Next Steps in Development for AMX0114: An Antisense Oligonucleotide Targeting Calpain-2, a Critical Effector of Axonal Degeneration

Poster M199

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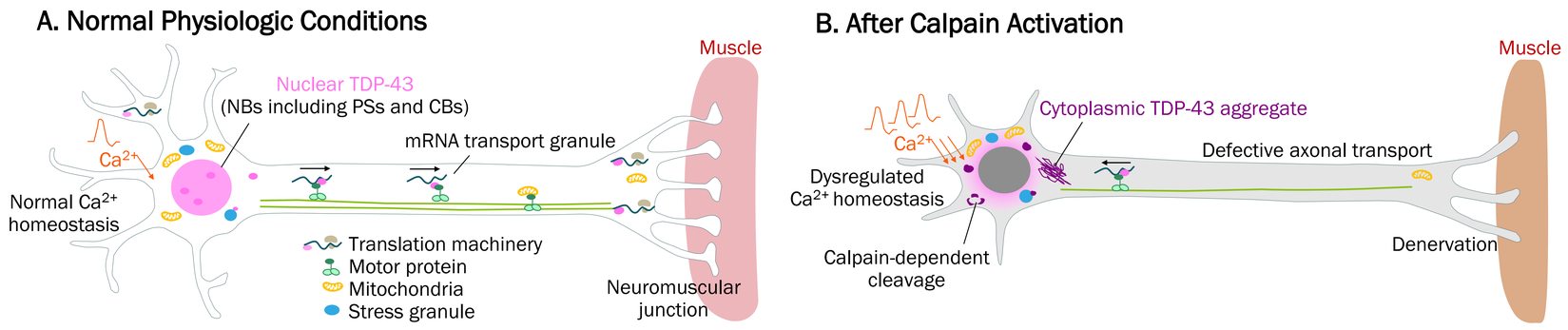
BACKGROUND/RATIONALE

- Axonal degeneration has been recognized as a key early contributor to the clinical presentation and pathogenesis of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases[1,2]
- Activation of calpain-2 is proposed as a critical effector of axonal degeneration (**Figure 1**)[2,3]
- Calpain-2 has been 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
- 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 (*CAPN2*)

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[3,7]

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

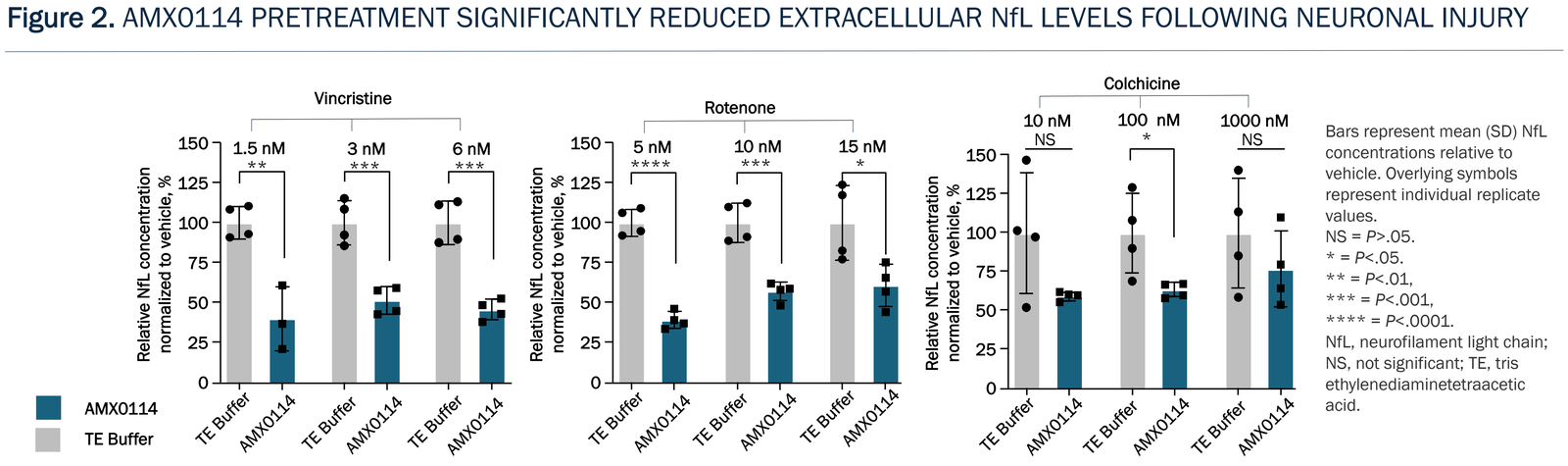
Lead ASO identification and characterization

