The Integrated Stress Response is modulated by eIF2B agonist DNL343: Results from Preclinical, Phase 1 Healthy Participant, and Phase 1b ALS Patient Studies

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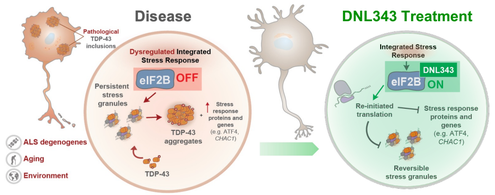
DNL343 is an investigational drug and has not been approved by any Health Authority

BACKGROUND

In ALS, TDP-43 pathology is linked to cellular dyshomeostasis resulting from chronic activation of the Integrated Stress Response (ISR) via inactivation of the eukaryotic initiation factor 2b (eIF2B)

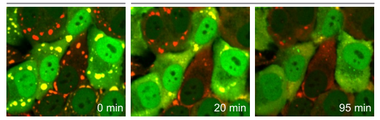
eIF2B activation has potential to slow neurodegeneration in ALS



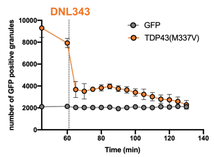
DNL343 is a novel therapeutic designed to inhibit the ISR and restore cells to a healthy state

Acronyms: TDP-43: transactive response DNA binding protein 43 kDa, ATF4: Activating Transcription Factor 4, CHAC1: ChaC Glutathione Specific Gamma-Glutamylcyclotransferase 1.

DNL343 EFFECTS IN NEURONS



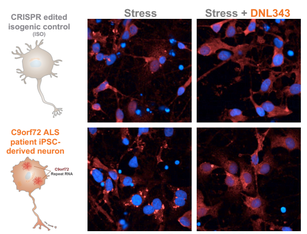
G3BP1 (Stress Granules) GFP-TDP-43$^{NLS, M337V}$
Co-localization of TDP-43 in Stress Granules

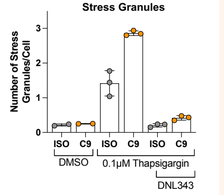


During cell stress TDP-43 localizes to stress granules in neuroglioma H4 cells and **DNL343** dissolves these structures

Acronyms: NLS, nuclear localization sequence
G3BP1, Ras GTPase-activating protein-binding protein 1

DNL343 EFFECTS IN iPSC-DERIVED NEURONS





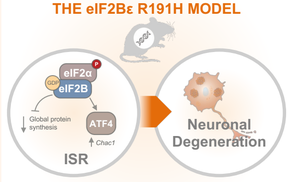
C9orf72 patient-derived neurons have increased stress granules. **DNL343** prevents this effect.

IN VITRO STUDY DESIGN

- GFP-TDP43$^{NLS, M377V}$/mCherry-G3BP1-expressing H4 cells were treated with sodium arsenite for 1h followed by addition of 1μM DNLS or DMSO. Cells were imaged every 5 mins and the number of GFP$^+$ puncta were quantified
- Forebrain neurons were differentiated from C9orf72-repeat containing patient iPSCs or isogenic control and matured for 2 weeks. Cells were pretreated with either DMSO or 1 μM DNL343 for 30 min followed by 2h thapsigargin treatment. Cells were then fixed and stained for G3BP1 and stress granules were quantified.

IN VIVO PHARMACOLOGY, PHARMACODYNAMICS AND MOTOR FUNCTION

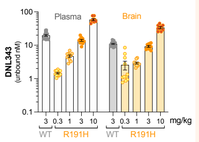
THE eIF2Bε R191H MODEL



The EIF2Bε R191H mouse models the consequences of ISR activation and is an ideal model to test DNL343 mechanism of action

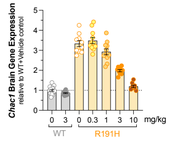
Acronyms: eIF2α, eIF2B, eukaryotic translation initiation factor 2α and 2B respectively; ISR, integrated stress response

DNL343 EXPOSURE



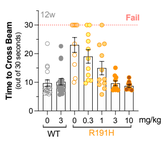
DNL343 achieved comparable exposure in the brain & plasma

ISR PATHWAY



Dose dependent modulation of brain ISR gene marker

MOTOR FUNCTION



Dose dependent restoration of motor function

DNL343 is BBB penetrant and achieved CNS pathway modulation in association with functional correction in an in vivo model

IN VIVO STUDY DESIGN

Wild-type and R191H mice self-administered chow-formulated DNL343 (*ad libitum*) for 13 weeks at doses ranging from 0.3 to 10 mg/kg daily, which led to a dose-dependent increase in exposures in the plasma and brain. Pathway modulation and functional effects were evaluated at the end of the dosing period. Data are presented as mean +/- SEM

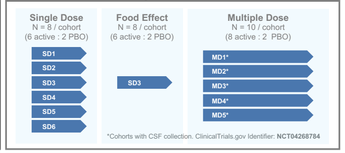
PHASE 1 STUDY IN HEALTHY PARTICIPANTS

PHASE 1 STUDY DESIGN

Study Overview

Type	Phase 1 study in healthy participants
Population	95 healthy participants (18-50 years, SAD=48; MAD=47)
Key Endpoints	**Safety:** • AEs • Safety labs • ECGs • Vital signs • C-SSRS (MAD) **PK (± food):** • Plasma • CSF **Pathway engagement in PBMCs:** • ATF4 protein, *CHAC1* gene expression **Other exploratory biomarkers of ISR**

