Scalable and Efficient Generation of iPSC-derived Human Sensory Neurons for CIPN and Pain Research

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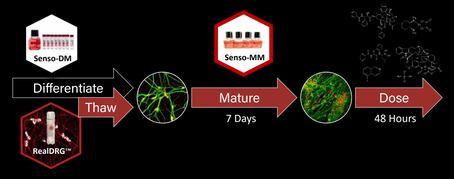
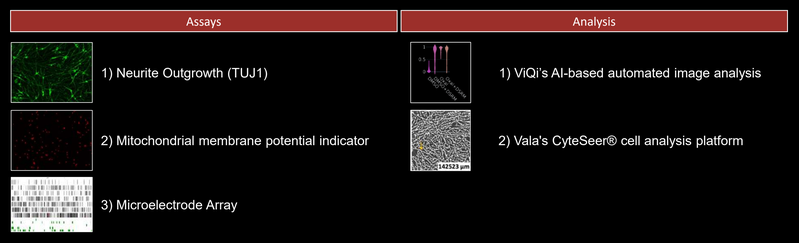
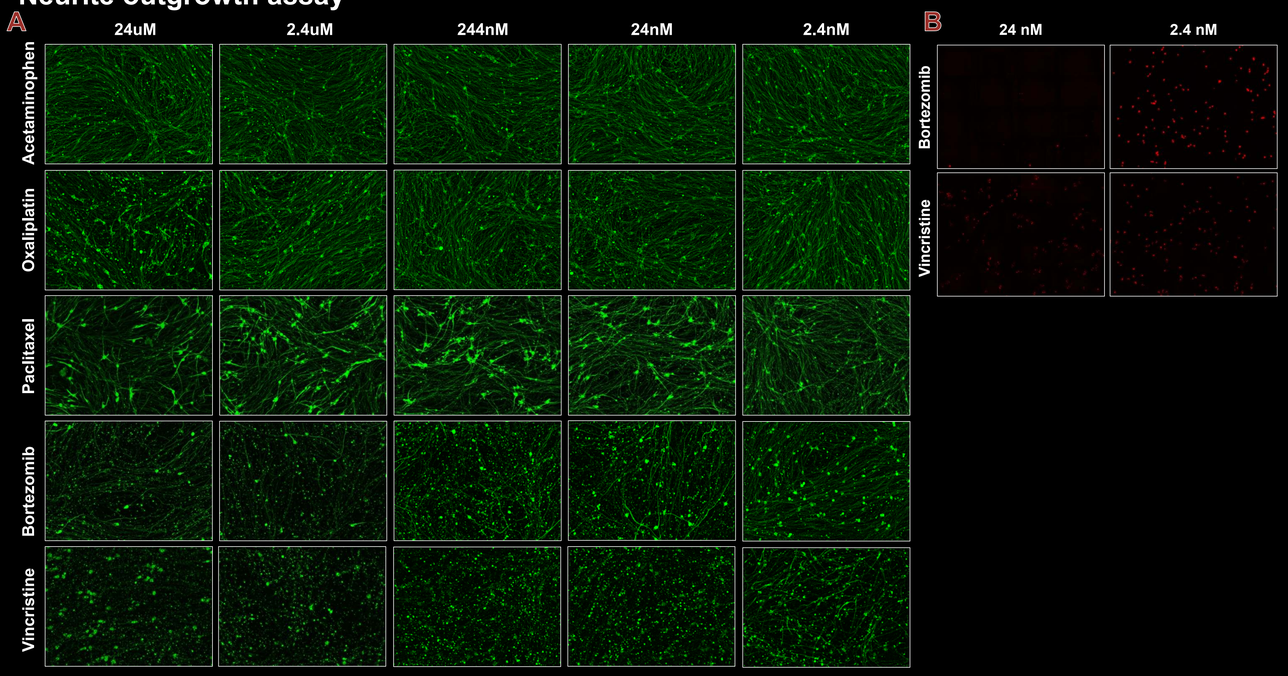
Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of cancer treatment that results in damage to the peripheral nerves. This can significantly impact a patient's quality of life and can even lead to dose reductions or treatment discontinuation. In order to develop treatments and/or preventative measures for CIPN, there is a need for high-throughput, reproducible peripheral nerve disease models that give insight into the interplay between chemotherapeutics, axonal morphology, function, and potential neuroprotective compounds. In a phenotypic study, we treated human induced pluripotent stem cell-derived sensory neurons (hiPSC-SN) that are functionally and molecularly similar to primary DRG with four chemotherapy drugs (bortezomib, oxaliplatin, paclitaxel, and vincristine) in dose response with and without pre-treatment of the SARM1 inhibitor DSRM-3716. After 48 hours of treatment, axonal and mitochondria health was analyzed via beta-III tubulin (TUJ1) and tetramethylrhodamine, methyl ester (TMRM) staining. Paclitaxel altered soma morphology and axonal branching, while oxaliplatin showed morphology changes at the highest dose. Both showed minimal mitochondrial membrane dysfunction. Bortezomib and vincristine greatly degraded axons while also reducing mitochondrial activity. Vincristine-treated mitochondria exhibited a “fragmented” morphology. Using an AI-based high content analysis software, AutoHCS™, images from the study were automatically scored to identify dose-dependent phenotypic responses to the drugs and the neuroprotective effects of DSRM-3716. Here, it is demonstrated that DSRM-3716 could have a protective effect on sensory neurons treated with oxaliplatin. To study how sensory neuron function could be affected by chemotherapeutics, sensory neurons were cultured on microelectrode arrays for 21 days. Vincristine induced hyperactivation after one hour of application. When co-treated with gabapentin after 48 hours, there was a rescue effect where spike train parameters returned to control levels. Together, these findings demonstrate the ability to phenotypically and functionally screen CIPN-related and potential neuroprotective compounds in human nociceptors in high throughput systems.



