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October 8-11, 2025 • Charlotte, NC

Design of a Pivotal Trial Evaluating the Safety, Tolerability, and Efficacy of Zilganersen, an Investigational RNA-Targeted Antisense Therapy, in People Living With Alexander Disease

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Disclosures

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- Amy T Waldman's disclosures are as follows:
  - Received grant funding from the National Institutes of Health (U54NS115052)
  - Received research funding for investigator-initiated research from Elise's Corner, Grayson's Ladder, Ionis, and the United Leukodystrophy Foundation
  - Received clinical trial support from Ionis, and (unrelated to this work) Novartis, Passage Bio, Pfizer, Roche/Genentech, and Sarepta Therapeutics
  - Served as a nonremunerated scientific advisor for Elise's Corner, EndAxD, and The Calliope Joy Foundation
  - Receives honoraria from MedLink Neurology and UpToDate
  - Received personal compensation for serving on a data safety monitoring board for SwanBio Therapeutics

Alexander Disease (AxD) is a Rare, Genetic, Progressive Astrocytopathy[1]

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- Prevalence is estimated at ~1 case per 1 to 3 million individuals[2-4] and may be underreported
- AxD is associated with pathogenic variants in the *GFAP* gene[1,5] that affect astrocytes[5-7]
- AxD is a clinically heterogenous disease[8,9] characterized by progressive neurologic deterioration[8,10]
- Symptoms often include impairment in gross motor function[8,10]

Common clinical features of AxD based on age of onset[9,11]	
Earlier age of onset	**Later age of onset**
Gross motor delay	Gait disturbance
Spasticity	Ataxia
Hyperreflexia	Muscle weakness
Developmental delay	Spastic para- or tetraparesis
Macrocephaly	Bulbar signs
Seizures	Palatal myoclonus
Cognitive dysfunction	Autonomic dysfunction
Failure to thrive	Ocular motor abnormalities

AxD, Alexander disease; GFAP, glial fibrillary acidic protein.

1. Brenner M, et al. *Nat Genet*. 2001;27(1):117-20. 2. Barczykowski AL, et al. *Am J Med Genet A*. 2012;158A(11):2835-42. 3. Stellitano LA, et al. *Dev Med Child Neurol*. 2016;58(7):680-9. 4. Yoshida T, et al. *J Neurol*. 2011;258(11):1998-2008. 5. Lin NH, et al. *J Biol Chem*. 2024;300(7):107402. 6. Sosonuv AA, et al. *Acta Neuropathol Commun*. 2017;5(1):27. 7. Hagemann TL, et al. *Hum Mol Genet*. 2005;14(16):2443-58. 8. Vaia Y, et al. *Mol Genet Metab*. 2023;138(3):107540. 9. Prust M, et al. *Neurology*. 2011;77(13):1287-94. 10. Pareyson D, et al. *Brain*. 2008;131(Pt 9):2321-31. 11. Li R, et al. *Ann Neurol*. 2005;57:310-26.

Zilganersen is the First Potential Disease-Modifying Therapy for AxD Under Investigation in Clinical Trials

