QurAlis

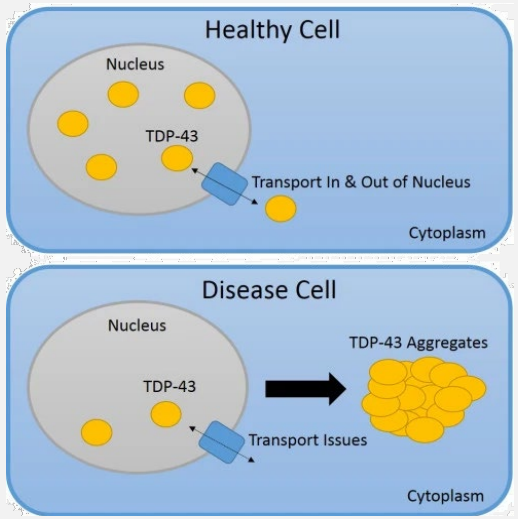
Biomarkers for Patient Stratification and Target Engagement in Patients with TDP-43 Pathology

Sandy Hinckley, Ph.D.
Head of Biology

STMN2: A Genetic Target For The Sporadic ALS Population and TDP43opathies

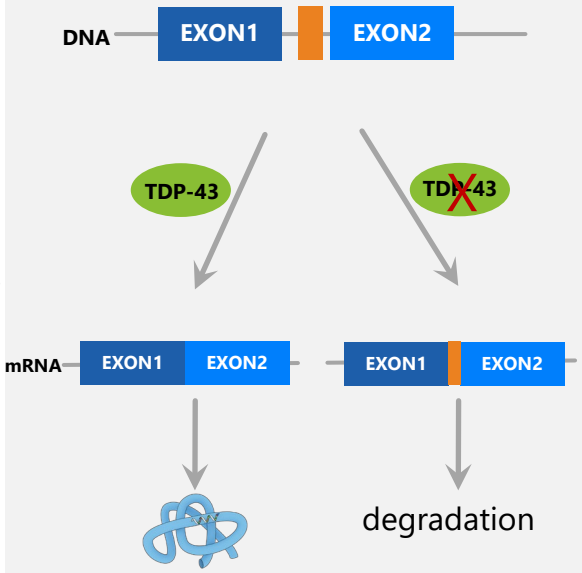
QurAlis Therapeutic Strategy

In ALS motor neurons TDP-43 leaves the nucleus



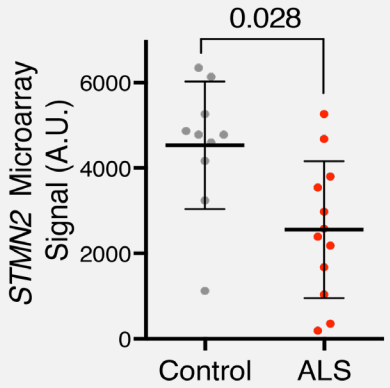
QurAlis niche

Loss of TDP-43 controlled cryptic exon splicing



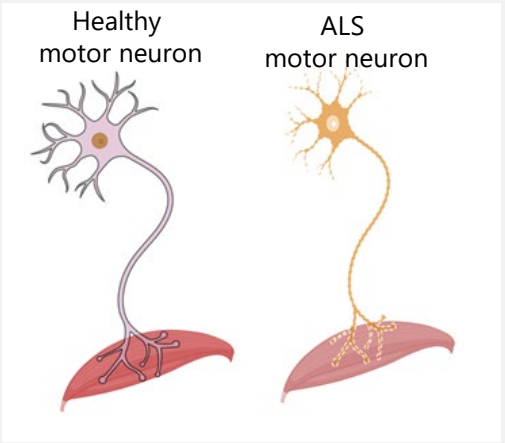
TDP-43 regulation of *STMN2*

