TDP-43 condensate modulation rescues TDP-43 loss of function in ALS patient-derived motor neurons and mouse models of TDP-43 proteinopathy

Gwyneth Welch[1], Jesse Lai[1], Marty Fernandez[1], Christina Curran[1], Miook Cho[1], Eric Riguet[2], Eduardo Martinez[1], Michael Wagner[2], Isaac A. Klein[1] and Violeta Yu[1]
[1] Dewpoint Therapeutics, Boston MA, USA | [2] Dewpoint Therapeutics GmbH, Frankfurt, Germany

Introduction

TAR DNA-binding protein 43 (TDP-43) is a highly conserved and ubiquitously-expressed RNA/DNA-binding protein involved in RNA processing. Cytoplasmic aggregates of TDP-43 occur in >97% of amyotrophic lateral sclerosis (ALS) cases.[1] Stress-induced formation of cytoplasmic TDP-43 condensates represent an intermediate, reversible, pre-pathological state in neurons.[2] Over time, TDP-43-containing condensates lose their fluid-like properties and potentially convert into irreversible, toxic aggregates.[3]

We aim to treat the majority of ALS patients by preventing and reversing cytoplasmic TDP-43 condensates. The role of condensate dysfunction in ALS pathogenesis is supported by the discovery of condensate proteins as genetic modifiers of ALS (e.g. TAF15, EWSR1, TIA1, HNRNPA1, HNRNPA2B1), as well as co-localization of C9ORF72 ALS G4C2 expansion-derived dipeptide repeats (DPRs) and stress granule proteins with TDP-43 inclusions in preclinical models.[2,4,5] Nuclear depletion of TDP-43 into cytoplasmic condensates leads to toxic loss of splicing function in motor neurons. Here, we propose using a small molecule condensate modifying drug (c-mod)[6] that directly modulates the TDP-43 condensates to treat diverse forms of ALS.

C-mods de-partition TDP-43 from stress granules and rescue stress-induced mis-splicing in motor neurons derived from a genetically diverse library of patient lines. Furthermore, c-mods can mitigate key neurodegeneration and neuroinflammation biomarkers in mouse models of TDP-43 proteinopathy.

C-mod proposed mechanism of action

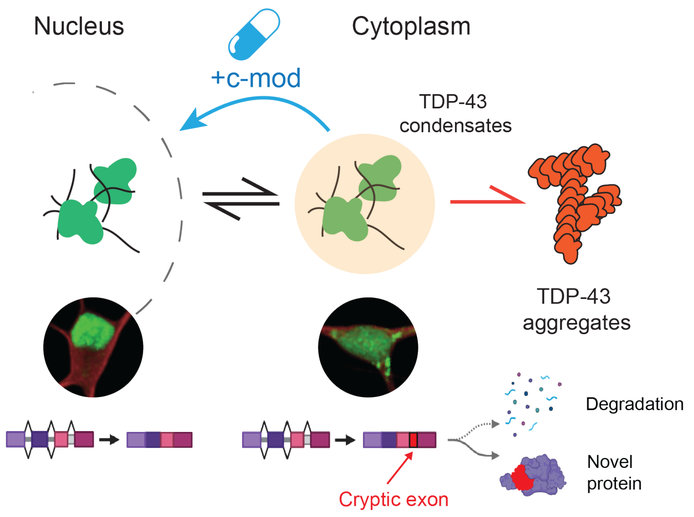
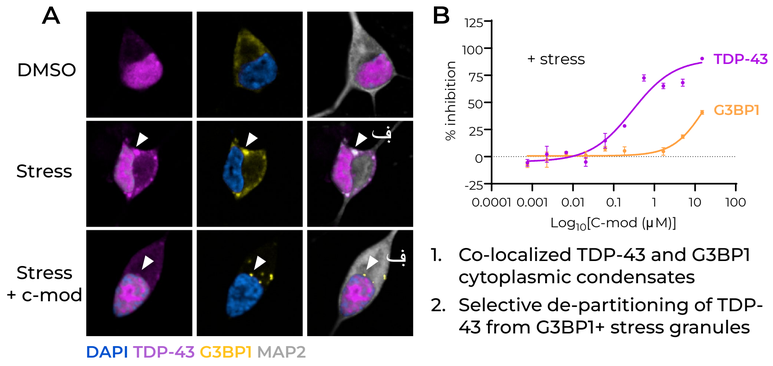


Figure 1. Proposed therapeutic hypothesis. C-mods target TDP-43 condensates to bring TDP-43 back into the nucleus, correcting splicing loss of function and preventing the formation of toxic aggregates.

