints	Deleted "and compared to normal subjects" from the Pupillometry (PLR) description	There are no plans to compare data in this study to normal subjects
7.3 Exclusion Criteria	Added "systemic" to "immunosuppressive medications"	To clarify that inhaled or topical corticosteroids are permitted

Section	Description of Change	Brief Rationale
Clinical Study Summary and 8.2.1 Prednisone	Amended from “Prednisone PO 1 mg/kg (up to a maximum dose of 80 mg) administered in the morning (approximately 8AM) given on D-1, D1, D2, and D3” to “1 mg/kg/day PO prednisone (up to a maximum dose of 80 mg) administered in the morning (approximately 8AM) given on D-1, D1, D2, D3, D4, D5, and D6; followed by 0.5 mg/kg/day PO prednisone for seven days; followed by 0.25 mg/kg/day PO prednisone for four days; and followed by 0.125 mg/kg/day PO prednisone for three days (21 days in total).”	In light of the 17-day steroid regimen course used in the unilateral eye treatment studies for the now FDA-approved retinal gene therapy voretigene neparvovec and in review of other publicly available current day ocular AAV gene therapy protocols, it was decided to extend the oral prednisone coverage for subjects from four days in the current protocol to a 21-day regimen with a slower taper. The risk of oral steroids for a short 21-day course is quite low in comparison to the benefit of reducing the risk of development of ocular inflammation post treatment.
9.1.1.3 Exploratory Parameters	Amended to reflect revision in optical coherence tomography parameters	To provide for additional clarity
9.2.2 Safety Assessment at Screening, Baseline, and During the Study	Amended information on the archival blood sample and anti-AAV5 and anti-RetGC1 sample to align with the laboratory manual; updated the approximate sampled blood volume table to account for changes in the archival blood sample volume, anti-AAV5 and anti-RetGC1 blood sample volume, and blood sample collection at Weeks 156, 208, and 260	To ensure consistency between the protocol and the laboratory manual, and to reflect an increased total blood volume due to blood samples required at Weeks 156, 208, and 260
10.4.2 Guidelines for Reporting Serious Adverse Events	Amended to reflect current safety reporting requirements	To provide for additional clarity
10.5 Obligations of the Sponsor	Amended to reflect current Sponsor obligations	To provide for additional clarity
12.1.2 Baseline Procedures	Changed the title and reworded to clarify that this section is referring to the Baseline visit	To provide for additional clarity
13.2.1 Disposition of Subjects	Deleted the “Evaluable population”	The inclusion of this population does not provide additional information beyond that already provided by the other specified populations

Section	Description of Change	Brief Rationale
Clinical Study Summary, 13.3 Analysis Population, 13.5 Extent of Study Treatment Exposure and Compliance	Amended the definitions of the safety population and the activity/efficacy population; updated the planned extent of exposure and compliance analyses to be based off of the activity/efficacy population	The safety population should include all subjects who sign informed consent to align with AE collection language; the activity/efficacy population should include only those subjects who receive IMP
13.8.5 Optical Coherence Tomography	Amended to reflect revision in optical coherence tomography statistical analysis	To provide for additional clarity

Amended Protocol 04, 25 September 2020

This amended protocol (amendment 04) is not considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

OVERALL RATIONALE FOR THE AMENDMENT

The primary driver for this protocol amendment is to include details that reflect a change in study sponsorship.

Section	Description of Change	Brief Rationale
Names and Addresses	Added new Sponsor personnel contact details.	Updated new Sponsor contact details.
Section 10.4.2	Revised to reflect the SAE reporting process as it pertains to Atsena Therapeutics, Inc. as the new Sponsor of the study. Added Sponsor email address for reporting SAE-related documentation.	To update the protocol with Atsena Therapeutics, Inc. as the Sponsor responsible for receiving and reporting SAE reports.
Appendix C	Added Principal Investigator's Agreement page	To indicate the Principal Investigator has read and understood the contents of this clinical protocol for Study No. ATSN-101-1

Amended Protocol 03, 11 May 2020

This amended protocol (amendment 03) is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

OVERALL RATIONALE FOR THE AMENDMENT

The primary driver for this protocol amendment is to add pre-operative and post-operative fundus autofluorescence imaging based on the request of the data monitoring committee for the trial following review of the data from Cohort 1.

