Gene-Therapy Mediated Targeted Protein Degradation in Neurodegenerative Disease Models

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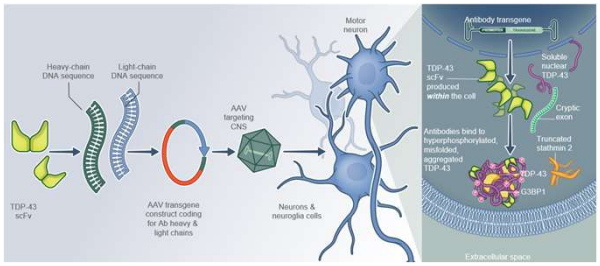
Introduction



Results

Figure 1. Schematic Representation of Targeting proteins to degradative pathways

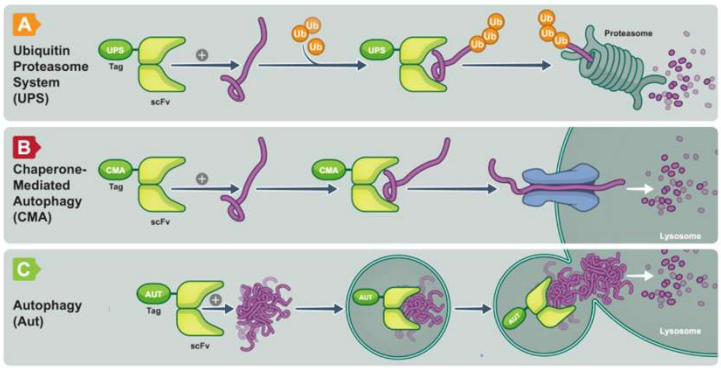


Table I

VecTron ID	Pathway
51	backbone
104 - 116	UPS
117 - 120	CMA
121-124	Autophagy

Figure 1A-C. A Three schematic representations of degradative pathways that the VecTron designs can utilize. VecTrons are designed by combining a single chain Fv (scFv). Three pathways have been utilized in the designs: **A**, The ubiquitin proteasome system (UPS), **B**, Chaperone mediated autophagy (CMA) and **C**, macroautophagy (AUT). **Table I**. The table shows an overview of the 21 Vectron designs and which pathways they target.

Figure 2. TDP-43 aggregate reduction mediated by VecTrons

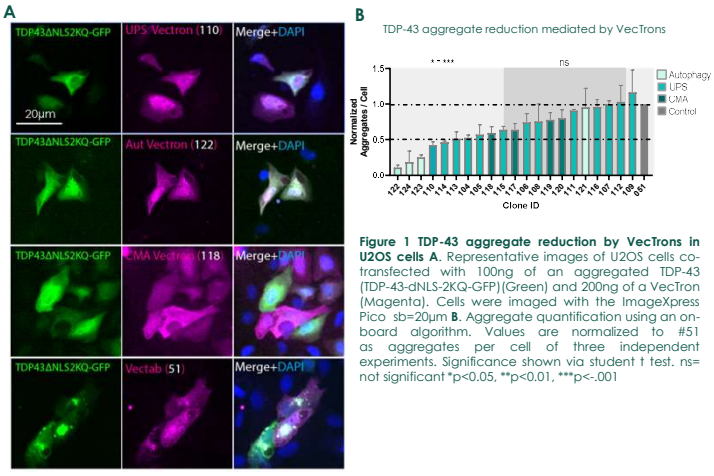


Figure 1 TDP-43 aggregate reduction by VecTrons in U2OS cells A. Representative images of U2OS cells co-transfected with 100ng of an aggregated TDP-43 (TDP-43-dNLS-2KQ-GFP)(Green) and 200ng of a VecTron (Magenta). Cells were imaged with the ImageXpress Pico sb=20µm **B**. Aggregate quantification using an on-board algorithm. Values are normalized to #51 as aggregates per cell of three independent experiments. Significance shown via student t test. ns= not significant *p<0.05, **p<0.01, ***p<-.001

