Exploring the Effects of SARM1 Inhibition in Preventing Neurodegeneration in Human and Rodent Neuropathic Pain Models

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Background

Neuropathic pain affects ~10% of adults in the US. Chemotherapy induced peripheral neuropathy (CIPN) is a subtype associated with axonal degeneration, metabolic dysfunction, & ion-channel dysregulation[1]. Chemotherapies like vincristine depolymerize microtubules and cause the degeneration of sensory neurons in the epidermis that pathogenically manifest CIPN pain. Mechanistically, vincristine causes an elevation in intracellular calcium that activates the protease calpain, leading to impaired axonal transport and cytoskeletal destruction. While exploring other calpain dependent mechanisms of axonal protection, we wanted to investigate whether an upstream regulator, Sterile Alpha and TIR Motif Containing 1 (SARM1), may be as efficacious a target. SARM1 is a metabolic sensor that is triggered by an increase in the NMN:NAD+ ratio, as is seen in CIPN. As a result, SARM1 further hydrolyzes NAD+ in a feed-forward manner, leading to metabolic failure. SARM1 also converts NAD+ into cyclic ADP-ribose (cADPR), which can trigger calcium release from intracellular stores, leading to calpain activation and axon degeneration. The objective of this study was to explore the activity and efficacy of SARM1 and calpain inhibition across *in vitro* & *in vivo* models of neurodegeneration and pain.

Methods

- SARM1 and calpain inhibitors' IC50s (**Table**) were measured in a rat primary neuron degeneration model. Neurons were then fixed, stained with TUJ1 for confocal microscopy, and neurite degeneration was then measured using NIS Elements software
- Vacor-induced neurodegeneration was tested by dosing mice (n=4/group) with SARM1 inhibitors 1hr before & 7hr after 30mpk vacor (PO) exposure (**Fig. 2**). Plasma was collected after 24h.
- SARM1 inhibition was tested in mouse models for neurodegeneration. In a sciatic nerve transection (SNT) model, mice were dosed (n=4/group, IP, BID) with inhibitor 1hr before and 7hr after surgically removing ~1mm of nerve. (**Fig. 3**)
- Vincristine-induced CIPN was tested by exposing mice (n= 8/group) to two 1.5 mpk IP doses of vincristine over 3 days. Mice were BID dosed with inhibitors for 20 days, and thermal pain sensitivity was evaluated with a Hargreaves instrument (**Fig. 4**).
- Human induced pluripotent stem cell (iPSC) derived sensory neuron cultures were generated following Deng *et al* procedure[2], relying on a combination of small molecules (CHIR98014, A83-01, DBZ, PD173074, PD0332991), supplements (N2 & B27), and recombinant proteins (BDNF, GDNF, NGF, NT-3) over 60 days and were characterized by BRN3A, PRPH, NF200, & TUJ1 staining.
- Cultures were pre-treated with SARM1 (NB-3, W8) or calpain (alicapistat) inhibitors then treated with vincristine for 24hr to induce neurite degradation (**Fig. 5**).
- Plasma from all experiments were analyzed for degenerated neurofilament-light (NF-L) protein via ELISA (Quanterix). Differences across groups were tested with one-way ANOVAs.

References

1. Park SB, Cetinkaya-Fisgin A, Argyriou AA, Alberti P, *et al. Axonal degeneration in chemotherapy- induced peripheral neurotoxicity: clinical and experimental evidence.* J Neurol Neurosurg Psychiatry. 2023.
2. Deng T, Jovanovic VM, Tristan CA, Singeç I, *et al. Scalable generation of sensory neurons from human pluripotent stem cells.* Stem Cell Reports. 2023.
3. Miyamoto T, Kim C, Chow J, Kane LA, et al. *SARM1 is responsible for calpain-dependent dendrite degeneration in mouse hippocampal neurons.* J Biol Chem. Feb 2024.