Loss of full length *STMN2*



Cryptic splicing-ASO approach to restore STMN2

Axonal degeneration and impaired repair



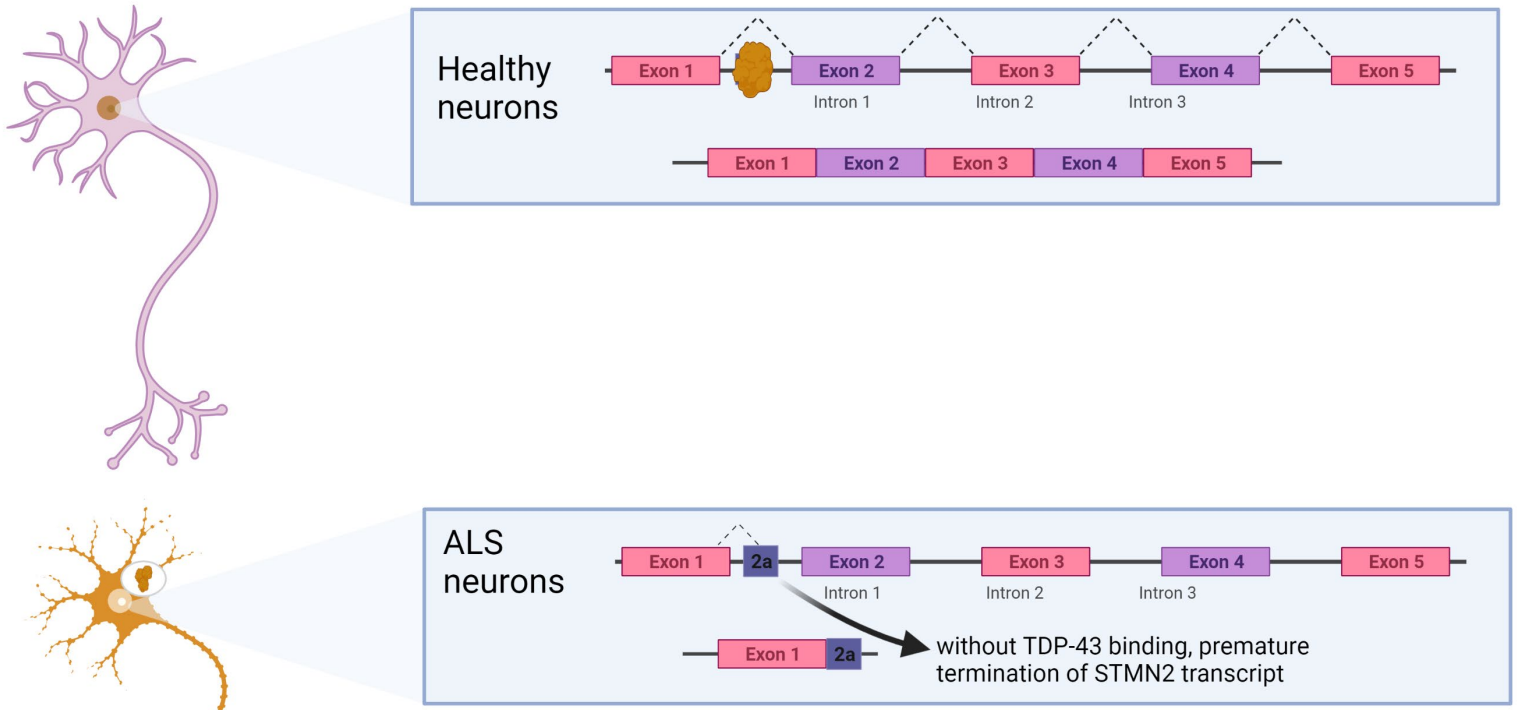
Rescue of axonal stability and repair

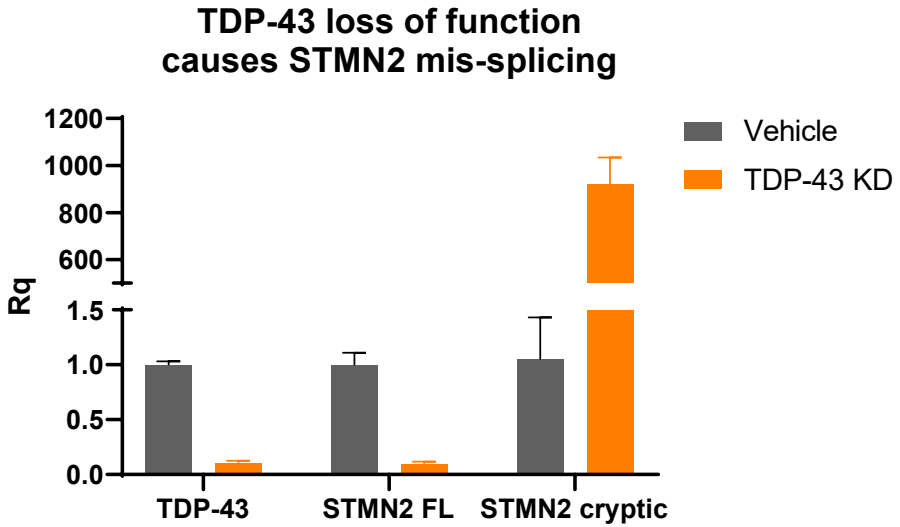
Nat. Neurosci. Feb 2019, Eggan ALS One 2020, Li et al., 2009, Morii et al., 2006, Shin et al., 2012, Shin et al., 2014, Xu et al., 2010, Rabin et al., 2009

QurAlis

STMN2 is a downstream target of TDP-43 function

- TDP-43 binding to exon 2a in the STMN2 pre-mRNA controls splicing and expression of STMN2 full length transcripts

