Preclinical Development of AMX0114, an Antisense Oligonucleotide Targeting Calpain-2

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Amylyx Pharmaceuticals, Inc.

July 10, 2024

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

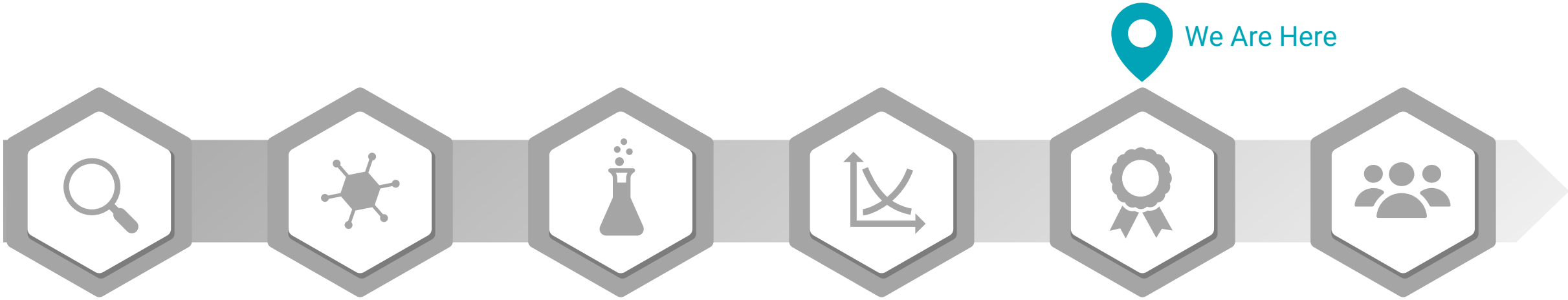
Disclosures/Disclaimers

- Evan Mizerak is a full-time employee of and has stock ownership options in Amylyx Pharmaceuticals
- This presentation is intended to provide scientific information about antisense oligonucleotides targeting calpain-2
- AMX0114 is an investigational agent not approved for use by the FDA or any other regulatory agency

AMYLYX

Our mission is to one day end the suffering caused by neurodegenerative diseases. Every day, we strive for better therapies.

AMX0114 Development Program in ALS



Scientific Rationale, Bioinformatics and Discovery

- Science supporting calpain-2 inhibition in ALS

Screening and Lead ID

- Development of AMX0114

Preclinical Proof-of-Concept

- Preclinical efficacy in ALS-relevant *in vitro* models

IND-Enabling Studies and CMC Scale-Up

- Toxicology, safety pharmacology, pharmacokinetics, etc.

(Preparation for) IND Filing

First-in-Human

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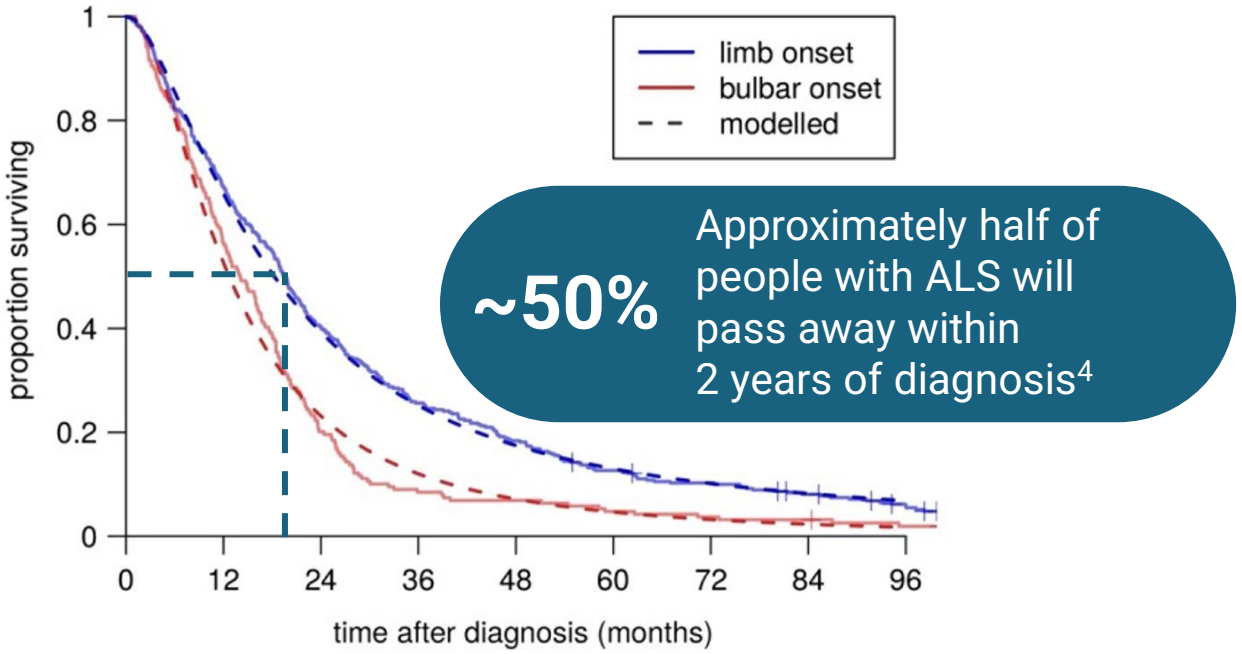
ALS is a Relentlessly Progressive, Debilitating, and Universally Fatal Disease Caused by Motor Neuron Degeneration[1]