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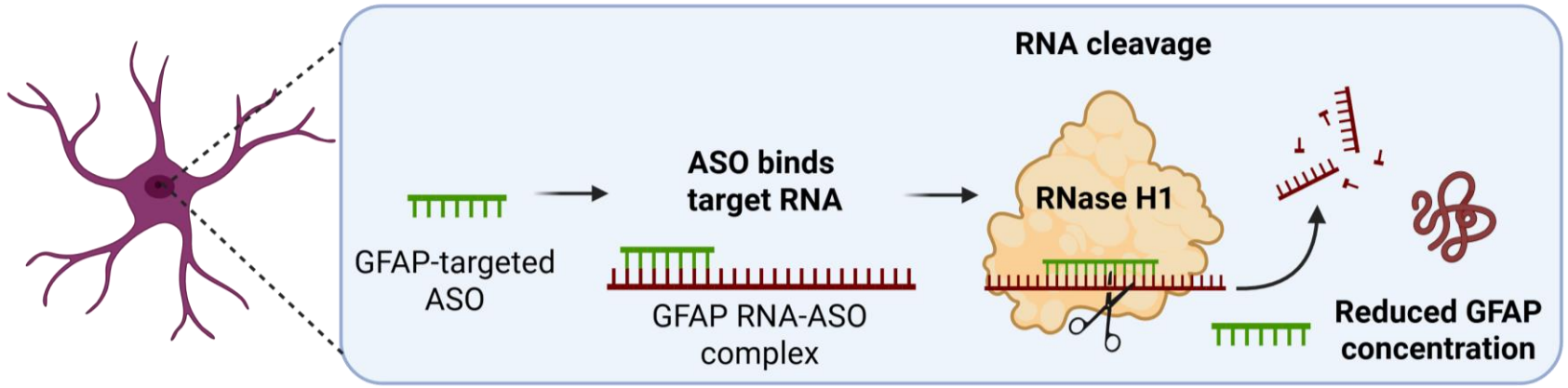
- Current approaches to disease management can provide care for symptoms but do not address the underlying cause or modify disease progression[1]
- Zilganersen is an investigational GFAP RNA–targeted antisense therapy designed to reduce GFAP production to slow or halt disease progression in people with AxD[2]



Zilganersen is an investigational treatment and has not been evaluated for safety or efficacy by any health authority.
ASO, antisense oligonucleotide; AxD, Alexander disease; GFAP, glial fibrillary acidic protein; RNase H1, ribonuclease H1.
1. Adang LA, et al. *Mol Genet Metab*. 2017;122(1-2):18-32. 2. Ionis announces positive topline results from pivotal study of zilganersen in Alexander disease. Accessed September 24, 2025. https://ir.ionis.com/news-releases/news-release-details/ionis-announces-positive-topline-results-pivotal-study.

Phase 1–3 Pivotal Trial Overview

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- This is an ongoing pivotal, phase 1–3, global, multicenter, randomized, controlled study (NCT04849741) evaluating zilganersen in individuals 2 to 65 years of age with genetically confirmed AxD
  - The study is assessing efficacy, safety, and tolerability

Key inclusion criteria

1. Aged 2 to 65 years[a]
2. Clinical phenotype and brain imaging consistent with a diagnosis of AxD
3. Documented genetic variant in the *GFAP* gene

Key exclusion criteria

1. Clinically significant abnormalities in medical history or physical examination
2. Any contraindication or unwillingness to undergo MRI
3. History of gene therapy or cell transplantation or any other experimental brain surgery
4. Current obstructive hydrocephalus
5. Presence of a functional ventriculoperitoneal shunt

[a]Participants <2 years of age at screening are included in an open-label substudy.
AxD, Alexander disease; GFAP, glial fibrillary acidic protein; MRI, magnetic resonance imaging.
ClinicalTrials.gov identifier: NCT04849741. Updated December 16, 2024. Accessed April 30, 2025. https://clinicaltrials.gov/ct2/show/NCT04849741.

Clinical Trial Design of the Main Study[1,2]

