**Targeting of misfolded, pathogenic TDP-43 with rationally designed antibodies**

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**INTRODUCTION**

**Background**

• Misfolded protein aggregates of TDP43 are a pathological hallmark of ALS and FTLD
• The pathological infiltration of TDP43 pathology in ALS and FTLD shows a spreading pattern consistent with progressive dissemination from source to receptor
• Misfolded TDP43 aggregates are toxic to neural cells
• Antibodies to the A53T mutant allele of TDP43 have been demonstrated in cell culture and animal models

• We have previously reported that pathogenic TDP43 induces misfolding of SOD1 and we recently determined that a tryptophan (Trp68) in the N-terminal domain (NTD) participates in the cross-fermenting disease process

• We have previously reported that pathogenic TDP43 includes misfolding of SOD1 and we recently determined that a tryptophan (Trp68) in the N-terminal domain (NTD) participates in the cross-fermenting disease process

**Goal**

Generate antibodies selective for misfolded disease-associated TDP-43 through immunization of rabbits with an N-terminal domain linear epitope including Trp68 (anti-NTD).

**METHODS**

**Surface Plasma Resonance**

TDP43 NTD (0.5 mg/ml) was immobilized on sensor chips at a very low concentration. The NTD epitope is recognized by antibodies in the picomolar range. Rabbit mAbs to misfolded TDP-43 NTD react with mislocalized, aggregated ΔNLS-TDP-43, while control antibodies do not recognize ΔNLS-TDP-43.

**Western Blot:**

SDS and Native gels were carried out using the Novex Bis-Tris system. Western blotting was carried out using the SuperSignal West Femto (Thermo Scientific, USA) substrate with donkey anti-Rabbit IgG HRP-labelled secondary antibodies (GE Healthcare Life Sciences, USA). TDP-43 NTD (0.5 mg/ml) was denatured by liquid chromatography instrument on a Superdex 75 (10/300) HPLC column (GE Healthcare, USA). The SuperSignal West Femto (Thermo Scientific, USA) substrate with donkey anti-Rabbit IgG HRP-labelled secondary antibodies (GE Healthcare Life Sciences, USA) was used according to the manufacturer’s instructions.

**Immunization with a predicted NTD epitope of misfolded TDP-43 produced a family of antibodies sensitive to solvent exposure of NTD TDP43 (anti-NTD)**

**REFERENCES**


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**CONCLUSION**

• Immunization with a predicted NTD epitope of misfolded TDP-43 produced a family of antibodies sensitive to solvent exposure of NTD TDP43 (anti-NTD)

• Anti-NTD displayed high epitope binding affinity in the picomolar range

• Selecting antibodies with high specificity for elemental pathogenic TDP43 aggregates in a HeLa cell model, including Trp68, normal, essential TDP43 function

• The same antibody did not show binding to physiological stress granules = preserved stress protection function

• Blocking 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

**WM = White matter   GM = Grey matter**