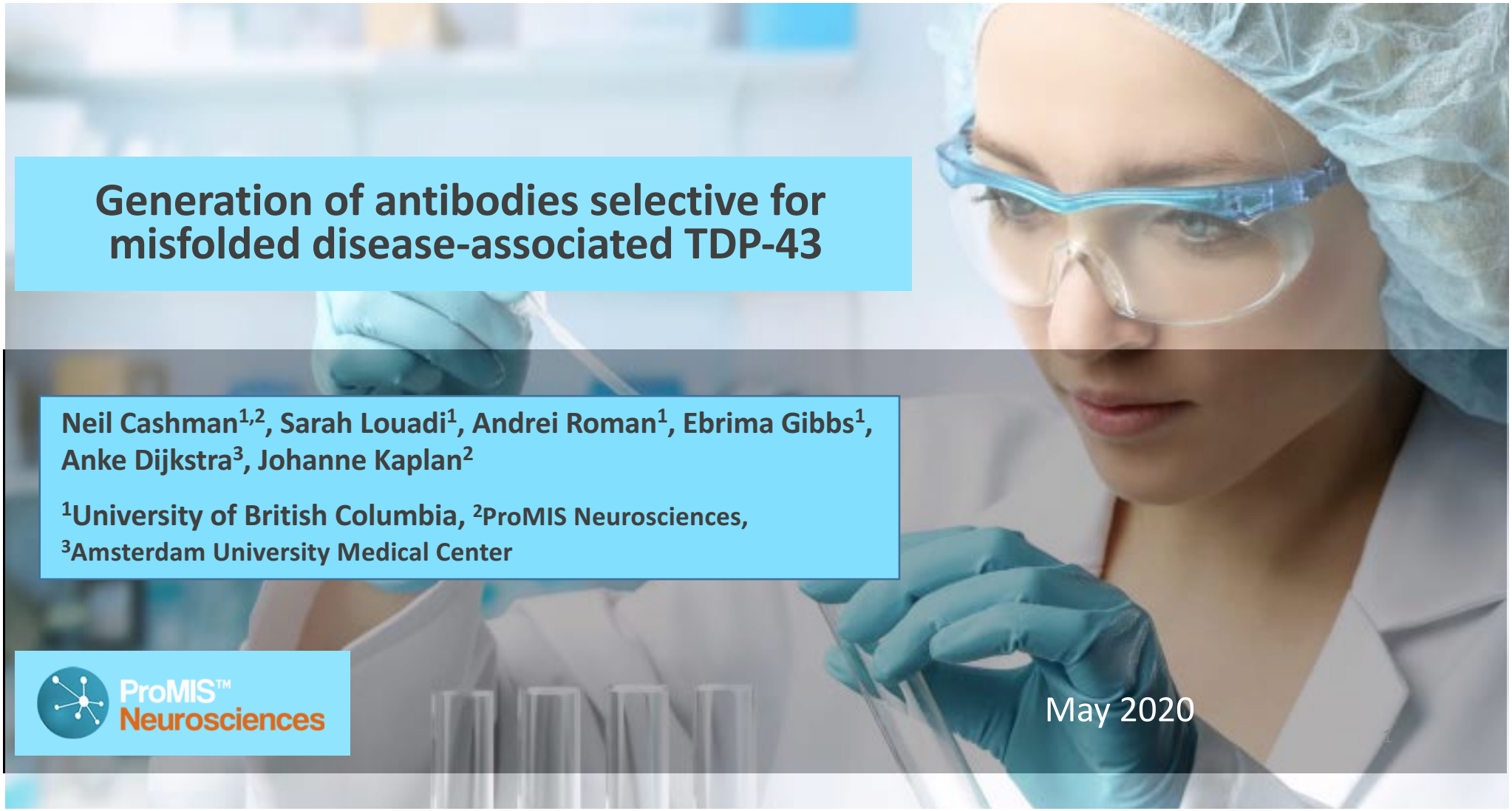

Generation of antibodies selective for misfolded disease-associated TDP-43

Neil Cashman[1,2], Sarah Louadi[1], Andrei Roman[1], Ebrima Gibbs[1], Anke Dijkstra[3], Johanne Kaplan[2]

[1]University of British Columbia, [2]ProMIS Neurosciences, [3]Amsterdam University Medical Center