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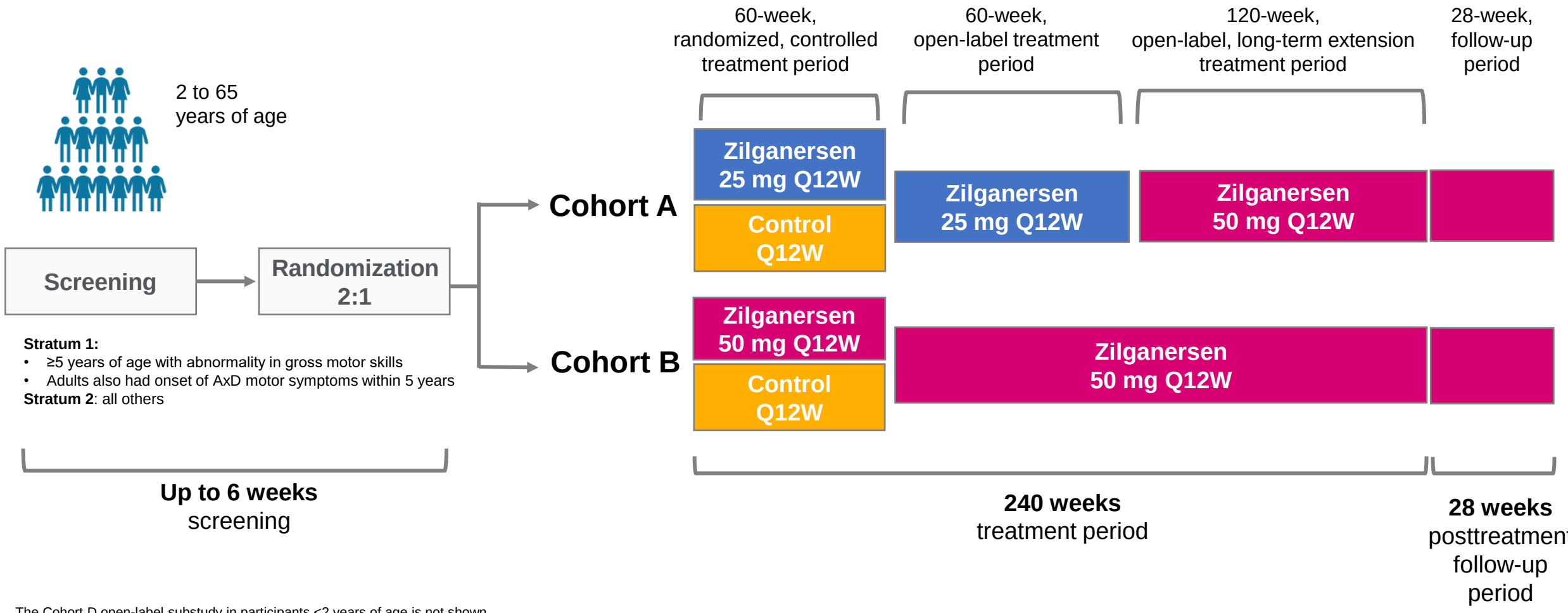

The Cohort D open-label substudy in participants <2 years of age is not shown.
AxD, Alexander disease; Q12W, every 12 weeks.

1. ClinicalTrials.gov identifier: NCT04849741. Updated December 16, 2024. Accessed April 30, 2025. https://clinicaltrials.gov/ct2/show/NCT04849741. 2. Ionis announces positive topline results from pivotal study of zilganersen in Alexander disease. Accessed September 24, 2025. https://ir.ionis.com/news-releases/news-release-details/ionis-announces-positive-topline-results-pivotal-study.

Primary Endpoint: Motor Function[1]

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- The 10MWT is an assessment of gait speed (m/s) on a standardized 10-meter course
  - The 10MWT is a **construct-valid measure** for capturing ambulatory disability and impairments in balance, motor development, and fine and gross motor function **in people with AxD[2]**

Participants ≥5 years old[1]

Assess gait speed at Week 61 vs baseline

Analysis from the NHS in AxD[2]

The 10MWT demonstrated construct validity to capture motor impairment and moderate to strong correlations with numerous other COAs that capture impairments in ambulatory and motor function in AxD

- **GMFM-88**
- **Disability scales**
- **GMFCS**
- **Manual Ability Scale**
- **Balance**

Health authorities endorsed use of the 10MWT in this trial given its construct validity in multiple sclerosis. This COA can be used to power a controlled trial in very rare disease, which was a regulatory requirement

10MWT, 10-Meter Walk Test; AxD, Alexander disease; COA, clinical outcome assessment; GMFM-88, Gross Motor Function Measure-88; GMFCS, Gross Motor Function Classification Scales; NHS, Natural History Study.

