Amylyx Update on Development of AMX0114, an Antisense Oligonucleotide Targeting Calpain-2

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In memory of Mick, a husband and father, who was a gifted tattoo artist and musician.

Please Note

- AMX0114 is an investigational drug and has not been approved by any health authority.
- This presentation is intended to provide scientific information about AMX0114. The statements and content shared in this presentation have not been evaluated by any health authority.

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Agenda

- Scientific Rationale for AMX0114: Calpain-2 in Axonal Degeneration and ALS
- About AMX0114
- AMX0114 Preclinical Data
- LUMINA Study Design

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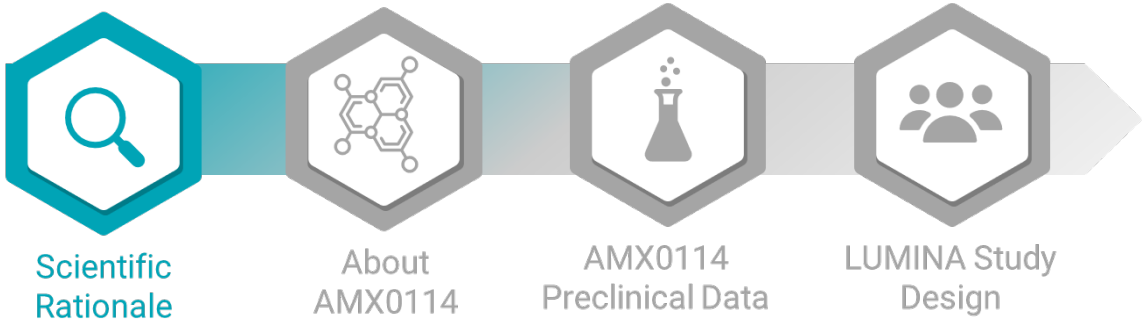
Scientific Rationale for AMX0114: Calpain-2 in Axonal Degeneration and ALS



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Calpain-2 is a Cysteine Protease Activated by Calcium

Calpains are a family of non-lysosomal proteolytic enzymes

- Calpains are involved in cytoskeletal remodeling, signal transduction, cell differentiation, embryonic development, vesicular trafficking, apoptosis, and necrosis[1,2]
- 15 calpain isoforms identified: calpain-1 and calpain-2 are ubiquitously expressed, including in the brain[1,2]
  - Calpain-1 largely associated with neuroprotection, while calpain-2 is implicated in axonal degeneration
  - Activation tightly regulated by intracellular **calcium** levels
- Unlike other proteases that cause complete breakdown of substrate, calpains cleave protein substrates at limited and specific sites[2]
- Endogenous calpain inhibitor = calpastatin[1,2]

Calpain-2 substrates include:[1-5]

- Cytoskeletal proteins
  - αII-spectrin
  - NfL
  - Microtubule-associated proteins (tau)
- TDP-43
- Cell death pathway signaling proteins

AMYLYX **1.** Ono Y, Saido TC, Sorimachi H. Nat Rev Drug Discov. 2016;15(12):854-876. **2.** Ma M. Neurobiol Dis. 2013;60:61-79. **3.** Ma M, et al. Neurobiol Dis. 2013;56:34-46. **4.** Yamashita, T et al. Nat Commun. 2012; 3:1307. **5.** Wang Y, et al. Cells.2020;9(12):2698.

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Calpain-2 Contributes to Axonal Degeneration

- Axonal degeneration is one of the earliest cellular processes leading to ALS[1]
  - > Calpain-2 activation can lead to axonal degeneration in multiple ways: activating or deactivating cellular pathways, reducing the integrity of cellular structures, and more[1-3]

Evidence for Targeting Calpain-2 in ALS[4-7]

- Calpain-2 levels are elevated in people with ALS

- Inhibition of calpain-2 has shown benefit in ALS mouse model

- Calpain-2 substrates include neurofilament and TDP-43

- AMX0114 has shown efficacy in pre-clinical ALS models

AMYLYX **1.** Fischer LR, Glass JD. Neurodegenerative Dis 2007;4:431–442. **2.** Ono Y, Saido TC, Sorimachi H. Nat Rev Drug Discov. 2016;15(12):854-876. **3.** Ma M. Neurobiol Dis. 2013;60:61-79. **4.** Ueyama H et al. J Neurol Sci. 1998;155(2):163-169. **5.** Yamashita T et al. Nat Commun. 2012;3:1307. **6.** Rao MV, et al. J Neurochem. 2016;137(2):253-65. **7.** Ma M, et al. Neurobiol Dis. 2013;56:34-46.