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May 2020

Disclosure

- Chief Scientific Officer of ProMIS Neurosciences

Generation of antibodies selective for misfolded disease-associated TDP-43

Background

- Misfolded protein aggregates of TDP43 are a pathological hallmark of ALS and FTLD[1]
- The stereotypical distribution of TDP43 pathology in ALS and FTD shows a spreading pattern consistent with progressive dissemination from neuron to neuron[2,3]
- Misfolded aggregates of TDP43 are toxic to neural cells[4,5]
- Prion-like cell to cell propagation of TDP43 aggregates has been demonstrated in cell culture[5,6] and animal models[7]
- We have previously reported that pathogenic TDP43 induces misfolding of SOD1[8] and we recently determined that a tryptophan (Trp68) in the TDP-43 N-terminal domain (NTD) participates in this cross-seeding despite being inaccessible in the natively folded NTD[9]

Hypothesis

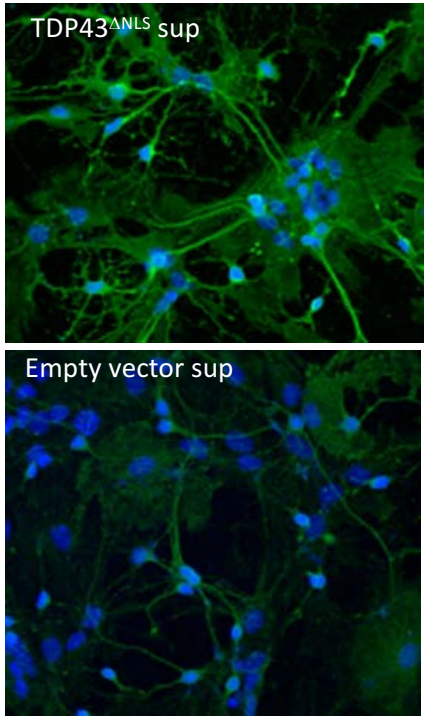
- NTD Trp68 becomes exposed when TDP-43 is cytosolically mislocalized/aggregated

Goal

- Generate antibodies selective for misfolded disease-associated TDP-43 through immunization of rabbits with an unfolded NTD linear epitope including Trp68

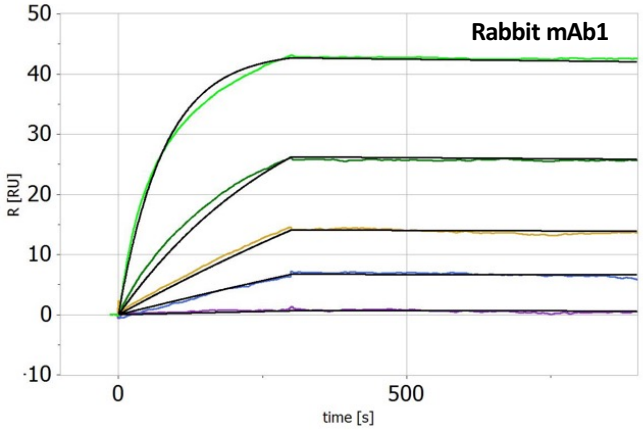
[1]Neumann et al, 2006, Science; [2]Braak et al, 2013, Nat Rev Neurol; [3]Brettschneider et al, 2014, Acta Neuropatholl; [4]Wang et al, 2016, Nat Med; [5]Nonaka et al, 2013, Cell Reports; [6]Feiler et al, 2015, J Cell Biol; [7]Porta et al, 2018, Nature Commun; [8]Pokrishevsky et al, 2016, Scientific Reports; [9]Afroz et al, 2017, Nature Commun

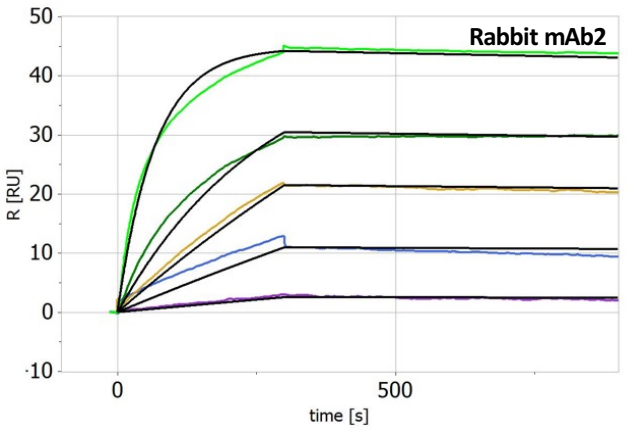
Induction of SOD1 mis-folding by pathogenic TDP43[8]