1. ClinicalTrials.gov identifier: NCT04849741. Updated December 16, 2024. Accessed April 30, 2025. https://clinicaltrials.gov/ct2/show/NCT04849741. 2. Yarlas A, et al. Poster at the AAN Annual Meeting 2024; April 13-18; Denver, CO, USA.

Secondary and Safety Endpoints

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- Secondary endpoints are classified by 4 disease domains capturing AxD symptoms[1-3]

Disease domains

Motor symptoms[1,2]	Cognitive symptoms[2]	Gastrointestinal symptoms[1-3]	Miscellaneous symptoms[1]
Weakness, spasticity, gait abnormalities, dystonia, tremor, ataxia, myoclonus, fine motor impairments	Speech problems, intellectual disability, language acquisition delays, behavior problems	Dysphagia, nausea, vomiting, reflux, constipation, weight loss	Urinary and sleep problems, orthostatic hypotension seizures, headaches, autonomic dysfunction

Secondary endpoints

Motor symptoms[1,2]	Cognitive symptoms[2]	Gastrointestinal symptoms[1-3]	Miscellaneous symptoms[1]
9-Hole Peg Test[4]	Vineland-3 Adaptive Behavior Assessment[4]	Pediatric QOL Inventory Gastrointestinal Symptoms Scale[4]	Composite Autonomic Symptom Score 31[4]
GMFM-88[4]			
Vineland-3 Motor Skills[4]			

Patient-reported change in the AxD domain reported at baseline as most bothersome[4]

Safety and tolerability including incidence and severity of TEAEs, SAEs, vital signs, and laboratory tests[4]

AxD, Alexander disease; GMFM-88, Gross Motor Function Measure-88; QOL, quality of life; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

1. Pareyson D, et al. *Brain*. 2008;131(Pt 9):2321-31. 2. Zampini L, et al. *Dev Neurorehabil.* 2023;26(4):253-61. 3. Li R, et al. *Ann Neurol*. 2005;57:310-26. 4. ClinicalTrials.gov identifier: NCT04849741. Updated December 16, 2024. Accessed April 30, 2025. https://clinicaltrials.gov/ct2/show/NCT04849741.

Baseline Demographics and Motor Function Were Generally Comparable Between Groups

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Safety set n (%)	Cohort A + B	
	Zilganersen 25 mg or 50 mg Q12W n = 32	Control n = 17
Age, years, median	11	10
2–18 years	29 (91)	15 (88)
Sex		
Female	22 (69)	10 (59)
Male	10 (31)	7 (41)
10MWT (m/s), ≥5 years, n	27	14
Mean (SD)	1.3 (0.6)	1.1 (0.5)
Median (min, max)	1.4 (0.0, 2.8)	0.9 (0.2, 2.1)

Safety set includes all patients who were randomized and received at least 1 dose of the study drug (zilganersen or control).
Per protocol, stratum 1 of Cohort B that received zilganersen 50 mg contributed n = 17 patients to the primary analysis set.
The Cohort D open-label substudy in participants <2 years of age is not shown (n = 5).
10MWT, 10-Meter Walk Test; max, maximum; min, minimum; Q12W, every 12 weeks; SD, standard deviation.
Ionis announces positive topline results from pivotal study of zilganersen in Alexander disease. Accessed September 24, 2025. https://ir.ionis.com/news-releases/news-release-details/ionis-announces-positive-topline-results-pivotal-study.

