Characterizing the CSF Biomarker Signature of Calpain-2 Activity in ALS and the Biomarker Impact of Calpain-2 Inhibition in a Preclinical Model of ALS

POSTER #11

Robert Bowser, PhD[1,2]; Jiyan An[1,2]; Pavol Zelina[3]; Carola Kusserow[3]; R. Jeroen Pasterkamp[3]; Lauren Kett, MD, PhD[4]; Evan Mizerak[4]; Jamie Timmons[4]; Sabrina Paganoni, MD, PhD[5,6]

[1]Barrow Neurological Institute, Phoenix, Arizona, USA; [2]nVector, Inc., Phoenix, Arizona, USA; [3]Department of Translational Neuroscience, UMC Utrecht Brain Center, Utrecht, The Netherlands; [4]Amylyx Pharmaceuticals, Inc., Cambridge, Massachusetts, USA; [5]Sean M. Healey and AMG Center for ALS & the Neurological Clinical Research Institute, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA; [6]Spaulding Rehabilitation Hospital, Harvard Medical School, Boston, Massachusetts, USA

BACKGROUND

About Calpain-2[1-3]

- Axonal degeneration is a key contributor to amyotrophic lateral sclerosis (ALS)
  - One critical effector of axonal degeneration and neuronal cell death is activation of the calcium-dependent protease calpain-2
- Based on evidence supporting a potential benefit of calpain-2 modulation, Amylyx Pharmaceuticals developed AMX0114, an antisense oligonucleotide (ASO) inhibitor of calpain-2, currently under study in the phase I, multiple ascending dose LUMINA trial

Calpain-2 is a calcium-dependent cysteine protease that targets multiple substrates within the axonal cytoskeleton including alpha II-spectrin and neurofilament light chain (NfL)[2]

About Alpha II-Spectrin and SBDP-145[4-6]

- Alpha II-spectrin is a cytoskeletal protein abundant in axons
- Extensive literature supports that alpha II-spectrin is cleaved by calpain-2 into 150 and 145 kDa breakdown products (SBDP-150 and SBDP-145); thus SBDP-145 is a calpain-specific cleavage product of alpha II-spectrin
- SBDP-145 may, therefore, serve as a biomarker of AMX0114 target engagement and marker of axonal degeneration in the LUMINA trial

SBDP-145 is a biomarker of interest in the LUMINA trial as it may reflect both calpain-2 activity – and thus AMX0114 target engagement – and serve as a marker of axonal degeneration

OBJECTIVES

- Characterize the cerebrospinal fluid (CSF) biomarker signature of calpain-2 activity in ALS as related to SBDP-145
- Evaluate the impact of calpain-2 inhibition with AMX0114 on SBDP-145 levels in an in vitro preclinical model of ALS

Contact Info: Lauren Kett, MD, PhD
Lauren_Kett@amylyx.com

AMX0114 is an investigational agent and has not been approved for use by any health authority (e.g., the FDA and EMA)

References
1. Suzuki N, et al. *Front Neurosci.* 2020;14:194. **2.** Ma M. *Neurobiol Dis.* 2013;60:61-79. **3.** Asakawa K, et al. *Cell Mol Life Sci.* 2021;78(10):4453-4465. **4.** Czogalla A, Sikorski AD. Cell *Mol Life Sci.* 2005. 62:1913-1924. **5.** Vanderklish PW, Bahr BA. *Int J Exp Pathol.* 2000:81(5);323-339. **6.** Liu MC, et al. *Biochem J.* 2006 Mar 15;394(Pt 3):715-25.

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Abbreviations
RT-qPCR: Reverse transcription quantitative polymerase chain reaction

Disclosures
LK, **EM,** and **JT** are or were full-time employees of and have stock option ownership in Amylyx Pharmaceuticals, Inc. **RB** and **JA** are employees of nVector, Inc. and **PZ**, **CK**, and **RJP** are employees of UMC Utrecht Brain Center which were contracted by Amylyx to perform the experiments described herein. **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.

EXPERIMENTS

Cerebrospinal Fluid (CSF) SBDP-145 Biomarker Study (*nVector, Inc.*)

METHODS: Quantified SBDP-145 levels in CSF from people living with ALS and healthy controls (HC) using enzyme-linked immunosorbent assay (ELISA) (Biomatik)

- A total of 107 CSF samples were analyzed across two biorepositories (NEALS Biorepository, n=47, and Target ALS Biorepository, n=60)
- Final cohort breakdown:
  - Healthy Controls (HC): n=42
  - Sporadic ALS (sALS): n=44
  - Familial ALS (fALS): n=9, including 7 C9orf72, 1 SOD1, and 1 TAF15 mutation carriers
- All samples were run in duplicate, with standard curves (0–40 ng/mL) generated per plate. Individual values and median levels were calculated for each diagnostic group

SBDP-145 levels were significantly elevated in the CSF of individuals with ALS compared to healthy controls (p = 0.0029), with particularly high levels observed in sALS vs controls (p = 0.0008), and a trend toward higher levels in sALS vs fALS

Figure 1. CSF SBDP-145 Levels in ALS vs. Healthy Controls

Pre-Clinical Studies with AMX0114 (*Pasterkamp Lab, UMC Utrecht Brain Center*)

METHODS: In vitro models and lentiviral expression systems for wild type and mutant *CAPN2* (CAPN2-I530V) were used to investigate the impact of AMX0114; bars represent mean (+ standard deviation)