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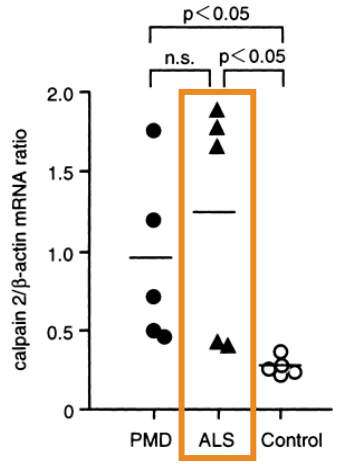
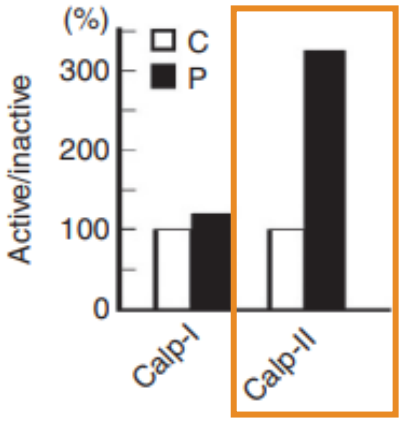
Why Target Calpain-2 in ALS?

Calpain-2 Levels are Upregulated in ALS[1,2]

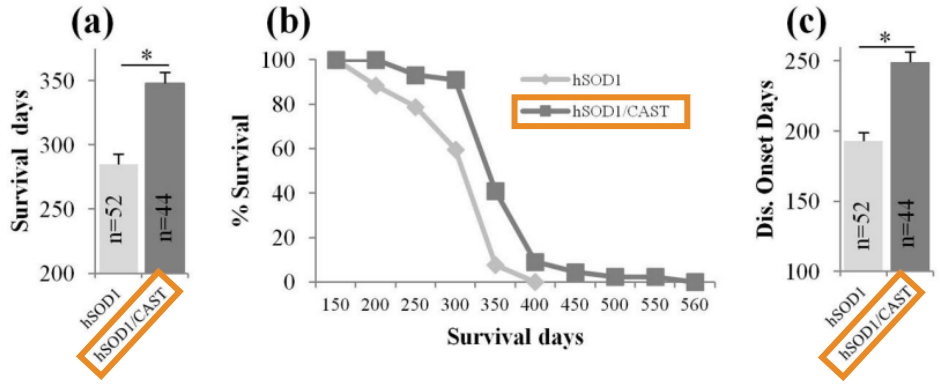


CAPN2 mRNA is upregulated in biopsied muscle samples of people with ALS[1]



Ratio of active to inactive calpain-2 in post-mortem spinal cord tissue of people with ALS (P) is ~3x higher than that in healthy controls (C)[2]

Calpain-2 Inhibition has Demonstrated Benefit in ALS Mouse Model[3]



In the transgenic ALS SOD1G93A mouse model, neuron-specific overexpression of calpain inhibitor calpastatin (hSOD1/CAST) increases overall survival (a-b) and delays disease onset (c)[3]

TDP-43 and Neurofilament are Substrates of Calpain-2[2,4]

- Calpain-specific TDP-43 cleavage products detected in spinal cords and primary motor cortices of patients with ALS-FTD in which there was also an increase in the levels of activated calpain-I and calpain-II compared with controls[2]
  - Mechanistic linkage between calpain-2 and one of the pathologic hallmarks of ALS
- Calpain-2 is considered the primary protease mediating cleavage of neurofilament[5]
  - CSF NfL levels in ALS may reflect calpain activity

1. Ueyama H et al. *J Neurol Sci*. 1998;155(2):163-169. **2.** Yamashita T et al. Nat Commun. 2012;3:1307. **3.** Rao MV, et al. J Neurochem. 2016;137(2):253-65. **4.** Ma M, et al. Neurobiol Dis. 2013;56:34-46.