- 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

- In subsequent preclinical efficacy studies, human iPSC-derived motor neurons were incubated with varying concentrations of neurotoxic triggers including vincristine, rotenone, and colchicine after preincubation with AMX0114 for 48 hours
- Pretreatment with 20 µM AMX0114 significantly reduced extracellular neurofilament light chain (NfL) levels following neuronal injury (**Figure 2**)

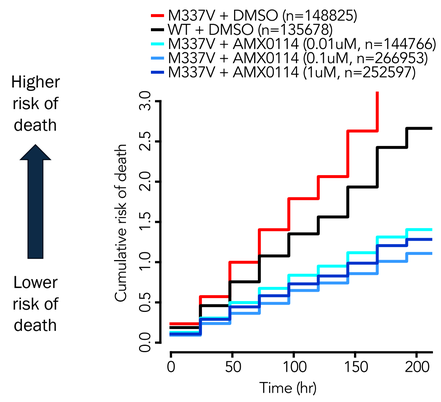
Figure 2. AMX0114 PRETREATMENT SIGNIFICANTLY REDUCED EXTRACELLULAR NfL LEVELS FOLLOWING NEURONAL INJURY



Bars represent mean (SD) NfL concentrations relative to vehicle. Overlying symbols represent individual replicate values.
NS = *P*>.05.
* = *P*<.05.
** = *P*<.01,
*** = *P*<.001,
**** = *P*<.0001.
NfL, neurofilament light chain; NS, not significant; TE, tris ethylenediaminetetraacetic acid.

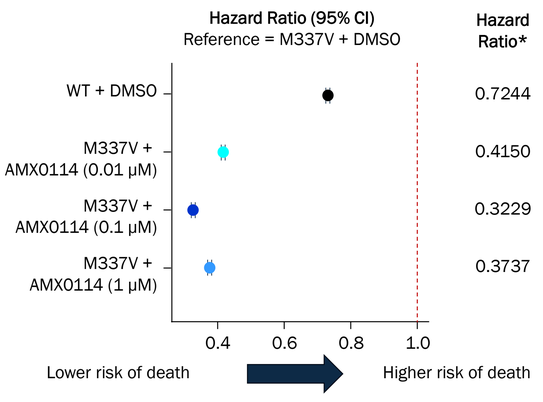
- An *in vitro* efficacy assessment study was conducted in wild-type and mutant TDP-43 (M337V) iNeurons treated with AMX0114 to test the ability of AMX0114 to prevent neurodegeneration
- A dose-dependent improvement in survival was achieved with AMX0114 treatment, demonstrating that robust knockdown achieved by AMX0114 translated to dose-dependent improvements in survival in an *in vitro* model of ALS with TDP-43 pathology (**Figure 3**)

Figure 3. AMX0114 TREATMENT YIELDED A DOSE-DEPENDENT IMPROVEMENT IN SURVIVAL IN AN *IN VITRO* ALS MODEL



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

Survival analyses performed in the lab of Dr Sami Barmada at the University of Michigan Medical School by Dr Michael Bekier



Forest plot illustrating hazard ratio for iNeurons treated with AMX0114, in comparison to TDP-43 (M337V) iNeurons treated with vehicle control (DMSO; dashed red line).

*All *P*<.0001 compared with reference. Hazard ratios and *P* values calculated using Cox proportional hazards analysis. n=number of neurons. Data integrated from 6 technical and 3 biological replicates/condition. DMSO, dimethyl sulfoxide; WT, wild-type.

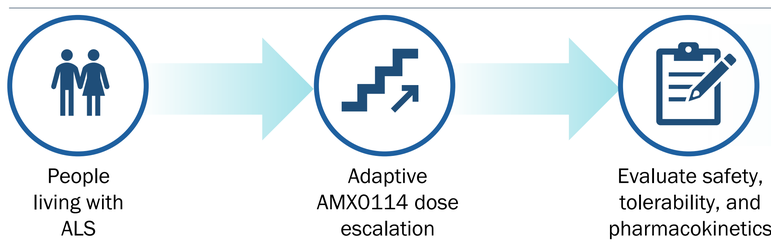
IND-enabling studies

- Investigational new drug (IND)–enabling studies (toxicology, safety pharmacology, pharmacokinetics, etc.) are underway and are scheduled to be completed in 2024

First-in-human trial

- A phase 1, first-in-human study of AMX0114 in people living with ALS is planned for initiation in 2024 (**Figure 4**)

Figure 4. 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 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. **RM, 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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