CIPN Axon Degeneration

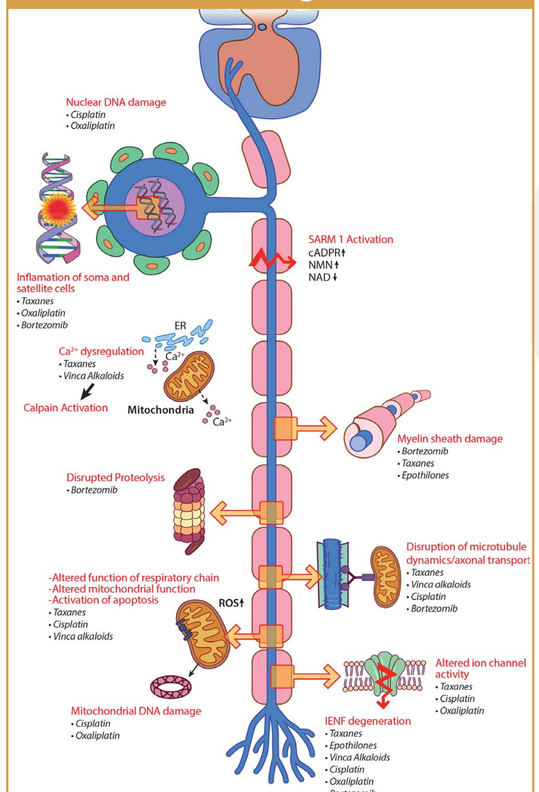
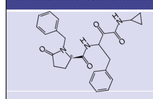
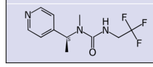


Figure 1. Schematic of CIPN pathophysiological processes underlying biomolecular mechanisms relevant to CIPN; repurposed from Park *et al* 2023[1].

Inhibitor Structure	Name/Company	Target	Rat *in vitro* IC50
	Alicapistat AbbVie	Calpain	133nM
NA	WO2025054368 Tenvie Tx	SARM1 (Allosteric Inhibitor)	374nM
	NB3 NuraBio	SARM1 (Base Exchange Inhibitor)	9nM

In Vivo SARM1 Validation

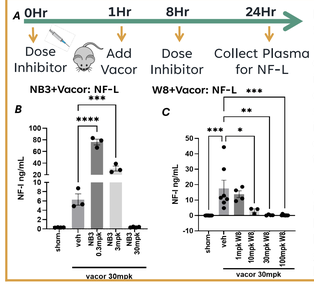


Figure 2. *In vivo* validation of SARM1 mechanism for Wallerian nerve degeneration. *A* Workflow of treating mice with SARM1 inhibitors and vacor (SARM1 activator). Vacor is structurally similar to nicotinamide and when introduced into the body is converted into a toxic metabolite that activates SARM1. *B* Graph of plasma degenerated NF-L levels across NB3 doses. Low doses increase NF-L while high dose lower NF-L . Low dose activation limited NB3 use the rest of the study. *C* Graph of plasma NF-L levels across W8 concentrations.

Nerve Damage + SARM1 Model

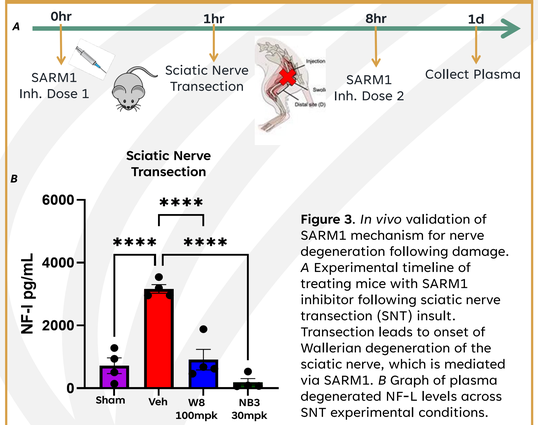


Figure 3. *In vivo* validation of SARM1 mechanism for nerve degeneration following damage. *A* Experimental timeline of treating mice with SARM1 inhibitor following sciatic nerve transection (SNT) insult. Transection leads to onset of Wallerian degeneration of the sciatic nerve, which is mediated via SARM1. *B* Graph of plasma degenerated NF-L levels across SNT experimental conditions.