Zilganersen Resulted in Significant and Clinically Meaningful Stabilization of Primary Endpoint Compared With Control

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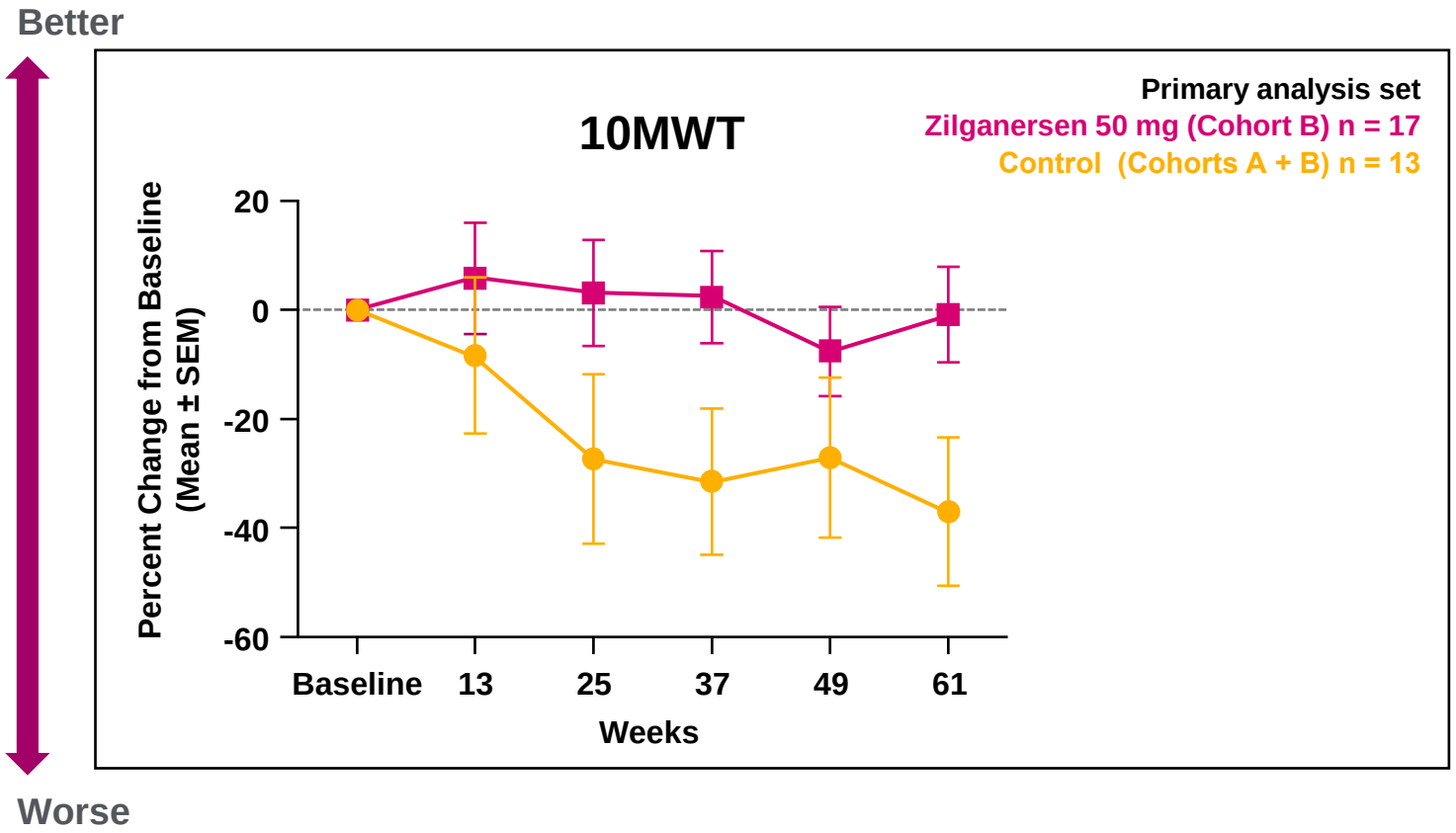

33.3% LSM Difference at Week 61 (*P* = 0.041; SE, 15.6)

Positive treatment effect of zilganersen exceeds the threshold of clinically meaningful difference in the 10MWT*,[2-5]