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About AMX0114

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AMX0114: An Antisense Oligonucleotide (ASO) Targeting Calpain-2

Selectivity of the ASO modality may offer distinct advantages over earlier, small molecule-based approaches to targeting calpain-2

- Specifically inhibits calpain-2 without disrupting the function of other calpains or calpastatin
- Designed to downregulate expression of the calpain-2 gene (*CAPN2*)
- Targets an exon in the active site of the calpain-2 protease
- Lowers levels of *CAPN2* mRNA transcript through RNase H-mediated degradation, subsequently lowering levels of functional calpain-2 protein in the cell

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AMX0114 Preclinical Data

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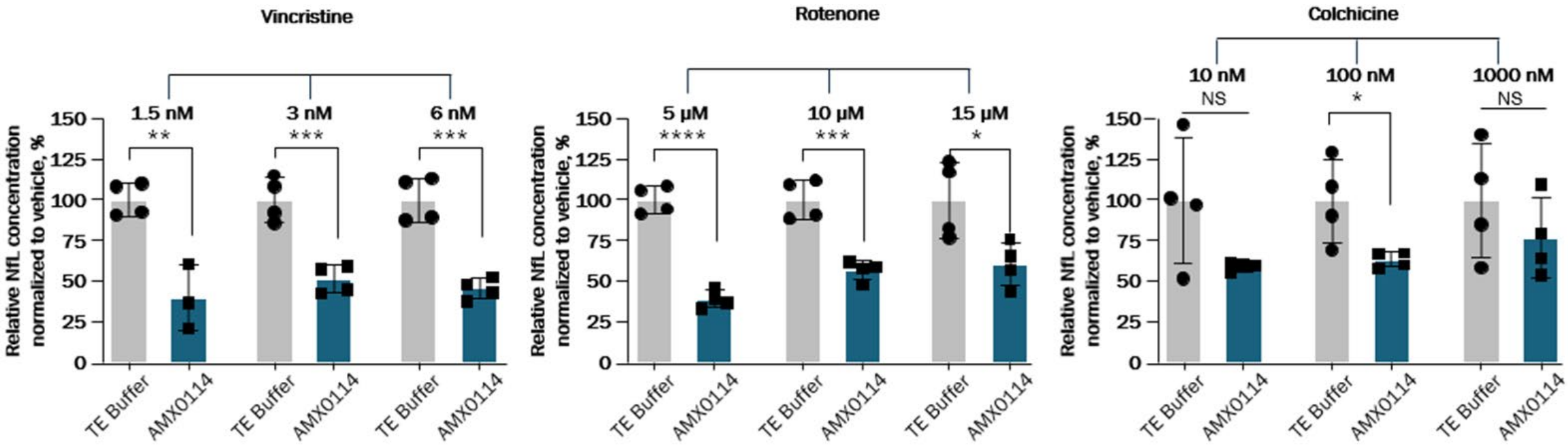
AMX0114 Reduces Extracellular NfL Levels in Model of Trigger-Induced Neuronal Injury

Charles River Labs

- NfL levels measured after induced pluripotent stem cell (iPSC)-derived motor neurons were exposed to varying concentrations of the neurotoxic compounds vincristine, rotenone, and colchicine after pretreatment with AMX0114



- Bars represent mean (±SD) NfL concentrations relative to vehicle.
- Overlying symbols represent individual replicate values (circles for vehicle and triangles for AMX0114)
- NS = *P*>.05. **P*<.05. ***P*<.01, ****P*<.001, *****P*<.0001.

AMX0114 **TE Buffer**

AMYLYX NfL, neurofilament light chain; NS, not significant; TE, tris EDTA.