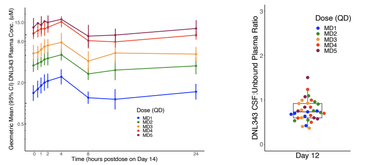
Study Schematic



*Cohorts with CSF collection. ClinicalTrials.gov Identifier: **NCT04268784**

PHASE 1 STUDY DESIGN Single-center, randomized, placebo-controlled, double-blind study of DNL343 in healthy volunteers (**NCT04268784**) with single ascending dose (SAD) and once daily, 14-day multiple ascending dose (MAD) cohorts. DNL343 administered orally.

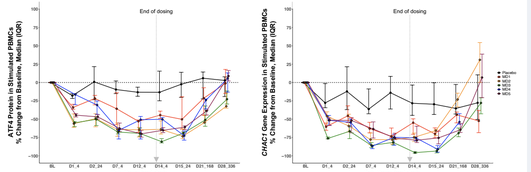
PHARMACOKINETICS



- DNL343 plasma concentration increased in a dose-dependent manner
- Mean plasma half-life of ~40-50 hours
- Mean CSF-to-unbound plasma concentration ratio ranging from 0.66 to 0.92 across the dose range

Dose-related increases in plasma exposure with extensive distribution to the CSF in healthy participants

PHARMACODYNAMICS



DNL343 achieved robust inhibition of ATF4 protein levels and *CHAC1* gene expression at all multiple doses studied in healthy participants

SAFETY PROFILE IN HEALTHY PARTICIPANTS

	PBO n=13	Total DNL343 n=34	MD1 n=6	MD2 n=7	MD3 n=7	MD4 n=7	MD5 n=7
≥1 TEAE, n (%)	12 (92.3)	31 (91.2)	6 (100)	7 (100)	7 (100)	5 (71.4)	6 (85.7)
≥1 TEAE reported in ≥2 participants receiving PBO or DNL343, n (%)							
Headache	7 (53.8)	19 (55.9)	4 (66.7)	3 (42.9)	5 (71.4)	2 (28.6)	5 (71.4)
Procedural pain	5 (38.5)	12 (35.3)	4 (66.7)	1 (14.3)	4 (57.1)	1 (14.3)	2 (28.6)
Fatigue	2 (15.4)	9 (26.5)	–	–	5 (71.4)	2 (28.6)	2 (28.6)
Dizziness postural	2 (15.4)	6 (17.6)	1 (16.7)	1 (14.3)	–	1 (14.3)	3 (42.9)
Dizziness	–	3 (8.8)	–	1 (14.3)	1 (14.3)	1 (14.3)	–
Myalgia	–	3 (8.8)	1 (16.7)	1 (14.3)	–	–	1 (14.3)
Muscle spasms	–	2 (5.9)	–	–	–	1 (14.3)	1 (14.3)
Post lumbar puncture syndrome	3 (23.1)	1 (2.9)	1 (16.7)	–	–	–	–
Nausea	2 (15.4)	1 (2.9)	–	–	1 (14.3)	–	–
Blood CPK increased	2 (15.4)	1 (2.9)	–	–	–	1 (14.3)	–
Dermatitis contact	2 (15.4)	–	–	–	–	–	–

CPK, creatine phosphokinase; PBO, placebo; TEAE, treatment-emergent adverse event.

DNL343 Safety Profile in Healthy Volunteers supported Further development in a Phase 1b Study

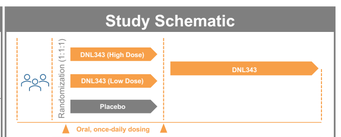
PHASE1b STUDY IN PARTICIPANTS WITH ALS

PHASE 1B STUDY DESIGN

Study Overview

Type	Phase 1b study in participants with ALS
Population	30 ALS participants (18-80 years) Interim Analysis of 20 participants Key criteria: • Disease onset < 4 years • SVC > 50%
Key Endpoints	**Safety:** • TEAE incident • Safety Labs • ECGs • Vital signs **PK:** • Plasma • CSF **Pathway engagement in PBMCs:** • ATF4 protein, *CHAC1* gene expression **Exploratory** • Additional ISR Biomarkers