In Vivo CIPN + SARM1 Model

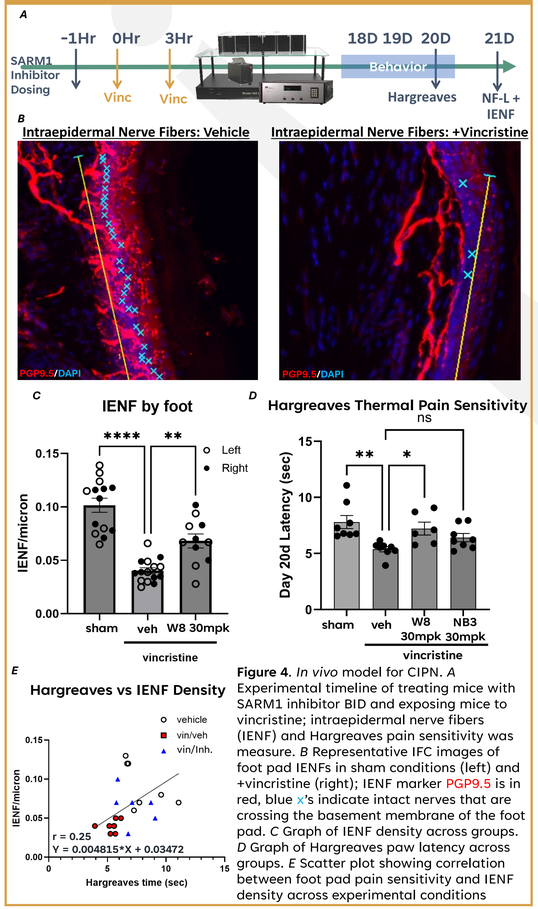


Figure 4. *In vivo* model for CIPN. *A* Experimental timeline of treating mice with SARM1 inhibitor BID and exposing mice to vincristine; intraepidermal nerve fibers (IENF) and Hargreaves pain sensitivity was measure. *B* Representative IFC images of foot pad IENFs in sham conditions (left) and +vincristine (right); IENF marker PGP9.5 is in red, blue x's indicate intact nerves that are crossing the basement membrane of the foot pad. *C* Graph of IENF density across groups. *D* Graph of Hargreaves paw latency across groups. *E* Scatter plot showing correlation between foot pad pain sensitivity and IENF density across experimental conditions