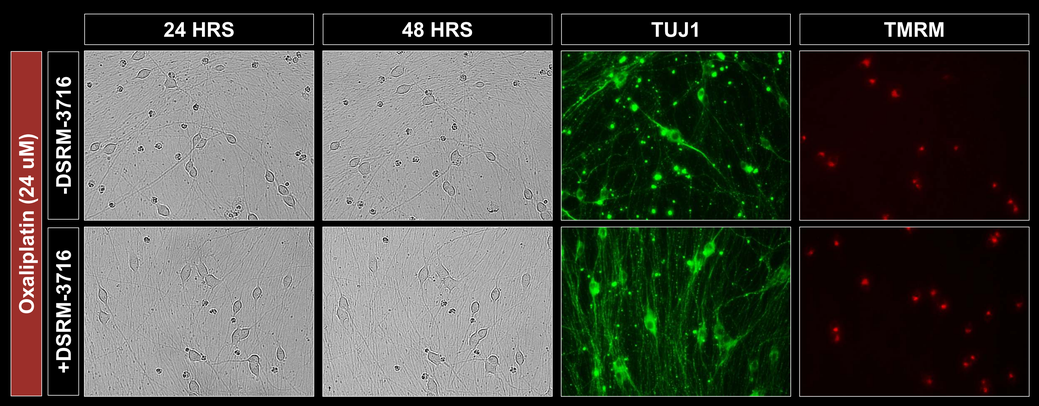


Neurite outgrowth assay



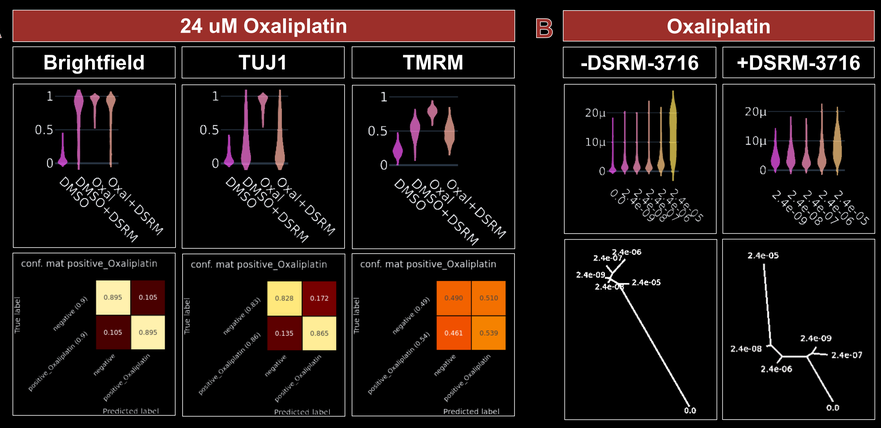
Scalable RealDRG™ hiPSC-derived sensory neurons were produced in seven days using a novel directed differentiation method (Senso-DM™) and banked. **A)** With the goal of generating a human DRG CIPN model, we tested four common CIPN-related compounds (bortezomib, paclitaxel, vincristine, and oxaliplatin) for 48 hours on day 7 cultures. Vehicle (0.1% DMSO) and acetaminophen served as negative controls. Cultures were then stained the TUJ1 for analysis. Acetaminophen did not affect axonal morphology or density. Oxaliplatin at induced noticeable morphological changes at the highest concentration and paclitaxel change soma morphology while decreasing neurite density. Bortezomib and vincristine showed toxicity at concentrations higher than 2.4 nM. **B) Bortezomib and vincristine hinder mitochondrial function.** Mitochondrial dysfunction is a primary driver indicated in CIPN and is related to a dysregulation of pathways involving calcium, reactive oxygen species, leading to consequent cell death and axonal network degradation (Canta et al. 2015). TMRM is a mitochondrial membrane potential stain in which brighter staining equates to a higher potential and therefore better functioning mitochondria. After 48 hours of chemotherapy drug dosage, cells were stained with TMRM and imaged. Even at low doses, both bortezomib and vincristine showed marked reductions in mitochondria function. For bortezomib, doses higher then 2.4 nM had an almost entire depletion of membrane potentials whereas for vincristine, there is a “fragmented” morphology which aligns with previously published observations (Berbusse et al. 2016).