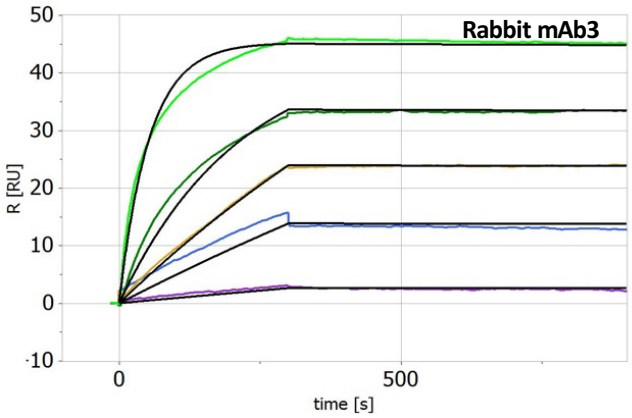


Mis-folded SOD1 (green) in primary spinal cord cultures exposed to supernatant from HEK293 cells transduced with TDP43$^{\Delta NLS}$ (misfolded) but not empty vector

Rabbit monoclonal antibodies raised against NTD epitope of misfolded TDP-43 display picomolar affinity for their cognate peptide epitope as assessed by surface plasmon resonance (SPR)





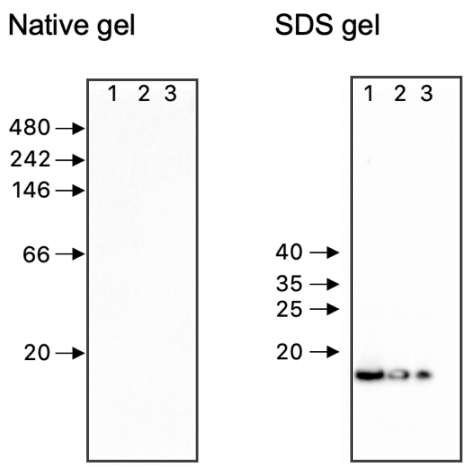


mAb	k_{ON} ($M^{-1}s^{-1}$)	k_{OFF} (s^{-1})	K_D (M)
Rabbit mAb1	4.03E+05	2.50E-05	6.20E-11
Rabbit mAb2	4.70E+05	4.32E-05	9.19E-11
Rabbit mAb3	5.99E+05	9.04E-06	1.51E-11

- TDP43 NTD epitope-BSA conjugate immobilized on sensor chips at a very low density of ~50 RUs
- Rabbit mAbs diluted 4-fold (31.25nM, 7.81nM, 1.95nM, 0.98nM, 0.24nM) and injected over the surface
- Binding curves fitted to a Langmuir 1:1 interaction model

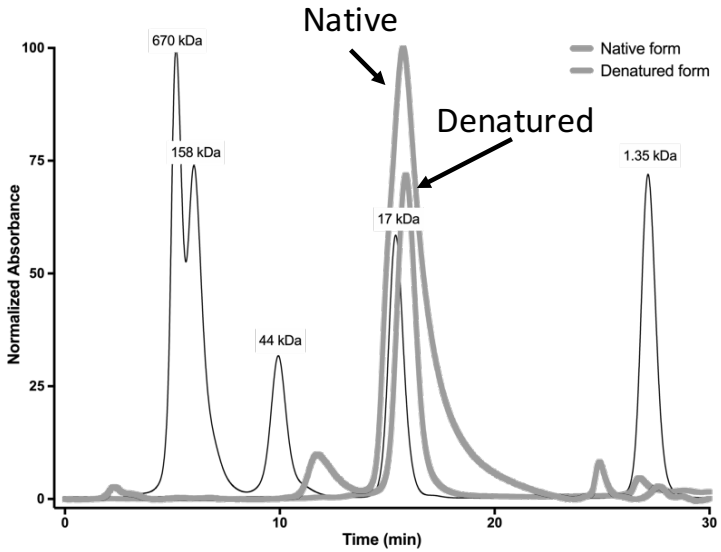
The epitope is recognized by antibody in denatured but not natively folded TDP43 N-terminal domain