Human *In Vitro* CIPN + Calpain + SARM1 Model

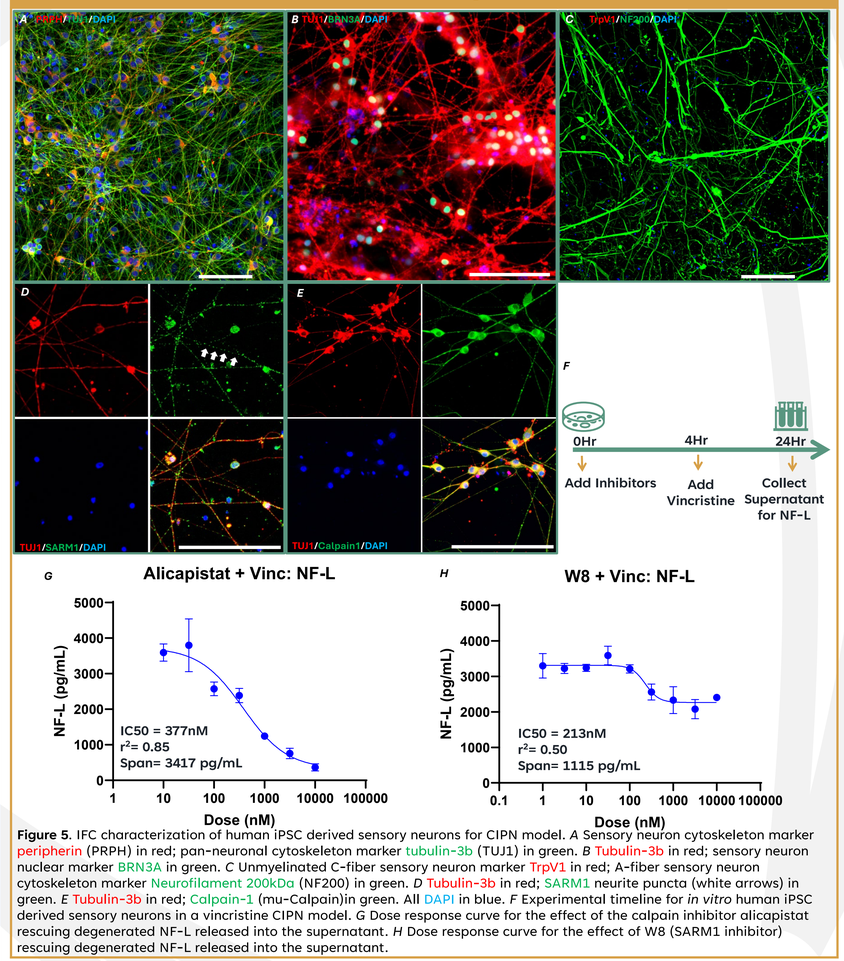


Figure 5. IFC characterization of human iPSC derived sensory neurons for CIPN model. *A* Sensory neuron cytoskeleton marker peripherin (PRPH) in red; pan-neuronal cytoskeleton marker tubulin-3b (TUJ1) in green. *B* Tubulin-3b in red; sensory neuron nuclear marker BRN3A in green. *C* Unmyelinated C-fiber sensory neuron marker TrpV1 in red; A-fiber sensory neuron cytoskeleton marker Neurofilament 200kDa (NF200) in green. *D* Tubulin-3b in red; SARM1 neurite puncta (white arrows) in green. *E* Tubulin-3b in red; Calpain-1 (mu-Calpain)in green. All DAPI in blue. *F* Experimental timeline for *in vitro* human iPSC derived sensory neurons in a vincristine CIPN model. *G* Dose response curve for the effect of the calpain inhibitor alicapistat rescuing degenerated NF-L released into the supernatant. *H* Dose response curve for the effect of W8 (SARM1 inhibitor) rescuing degenerated NF-L released into the supernatant.

Conclusions

- Prior studies have shown that SARM1 activates calpain-dependent neurodegeneration[3], which we explored via *in vitro* and *in vivo* pain models.
- The allosteric SARM1 inhibitor W8 rescued vacor-induced neurodegeneration *in vivo* in a dose dependent manner, while the base exchange inhibitor NB3 had deleterious low dose activation.
- Elevated degenerated NF-L caused by SNT can be ameliorated by SARM1 inhibitors.
- *In vivo* vincristine-induced CIPN caused loss of IENFs in mice foot pads with a decrease in Hargreaves thermal pain latency. SARM1 inhibitor treatment could rescue both deficiencies. Foot pad pain sensitivity correlated with IENF density in CIPN model.
- Human iPSC derived sensory neuron cultures expressed PRPH, TUJ1, MAP2, BRN3A, and NF200, SARM1, and Calpain.
- Human iPSC derived sensory neurons treated with vincristine degenerated *in vitro* and released NF-L (a measure of neurodegeneration) into the supernatant, which SARM1 inhibition lessened in a dose dependent manner
- Calpain inhibition lessened human sensory neuron degeneration (as measured by NF-L) in a dose dependent manner, but to an extent greater than SARM1 inhibition.
- These findings further validate calpain as a target for neuropathic pain disorders and highlights SARM1 as a possible therapeutic target, provided robust inhibitors can be designed without adverse low dose effects.