Updates on Development of AMX0114: An Antisense Oligonucleotide Inhibitor of Calpain-2, a Critical Effector of Axonal Degeneration

Poster 287

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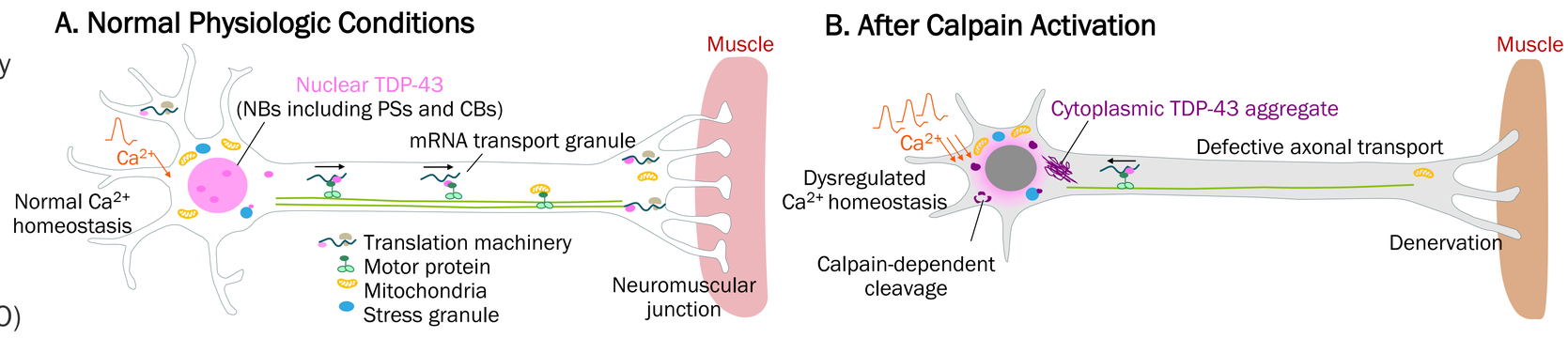
BACKGROUND/RATIONALE

- Axonal degeneration is a key contributor to the clinical presentation and pathogenesis of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases[1,2]
- Activation of the calcium-dependent protease calpain-2 is a critical effector of axonal degeneration and neuronal cell death (**Figure 1**)[2,3]
- Calpain-2 is implicated in the pathogenesis of ALS based on:
  - Findings of elevated calpain-2 messenger RNA (mRNA) in muscle samples[4] and calpain-specific transactive response DNA-binding protein 43 (TDP-43) cleavage product concentrations in postmortem spinal cord[3,5] and brain[3] samples from people with ALS
  - Calpain-dependent TDP-43 cleavage promotes aggregation of TDP-43, a pathologic hallmark in ALS and other neurodegenerative diseases[3]
  - Therapeutic benefit of calpain-2 activity modulation in animal models of ALS[6]
  - The role of calpain-2 in cleaving neurofilament, a component of the axonal cytoskeleton[2] and a broadly researched biomarker in ALS
- Activation of calpain-2 has been implicated in neuronal death resulting from acute neuronal injury[7]
- Based on evidence supporting a potential benefit of calpain-2 modulation in ALS and other neurodegenerative diseases, Amylyx Pharmaceuticals developed AMX0114, an antisense oligonucleotide (ASO) inhibitor of calpain-2 (encoded by the *CAPN2* gene)

What Are Calpains?

- Calpains are a family of calcium-dependent cysteine proteases that target multiple substrates within the axonal cytoskeleton[2]
- There are >12 calpain isoforms. Of the 2 main isoforms (calpain-1 and calpain-2), calpain-1 is generally believed to play a neuroprotective role, while activation of calpain-2 is associated with axonal degeneration[3,8]

Figure 1. CONSEQUENCES OF CALPAIN ACTIVATION FOR MOTOR NEURON FUNCTION[3]



Ca2+, calcium; CB, Cajal body; mRNA, messenger ribonucleic acid; NB, nuclear body; PS, paraspeckle; TDP-43, transactive response DNA-binding protein 43.