ProMIS pAb reacts with denatured TDP-43 (SDS gel) but not native TDP-43



Lanes 1, 2, 3 contain 0.6, 0.3, 0.15 ug TDP-43 protein/lane, respectively
pAb: Polyclonal rabbit antibody

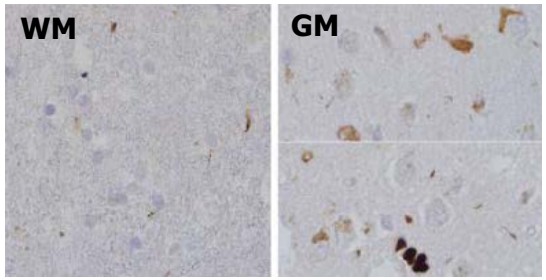
Size exclusion chromatography confirms integrity of TDP-43 protein



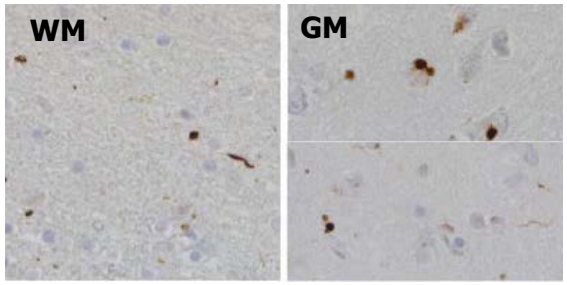
MW weight markers denote MW range

NTD epitope of misfolded TDP-43 is recognized by antibody in brain tissue from FTLD patients with pathology subtypes A,B,C,E and in spinal cord from ALS patients