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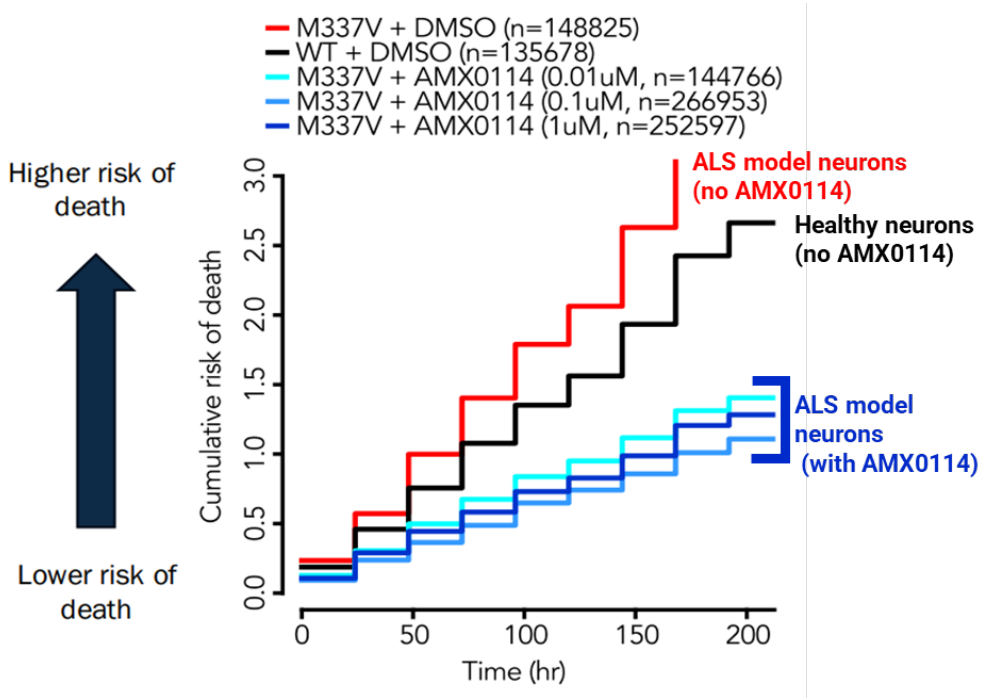
AMX0114 Improved Survival and Reduced Extracellular NfL Levels in Model of TDP-43 ALS

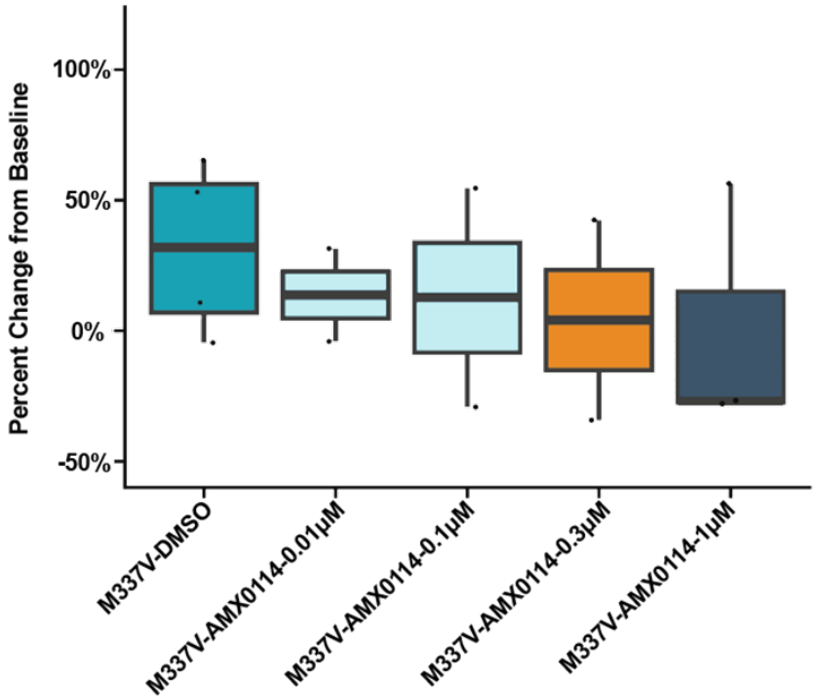
Barmada lab

- Assessment of survival and extracellular NfL levels at multiple AMX0114 concentrations were evaluated in iPSC-derived neurons harboring the ALS-linked TPD-43(M337V) mutation

Dose-Dependent Improvement in Survival with AMX0114 in Model of TDP-43 ALS



Reduction in Extracellular NfL with AMX0114 in Model of TDP-43 ALS



DMSO, dimethyl sulfoxide.

