51. Tonabersat as a Neuroprotective Compound

(Johns Hopkins University)

Asset Overview

Product Type	Small Molecule
Disease Area	Musculoskeletal Disease
Indication	Amyotrophic Lateral Sclerosis
Current Stage	Lead Optimization
Target	Cx43 (One component of the gap junctions)
MoA	Tonabersat specifically targets and inhibits Cx43
Brief Description	• Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons, and results in muscle atrophy and loss of motor control. • Tonabersat (SB-220453) is a novel cis benzopyran derivative molecule that specifically targets and inhibits Cx43. Compared to the non-specific gap junction inhibitors, tonabersat demonstrates better target specificity, thus minimizing the potentially undesirable effects from broad gap junction inhibition. Additionally, unlike large peptide blockers that have difficulty crossing the blood-brain barrier, tonabersat's small size enables it to easily penetrate the blood-brain barrier to target astroglial cells in the central nervous system. • Also, as an orally available compound, tonabersat has an excellent safety profile and is already shown to be well-tolerated in studies for patients with migraine. In vitro studies have demonstrated that tonabersat can protect motor neurons from astrocyte-induced toxicity, which may slow the process of motor neuron degeneration during ALS's disease progression.
Intellectual Property	US20200352902A1
Publication	Cx43 hemichannels contribute to astrocyte-mediated toxicity in sporadic and familial ALS. PNAS, (2022)
Inventors	Nicholas J. Maragakis

Highlights

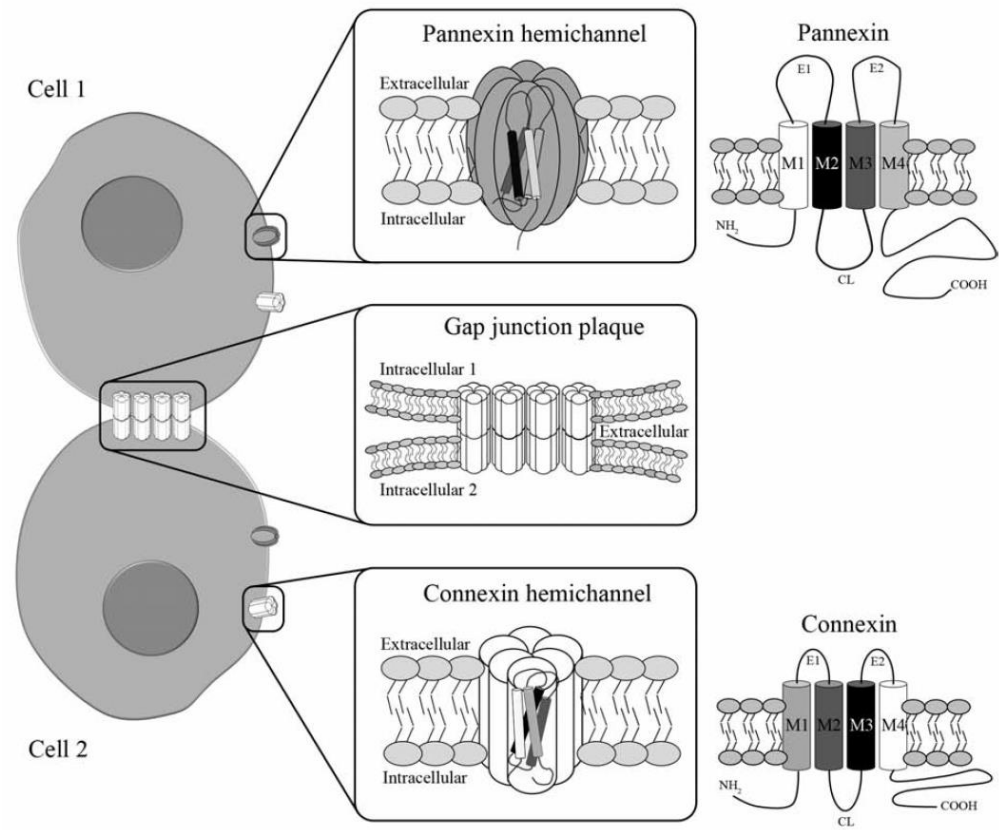
- Astrocyte-specific deletion of Cx43 improves survival and slows caudal rostral progression in SOD1G93A mice.
- Human ALS iPSC-A have increased Cx43 HC levels associated with increased cell permeability, which can be blocked by Gap19.
- Human iPSC-MN toxicity is mediated by Cx43 HC from FALS as well as SALS hiPSC-A.
- The Cx43 HC blocker tonabersat provides dose-dependent neuroprotection to hiPSC-MN.
- MEA recordings from hiPSC-Astro/MN cultures demonstrate that tonabersat Cx43 HC-mediated effects on hiPSC-MN electrophysiology correlates with neuroprotection.