AMX0114 DEVELOPMENT PROGRAM

Lead ASO identification and characterization

- AMX0114 was initially identified via a preliminary screen of 80 candidate ASOs targeting *CAPN2*, showing substantial reduction in *CAPN2* mRNA expression and no measurable cytotoxicity
- Subsequent dose response and kinetic profiling experiments demonstrated that AMX0114 achieved potent, dose-dependent, and durable knockdown of *CAPN2* mRNA expression and calpain-2 protein levels in human induced pluripotent stem cell (iPSC)–derived motor neurons for ≥21 days following a 48-hour treatment period

Preclinical efficacy studies

- Assessment of survival at multiple AMX0114 concentrations was evaluated in iPSC-derived neurons harboring the ALS-linked TPD-43(M337V) mutation
- Robust knockdown achieved by AMX0114 translated to dose-dependent improvements in survival (**Figure 2**)
- Treatment with 0.1 µM AMX0114 resulted in ~60% decrease in extracellular neurofilament light chain (NfL) above baseline relative to DMSO (vehicle)-treated M337V controls (**Figure 3**)
- Decreases in NfL levels from baseline 11 days following treatment with AMX0114 were correlated with decreased risk of death
- In subsequent efficacy studies, AMX0114 was evaluated in a model of oxidant-induced cell death
- 96 hours after first exposure to hydrogen peroxide (H_2O_2) (120-hour timepoint), 77.2% cell body area remained in the AMX0114-treated neurons, relative to only 12.9% in controls (p=0.0153) (**Figure 4**)
- At the latest timepoint tested (192 hours), AMX0114-treated neurons preserved 56% of their initial cell body area, relative to only 11.8% in controls (p=0.0183) (**Figure 4**)
- Pre-treatment with AMX0114 achieved potent calpain-2 inhibition and resulted in statistically significant neuroprotection in a model of oxidative stress-induced axonal degeneration (**Figures 4 and 5**)

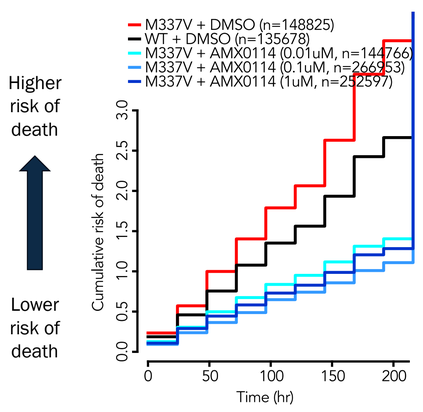
IND-enabling studies

- Investigational new drug (IND)–enabling studies (toxicology, safety pharmacology, pharmacokinetics (PK), etc.) have concluded

First-in-human trial

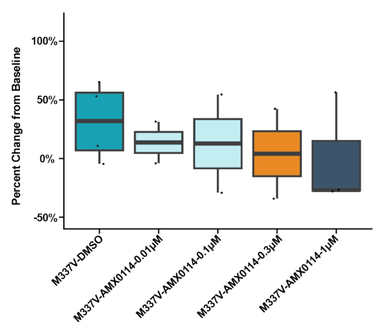
- A phase 1, first-in-human study of intrathecal AMX0114 in people living with ALS is planned for initiation in 2024 (**Figure 6**)

Figure 2. Dose-Dependent Improvement in Survival with AMX0114



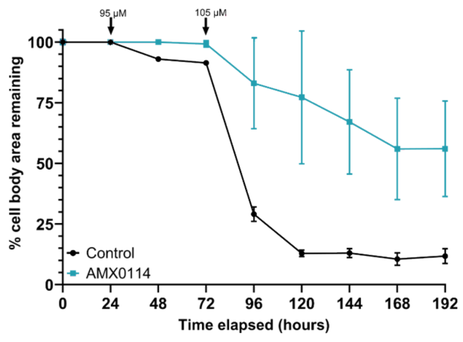
Cumulative risk of death plot for wild-type (WT) and TDP-43 mutant (M337V) human iNeurons treated with AMX0114 (0.01-1µM).