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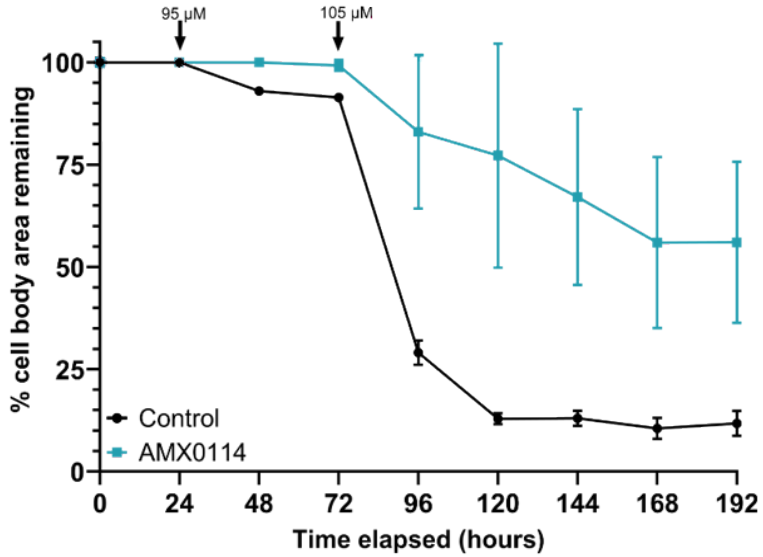
AMX0114 Pre-Treatment Resulted in Neuroprotection in Model of Oxidant-Induced Cell Death

Manfredi lab

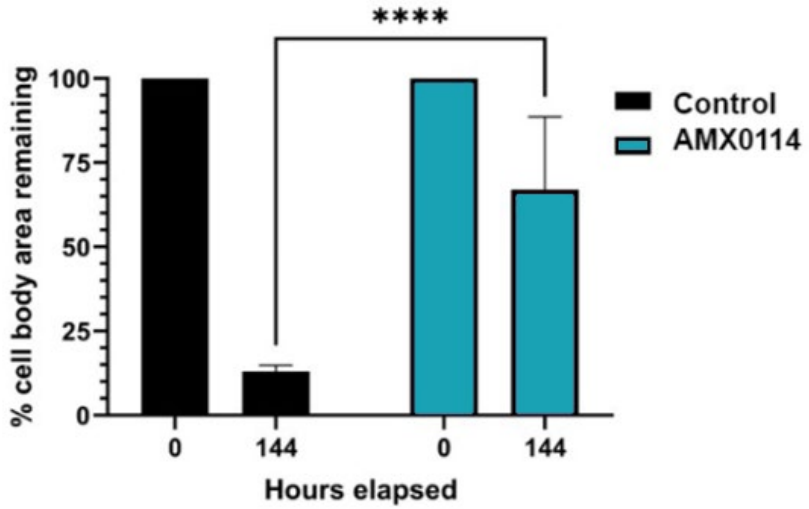
- Neuronal cells were pre-treated with AMX0114 and compared to untreated controls after exposure to hydrogen peroxide [24 hours (95 µM) and 72 hours (105 µM)]. Cell body area was measured every 24hr from timepoints 0 to 192hr.

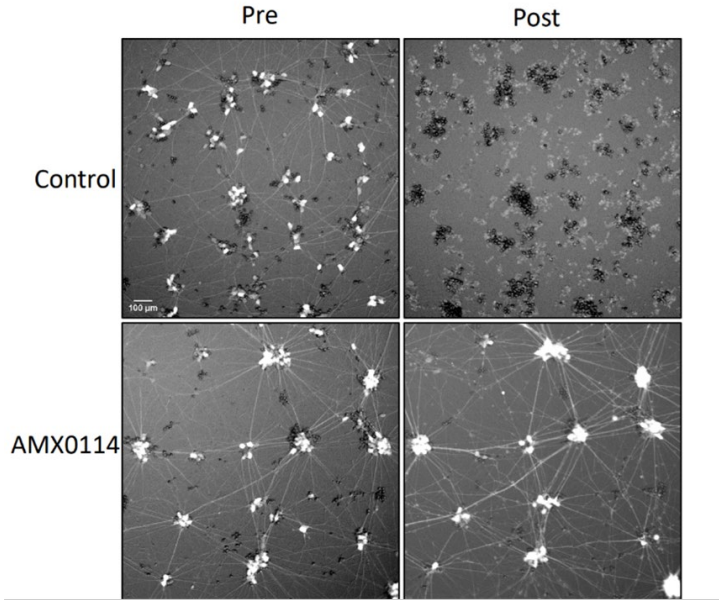
Calpain-2 Knockdown with AMX0114 Translated to Improved Survival Following Exposure to Hydrogen Peroxide

