Onset of CIPN Delayed Through SARM1 Inhibition in Human NerveSim Preclinical Drug-Discovery Platform

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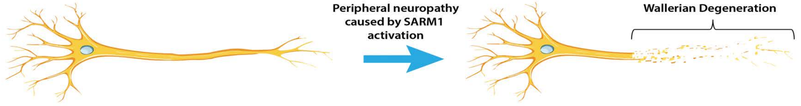
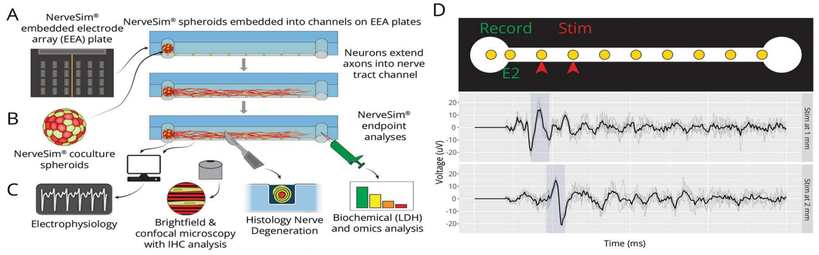
Overview

- AxoSim has developed a novel microphysiological human Nerve-on-a-Chip system, or NerveSim®, comprised of an iPSC-derived sensory neuron and primary human Schwann cell 3D coculture system to model peripheral nerves *in vitro*.
- The system uses a 24-well Embedded Electrode Array (EEA) culture plate to provide high-throughput electrophysiological characterization to perform drug screening for neurotoxicity and neuroprotection applications on a clinically relevant nerve model.
- In this work, we screened multiple SARM1 inhibitors co-administered with the chemotherapeutic vincristine to quantify the neuroprotection by correlating changes in compound action potentials (CAPs) and axonal morphology.

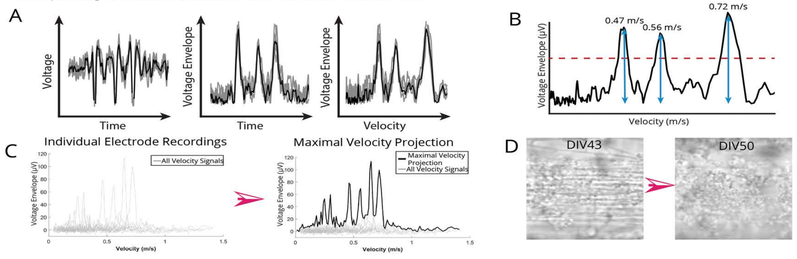


Chemotherapeutics commonly cause off-target damage to peripheral nerves causing chemotherapy-induced peripheral neuropathy (CIPN). CIPN is typically characterized by a Wallerian degeneration-like morphology where distal axonal segments begin to degenerate. SARM1, a protein expressed in peripheral neurons, has been identified as the central executioner for Wallerian degeneration and a potential target for preventing or limiting CIPN.

Methods



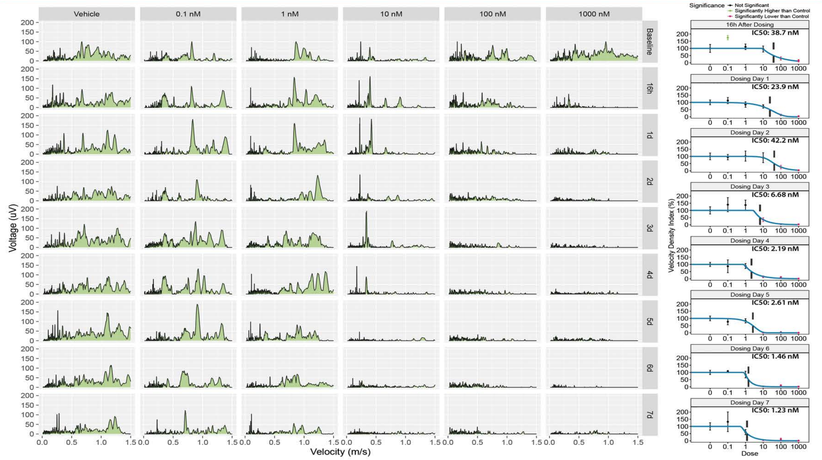
NerveSim® Overview: Coculture spheroids (B) are formed and placed into NerveSim® EEA plates plates (A). Neurites bundle and grow down the channel creating a three dimensional nerve model. Once developed, cultures are stimulated for evoked electrophysiology, imaged for degeneration measurement, and media is collected to estimate cytoxicity (C). Raw recordings from a human iPSC NerveSim® stimulated at electrodes 3 and 4 with 48 uA. The evoked responses on electrode 2 show latency shifts corresponding to stimulation distance with consistent velocities (D).