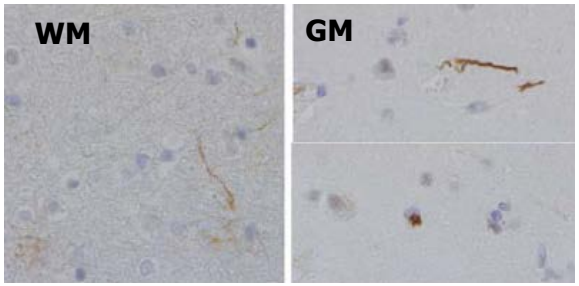
FTD brain, TDP-43 pathology type A



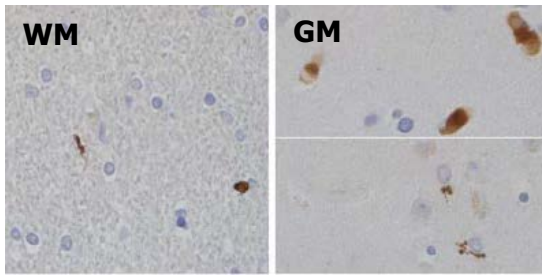
FTD brain - TDP-43 pathology type B



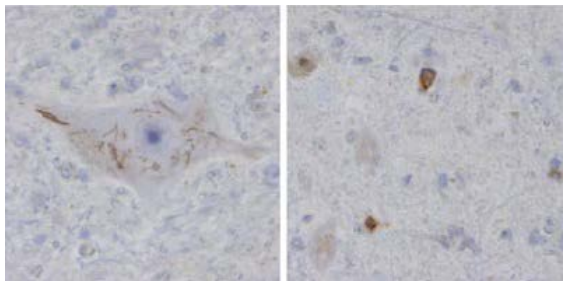
FTD brain - TDP-43 pathology type C



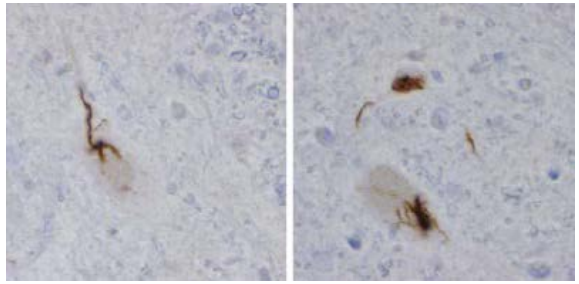
FTD brain - TDP-43 pathology type E



ALS patient 1 – Spinal cord



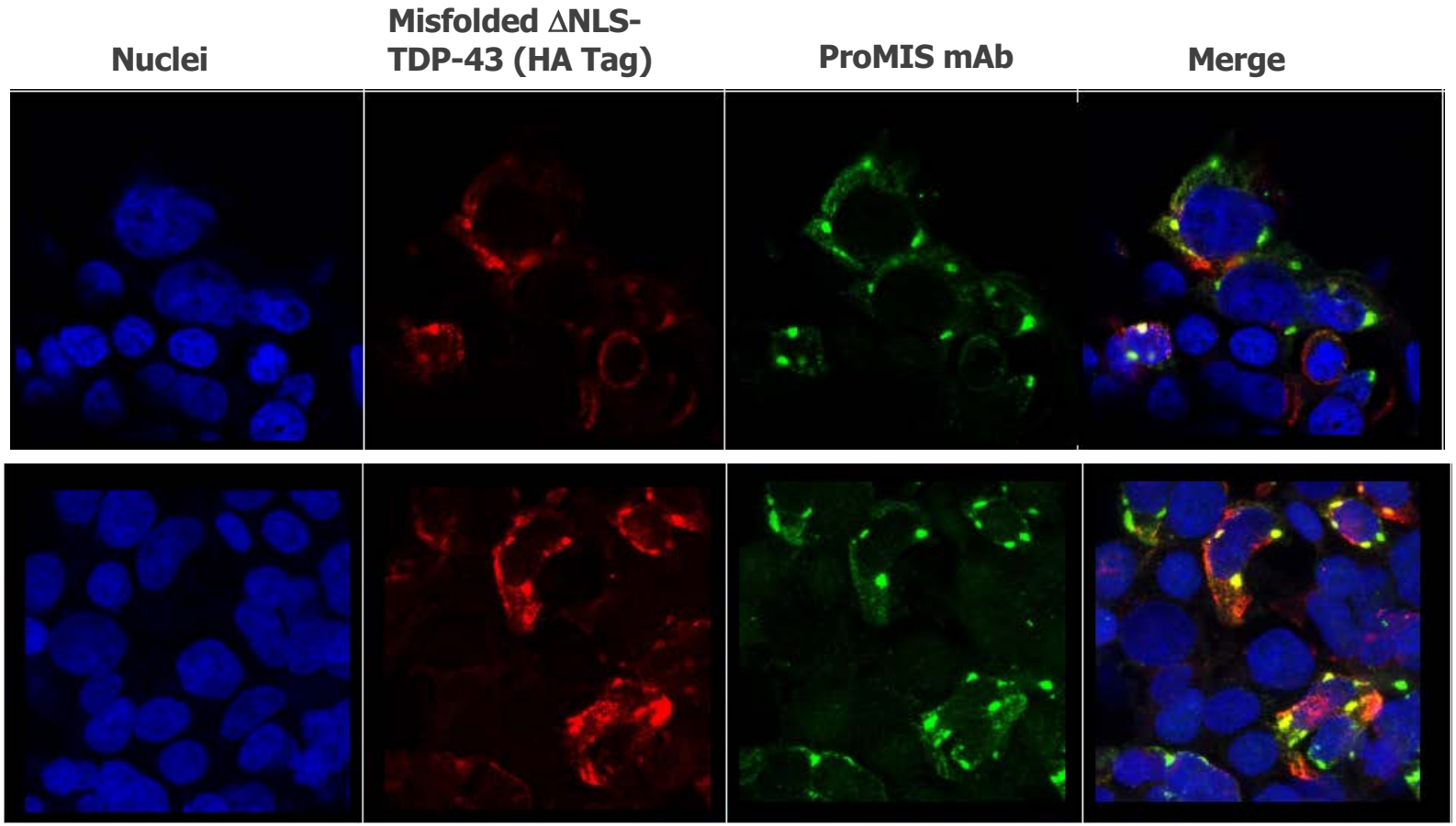
ALS patient 2 – Spinal cord



WM – White matter
GM = Grey matter

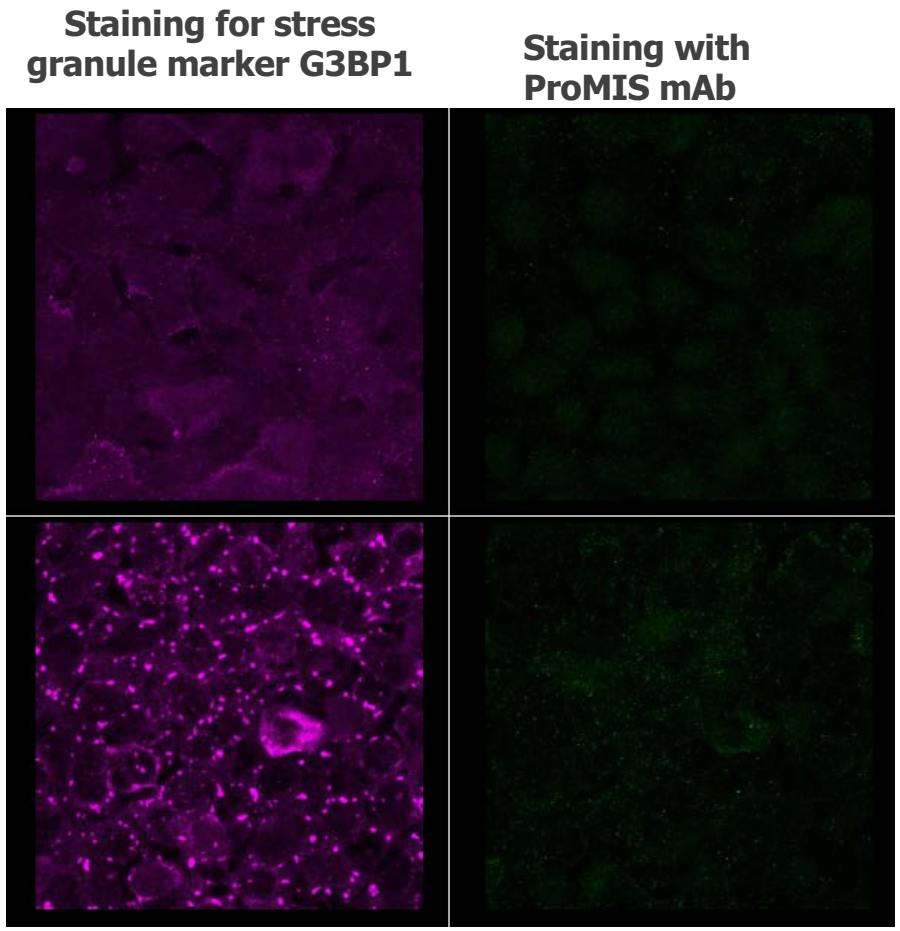
Staining with ProMIS rabbit pAb to TDP-43 NTD
Performed by Dept. of Pathology, Amsterdam Neuroscience, VU University Medical Center, Amsterdam, The Netherlands

mAbs to misfolded TDP-43 NTD react with mislocalized, aggregated ΔNLS-TDP-43 but not nuclear wild type TDP-43



- HEK-293 cells transfected with ΔNLS TDP-43 lacking a functional nuclear localization signal
- Cells stained for HA tag (red) of overexpressed ΔNLS TDP-43 or with rabbit mAb to NTD epitope at 2 μg/ml (green).
- Nuclei stained with DAPI (blue)
- Images analyzed by confocal microscopy (Z-stacks)

mAbs to misfolded TDP-43 NTD do not recognize TDP43 in physiological stress granules