Protocol Amendment Summary of Changes Table

Section No.	Description of Change	Brief Rationale
Section 1.2	Added fundus autofluorescence imaging to the flow chart at baseline and D28.	To add fundus autofluorescence imaging per the DMC request.
Section 4.2.4, Section 9.2.2	Added fundus autofluorescence imaging to the ocular examinations.	To add fundus autofluorescence imaging per the DMC request.
Appendix B	Added this appendix to document protocol amendment history.	To document protocol amendment history.

Amended Protocol 02, 15 January 2019

This amended protocol (amendment 02) is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

OVERALL RATIONALE FOR THE AMENDMENT

The primary driver for this protocol amendment is to change the study design such that only patients with best corrected visual acuity (BCVA) of 20/200 or worse in the eye to be injected are included in Cohorts 1-3, incorporating feedback received from the FDA during the IND review.

Protocol Amendment Summary of Changes Table

Section No.	Description of Change	Brief Rationale
Clinical Trial Summary	Inclusion criteria modified such that for cohorts 1-3, BCVA of 20/200 or worse in the eye to be injected is required	To require advanced vision impairment in the eye to be injected in all cohorts of Part A
Section 4.2.2, Rationale for study design	Changed to reflect modification in inclusion criteria: that for cohorts 1-3, BCVA of 20/200 or worse in the eye to be injected is required.	To require advanced vision impairment in the eye to be injected in all cohorts of Part A.
Section 4.2.2, Rationale for Population	Changed to reflect modification in inclusion criteria: that for cohorts 1-3, BCVA of 20/200 or worse in the eye to be injected is required.	To require advanced vision impairment in the eye to be injected in all cohorts of Part A.

Section 6.1, Description of the study	Added the following statement from Section 4.2.5.3 into Section 6.1, Description of the study: "Before enrolling pediatric subjects (Cohort 5), a preliminary summary of safety and biological activity data of Part A along with a rationale for the dose to be used in Cohort 5 will be provided to the DMC for review. This information will also be submitted to the Regulatory Authorities and other review committees as required by local site requirements."	To clarify that before enrolling pediatric subjects (Cohort 5), a preliminary summary of safety and biological activity data of Part A along with a rationale for the dose to be used in Cohort 5 will be provided to the DMC for review. To also clarify that this information will be submitted to the Regulatory Authorities and other review committees as required by local site requirements.
Section 7.2, Inclusion Criteria	Inclusion criteria modified such that for cohorts 1-3, BCVA of 20/200 or worse in the eye to be injected is required	To require advanced vision impairment in the eye to be injected in all cohorts of Part A

Amended Protocol 01, 07 January 2019

This amended protocol (amendment 01) is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

OVERALL RATIONALE FOR THE AMENDMENT

The primary driver for this protocol amendment is to increase monitoring for elevated intraocular pressure and ocular inflammation, incorporating feedback received from the FDA during the IND review.

Protocol Amendment Summary of Changes Table

Section No.	Description of Change	Brief Rationale
Names and Addresses	Changed Investigator to Primary Investigator	To clarify that Dr. Samuel Jacobson will be the Primary Investigator for the trial.
Clinical trial summary	Increased the interval between the second and third patients for Cohorts 1 and 5 to 14 days	This interval was extended to increase the monitoring period for inflammation prior to treatment of the third patient in these two cohorts.
Clinical trial summary	Specified that the DMC will meet at least yearly during the course of the trial	To clarify the frequency of DMC meeting throughout the entire duration of the trial
Clinical trial summary	Specified that for patients who enroll in the separate long-term follow-up study, the end of study visit would be at Week 52	To clarify the end of study visit for patients who enroll in the separate long-term follow-up study