Results

C-mod selectively inhibits TDP-43 condensates



1. Co-localized TDP-43 and G3BP1 cytoplasmic condensates
2. Selective de-partitioning of TDP-43 from G3BP1+ stress granules

Figure 2. A) Co-treatment of stress and TDP-43 c-mod (1 μM) in ALS patient-derived iPSC-motor neurons. White arrows show stress granule and TDP-43 overlap. B) C-mod dose-response curve selectively inhibits TDP-43 condensates whilst leaving G3BP1+ stress granules intact.

Results

C-mods prevent dysregulation of alternative splicing & expression of TDP-43-dependent genes

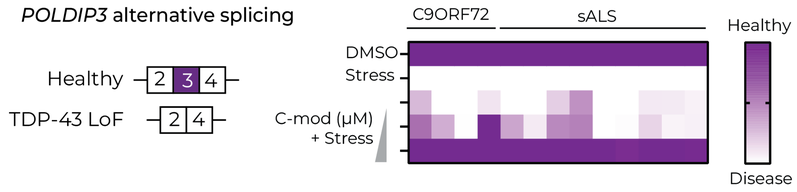


Figure 3. RT-qPCR of stressed ALS iPSC-MNs following c-mod treatment shows a prevention of changes in alternative splicing of *POLDIP3* in 9 sporadic ALS lines and 4 *C9ORF72* repeat expansion ALS lines.

C-mods restore ALS iPSC-MN TDP-43-dependent transcriptional profile and correct cryptic exon expression

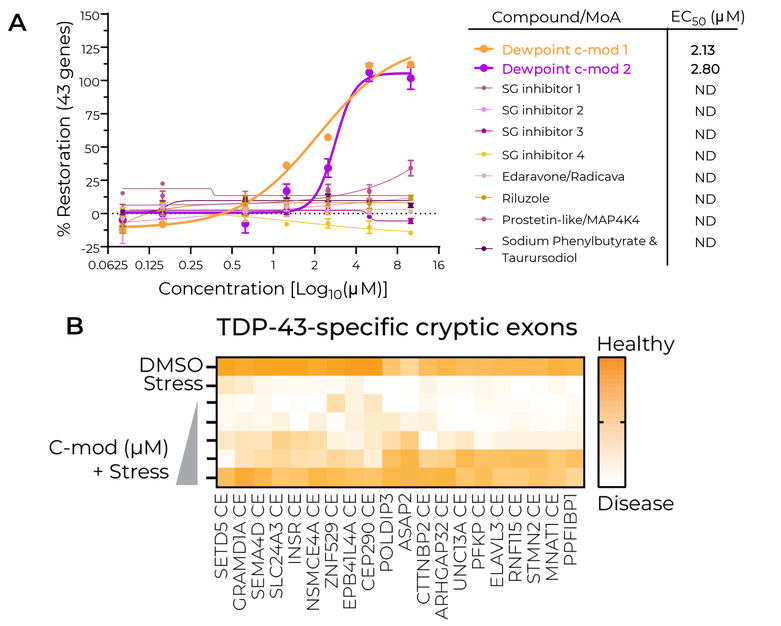


Figure 4. A) Rescue of stress-induced TDP-43-dependent gene expression in ALS iPSC-MNs treated with c-mod. Stress granule inhibitors as well as ALS standard-of-care molecules were also tested. B) C-mod treatment also corrects the stress-induced expression of 20 TDP-43-dependent cryptic exons in a concentration-dependent manner.

C-mods extend survival of stressed motor neurons

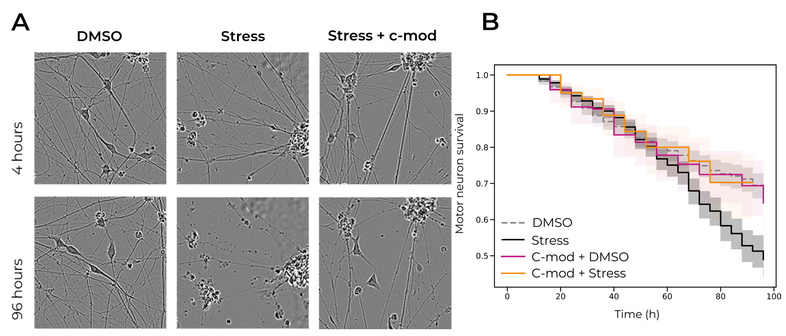


Figure 5. A) Stress-induced neurite retraction and cell death over the course of 96 hours in ALS iPSC-MNs. B) Single-cell Kaplan-Meyer plot depicting the extended survival of stressed motor neurons treated with c-mod.