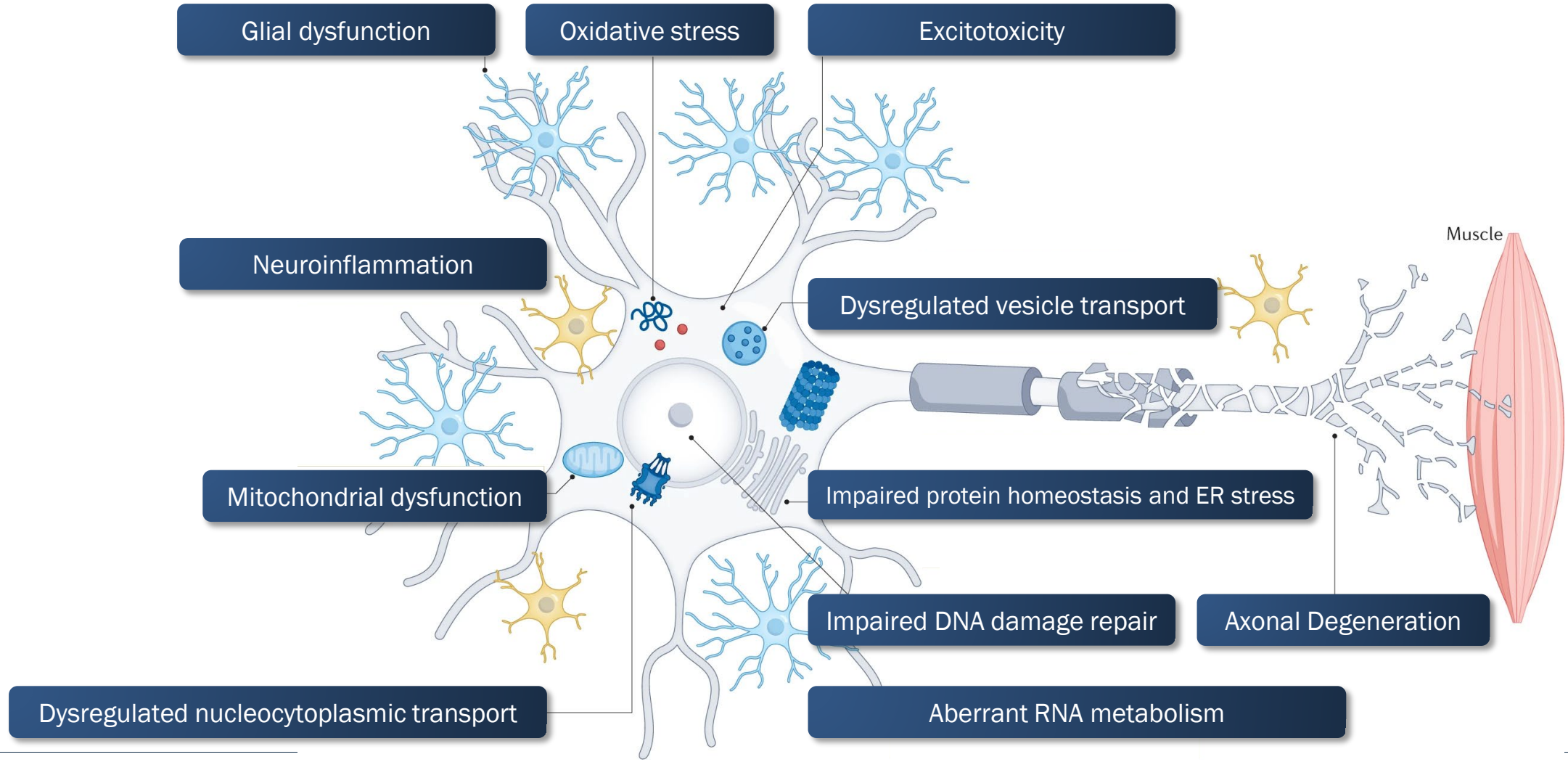
- ALS leads to deteriorating muscle function, inability to move and speak, respiratory paralysis, and death[1,2]
- Age of onset ranges from 40-65 years old[3]
- >90% of people with ALS have no family history of the disease[1]



~50% Approximately half of people with ALS will pass away within 2 years of diagnosis[4]

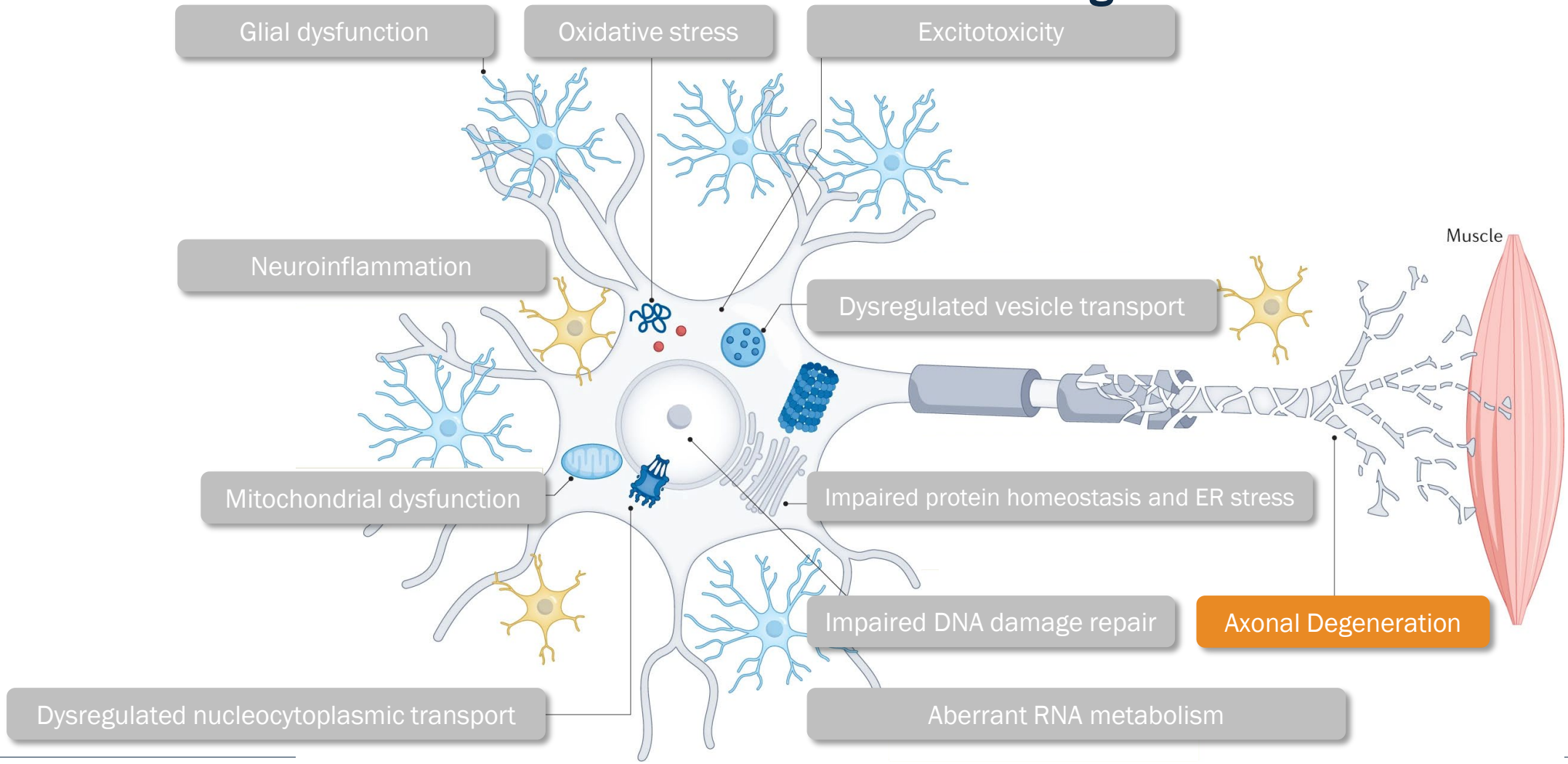
1. Brown RH, et al. *N Engl J Med*. 2017;377(2):162-172. **2.** Al-Chalabi A, et al. *Lancet Neurol*. 2016;15(11):1182-1194. **3.** Masrori P, et al. *Eur J Neurol.* 2020 Oct;27(10):1918-1929. **4.** Knibb JA, et al. *J Neurol Neurosurg Psychiatry*. 2016;87(12):1361-1367.