51. Tonabersat as a Neuroprotective Compound

(Johns Hopkins University)

Key Data

The role of connexin in astrocytes



Scheme showing the membrane topology of pannexin, connexin, hemichannels, and gap junction channels. *Top and bottom right* correspond to pannexin and connexin in a plasma membrane, respectively. Both protein types have four transmembrane domains (M1–4) with amino (¶NH2) and carboxy (¶COOH) termini on the cytoplasmic side, two extracellular loops (E1 and E2), and one cytoplasmic loop (CL). *Top and bottom center* show hemichannels formed of six pannexin or connexin subunits each. The *middle center* shows an aggregate of connexin gap junction channels, a section through a gap junction "plaque", at a close contact between cells 1 and 2, as shown in the *left*. Each gap junction channel is formed by two hemichannels docked together (and rotated 30° with respect to one another). Each cell contributes one of hemichannels.

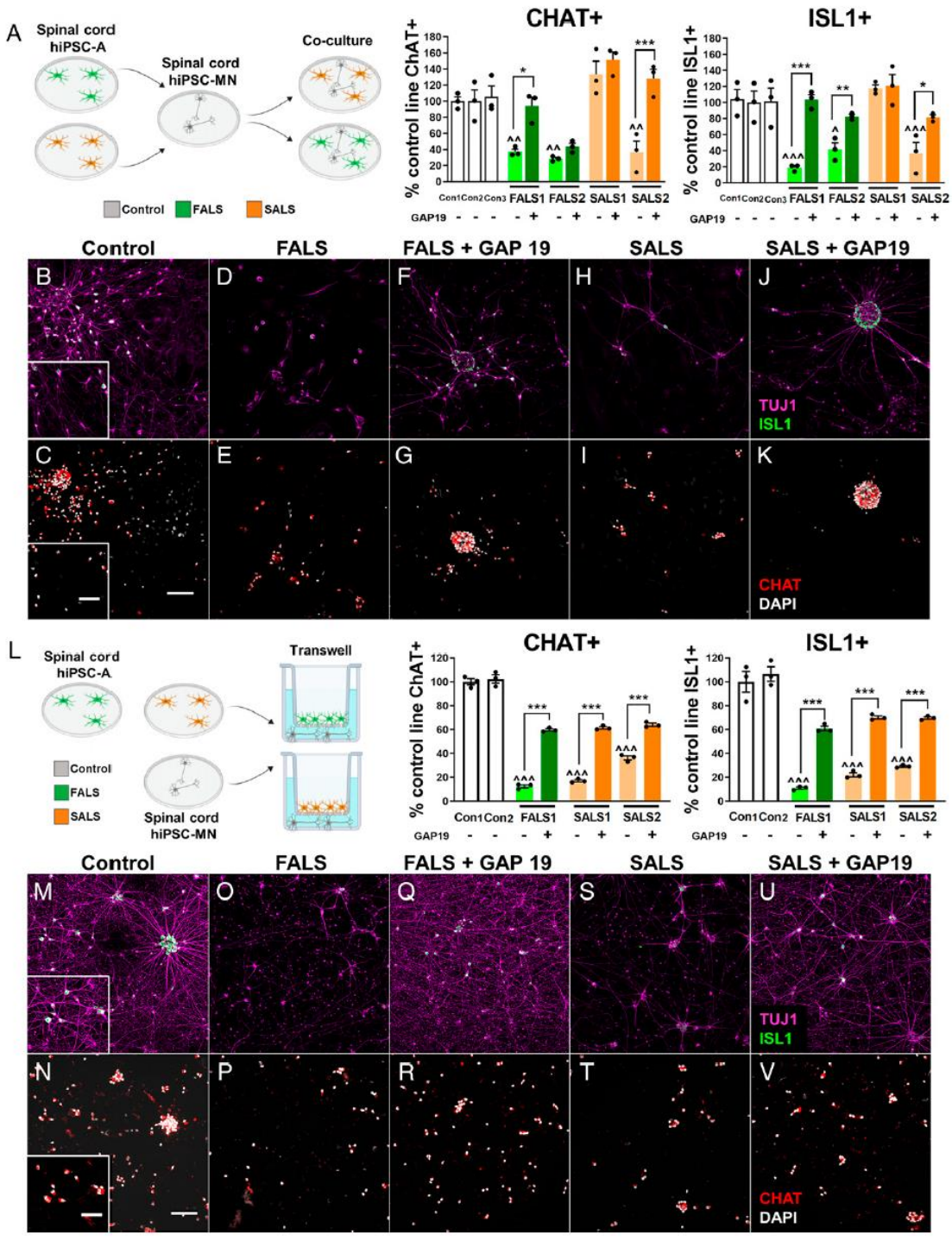
Source: Orellana et al., 2009

51. Tonabersat as a Neuroprotective Compound

(Johns Hopkins University)

Key Data

Human iPSC-MN toxicity is mediated by Cx43 HC from FALS as well as SALS hiPSC-A



To be continued

51. Tonabersat as a Neuroprotective Compound