- SHSY5Y cells were transduced with the pLenti-Puro lentiviral construct to express *CAPN2*
- AMX0114 was applied at 5 µM on the day of plating to enable gymnosis-based uptake; lentivirus was introduced 72 hours later
- Cells were processed 7 days after viral transduction; media (including ASO) was refreshed every 48–72 hours, with a final change 48 hours before processing
- Levels of SBDP-145 secreted into the culture medium were quantified using ELISA (MyBioSource)

No AMX0114 | With AMX0114

Figure 2. *CAPN2* mRNA Levels Reduced with AMX0114

N=3 independent RT-qPCR experiments

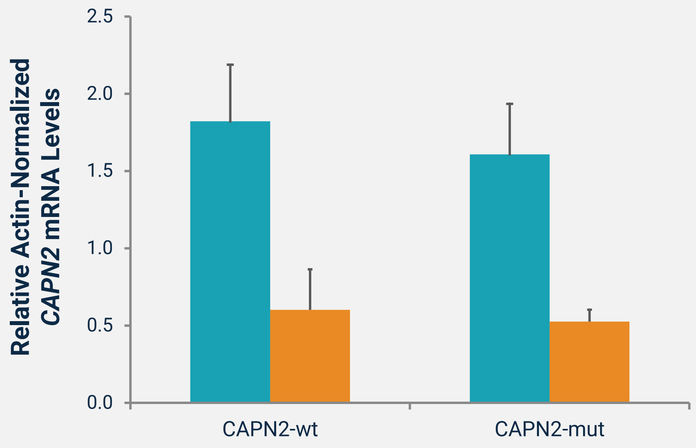


Figure 3. Calpain-2 Protein Levels Reduced with AMX0114

N=5 independent western blot experiments

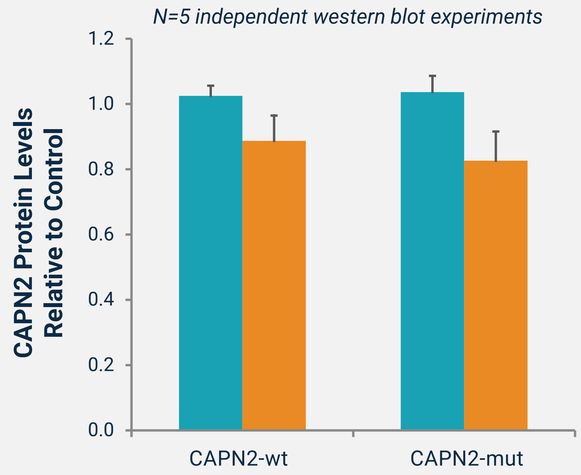


Figure 4. SBDP-145 Levels Reduced with AMX0114

N=6 independent ELISA experiments

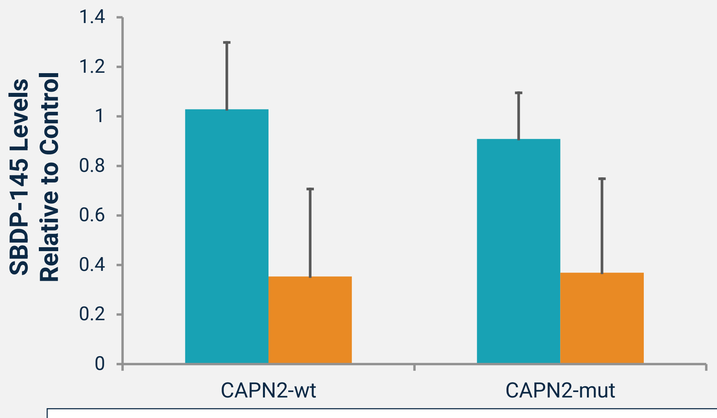
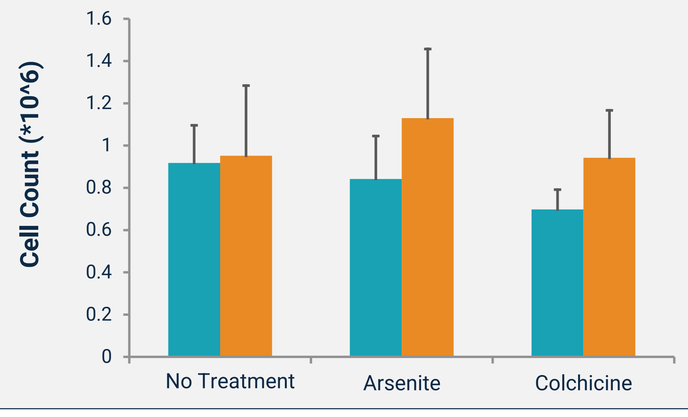


Figure 5. Cell Viability Under Stress Improved with AMX0114

N=5 independent experiments in non-transfected SHSY5Y cells



In preclinical models, AMX0114 reduced SBDP-145 levels and improved cell viability under stress

CONCLUSIONS

- **SBDP-145 levels were significantly elevated in ALS CSF, particularly sALS, compared to healthy controls (Fig. 1)**
- **AMX0114 significantly reduced SBDP-145 levels in pre-clinical studies**, indicating decreased alpha II-spectrin breakdown and effective calpain-2 inhibition **(Fig. 2-4)**
- Together these results support **SBDP-145 as a potential biomarker of calpain-2 activity, axonal injury, and AMX0114 target engagement**
- **AMX0114 improved cell viability** under stress in differentiated SHSY5Y cells, indicating neuroprotective potential **(Fig. 5)**
- **AMX0114 shows therapeutic potential in ALS;** the ongoing phase I, multiple ascending dose LUMINA trial is evaluating its safety, tolerability, and effects on biomarkers of target engagement and disease progression, and additional research is also underway to assess its effects on NfL and other biomarkers

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