Complex ALS Pathophysiology Provides Multiple Pathways to Target



Mead RJ, et al. *Nat Rev Drug Discov*. 2023;22(3):185–212.

Axonal Degeneration Has Been Increasingly Recognized as a Key Early Contributor to the Clinical Presentation and Pathogenesis of ALS[1,2]

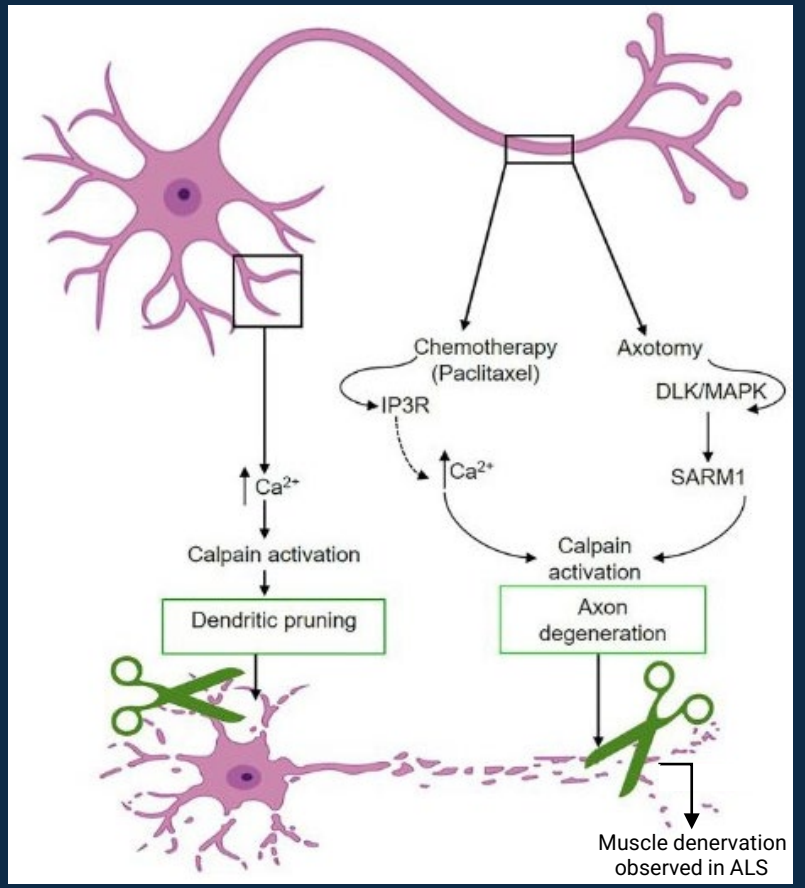


1. Moloney EB, et al. *Front Neurosci*. 2014;8:252. **2.** Ma M. *Neurobiol Dis*. 2013;60:61-79.

Activation of Calpain-2 is Proposed as One of the Critical Effectors of Axonal Degeneration[1-3]

- Calpains are a family of calcium (Ca^{2+})-dependent proteases that target substrates within the axonal cytoskeleton[2]
- There are over a dozen calpain isoforms, but activation of calpain-2 has shown the clearest association with axonal degeneration[4]
- Following injury-induced Ca^{2+} dyshomeostasis, proteolysis mediated by calpain-2 results in cytoplasmic TDP-43 aggregates, defective axonal transport, and ultimately muscle denervation observed in ALS[3,5]

Mechanisms of Axonal Degeneration[4]

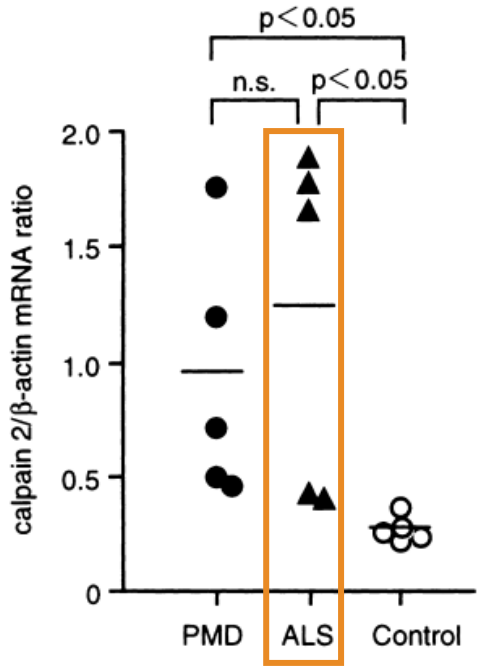


Multiple injury paradigms and hypotheses of axonal degeneration converge on calpain-2