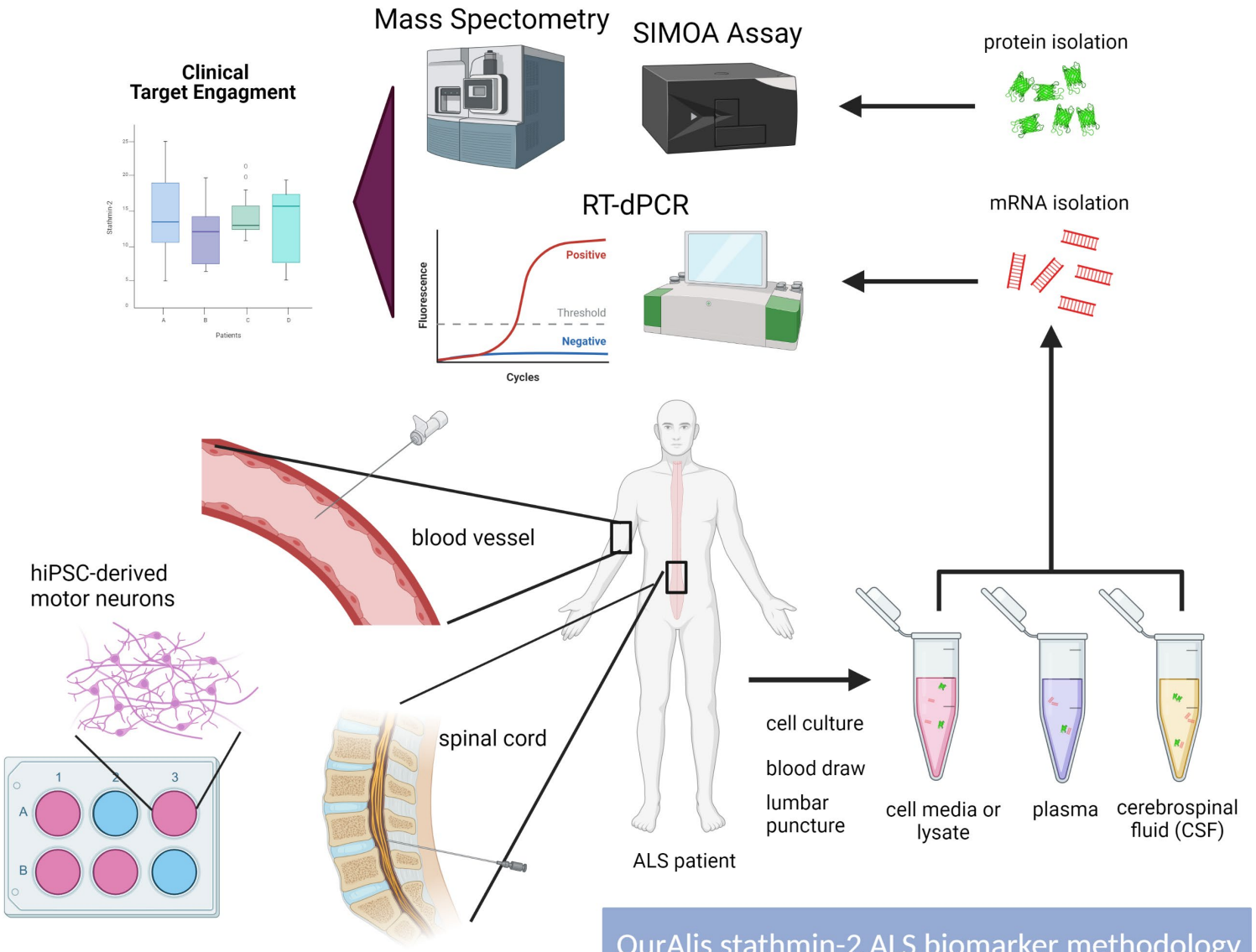




- QurAlis has discovered a therapeutic ASO that restores *STMN2* mis-splicing due to TDP-43 pathology
- We are developing biomarkers that can be used to identify patients with TDP-43 pathology and measure STMN2 target engagement