SARM1 inhibitor DSRM-3716 phenotypically mitigates axonal degradation



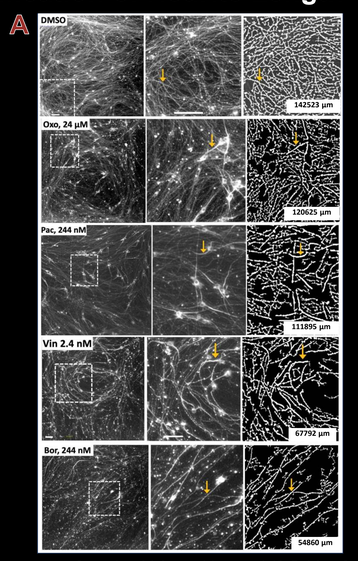
It has been recently demonstrated that a class of small molecule SARM1 NADase inhibitors can protect axonal structure and function, both in vitro and in animal models of CIPN (Bosanac et al. 2021). DSRM-3716 is a potent and selective inhibitor of SARM1 NADase (IC50 value of 75 nM) that recapitulates the SARM1-/- phenotype and protects axons from degeneration induced by axotomy or mitochondrial dysfunction. In a follow-up study, cultures were pre-treated with 75 nM DSRM-3716 before treatment with CIPN-related compounds in dose response for 48 hours. Daily brightfield images were taken and TMRM staining was performed before fixation and TUJ1 immunocytochemistry was performed. Via brightfield and TUJ1 staining images, axonal morphology was spared with DSRM-3716 pre-treatment in oxaliplatin 24 uM conditions while there were no discernable differences in mitochondrial membrane potential.