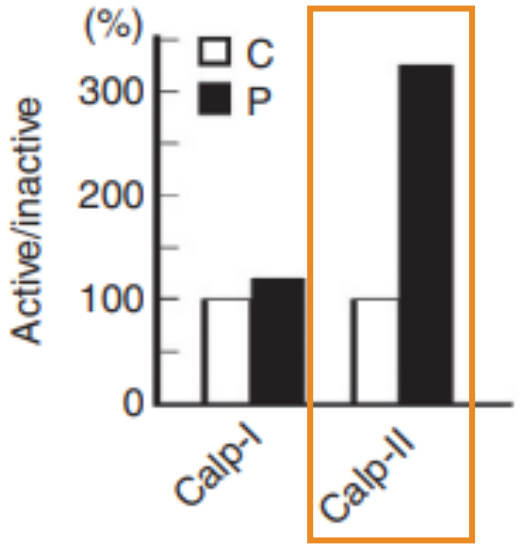
TDP-43, transactive response DNA-binding protein 43.

1. Moloney EB, et al. *Front Neurosci*. 2014;8:252. **2.** Ma M. *Neurobiol Dis*. 2013;60:61-79. **3.** Asakawa K, et al. *Cell Mol Life Sci*. 2021;78(10):4453-4465. **4.** Wang Y, et al. *Cells*. 2020; 9(12): 2698. **5.** Metwally E et al. *Front Vet Sci*. 2023 Sep 5;10:1235163.

Calpain-2 Levels Are Upregulated in ALS



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



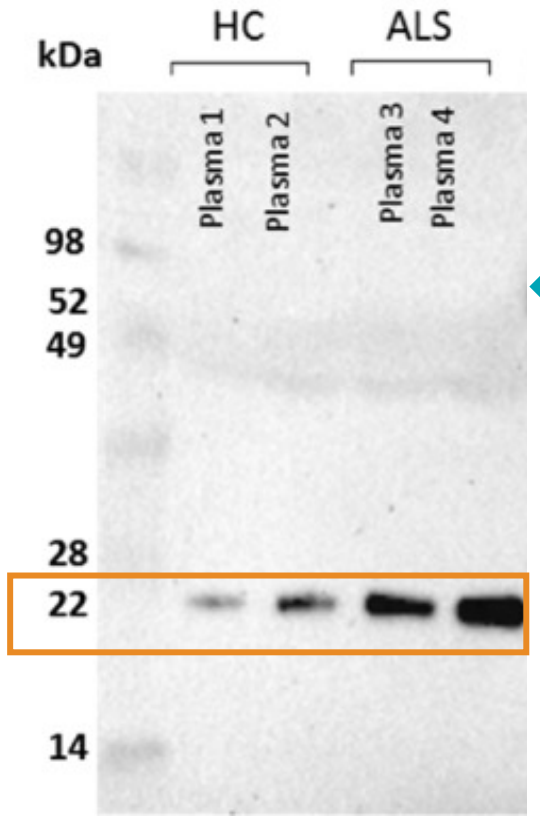
Ratio of active to inactive calpain-2 in post-mortem brain tissue of people with ALS (P) is ~3x higher than that in healthy controls (C)[2]

CAPN2, calpain-2 gene encoding.

1. Ueyama H et al. *J Neurol Sci*. 1998;155(2):163-169. **2.** Yamashita T et al. Nat Commun. 2012;3:1307.

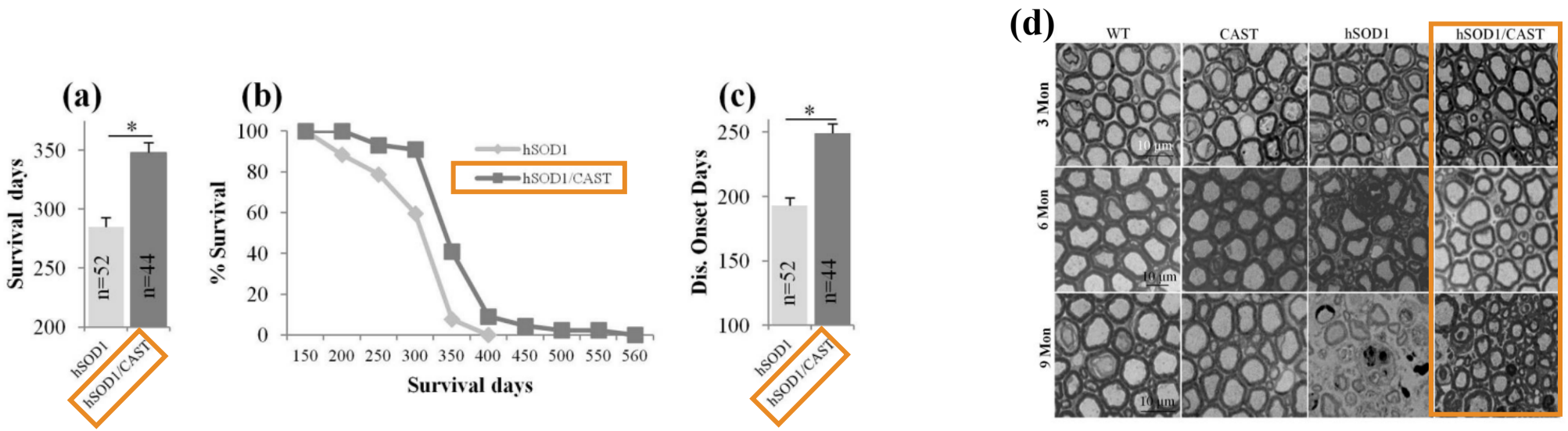
Calpain-2 Activation Leads to Neurofilament Proteolysis