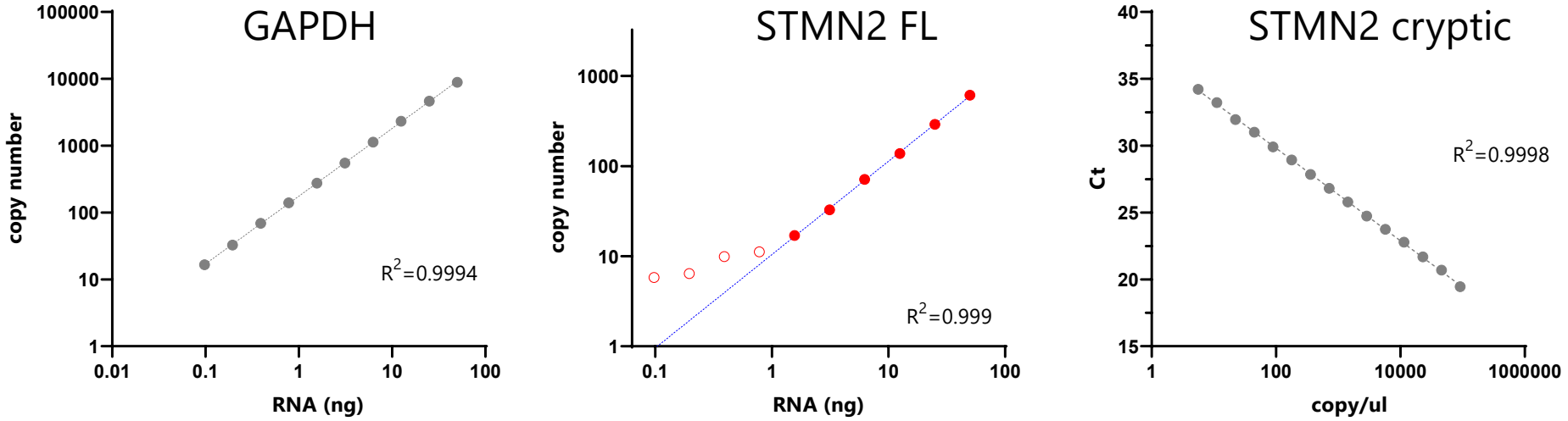
QurAlis

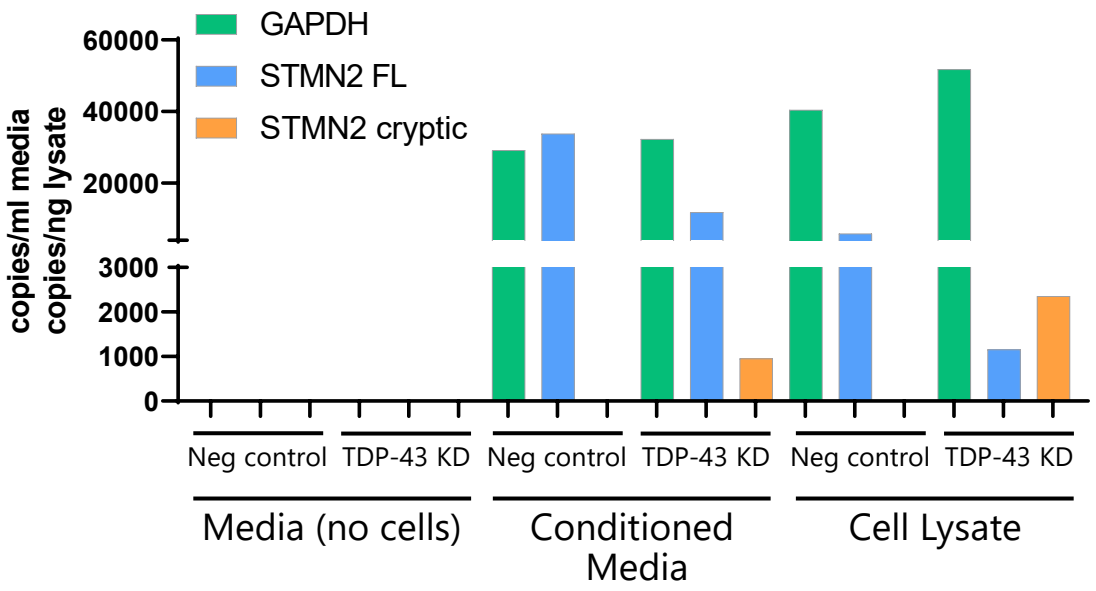
STMN2 biomarker development



QurAlis stathmin-2 ALS biomarker methodology

Detection of STMN2 transcripts in hiPSC-derived motor neuron cultures





- ddPCR for GAPDH and STMN2 FL shows assay linearity down to 10 copies
- STMN2 cryptic assay was evaluated using G-block DNA and qPCR testing shows assay linearity