(Johns Hopkins University)

Key Data

Human iPSC-MN toxicity is mediated by Cx43 HC from FALS as well as SALS hiPSC-A

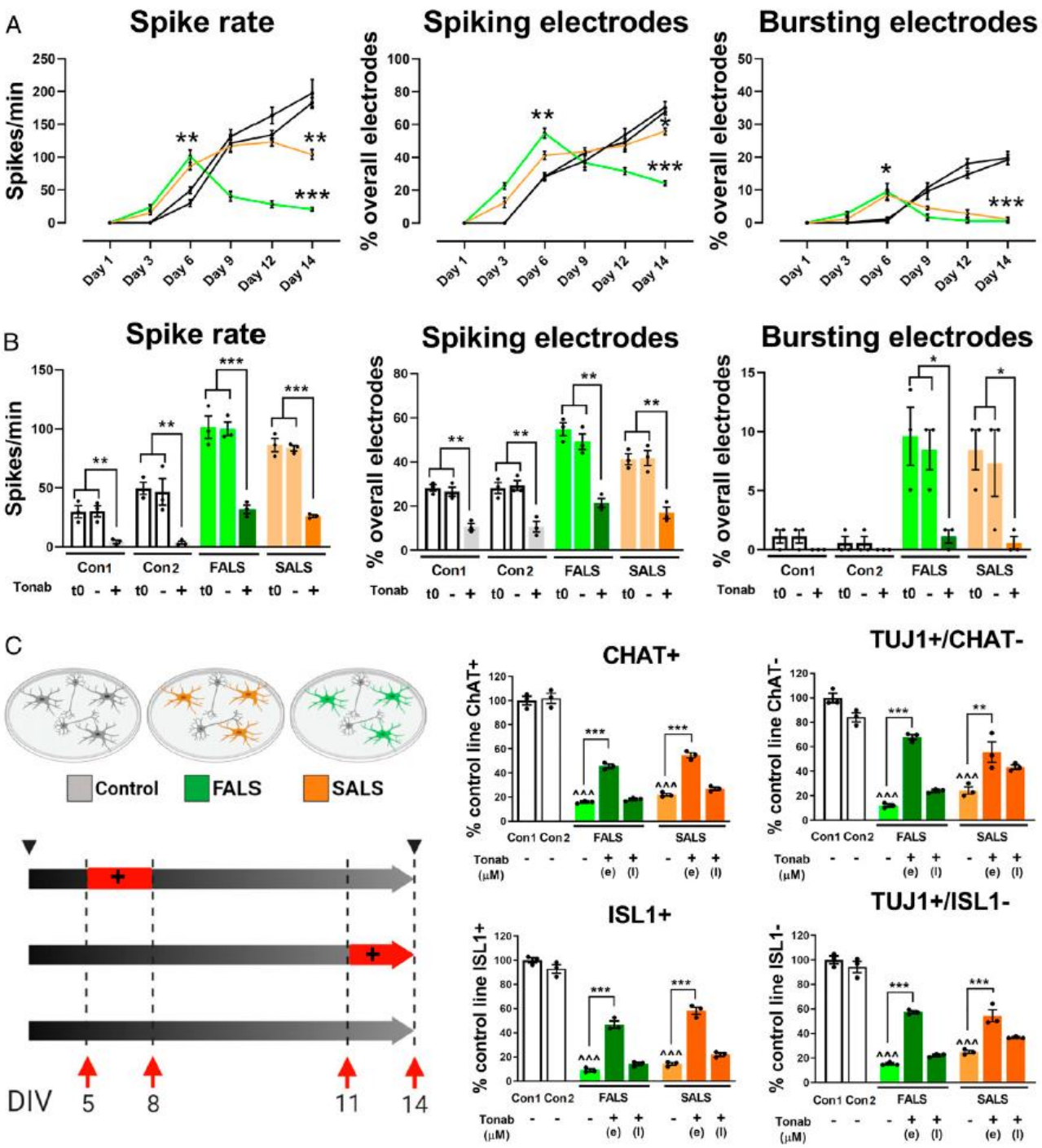
(A–K) MNs derived from control hiPSC were plated with hiPSC-A from either control, SALS, or FALS (SOD1A4V) patients. The number of hiPSC-MN (ChAT+ and Isl-1+) plated on FALS and SALS hiPSC-A decreased over a period of 14 d compared to MN plated with control hiPSC-A. Cocultures containing ALS hiPSC-A treated with Gap19 show an increase in hiPSC-MN survival. (L–V) The coculture of hiPSC-A/-MN in a transwell system allowed neurons and astrocytes to share the same medium, preventing direct cell contact. FALS (SOD1A4V) and SALS hiPSC-A shows ChAT+ and Isl-1+ hiPSC-MN toxicity rescued by Gap19. Con1 = CIPS, Con2 = JH082, Con3 = GM01582, FALS1 = GO013, FALS2 = GO002, SALS1 = JH040, SALS2 = JH058 (n = 3 coverslips per condition). Significant comparisons (one-way ANOVA) between untreated control and ALS cocultures are marked with "^"; significant effects of Gap19 on cocultures containing ALS astrocytes are marked with "*". *P < 0.05 and ^P < 0.05; **P < 0.01 and ^^P < 0.01; ** P < 0.001 and ^^^P < 0.001. (Scale bars, 50 μm main panel; Inset is 20 μm.) Data are represented as mean ± SEM.

