

Supplementary Materials: Safe and Effective Cynomolgus Monkey GLP—Tox Study with Repetitive Intrathecal Application of a TGFBR2 Targeting LNA-Gapmer Antisense Oligonucleotide as Treatment Candidate for Neurodegenerative Disorders

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Supplementary information

Table S1. Timepoints of plasma sampling.

Overview of the different time points for plasma collection.

| Group/Sex | Time Points | Sample Time (Hours Postdose) | | | | | | | | |
|-----------|---------------|------------------------------|---|---|---|---|----|----|----|-----|
| | | 0 | 1 | 2 | 4 | 8 | 12 | 24 | 48 | 168 |
| 1 M/F | Days 1 and 85 | X | X | X | X | X | X | X | X | X |
| 2 M/F | Days 1 and 85 | X | X | X | X | X | X | X | X | X |
| 3 M/F | Days 1 and 85 | X | X | X | X | X | X | X | X | X |
| 4 M/F | Days 1 and 85 | X | X | X | X | X | X | X | X | X |

F = Females; M = Males.

| Group/Sex | Time Points | Sample Time (Before Dosing) | | | | | | | | |
|-----------|---------------------|-----------------------------|--|--|--|--|--|--|--|--|
| | | | | | | | | | | |
| 1 M/F | Days 15, 29, and 57 | X | | | | | | | | |
| 2 M/F | Days 15, 29, and 57 | X | | | | | | | | |
| 3 M/F | Days 15, 29, and 57 | X | | | | | | | | |
| 4 M/F | Days 15, 29, and 57 | X | | | | | | | | |

F = Females; M = Males.

| Group/Sex | Time Points | Sample Time | | | | | | | | |
|-----------|--|-------------|--|--|--|--|--|--|--|--|
| | | | | | | | | | | |
| 1 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X | | | | | | | | |
| 2 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X | | | | | | | | |
| 3 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X | | | | | | | | |
| 4 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X | | | | | | | | |

F = Females; M = Males.

Table S2. Timepoints of CSF sampling.

Overview of the different time points for CSF collection.

| Group/Sex | Time Points | Sample Time (Hours Postdose) | | |
|-------------|-------------------------------|------------------------------|---|-----------------|
| | | 0 | 8 | 24 |
| 1 M/F | Days 1, 15, 29, 57, and 85 | X | X | X |
| 2 M/F | Days 1, 15, 29, 57, and 85 | X | X | X |
| 3 M/F | Days 1, 15, 29, 57, and 85 | X | X | X |
| 4 M/F | Days 1, 15, 29, 57, and 85 | X | X | X |
| 1 to 4 M/F | Days 43 and 71 (± 1 day) | X | X | X |
| 1 to 4 M/Fa | Day 92 | | | Before Necropsy |

F = Females; M = Males.

a Only for terminal sacrifice animals.

| Group/Sex | Time Points | Sample Time |
|-----------|--|-------------|
| | | |
| 1 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X |
| 2 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X |
| 3 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X |
| 4 M/F | Days 98, 105, 112, 119, 133, 147, 175, and 182 | X |

F = Female; M = Male.

Table S3. Neurobehavioral Observations.

Overview of the relevant parameters for neurobehavioral observations

| Parameters | Explanation | Score | Range |
|----------------------------|---|----------|------------|
| Part 1 | | | |
| Checking Vocalization | Mouth partially closed, with abdominal contractions | -1 to +1 | 0 = normal |
| Communicative Vocalization | Mouth open, often with increasing volume | -1 to +1 | 0 = normal |
| Grooming | Auto-grooming behavior | -1 to 0 | 0 = normal |
| Posture | Head and/or body posture | -2 to 0 | 0 = normal |
| Balance/Coordination | Limb coordination and/or postural balance | -4 to 0 | 0 = normal |
| Locomotor Activity | Activity level within the home cage | -1 to +1 | 0 = normal |
| Tremors | Involuntary muscle contractions that cause oscillatory movement of the body, head, or limbs | 0 to +2 | 0 = absent |
| Twitches/Jerks | Involuntary muscle contractions that cause the animal to abruptly jerk or twitch | 0 to +1 | 0 = absent |
| Muscle Cramping | Involuntary muscle contractions that persist for seconds to minutes | 0 to +1 | 0 = absent |