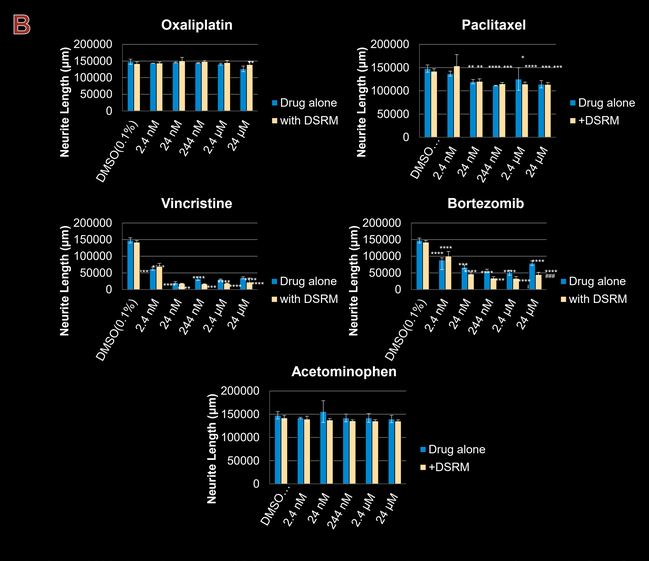
ViQi AI-based automated image analysis



A) A binary AI was trained on two classes: I) 0.1% DMSO (Vehicle control) and DMSO + DSRM-3716 combined and II) the highest concentration of CIPN-related compound. The performance of this AI is represented by the similarity matrix. This similarity matrix shows how well the AI can distinguish between these two classes. We then fed this trained AI four classes of data: I) DMSO, II) DMSO+DSRM-3716, III) Compound, and IV) Compound+DSRM-3716. Represented above is 24 uM oxaliplatin. The predictions this AI makes, represented by the violin plot, demonstrates whether each class looks more similar to the negative control (DMSO & DMSO+DSRM-3716) or the compound without DSRM-3716 treatment. If the violin plot is skewed towards the bottom, then that class looks more like the negative control condition than the compound without treatment condition. The distribution of sample points can be interpreted as the precision of the AI in its classification. Preliminary confirmations from ViQi’s AI-based AutoHCS indicate that overall pre-treatment with DSRM-3716 before oxaliplatin dosing results in phenotypes more closely resembling baseline un-treated, though not completely recovered based on the image data set (brightfield, beta-III tubulin, or mitochondria function). Beta-III tubulin staining showed the most similarity to controls whereas brightfield and TMRM staining were less distinguishable. **B)** Each row is a single AI trained on classifying dosages of oxaliplatin +/- DSRM-3716. Each violin plot shows the true dose for each sample on the x axis and the predicted label for each sample on the y axis. The dendrograms represent how each dose (and DMSO) relate to each other in phenotypic space. With oxaliplatin pre-treated with DSRM-3716, most doses of the compound are closer in phenotypic space to the DMSO condition, implying that the DSRM treatment is, in fact, reducing the severity of the phenotype induced by the compound.

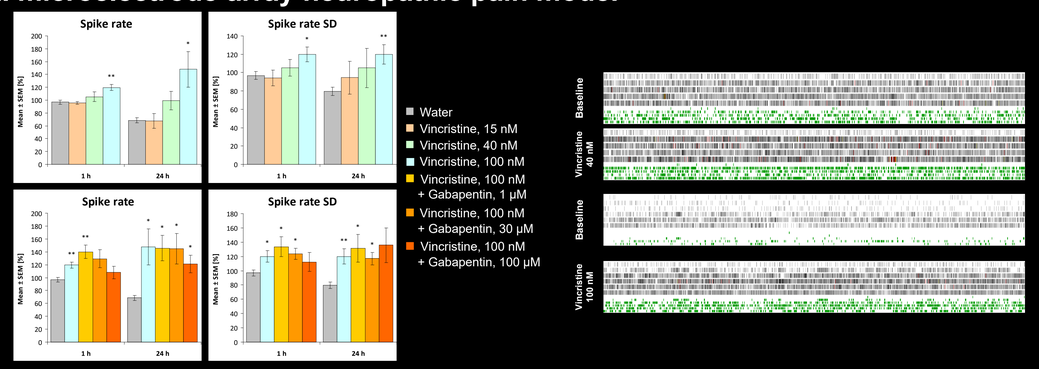
Quantification of Neurite Length





The plates were transferred to Vala and imaged (9 fields of a view) with a 10X objective on an IC200 instrument, and the images analyzed via CyteSeer® for Total Neurite Length (microns per well). **A)** Representative images depicting neurites and neurite mask. **B)** Quantitation of Total Neurite Length (microns per well summed for all fields of view). Each bar is the mean ± SD for n=15 wells for DMSO, DSRM (75 nM, by itself), and n=3 for all other conditions. *p<0.05,**p<0.01,***p<0.001,****p<0.0001 vs. DMSO, Dunnett’s test.

Functional microelectrode array neuropathic pain model



Vincristine was used as a model compound for neuropathic pain in neuronal cell cultures on microelectrode array 48-well plates from Axion Biosystems. Vincristine is suspected of up-regulating the transient receptor potential vanilloid 1, TRPV1 (Chiba et al. 2017). RealDRG hiPSC-derived sensory neurons were cultured for 21 days in vitro. Vincristine on hiPSC-derived sensory neurons showed excitatory effects (increased spike rate) after 1 hour and 24 hours post application at 100 nM concentration. Gabapentin, an inhibitor of neuronal hyperactivity, reduces spike rate after 24 hours when co-applied with vincristine.

Conclusion

- Sensory neurons rapidly mature and extend axons within seven days and phenotypically respond to common chemotherapeutic compounds
- Mitochondrial staining indicates a marked reduction in membrane potentials after 48 hour dosing with bortezomib and vincristine
- Pre-treatment with the SARM1 inhibitor DSRM-3716 phenotypically mitigates axonal degradation via TUJ1 staining and AI-based comparison
- Vincristine at 100nM induces hyperactivation after one and twenty-four hours of application
- Demonstration of both a phenotypic and functional model of CIPN using hiPSC-derived sensory neurons

References

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