51. Tonabersat as a Neuroprotective Compound

(Johns Hopkins University)

Key Data

Tonabersat Cx43 HC-mediated effects on hiPSC-MN electrophysiology correlates with neuroprotection



To be continued

51. Tonabersat as a Neuroprotective Compound

(Johns Hopkins University)

Key Data

Tonabersat Cx43 HC-mediated effects on hiPSC-MN electrophysiology correlates with neuroprotection

(A) MEA recording of neuronal activity from cocultures between control hiPSC-MN and control or ALS hiPSC-A. The presence of SALS and FALS astrocytes when compared to control astrocytes determines early increases in spiking and bursting activity followed, at later time points, by reduced electrophysiological activity. Significant time-point comparisons (i.e., day 6 and 14 of coculture) between control and ALS conditions are marked with an asterisk (*) (black line = control, orange line = SALS, green line = FALS). (B) MEA activity within 5 min after the application of 10 μM tonabersat (+) on cocultures with control or ALS hiPSC-A compared to baseline (t0) and vehicle (). MEA baseline activity at day 6 shown in A. Tonabersat shows significant inhibition (*) of neuronal spiking and bursting activity. (C) The effects of 10 μM tonabersat (+) on hiPSC-neuron survival in control and ALS cocultures was tested as outlined in the figure, but for a shorter time course of 3 d, either early (DIV 5 to 8, or "e") or late (DIV 11 to 14 or "l") during coculture. Significant neuroprotection was appreciated after early but not late exposure to tonabersat; evident for both MN (ChAT+ and ISL1+ cells) and non-MN cell types (TUJ1+/ChAT, TUJ1+/ISL1). Con1 = CIPS, Con2 = GM01582, FALS = GO013, SALS = JH058. Significant comparisons (one-way ANOVA) between untreated control and ALS cocultures are marked with (^), while significant effects of tonabersat on cocultures containing ALS astrocytes are marked with (*). *P < 0.05 and ^P < 0.05; **P < 0.01 and ^^P < 0.01; ***P < 0.001 and ^^^P < 0.001, n = 3 per condition. Data are represented as mean ± SEM.