- Quantitation of STMN2 FL and cryptic exon STMN2 mRNA isolated either from conditioned media or cell pellets of hiPSC-derived motor neurons
- Both STMN2 FL and STMN2 cryptic exon mRNA is detectable in conditioned media
- Fluid STMN2 FL and cryptic levels represent the cellular biology and show STMN2 FL decrease and STMN2 cryptic increase upon mis-splicing induction

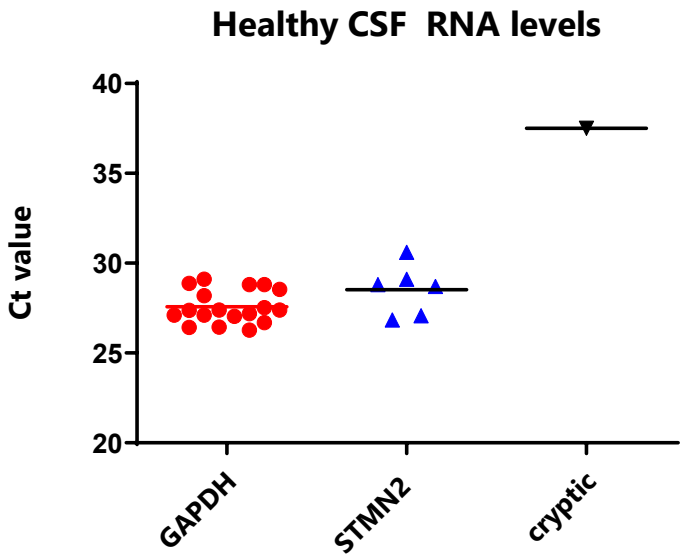
QurAlis

STMN2 RNA quantification in human cerebrospinal fluid (CSF)

1mL pooled CSF (healthy)

