# Update on AMX0114: An Antisense Oligonucleotide Targeting Calpain-2, a Critical Effector of Axonal Degeneration

Poster #14

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## BACKGROUND

- Axonal degeneration (also known as Wallerian 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 the calcium-dependent protease calpain-2 is proposed as a critical effector of axonal degeneration[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 and calpain-specific TAR DNA binding protein 43 cleavage product concentrations in postmortem ALS spinal cord samples[4,5]
  - Therapeutic benefit of calpain-2 activity modulation in animal models of ALS[6]
  - The role of calpain-2 in cleaving neurofilament, a broadly researched biomarker in ALS[2]
- Given the evidence supporting a potential benefit of calpain-2 modulation in ALS and other neurodegenerative diseases, we have developed AMX0114, an antisense oligonucleotide (ASO) targeting the gene encoding calpain-2 (*CAPN2*)

## OBJECTIVES

- Provide an overview and update on recent kinetic profiling experiments for AMX0114

## METHODS

### 1. Preliminary Efficacy Screen in Disease-Relevant Cell Line

- 80 candidate ASOs targeting *CAPN2* were evaluated in human induced pluripotent stem cell (iPSC)-derived glutamatergic neurons (ioGlutamatergic Neurons; bit.bio)
- All ASOs had 5-10-5 gapmer chemistry, 2'-O-methoxy-ethyl wing modifications and fully phosphorothioated backbones
- *CAPN2* mRNA levels were assessed by real-time quantitative polymerase chain reaction (RT-qPCR)
- Cytotoxicity was assessed by Hoechst (5 µg/mL) staining and imaging (2 days after ASO treatment)

### 2. Dose Response Studies

- The effects of varying concentrations of the identified ASO candidate on *CAPN2* mRNA levels were evaluated in iPSC-derived human spinal motor neurons (iCell Motor Neurons; FUJIFILM Cellular Dynamics, Inc)
- After 72 hours, *CAPN2* mRNA levels were assessed by RT-qPCR

### 3. Kinetic Profiling Assays

- Kinetic profiling was performed by incubating iPSC-derived human spinal motor neurons with AMX0114 for 48 hours and conducting RT-qPCR and size-based automated capillary Western blot assays (Jess Simple Western™, Biotechne) at 0, 3, 7, 10, 14, and 21 days after ASO removal
- Levels of *CAPN2* mRNA and calpain-2 protein were compared with a negative control ASO treatment at each time point

## RESULTS

### 1. Preliminary Efficacy Screen in Disease-Relevant Cell Line

*CAPN2 Expression in Human Glutamatergic Neurons*

**FIGURE 1A.** TOP 15 ASO CANDIDATES REDUCED *CAPN2* mRNA LEVELS BY ≥30%

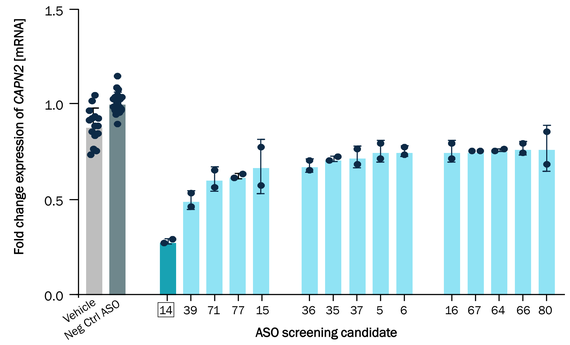



**FIGURE 1B.** TOP 15 ASO CANDIDATES DID NOT DISPLAY MEASURABLE CYTOTOXICITY IN GLUTAMATERGIC NEURONS

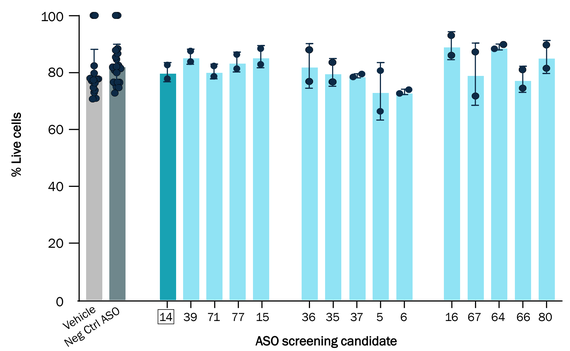



Figure showing top 15 best-performing test-ASOs sorted by fold-change of *CAPN2* expression (**A**) sorted from low to high fold change, and (**B**) percentage live cells. Blue bars on the right are ASO candidates identified by internal ID number. On the left, gray is vehicle (5% tris EDTA Buffer), blue-gray is negative control ASO. 5 µM of each ASO was used in the screen. Data represent mean ± SD of biological replicates; individual replicates are indicated by black dots.