Figure 3. VecTrons can reduce pTDP-43 levels in aggregate induced cell lines

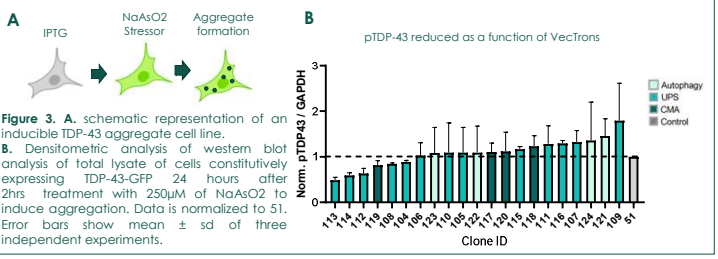


Figure 3. A. schematic representation of an inducible TDP-43 aggregate cell line.
B. Densitometric analysis of western blot analysis of total lysate of cells constitutively expressing TDP-43-GFP 24 hours after 2hrs treatment with 250µM of NaAsO2 to induce aggregation. Data is normalized to 51. Error bars show mean ± sd of three independent experiments.

Figure 4. VecTron levels are increased when their respective pathways are blocked Pathway by either MG132 (UPS) or Chloroquine (CMA / Autophagy)

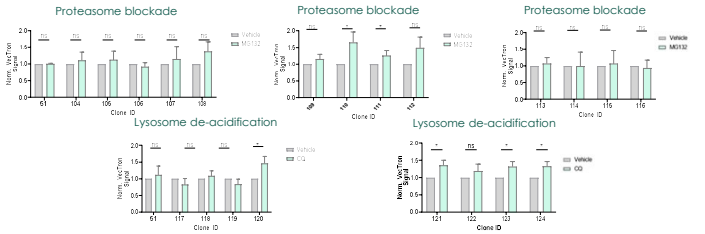


Figure 4. Densitometric quantification of VecTrons by western blot analysis of total lysate of cell transfected with VecTrons and either treated 8hrs with 10µM MG132 or 24 hrs with Chloroquine (CQ). Data was normalized to Vehicle. Graphs show the mean ± sd from three independent experiments. Significance shown via student t test. ns= not significant *p<0.05, ns = not significant

Discussion / Conclusion

The challenges of antibody therapies for neurodegenerative diseases are that the location of the pathology that is not accessible due to the BBB and the inherent difficulty of the removal of intracellular protein aggregates. We have demonstrated that large TDP-43 aggregates are removed by using the endogenous cellular protein degradation pathways – UPS, CMA or autophagy. Additionally, VecTrons likely inhibit and reduce the TDP-43 phosphorylation, a pathological hallmark of ALS. The pathway specificity of the VecTrons was further validated by inhibiting the interactions with relevant protein partners in the cell and expanding on the current degron library. Together, this initial data shows the first hallmarks of a promising therapeutic approach for ALS

At VectorY we aim to combine the next generation of gene therapy, antibody and degron technology to develop a strong platform that brings innovative therapies to the patient.

Reference

1 Scotter EL, Chen HJ, Shaw CE. TDP-43 Proteinopathy and ALS: Insights into Disease Mechanisms and Therapeutic Targets. Neurotherapeutics. 2015 Apr;12(2):352-63. doi: 10.1007/s13311-015-0338-x. Erratum in: Neurotherapeutics. 2015 Apr;12(2):515-8. PMID: 25652699; PMCID: PMC4404432.

2 Tamaki Y, Shodai A, Morimura T, Hikiami R, Minamiyama S, Ayaki T, Tooyama I, Furukawa Y, Takahashi R, Urushitani M. Elimination of TDP-43 inclusions linked to amyotrophic lateral sclerosis by a misfolding-specific intrabody with dual proteolytic signals. Sci Rep. 2018 Apr 16;8(1):6030. doi: 10.1038/s41598-018-24463-3. PMID: 29662239; PMCID: PMC5902603.

Take-Home Message & Future Directions

- ❑ Targeted protein aggregation can be accellerated by VecTrons – a vectorized antibody technology with a degron tag.
- ❑ Adding a degron to a scFv can further improve aggregate clearance
- ❑ Further steps will provide proof of concept for the degradation pathway specificity of the best VecTron candidates in vitro and in vivo