CAP Analysis (VDI): Raw signals were processed (A) and significant peaks were detected (B) for each electrode recording. Recordings from multiple electrodes in a single well were combined (C) into a maximal velocity projection (MVP) to quantify the population-level response to electrical stimulation for each individual NerveSim®. The area under the curve of the MVP was calculated to create a velocity density index (VDI).

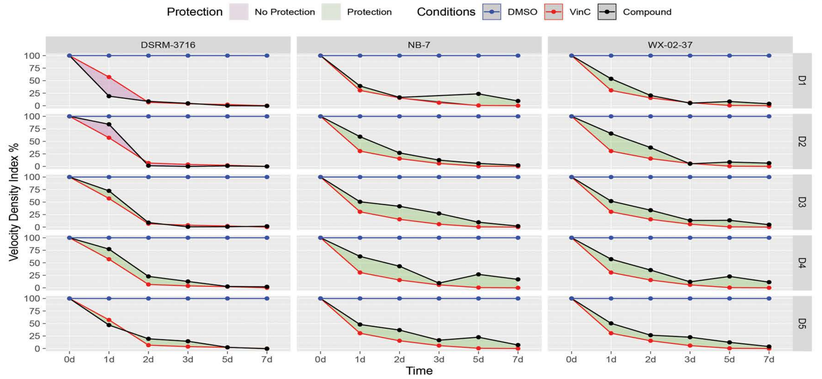
Bright Field Imaging for Degeneration: Live image of NerveSim® at 43 days in culture prior to dosing (D). Image is one slice of a z-stack showing aligned neurite growth. After 7 days of dosing with 100 nM Vincristine, the bundles are no longer visible and significant fragment of neurites is present . Quantification of degeneration is done by comparing changes to the same slice, well, and plate combination over time using a mask to highlight edges of processes.

Vincristine-Induced CIPN Model



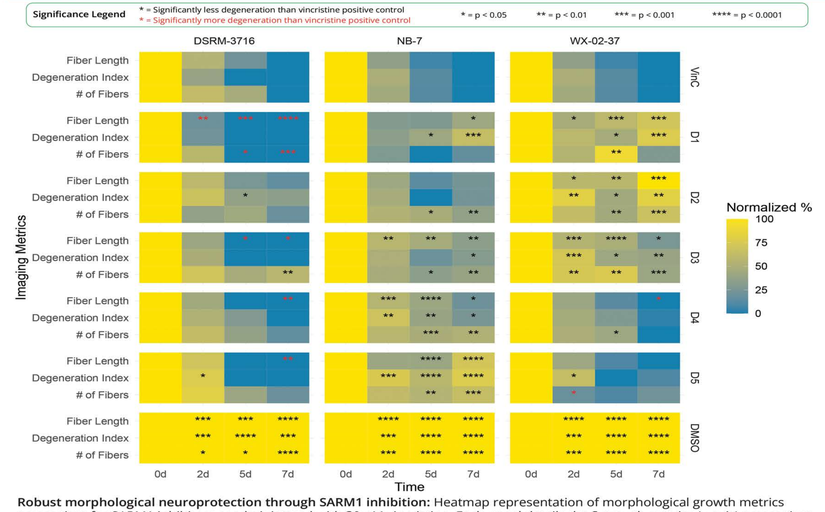
Vincristine-induced peripheral neuropathy (VIPN) Model: The small molecule chemotherapeutic vincristine was applied at 5 doses over the course of 1 week of dosing. Neurotoxicity was evaluated daily using functional electrophysiological recordings. Maximum velocity projections (left) showed substantial loss of function caused by high doses, which were confirmed and quantified using IC50 sigmoidal curve fitting (right). The IC50 curves showed a gradual shifting of IC50 from ~30 nM to ~1 nM over the course of 1 week of dosing, with significant deficits observed within 16 hours of initial dosing at the highest concentrations.