RNA purification

qPCR assays
GAPDH, STMN2, STMN2 cryptic



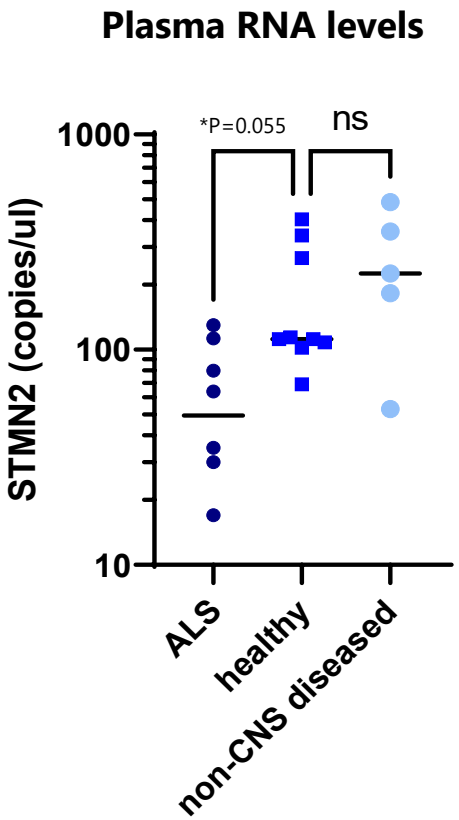
- Healthy CSF was assayed for the presence of *STMN2* RNA
- *STMN2* RNA was detected at high quantity in CSF about the same as the reference *GAPDH*
- Cryptic exon containing transcripts were mainly not detected, as expected in healthy samples

STMN2 RNA quantification in human plasma

2 mL plasma (ALS & healthy)

RNA purification

ddPCR assay
STMN2 FL



- ALS, healthy, and non-CNS disease plasma was assayed for the presence of *STMN2* RNA
- *STMN2* RNA was detected in 21/22 samples. 1/8 ALS sample had no measurable STMN2
- Trend toward decreased *STMN2* RNA level in ALS plasma

N=8 ALS
N=9 healthy
N=5 non-CNS disease controls
Kruskal-Wallis test
ANOVA
Dunn's MC test

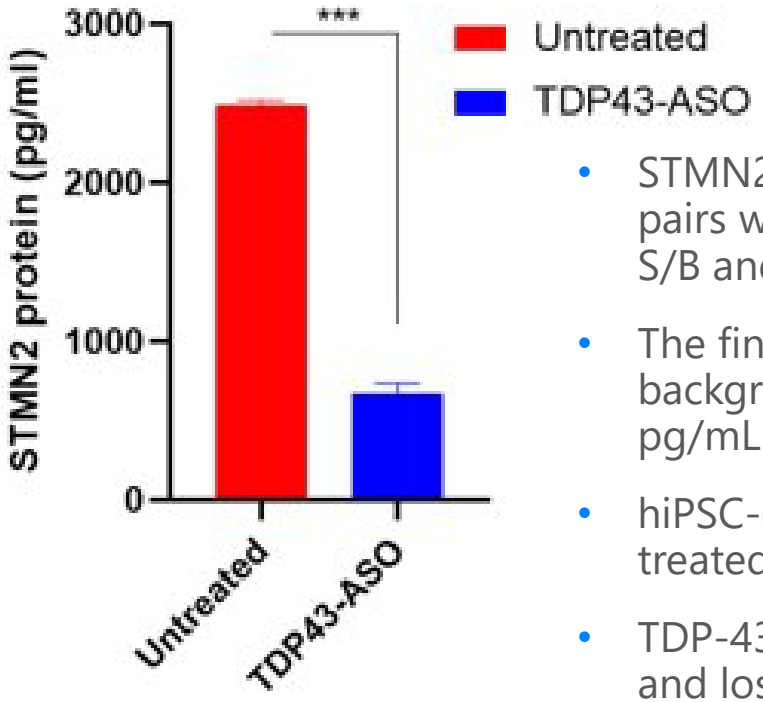
QurAlis

SIMOA assay for detection of STMN2 protein

Calibration Curve			
[stathmin-2] pg/mL	Average AEB	%CV	S/B
0.0	0.005	22%	
0.4	0.012	2%	2
1.5	0.034	8%	6
5.9	0.120	10%	22
23.4	0.450	13%	82
93.8	2.282	13%	417
375.0	8.023	0%	1466
1500.0	22.500	2%	4111