Section No.	Description of Change	Brief Rationale
Section 1.1, Graphical Study Design	Modified to indicate revised intervals between dosing of each patient for cohorts 1 and 5, and to clarify that the DMC will meet at least yearly during the course of the trial	To reflect the changes that were made in the study design
Section 1.2, Study Flow Chart	Added D12 and D14 study visits	To extend the close post-procedure monitoring period for intraocular pressure and ocular inflammation to 14 days
Section 1.2, Study Flow Chart	Specified that Week 104 is the end of study visit, and added a footnote indicating that for those patients who enroll in the separate long-term follow-up study, the end of study visit would be at Week 52	To clarify the end of study visit for patients who enroll in the separate long-term follow-up study
Section 4.2.2, Design Rationale	Increased the interval between the second and third patients for Cohorts 1 and 5 to 14 days, specified that the DMC will meet at least yearly during the course of the trial, and specified that for patients who enroll in the separate long-term follow-up study, the end of study visit would be at Week 52	To increase monitoring of intraocular pressure and ocular inflammation and clarify the end of study visit
Section 4.2.2, Design Rationale	Revised the “Rationale for peri-procedural medication regimen” to provide a more detailed justification for the use of corticosteroids administered via three routes.	To clarify the rationale for the peri-procedural corticosteroid regimen.
Section 6.1, Description of the study	Increased the interval between the second and third patients for Cohorts 1 and 5 to 14 days, specified that the DMC will meet at least yearly during the course of the trial, and specified that for patients who enroll in the separate long-term follow-up study, the end of study visit would be at Week 52	To increase monitoring of intraocular pressure and ocular inflammation and clarify the end of study visit
Section 6.2.1, Duration of study participation for each subject	Specified that for patients who enroll in the separate long-term follow-up study, the end of study visit would be at Week 52	To clarify the end of study visit for patients who enroll in the separate long-term follow-up study
Section 8.8, Treatment Accountability and Compliance	Removed the use of a patient diary	The patient diary has been removed from the study.

Section No.	Description of Change	Brief Rationale
Section 12.1.3, Description by type of visit	Specified that for patients who enroll in the separate long-term follow-up study, the end of study visit would be at Week 52	To clarify the end of study visit for patients who enroll in the separate long- term follow-up study

APPENDIX C. PRINCIPAL INVESTIGATOR'S AGREEMENT

I have read and understand the contents of this amended clinical study protocol 08 for Study No. ATSN-101-1, dated 13 Sep 2023 and agree that it contains all the information necessary to conduct the study as required. By my signature, I agree to personally supervise the conduct of this study at my study site and to ensure its conduct is in compliance with the protocol, informed consent, human research ethics committees (IRBs or IECs) procedures, the World Medical Association Declaration of Helsinki, ICH Guideline for Good Clinical Practice E6(R2), and local regulations governing the conduct of clinical studies.

I will maintain as confidential all written and verbal information provided to me by the Sponsor, including but not limited to, the protocol, case report forms, Investigator's Brochure, material supplied at Investigator Meetings, minutes of teleconferences, etc. Such material will only be provided as necessary to site personnel involved in the conduct of the study, the IRB or IEC, or local regulatory authorities.

I will obtain written informed consent from each prospective study subject or each prospective subject's legal representative prior to conducting any protocol-specified procedures. The consent form will have the approval of the local IRB or IEC.

I will maintain adequate source documents and record all observations, study drug, and procedures pertinent to study subjects in their medical records. I will accurately complete the case report forms supplied by the Sponsor in a timely manner. I will ensure that my facilities and records will be available for inspection representatives of the Sponsor, the local IRB or IEC, or local regulatory authorities. I will ensure that my staff are available to meet with representatives of the Sponsor during regularly scheduled monitoring visits.

I will notify the Sponsor within 24 hours of any serious adverse events. Following this notification, a written report describing the serious adverse event will be provided to the Sponsor as soon as possible, but no later than three days following the official notification.

Investigator's Name (Print)

Investigator's Signature

Date

Document Approvals
Approved Date: 13 Sep 2023

Task: Approvers Approval Verdict: Approve changes & release Approval to be made Effective	<div></div> 13-Sep-2023 15:31:51 GMT+0000
Task: QA Approval Verdict: Approve changes & release QA Approval to be made Effective	<div></div> 13-Sep-2023 20:16:25 GMT+0000