C-mods reduce CSF NfL and phospho-TDP-43 inclusions in the delta-NLS hTDP-43 mouse model

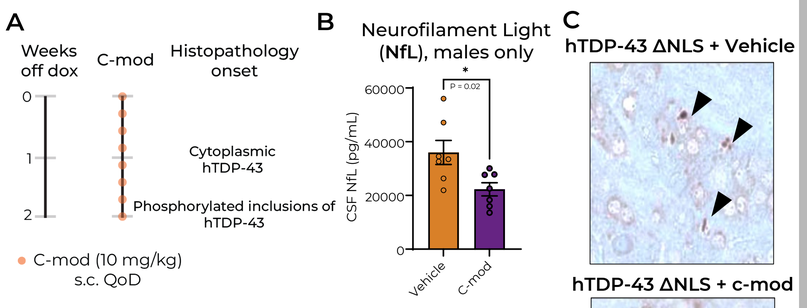


Figure 6. A) Dosing schedule for hTDP-43 ΔNLS mice treated with c-mod. Mice were injected subcutaneously with c-mod every other day for two weeks, coincident with the removal of doxycycline from their diet. B) Male hTDP-43 ΔNLS mice exhibited reduced NfL in their CSF following c-mod treatment (un-paired T-test). C) hippocampal phospho-TDP-43 IHC suggests reduced phospho-TDP-43 inclusions (black arrows) in c-mod treated mice.

Results

C-mods reduce cytoplasmic TDP-43 inclusions in a mouse model of traumatic brain injury (TBI)

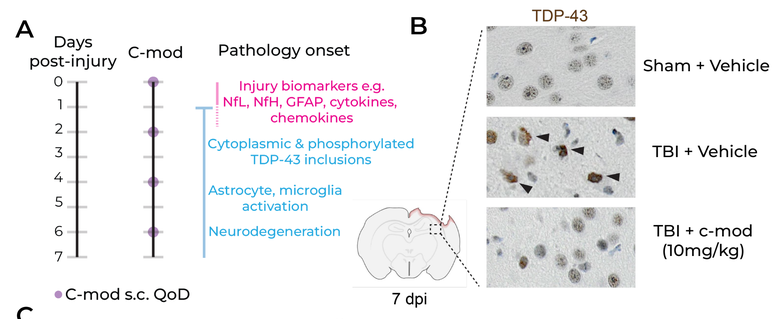


Figure 7. A) Dosing schedule for TBI mice treated with c-mod. Mice were injected subcutaneously with c-mod every other day for 7 days. The first injection of c-mod occurred just prior to injury on Day 0. B) TDP-43 IHC adjacent to the site of injury in TBI mice. Cytosolic TDP-43 is evident in TBI mice receiving vehicle. C) Quantification of TDP-43 cytoplasmic intensity in TBI mice. C-mod treatment reduces cytoplasmic TDP-43 intensity to that seen in the uninjured hemisphere. (One-way ANOVA, Dunnett's multiple comparisons test)

C-mods rescue neurodegeneration and neuroinflammation biomarkers in CSF and plasma of TBI mice

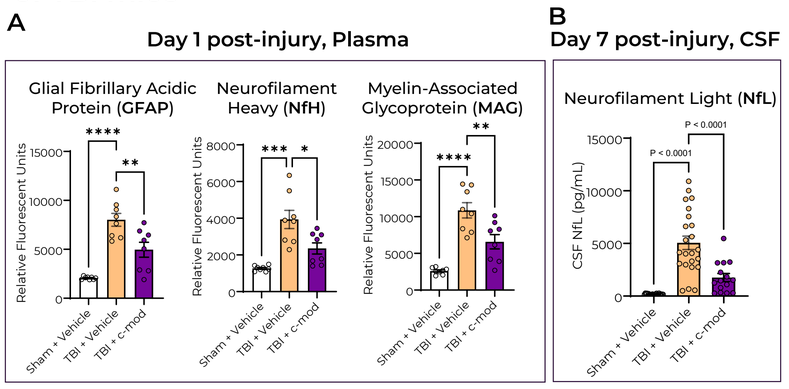


Figure 8. A) One day post-injury, TBI mice treated with c-mod had significantly reduced levels of GFAP, NfH, and MAG in their plasma compared to vehicle (One-way ANOVA, Šídák's multiple comparisons test). B) Seven days post-injury, TBI mice treated with c-mod also displayed significantly reduced NfL in CSF (One-way ANOVA, Dunnett's multiple comparisons test).

Conclusions

Our data demonstrates that modulation of TDP-43 condensates using c-mods effectively rescues TDP-43 LoF in ALS patient-derived motor neurons and reduces TDP-43 inclusions in mouse models of TDP-43 proteinopathy. By selectively de-partitioning TDP-43 from stress granules, c-mods restore proper RNA processing, reduce cytoplasmic TDP-43 inclusions, and mitigate neurodegeneration and neuroinflammation biomarkers. These findings highlight the therapeutic potential of targeting TDP-43 condensates to address the underlying pathogenesis of ALS. Importantly, we generated these results utilizing a wide range of patient lines, which holds promise for the development of c-mods that are effective for the majority of patients with ALS.

References

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