% Neuronal Cell Body Area Remaining Over Time



% Neuronal Cell Body Area Remaining After 144 Hours





Representative images of control (untreated) and AMX0114-treated iPSC-derived motor neurons before (pre) and after (post) exposure to H_2O_2

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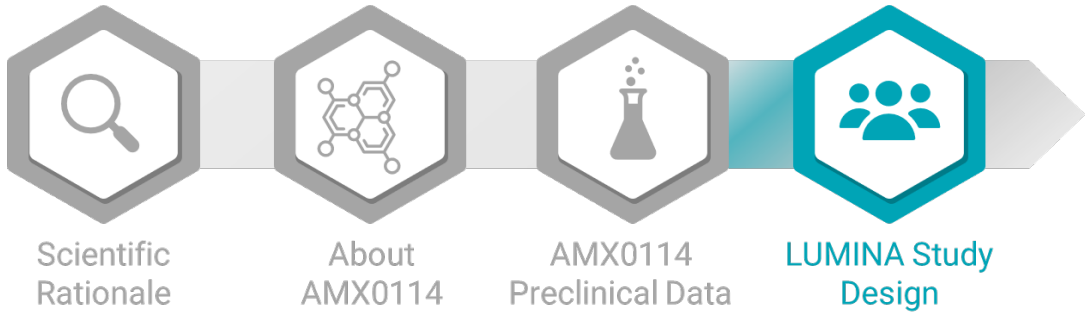
LUMINA Study Design



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LUMINA Study Design

- LUMINA is a phase 1, multicenter, randomized, placebo-controlled multiple ascending dose study in ~48 adult participants with ALS
- Four dose levels of study drug (AMX0114 or placebo) are planned to be examined sequentially

Objectives:

- To evaluate the safety and tolerability of AMX0114 administered intrathecally for a total of up to 4 doses per dose level
- To evaluate the pharmacokinetics of AMX0114 in CSF and plasma
- To assess effects of AMX0114 on levels of plasma and CSF biomarkers and functional measures of ALS disease progression

Key Trial Entry Criteria

- ✓ Adults ≥ 18 years
- ✓ Diagnosis of clinically definite or clinically probable ALS, based on El Escorial criteria
- ✓ Time since onset of first symptom of ALS <24 months
- ✓ Slow vital capacity (SVC) ≥ 75%
- ✓ Approved treatments for ALS are allowed if participant is on a stable dose for at least 30 days prior to baseline visit