- Neurofilaments are broadly researched biomarkers in ALS related to axonal degeneration and neurofilament was reported as a substrate for calpain-2 proteolysis as early as 1982[1]
- Calpain-2 cleaves neurofilament to produce 22 kDa, 40 kDa, and 55 kDa fragments[2]
- No full-length neurofilament light chain (NfL) is detected in ALS cerebrospinal fluid (CSF) or plasma, and the 22 kDa fragment is the NfL fragment which predominates in ALS[3]
  - This suggests a major role for calpain-2 in producing the NfL signal detected in ALS



1. Zimmerman UJ, et. al. *Biochemistry*. 1982; 17;21(17):3977. **2.** Ma M, et al. *Neurobiol Dis*. 2013; 56:34-46. **3.** Lombardi V, et al. *Sci Rep*. 2020;26;10(1):10774.

Calpain-2 Modulation Has Demonstrated Therapeutic Benefit in Preclinical Models



In the transgenic SOD1G93A mouse model of ALS, neuron-specific overexpression of calpastatin, an endogenous inhibitor of calpain-2 (hSOD1/CAST) increases overall survival (a-b), delays disease onset (c), and rescues motor neuron loss (d)

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First-in-Human

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

Selectivity of the ASO modality offers 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

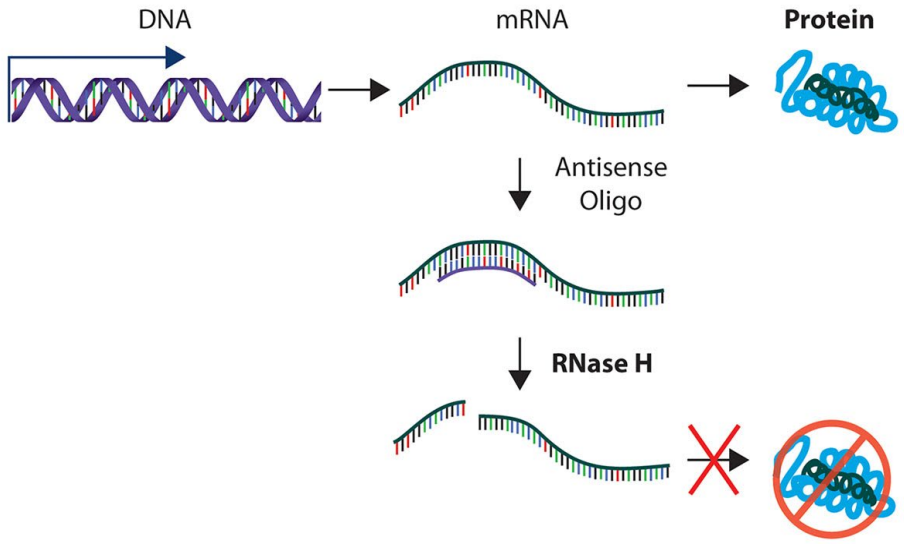


Image Adapted from *Online Biology Notes*

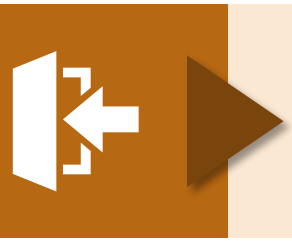
Advantages of RNase H Competent (Gapmer) Antisense Oligonucleotides

Distinct practical advantages over small molecule approach

- Highly targeted[1]
- Standardized, tunable approach to target evaluation and lead ID lends itself to platform building[1]

ASOs with comparable chemical modifications exhibit consistent properties

- Metabolites are predictable and relatively consistent across species[2]
- Tolerability and distribution are well-characterized[2]



Delivery

- Gymnotic uptake can circumvent the need for viral vectors or lipid nanoparticles[3,4]

1. Crooke S, et al. Nat Rev Drug Discov. *2021;20(6):427-453.* **2.** Takakusa H, et al. *Nucleic Acid Ther*. 2023;33(2):83-94. **3.** Koller E, et al. *Nucleic Acids Res*. 2011;39(11):4795-4807. **4.** Stein CA, et al. *Nucleic Acids Res*. 2010;38(1):e3.

AMX0114: An Antisense Oligonucleotide Targeting Calpain-2

Key Design Features of AMX0114

1

20-mer (5-10-5 gapmer)

Convenient size for synthesis, long enough to ensure uniqueness in the human genome[1]

2

2'-O-methoxyethyl (2'MOE) modifications on wing residues

Improve nuclease stability and binding ability[2,3]

3

Phosphorothioate (PS)-modified backbone

Increased binding to cellular proteins and components of the extracellular matrix compared to natural phosphodiester oligonucleotides[4]