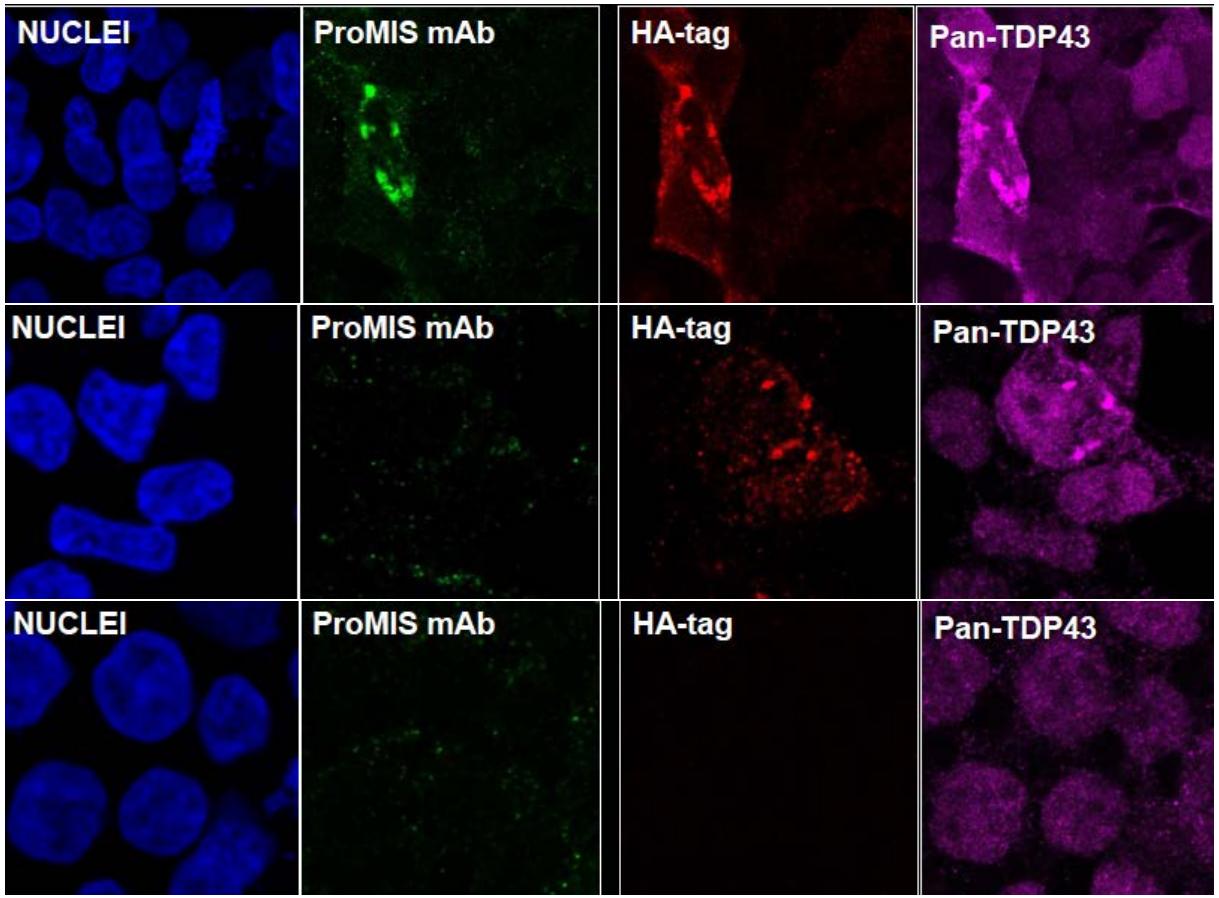
- HEK293 cells stressed by 60min exposure to 1mM sodium arsenite for 60 min
- Cells stained with ProMIS rabbit mAb at 10 μg/ml (green) or with antibody to G3BP1 (magenta)
- Images analyzed by confocal microscopy

Immunoreactivity of mAbs with ΔNLS-TDP-43 is abrogated by mutation of Trp68 to serine indicating immunodominance of Trp68 in misfolded TDP43 NTD epitope

ΔNLS-TDP-43
mAb recognition of misfolded cytoplasmic aggregates

ΔNLS-TDP-43-W68S
Loss of mAb reactivity with aggregates when Trp68 mutated

Empty vector
Negative control



- HEK-293 cells transfected with ΔNLS TDP-43 lacking a functional nuclear localization signal
- Cells stained for HA tag (red) of overexpressed ΔNLS TDP-43 or with rabbit mAb to NTD epitope at 2 μg/ml (green) or a pan-TDP-43 antibody (magenta).
- Nuclei stained with DAPI (blue)
- Images analyzed by confocal microscopy

Conclusions

- Immunization with a predicted NTD epitope of misfolded TDP-43 produced a family of antibodies sensitive to solvent exposure of NTD Trp68
- The mAbs displayed high epitope binding affinity in the picomolar range
- The antibodies showed reactivity and specificity for aberrant cytoplasmic TDP43 aggregates in a HEK293 cell model, and no reactivity with wild-type nuclear TDP43 -> preserves normal, essential TDP43 function
- The same antibodies did not show binding to physiological stress granules -> preserves stress-protective function
- Staining of TDP43 in human FTD and ALS samples confirmed reactivity with pathological TDP43
- These antibodies may find utility in biomarker and immunotherapy applications for TDP-43 associated diseases