Primary analysis set includes all patients who were randomized, received at least 1 dose of the study drug (zilganersen or control), and have at least 1 postbaseline primary efficacy assessment. Primary analysis set contains Stratum 1 only: (i) ≥ 5 years of age; and (ii) if ≥18 years of age, had onset of AxD motor symptoms/signs within 5 years of screening; and (iii) demonstrate an abnormality in gross motor skills.
*Due to lack of sufficient literature in AxD, MCID was based on multiple sclerosis literature.
10MWT, 10-Meter Walk Test; AxD, Alexander disease; LSM, least square mean; MCID, minimal clinically important difference; SE, standard error; SEM, standard error of the mean.
1. Ionis announces positive topline results from pivotal study of zilganersen in Alexander disease. Accessed September 24, 2025. https://ir.ionis.com/news-releases/news-release-details/ionis-announces-positive-topline-results-pivotal-study. 2. Kaufman M, et al. *Mult Scler*. 2000;6(4):286-290. 3. Hobart J, et al. *Neurology*. 2013;80:1509-17. 4. Cohen JA, et al. *JAMA Neurol*. 2014;71:1386-93. 5. Kragt J, et al. *Mult Scler*. 2006;12:594-8.

Zilganersen Significantly Reduced Plasma GFAP Levels Compared With Control

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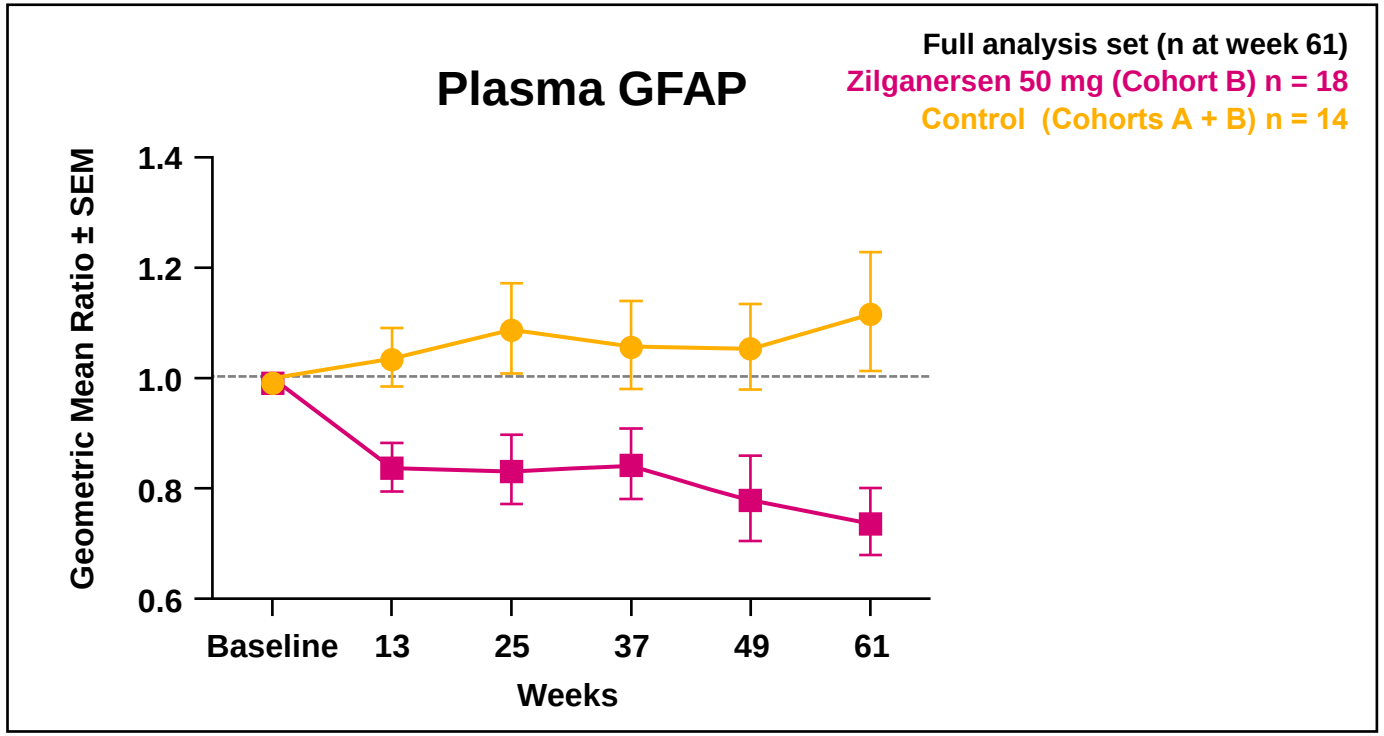
Increasing