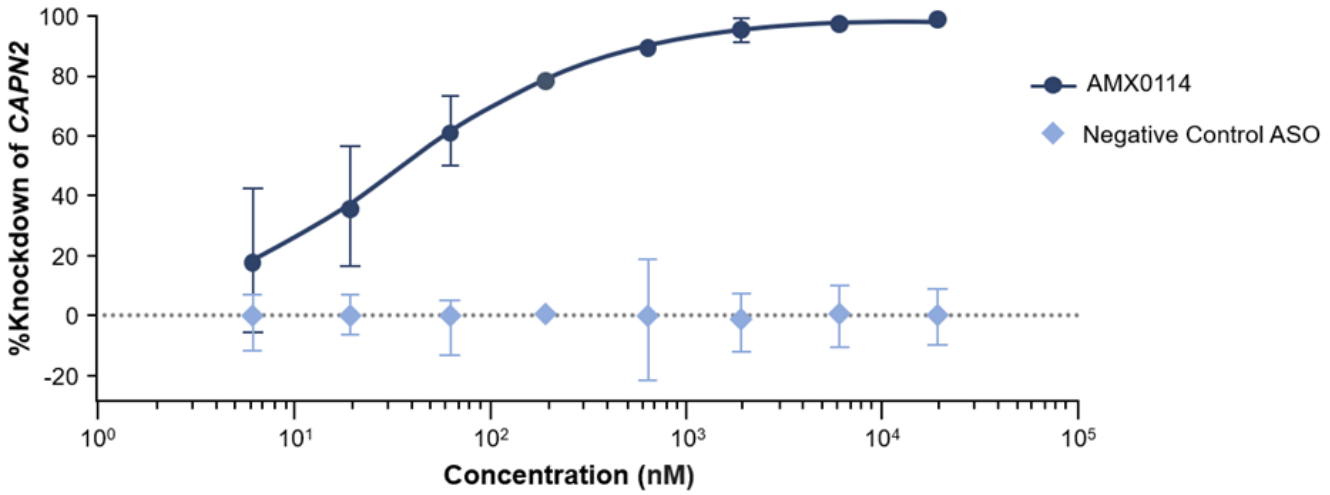
4

All C residues methylated

Prevents immune stimulation without affecting hybridization[5,6]

1. De Mesmaeker A, et al. *Acc Chem Res.*1995;28(9):366-374. **2.** Dhuri K, et al. *J Clin Med.* 2020;9(6):2004. **3.** Roberts TC, et al. *Nat Rev Drug Discov.* 2020;19(10):673-694. **4.** Iwamoto N, et al. *Nat Biotechnol.* 2017;35(9):845-851. **5.** Wan WB and Seth PP. *J Med Chem.* 2016 10;59(21);9645-9667. **6.** Dhuri K, et al. *J Clin Med.* 2020;9(6):2004.

AMX0114 Achieves **Potent**, **Dose-Dependent**, and **Durable** Knockdown of *CAPN2* mRNA and Calpain-2 Protein



Days After AMX0114 Removal	CAPN2 mRNA Knockdown (Reduction vs. Control)	Calpain-2 Protein Knockdown (Reduction vs. Control)
0	94.47%	3.25%
3	87.12%	23.32%
7	84.36%	45.47%
10	82.16%	31.39%
14	83.80%	51.07%
21	77.25%	40.75%

- mRNA knockdown >90% at a concentration of 20 µM in human motor neurons[1]
- Potency (half-maximal effective concentration or EC_{50}) ≈ 40-100 nM[1]

- Reduction in *CAPN2* mRNA and calpain-2 protein levels following treatment with AMX0114 is rapid, robust, and stable over at least 21 days in a disease-relevant cell model

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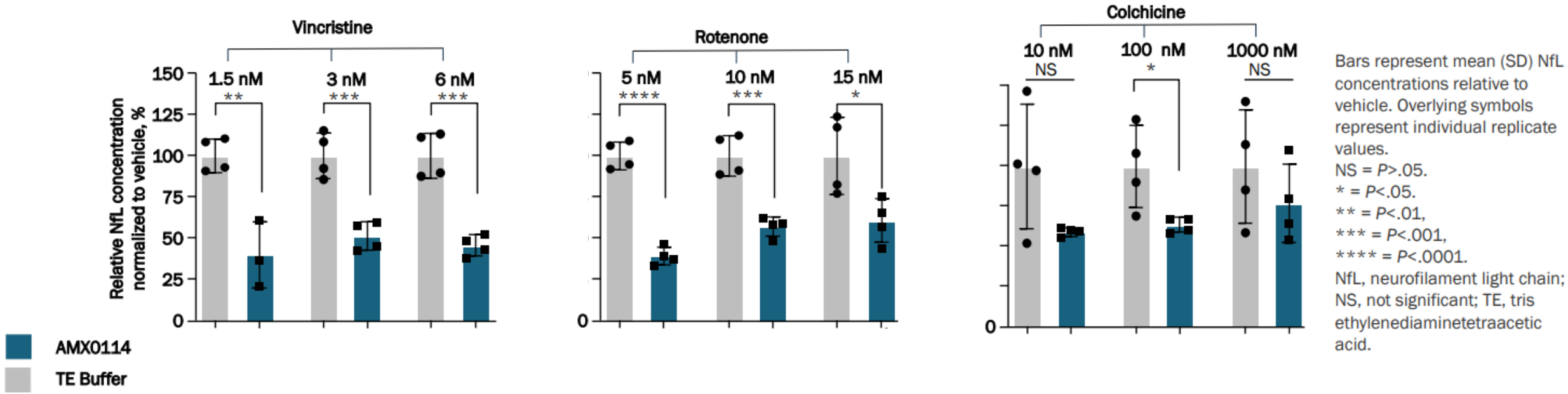
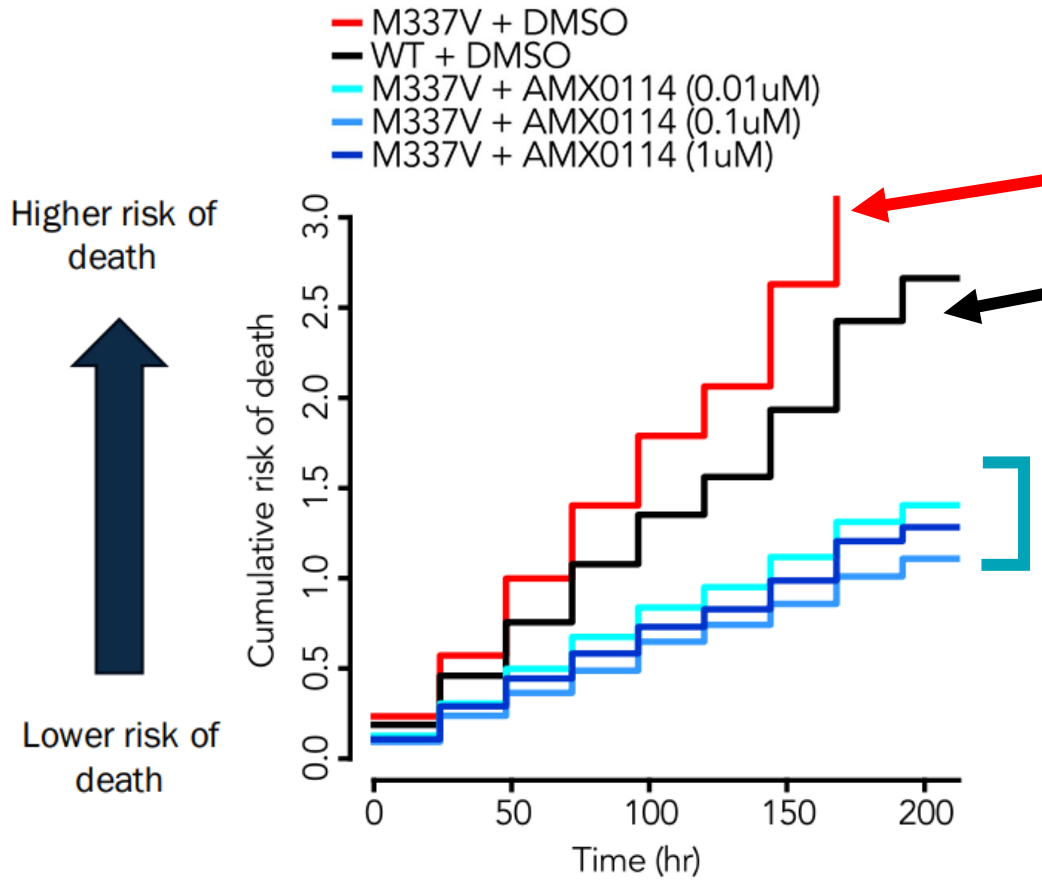
AMX0114 Reduces Extracellular NfL Levels in Multiple Models of Trigger-Induced Neuronal Injury