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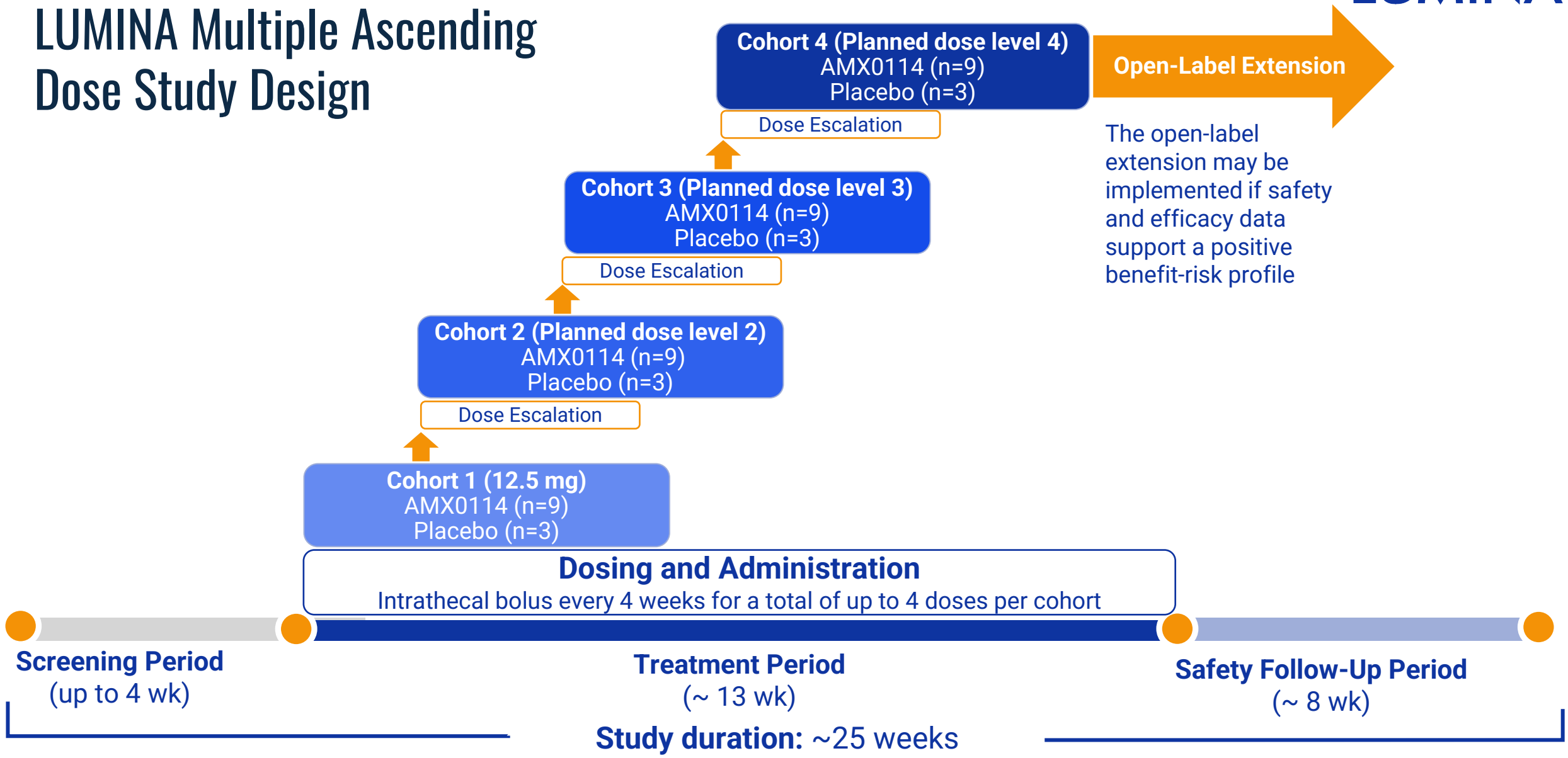
LUMINA Multiple Ascending Dose Study Design



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LUMINA Study Endpoints

Primary Endpoints

- Incidence of adverse events (AEs), serious adverse events (SAEs) and dose limiting toxicities (DLTs)
- Incidence of abnormalities in clinical laboratory assessments, vital signs, physical and neurological examinations and electrocardiograms

Secondary Endpoints

- Pharmacokinetic concentrations, including plasma and cerebrospinal fluid (CSF) levels of AMX0114

Tertiary Endpoints

Pharmacodynamics/Biomarkers

- Change from baseline of plasma and CSF pharmacodynamic measures of ALS and markers of target engagement (e.g., calpain-2 levels, NfL, SBDP-145)

ALS Progression Measures

- Change from baseline of ALS Functional Rating Scale – Revised (ALSFRS-R) and slow vital capacity (SVC)

NfL, neurofilament light chain; SBDP-145, spectrin breakdown product 145.

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Development of AMX0114: An Antisense Oligonucleotide Targeting Calpain-2

Calpain-2 is a critical effector of axonal degeneration, a key early contributor to the pathogenesis of ALS

AMX0114 is an antisense oligonucleotide (ASO) inhibitor of calpain-2 (encoded by the *CAPN2* gene)

AMX0114 has shown benefit on neuronal survival and reduction of neurofilament light chain across multiple disease-relevant cell types and preclinical models

LUMINA will be a phase 1 study evaluating the safety and tolerability of AMX0114 in adult participants with ALS by end of 2024 or in early 2025

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Q&A

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