Figure 3. Dose-Dependent Decrease in Extracellular NfL Levels Following Treatment with AMX0114



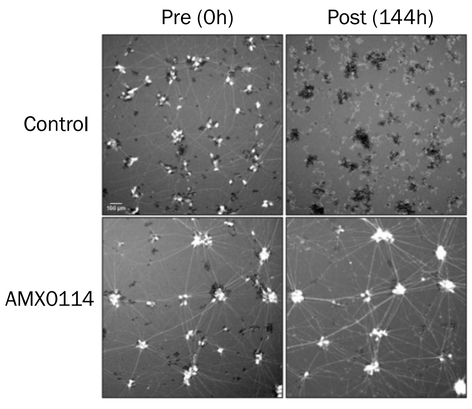
TDP-43 mutant (M337V) neuronal cultures were treated with a single dose of AMX0114 (0.01, 0.1, 0.3, or 1 µM) or vehicle (DMSO). Medium was harvested 10 days later and NfL levels were assessed by ELISA. "Baseline" refers to NfL levels in isogenic controls.

Figure 4. AMX0114 Treatment Results in Neuroprotection in a Model of Oxidative Stress



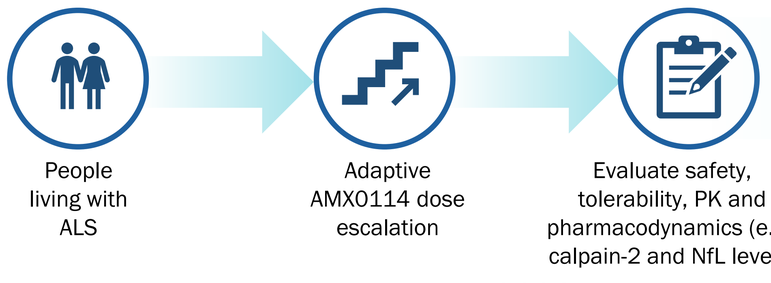
AMX0114 (10 µM) was added 48h and 72h prior to the start of the survival time series. H_2O_2 was added at 24 hours (95 µM) and 72 hours (105 µM). Area (µm²) was calculated as the sum of all cell bodies for each timepoint and expressed as fraction relative to Day 0. Entire curves were tested using repeated measures ANOVA (p=0.0122).

Figure 5. Pre-Treatment with AMX0114 Preserved Motor Neurons Post H_2O_2 Exposure



Motor neurons with or without AMX0114 pre-treatment pre- and post H_2O_2 exposure (144-hour timepoint).

Figure 6. DESCRIPTION OF FIRST-IN-HUMAN STUDY OF AMX0114



Potential for subsequent long-term extension providing continued access to AMX0114 for participants completing the study if data support a positive benefit-risk profile

Phase 1 study projected to begin in the second half of 2024
Further details will be provided once study design and timing are finalized

AMX0114 is an investigational drug and not approved for use by any health authority.

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Disclosures

JC and **JK** are co-CEOs of and own stock in Amylyx Pharmaceuticals, Inc. **LK, EM, JP, AH,** and **LM** are full-time employees of and may have stock option ownership in Amylyx Pharmaceuticals, Inc. **SP** reports research grants from Amylyx Therapeutics, Revalesio Corporation, Eledon, Alector, UCB Pharma, Biohaven, Clene Nanomedicine, Prilenia Therapeutics, Seelos, Calico, Denali, NIH, CDC, DoD, the ALS Association, the Muscular Dystrophy Association, Tambourine and reports consulting fees from Amylyx, Arrowhead, BMS, Clene, Iris, Eikonizo, and Cytokinetics. She has been a paid educational speaker for PeerView and Medscape. **JS** reports research funding from Amylyx, Biogen, Cytokinetics Incorporated, Ionis, Mitsubishi Tanabe Pharma America, Quralis, PTC, Sanofi, Wave, and Myolex. He reports personal compensation for consulting from Amylyx, Cytokinetics, Denali, GSK, Mitsubishi Tanabe Pharma America, Neurosense, Orthogonal, Pinteon, RRD, Acurastem, Revalasio, Apellis, Swanbio, Novartis, Sanofi, Eikonizo, and Sarepta. **LvdB** reports consulting fees from Amylyx, Argenx, Cytokinetics, Denali, Projenx, Sanofi, Takeda, and Verge.

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