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.

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

AMX0114 Improves Survival in a Model of TDP-43 ALS



ALS model neurons (no AMX0114)

Healthy neurons (no AMX0114)

ALS model neurons (with AMX0114)

- TDP-43(M337V) is an ALS-linked mutation resulting in **increased risk of cell death**
- Treatment with as little as 0.01 µM AMX0114:
  - Reduced cell death by nearly **60%** relative to untreated M337V neurons ($p<0.0001$)
  - Reduced cell death by **>30%** relative to untreated wild-type neurons ($p<0.0001$)

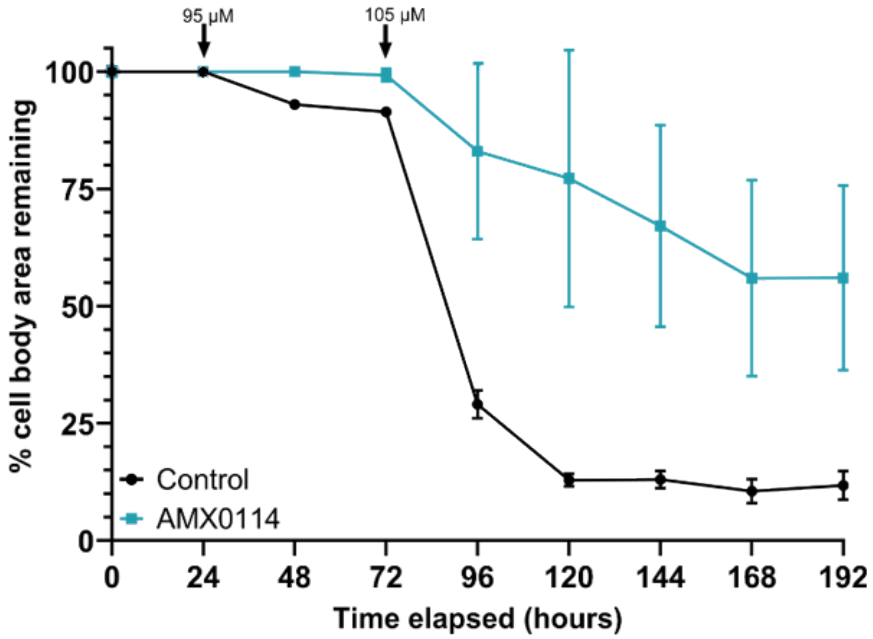
Survival analyses performed in the lab of Dr. Sami Barmada at the University of Michigan Medical School by Dr. Michael Bekier

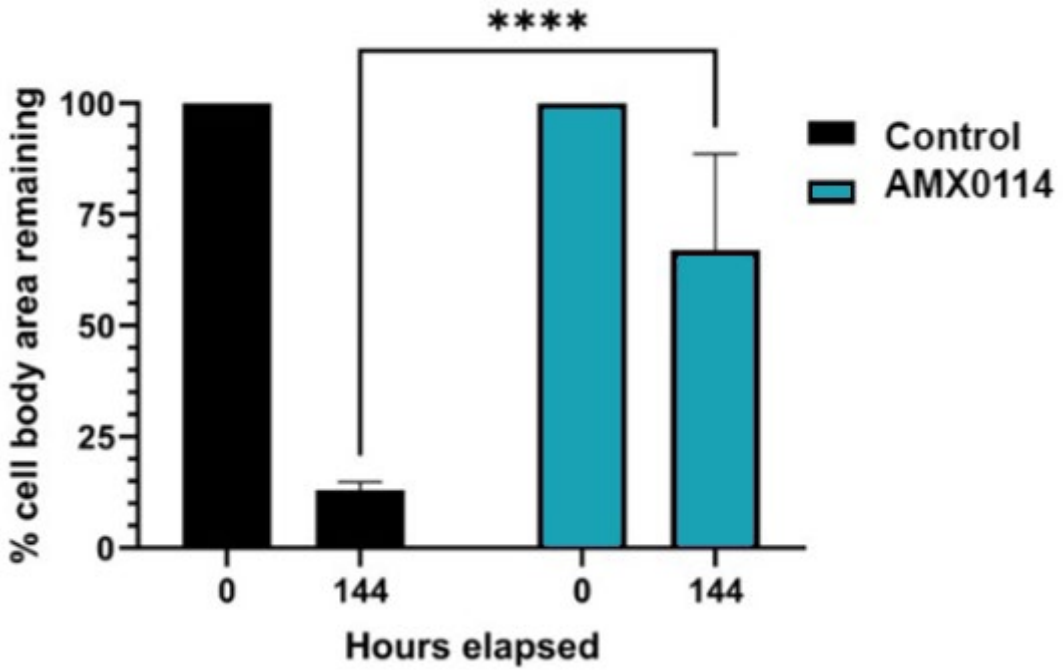
AMX0114 is Neuroprotective in a Model of Oxidant-Induced Cell Death