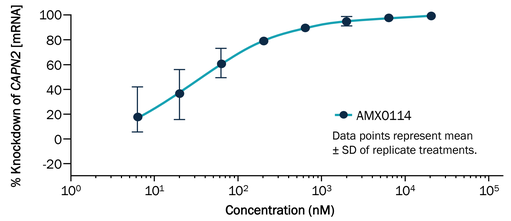
- The top 6 ASO candidates reduced *CAPN2* mRNA expression by ≥30%
- The lead ASO, AMX0114, reduced *CAPN2* mRNA expression by 72%
- AMX0114 was selected for further experiments based on robust preliminary *CAPN2* mRNA knockdown and absence of measurable cytotoxicity

## RESULTS (cont'd)

### 2. Dose Response Studies

*CAPN2 Expression in Human Motor Neurons*

**FIGURE 2.** AMX0114 POTENCY SCREEN

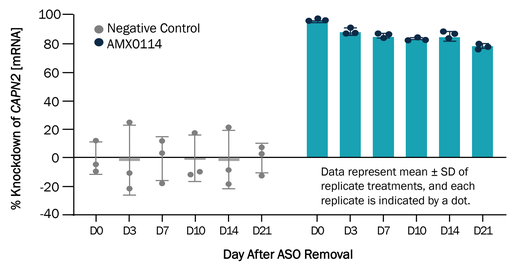



- AMX0114 reduced *CAPN2* mRNA levels in iPSC-derived human motor neurons in a dose-dependent manner, reaching ≥90% knockdown without observed cytotoxicity at the highest concentrations tested

### 3. Kinetic Profiling Assays

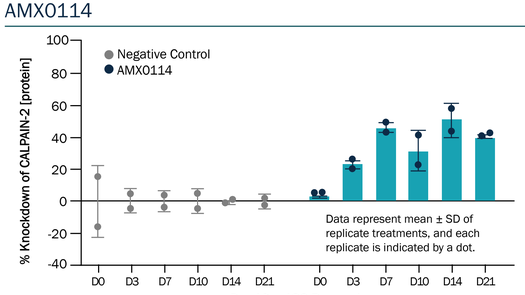
*CAPN2 mRNA Expression and Calpain-2 Protein Levels in Human Motor Neurons*

**FIGURE 3.** KINETICS OF *CAPN2* mRNA KNOCKDOWN BY AMX0114




- AMX0114 treatment achieved efficacious and robust mRNA knockdown at all time points
  - Maximum *CAPN2* mRNA knockdown of 94% was reached on Day 0 (48 hours after delivery) with a slight decrease over time to 77% on Day 21

**FIGURE 4.** KINETICS OF CALPAIN-2 PROTEIN KNOCKDOWN BY AMX0114




## RESULTS (cont'd)

- Stable knockdown of calpain-2 protein was also achieved with AMX0114 compared to the negative control ASO
  - The highest relative knockdown of calpain-2 protein was 51% on Day 14, and knockdown remained stable from Day 7 through Day 21

## CONCLUSIONS

- Amylyx has developed AMX0114, an ASO targeting *CAPN2*
- AMX0114 achieves efficacious and dose-dependent knockdown of *CAPN2* mRNA expression and calpain-2 protein levels in human motor neurons
- The extent of knockdown conferred by AMX0114 exhibits little decrease over time, suggesting a durable effect
- These findings will inform future *in vivo* dosing protocols in models of ALS
- AMX0114 is an investigational agent not approved for use by the Food and Drug Administration (FDA) or any other regulatory agency, but is currently in investigational new drug (IND)–enabling studies to support clinical study initiation
- IND-enabling studies are scheduled to complete in 2024 which may allow for initiation of a first-in-human study during 2024

**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.
- EM and JT are full-time employees of and may have stock option ownership in Amylyx Pharmaceuticals, Inc.
- TMC, SD, TSF, FvV, ME, MH, MBT, RdW, SdM, and RSR are employees of Charles River Laboratories, which was contracted by Amylyx Pharmaceuticals, Inc. to perform the experiments described herein.

### References

1. Suzuki N, et al. *Front Neurosci*. 2020;14:194.
2. Ma M. *Neurobiol Dis*. 2013;60:61-79.
3. Wang LH, et al. *J Neurosci.* 2022;42(25):5102-5114.
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):253-265.

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