Decreasing

33.6% lower plasma GFAP with zilganersen vs control at Week 61 in an exploratory analysis (*P* = 0.003; LSGM ratio difference [95% CI], 0.7 [0.5, 0.9])

Full analysis set includes all patients who were randomized, received at least 1 dose of the study drug (zilganersen or control), and have at least 1 postbaseline efficacy assessment in the double-blind treatment period
CI, confidence interval; GFAP, glial fibrillary acidic protein; LSGM, least square geometric mean; SE, standard error.
Data on file.

Zilganersen Demonstrated Favorable Safety and Tolerability at Week 61

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- Most TEAEs were mild or moderate
- No TEAEs led to treatment discontinuation in the double-blind treatment period
- Serious TEAEs in >1 patient included seizure, vomiting, influenza, and scoliosis
  - One TEAE with fatal outcome in the zilganersen group (disease progression, not related to the study drug)
- TEAEs of increased ICP and laboratory CSF protein >45 mg/dL were numerically lower with zilganersen than control
- No zilganersen-related effects on platelet counts, or renal or liver function were observed

Safety set n (%)	Cohort A + B	
	Zilganersen 25 mg or 50 mg Q12W n = 32	Control n = 17
Any TEAEs	32 (100)	17 (100)
Mild	7 (21.9)	3 (17.6)
Moderate	18 (56.3)	11 (64.7)
Severe	7 (21.9)	3 (17.6)
TEAEs leading to		
Treatment discontinuation	0	0
Treatment interruption	4 (12.5)	4 (23.5)
Any serious TEAEs (n >1)	12 (37.5)	8 (47.1)
Seizure	2 (6.3)	2 (11.8)
Vomiting	1 (3.1)	2 (11.8)
Influenza	2 (6.3)	0
Scoliosis	2 (6.3)	0
Increased ICP	1 (3.1)	3 (17.6)
CSF protein >45 mg/dL	6 (18.8)	5 (29.4)

Safety set includes all patients who were randomized and received at least 1 dose of the study drug (zilganersen or control). Increased ICP TEAE includes signs and/or symptoms of possible increased ICP and elevated CSF opening pressure.
CSF, cerebrospinal fluid; ICP, intracranial pressure; Q12W, every 12 weeks; TEAE, treatment-emergent adverse event; Ionis press release. https://ir.ionis.com/news-releases/news-release-details/ionis-announces-positive-topline-results-pivotal-study.

Conclusions

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Zilganersen **continues to be evaluated for long-term safety and efficacy** in an ongoing open-label trial, and the potential to initiate an Expanded Access Program in the US is being evaluated

Zilganersen **demonstrated target engagement, statistically significant** and **clinically meaningful stabilization** of the primary endpoint, and a **favorable tolerability and safety profile**

Zilganersen is the **first potential disease-modifying GFAP RNA–targeted therapy for AxD**

AxD, Alexander disease; GFAP, glial fibrillary acidic protein.

Acknowledgments

We thank all of the patients, family members, and staff who participated in the study

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