SARM1 Inhibition Electrophysiology



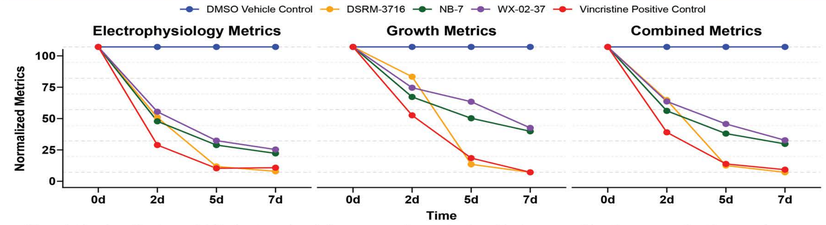
Limited functional neuroprotection through SARM1 inhibition: Different concentrations of the SARM1 inhibitors DSRM-3716, NB-7, and WX-02-37 were co-administered with a constant concentration of 30 nM vincristine, known to cause CIPN. Each panel shows the timecourse of functional electrophysiological responses for the vehicle control (blue; normalized to be 100% over time), positive control (red; 30 nM vincristine alone), and test condition (black; SARM1 inhibitor plus 30 nM vincristine). Each row of panels represents a single dose of SARM1 inhibitors with the highest doses at the bottom (e.g. D5). Neuroprotection is highlighted in green when the test condition lies above the positive control, suggesting preservation of function, while non-protection is highlighted in red. Overall, DSRM-3716 showed little neuroprotection while NB-7 and WX-02-37 showed neuroprotective qualities in the first few days of treatment. By day 7, all functional responses were suppressed completely regardless of dose or compound.

SARM1 Inhibition Growth Metrics



Robust morphological neuroprotection through SARM1 inhibition: Heatmap representation of morphological growth metrics across time for SARM1 inhibitors coadministered with 30 nM vincristine. Each panel details the 3 growth metrics (y-axis) across time (x-axis) normalized to fall between the vehicle control (bright yellow = 100%) and the positive control (dark blue = 0% at 7d dosing). Each row of panels shows a different dosing condition with positive control alone at the top, increasing doses of SARM1 inhibitors (highest dose is D5), and finally the DMSO vehicle control in the bottom row. Significant differences compared to the positive control are indicated by asterisks showing significant neuroprotection (black) or significant toxicity (red). In contrast with the functional neuroprotection data, the growth metrics showed significant neuroprotection for both NB-7 and WX-02-37 for up to 7 days. The highest doses of NB-7 and the middle doses of WX-02-37 showed the best neuroprotection. DSRM-3716 showed little to no morphological neuroprotection.

Conclusions



- Vincristine's effect on CAP electrophysiology was characterized in terms of both concentration and dosing time to develop an in vitro CIPN model for neuroprotection applications
- Three SARM1 inhibitors from literature were investigated via functional electrophysiological and morphological growth metrics:
  - DSRM-3716 showed lowest levels of protection in either electrophysiology or growth metrics
  - NB-7 showed limited dose- and time-dependent functional neuroprotection but significant morphological protection at higher doses
  - WX-02-37 showed limited dose- and time-dependent functional neuroprotection but significant morphological protection increasing with dose before dropping off at highest doses, which aligns well with cytotoxic effects noted in literature
- The NerveSim® model enables both neurotoxicity and neuroprotection assays focused on sensory peripheral nerves