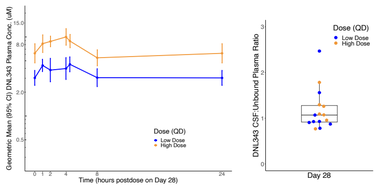
Study Schematic



ClinicalTrials.gov ID: **NCT05006352**

Phase1b Study Design A 28-day, multi-center, randomized, placebo controlled, double-blind study (DB) followed by an 18-month open label extension (OLE) in participants with ALS (NCT05006352). We report **Topline PK, PD and safety results** after 28 participants completed the double-blind period of the study (enrolled Aug-2021 to Nov-2022). DNL343 is administered orally or via gastrostomy tube once daily. The clinical cutoff date for this analysis is 21-Dec-2022. **Investigators** who contributed patients to this study include: Leonard van den Berg, MD, PhD (University Medical Center, Utrecht, NL), Geert Jan Groeneveld, MD (Center for Human Drug Research, Leiden, NL); Jonathan Katz, MD (California Pacific M.C., San Francisco, CA), John Ravits, MD (University of California, San Diego, CA), Christina Fournier, MD (Emory University, Atlanta, GA), Peter Creigh, MD (University of Rochester, Rochester), NY; Kevin Felice, MD (Hospital for Special Care, New Britain, CT)

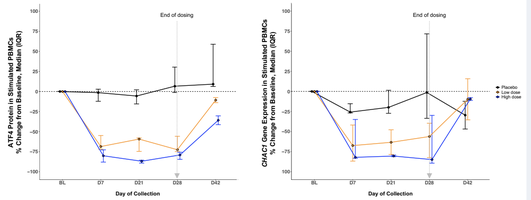
PHARMACOKINETICS



- DNL343 plasma concentration increased in a dose-dependent manner
- Low variability and low peak-to-trough fluctuation at steady state
- Distribution to CNS, with mean CSF-to-unbound plasma concentration ratios ~1.2 DNL343 demonstrated similar overall exposure in participants with ALS as in the healthy participants in the Phase 1 Study

DNL343 PK demonstrates extensive CSF distribution and supports once-daily oral administration

PHARMACODYNAMICS



- Fresh PBMCs were collected and stimulated ex vivo for each time point indicated for a subset of patients (per dose group: n=5-7 through day 28 and 2-3 for day 42)
- Experiments using cryopreserved PBMCs were also performed and showed similar results

DNL343 achieved robust inhibition of ATF4 protein levels and *CHAC1* gene expression at both doses studied in ALS participants

SAFETY PROFILE IN ALS PARTICIPANTS

Safety Population	Placebo (n = 9)	DNL343 Total (n = 19)	Low Dose (n = 10)	High Dose (n = 9)
>=1 TEAE	8 (88.9)	14 (73.7)	7 (70.0)	7 (77.8)
>=1 TEAE reported in ≥2 DNL343 participants, n (%)				
Headache	2 (22.2)	7 (36.8)	3 (30.0)	4 (44.4)
Fatigue	2 (22.2)	6 (31.6)	4 (40.0)	2 (22.2)
Post Lumbar Puncture Syndrome	1 (11.1)	3 (15.8)	1 (10.0)	2 (22.2)
Hypogeusia	0	2 (10.5)	1 (10.0)	1 (11.1)
Presyncope	2 (22.2)	2 (10.5)	1 (10.0)	1 (11.1)
Nausea	1 (11.1)	2 (10.5)	0	2 (22.2)

Ph1b Double Blind Period Safety

- Twenty-nine participants were randomized of which 28 received treatment with placebo or DNL343
- No SAEs, deaths, or AESIs
- All TEAEs were Grade 1 or 2
- One discontinuation due to rash (Grade 2) in high dose group, considered related
- Non-procedure related TEAEs that occurred in two or more DNL343 participants, and were more common with DNL343 vs. placebo were headache (all Grade 1 in the DNL343 groups), fatigue (all Grade 1), and hypogeusia (all Grade 1)
- No dose dependent or clinically meaningful trends in safety labs, ECG, vital signs or findings in the neurological exam or C-SSRS

CONCLUSION

Preclinical Experimental Models

- **DNL343 suppresses** increased **stress granules** in thapsigargin treated **C9orf72** patient-derived **iNeurons**
- **DNL343** was **BBB penetrant,** achieved **CNS pathway modulation** and **preserved motor function** in an **EIF2B** mutant mouse model

Phase 1 Summary and Phase1b Primary Analysis

- A **Phase 1** study (n=95) in healthy participants completed in Aug-2021
- A **Phase 1b** study (n=29) in participants with ALS completed the 28-day DB period in Dec-2022
- **DNL343** was **generally safe and well-tolerated** in **healthy Phase 1** and **ALS** participants during the **Phase 1b DB** period
- **Phase 1** and **Phase 1b (DB)** PK demonstrated **low variability,** predictable **dose-related increases** in exposure, and a half-life supporting **once-daily dosing**
- **Clinical and non-clinical PK data demonstrate extensive CNS distribution of DNL343**
- **Phase 1** and **Phase 1b (DB)** pharmacodynamics demonstrated **inhibition of two ISR biomarkers** (ATF4, *CHAC1*) in ex-vivo stimulated PBMCs with robust response at all doses
- No dose dependent or clinically meaningful trends in safety labs, ECG, vitals signs or findings in the neurological exam or C-SSRS during the DB period

In conclusion, DNL343 was generally safe and well-tolerated in healthy participants and participants with ALS supporting continued study of DNL343 as a once-daily investigational drug. An OLE for Phase 1b participants is ongoing, with median OLE exposure duration of 4 months and maximum duration of 12 months as of Dec-2022.