| Parameters | Explanation | Score | Range |
|------------------------------|---|----------|------------|
| Part 1 | | | |
| Stereotypy | Repetitive, apparently purposeless or ritualistic postures or movements (e.g., circling behavior or retropulsion) | 0 to +2 | 0 = absent |
| Alertness | Noticeable reaction to a technician approaching the cage | -3 to +1 | 0 = normal |
| Offensive Aggression | In response to a technician approaching the cage (moves to the front of the cage, bears teeth, and possibly vocalizes) | 0 to +2 | 0 = normal |
| Defensive Aggression | In response to a technician approaching the cage (moves to the back of the cage, bears teeth, and possibly vocalizes) | 0 to +2 | 0 = normal |
| Reaction to Visual Stimuli | Tracking of an object's movement in front of the cage | -1 to +1 | 0 = normal |
| Reaction to Acoustic Stimuli | Response to an acoustic stimulus from a hidden source | -1 to +1 | 0 = normal |
| Part 2 | | | |
| Muscle Tone | Assessed by palpation of limbs and the abdomen | -2 to +2 | 0 = normal |
| Grip Strength | Qualitatively assessed during removal of the animal from the cage or during further handling (e.g., ability to grip a part of the cage) | -3 to 0 | 0 = normal |
| Lacrimation | By visual inspection | 0 to +2 | 0 = absent |
| Salivation | By visual inspection | 0 to +2 | 0 = absent |
| Part 2 - Measurement | | | |
| Body Temperature | Rectal measurement (°C) | | |

Table S4. Summary of parameters tested within plasma, CSF, and urine.

Overview of the tested parameters for hematology, clinical chemistry, CSF parameters, and urinalysis.

Hematology Tests

| | |
|-------------------------------------|------------------------------|
| Reticulocyte Count (RETI.) | Lymphocytes (LYM.) |
| Absolute Reticulocyte Count (TRET.) | Monocytes (MONO.) |
| Red Blood Cell Count (RBC.) | Eosinophils (EOS.) |
| Hemoglobin (HGB.) | Basophils (BASO.) |
| Hematocrit (HCT.) | Absolute Neutrophils (ANEU.) |
| Mean Corpuscular Volume (MCV.) | Absolute Lymphocytes (ALYM.) |
| Mean Corpuscular Hemoglobin (MCH.) | Absolute Monocytes (AMON.) |

Mean Corpuscular Hemoglobin Concentration (MCHC.)

White Blood Cell Count (WBC.)

Neutrophils (NEUT.)

Absolute Eosinophils (AEOS.)

Absolute Basophils (ABAS.)

Platelet Count (PLT).

Clinical Chemistry Tests

Total Bilirubin (TBIL.)

Creatinine (CREA.)

Blood Urea (BU.)

Glutamate Dehydrogenase (GLDH.)

Aspartate Aminotransferase (AST.)

Alanine Aminotransferase (ALT.)

Alkaline Phosphatase (ALP.)

Gamma Glutamyl Transferase (GGT.)

Glucose (GLU.)

Total Cholesterol (CHOL.)

Triglycerides (TRIG.)

Inorganic Phosphorus (PHOS.)

Calcium (CA.)

Sodium (NA.)

Potassium (K.)

Chloride (CL.)

Total Protein (TP.)

Albumin (ALB.)

Globulin (GLOB.)

Albumin/Globulin Ratio (A:G.)

CSF Chemistry Tests

CSF Glucose (CGLU.)^a

CSF Inorganic phosphorus (CPHO.)^a

CSF Calcium (CCA.)^a

CSF Sodium (CNA.)^a

CSF Qualitative WBC (CQWBC.)

CSF Potassium (CSFK.)^a

CSF Chloride (CCL.)^a

CSF Total protein (CTP.)^a

CSF Albumin (CMIA.)^a

CSF Qualitative RBC (CQRBC.)

Urinalysis Tests

Urine Volume (UVOL.)

Urine Specific Gravity (USGR.)

Urine pH (UPH.)

Urine Leukocytes (ULEU.)^a

Urine Nitrite (UNIT.)^a

Urine Protein (UPRO.)^a

Urine Glucose (URGL.)^a

Urine Ketones (UKET.)^a

Urine Urobilinogen (UUBG.)^a

Urine Bilirubin (UBIL.)^a

Urine Occult Blood (UOBL.)^a

Urine Color (UCOL.)

Urine Clarity (UCLA.)