LOD: 0.087 pg/ml
LLOQ: 0.33 pg/ml

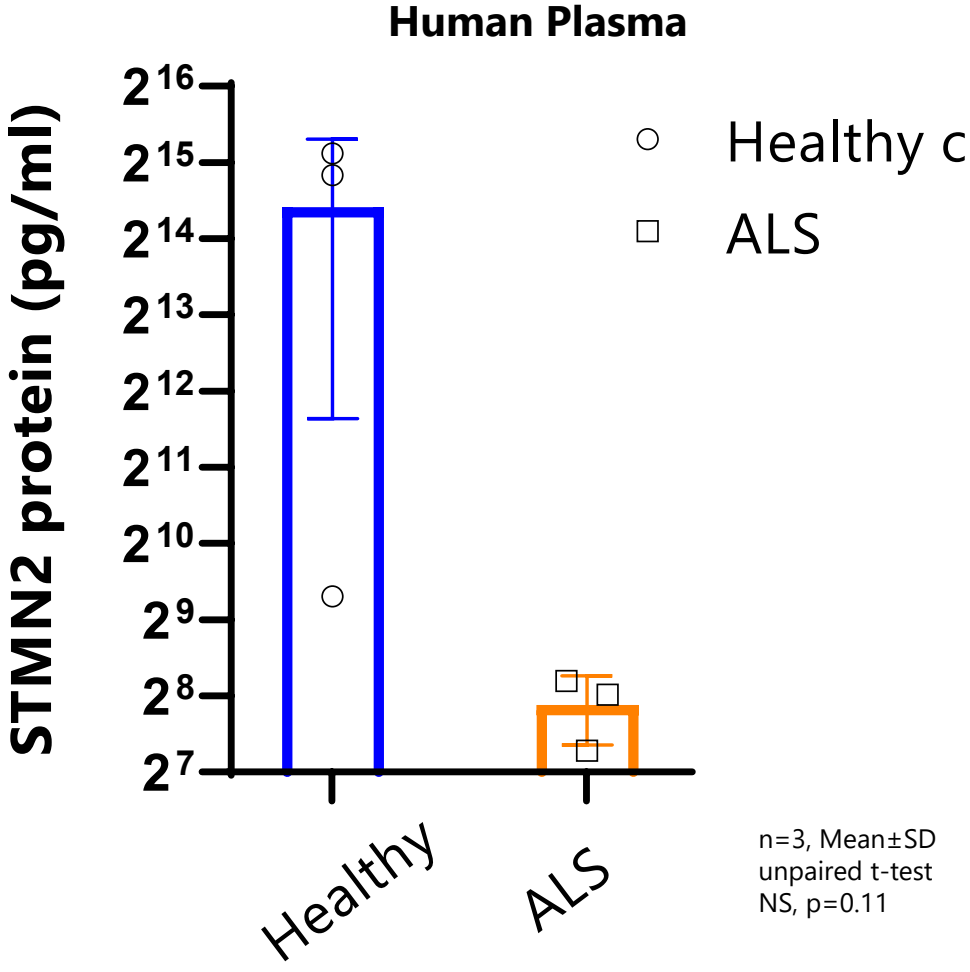
Motor Neuron Lysates



n=2, Mean±SD
unpaired t-test
***p<0.005

- STMN2 capture and detection antibody pairs were tested and the 2 with lowest S/B and dilution linearity were selected
- The final assay format shows low background levels (AEB<0.01) and sub-pg/mL LLOQ
- hiPSC-derived motor neurons were treated with/out TDP-43 gapmer ASO
- TDP-43 KD results in STMN2 mis-splicing and loss of STMN2 protein levels
- STMN2 SIMOA assay detects changes in human STMN2 levels due to TDP-43 loss of function

STMN2 protein quantification in human plasma



- Healthy and ALS plasma was tested in the STMN2 SIMOA assay (n=3 individuals each)
- Healthy control and ALS samples within the dynamic range of the assay
- ALS STMN2 levels are 50% reduced compared to the lowest healthy sample and 98% reduced compared by mean values

QurAlis

Conclusions

- We have developed both RNA and protein quantitation methods to detect STMN2 in human biofluids
- STMN2 biomarker assay allows the characterization of TDP-43 pathology and downstream target biology in living ALS patients
- Next steps include:
  - Assay qualification and validation for use as a clinical exploratory Biomarker
  - Characterization of larger patient cohorts for protein assay from annotated clinical libraries
  - Expansion of human data set into other TDP43opathies (FTD, AD, PD)

**check out QurAlis's other presentation by Taylor Gray on STMN2 biology

Acknowledgements

Contributions by:

Taylor Gray, Scientist

Hafiz Mohmmadabdul, Senior Scientist

Daniel Elbaum, Chief Scientific Officer

Research Support:

Work supported in part by Target ALS Industry Led Consortium Grant