Calpain-2 knockdown translates to **improved survival** following exposure to hydrogen peroxide (H_2O_2), a stressor meant to induce oxidative stress and cell death

LESS neuronal death

MORE neuronal death

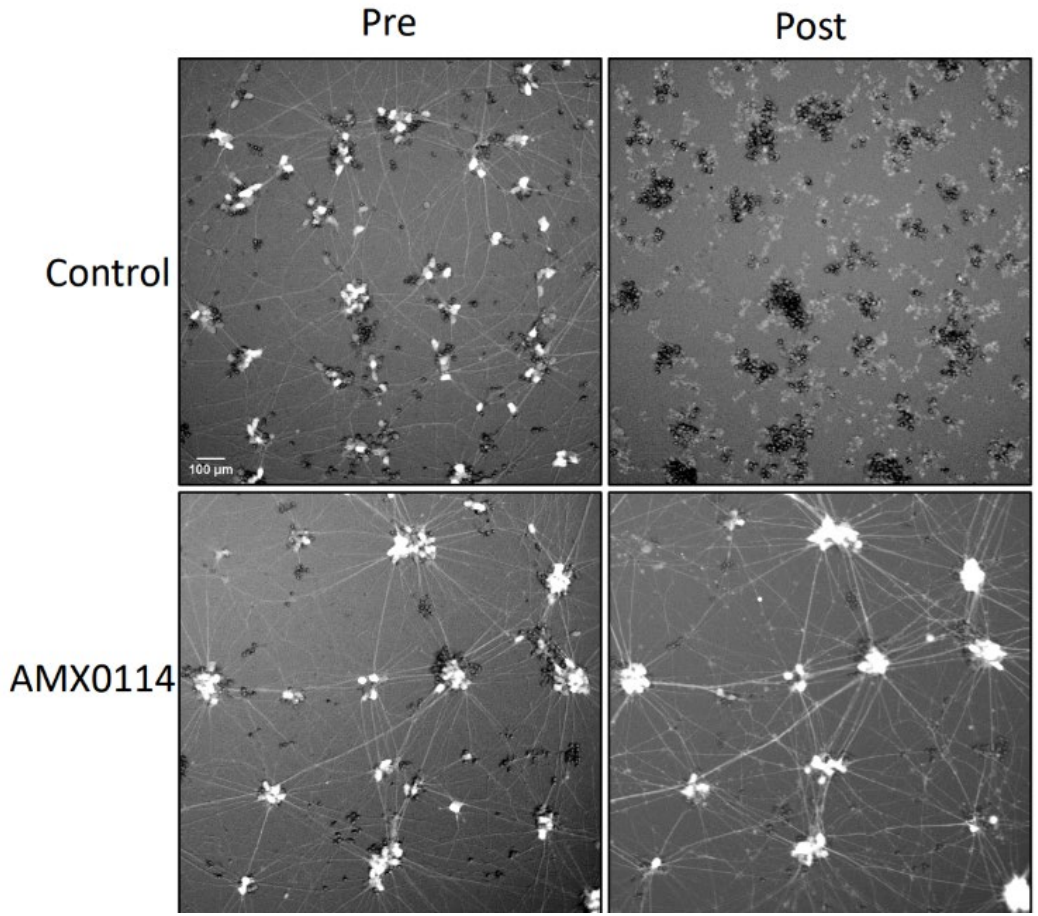




Survival analyses performed in the lab of Dr. Giovanni Manfredi at Weill Cornell Medicine by Dr. Kevin McAvoy

AMX0114 is Neuroprotective in a Model of Oxidant-Induced Cell Death

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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AMX0114 Development Program in ALS

- IND-enabling studies are concluding and reflect a favorable safety and tolerability profile of AMX0114

AMX0114 Development Program in ALS

- Targeting first participant dosed in first-in-human multiple ascending dose study in the second half of 2024

Calpain-2 Inhibition is a Promising Therapeutic Strategy in Many Disease Areas

AMX0114 Has Potential Applications Beyond ALS

The scientific literature has identified a role for calpain-2 in the pathophysiology of diseases including:

1. Gafni J et al. *J Neurosci*. 2002;22(12):4842-9. **2.** Kurbatskaya K, et al. *Acta Neuropathol Commun.* 2016;31;4:34. **3**. Guyton MK, et al. *J Neurosci Res.* 2010;15;88(11):2398-408. **4.** Adamec E, et al. *Acta Neuropathol.* 2004;104(1):92-104. **5.** Wang Y et al. *Expert Opin Ther Targets*. 2018;22(1):19-29. **6.** Stillger MN et al. *Cancer Cell Int*. 2023;23(1):49. **7**. Wang Y et al. *Neurobiol Dis.*2016;93:121-8.

AMX0114: An Antisense Oligonucleotide Targeting Calpain-2

- Activation of calpain-2 is a critical step in axonal degeneration, an early contributor to ALS
- Scientific literature supports a role for calpain-2 in ALS pathophysiology, including in the proteolysis of NfL

- Amylyx has developed AMX0114, an ASO inhibitor of calpain-2
- AMX0114 achieves potent and durable knockdown of *CAPN2* mRNA and calpain-2 protein

- AMX0114 has demonstrated functional efficacy in multiple preclinical models of ALS

- IND-enabling studies for AMX0114 reflect a favorable safety and tolerability profile

- Targeting first-in-human multiple ascending dose study initiation in the second half of 2024

- Calpain-2 inhibition is a promising therapeutic strategy in multiple disease areas

Thank you!

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