In Vitro Strategy for Preclinical to Clinical Translation in Pain: Sodium Channel

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Introduction

Voltage gated sodium channels are pivotal therapeutic targets implicated in a variety of disorders, including chronic pain, epilepsy, and cardiac arrhythmias. The discovery of modulators that selectively and effectively target specific sodium channel subtypes requires advanced screening methodologies capable of producing accurate and biologically relevant data. This report outlines a fully integrated in vitro platform designed to streamline and enhance the discovery of sodium channel modulators through a multi-step approach: 1). Preliminary Screening: State dependent assays to identify potent compounds with targeted activity; 2). Selectivity Profiling: Determination of isoform selectivity (Nav1.1–Nav1.8) and species-specific selectivity; 3). Secondary Screening: Use-dependent assays to provide biologically relevant insights into compound activity; 4). Primary Neuron-Based Assays: Evaluation of candidate compounds using dorsal root ganglion (DRG) neurons from mouse, rat, cynomolgus monkey and human models; 5). Safety Profiling: Comprehensive assessment of off-target liabilities using a safety panel. This Strategy enables the efficient identification and optimization of sodium channel modulators with superior specificity, efficacy, and safety profiles. By addressing critical challenges such as subtype selectivity and off-target effects, it facilitates the accelerated development of next-generation therapeutics targeting ion channels.

In Vitro Strategy in ICE lab for translation of the selective Nav1.8 inhibition into clinical efficacy and safety

1st: In-vitro ephys assay
- Screening against intended target
- Nanomolar potency range

2nd: Nav1.8 isoforms selectivity
- isoforms selectivity
- Off-targets screening for abuse
- potential panel and safety panel

3rd: DRG assay panel
- Compound effect on TTX-R current and action potential assay
- Multi-species portfolio: Mice，rat, dog, monkey and human

It has been suggested a preclinical strategy focused on NaV1.8 subtype selectivity and inhibition of pain signaling in human primary sensory neurons provides an effective and efficient model for preclinical to clinical translation in pain.

IPart-1: in vitro ephys assays for drug efficacy assessment

VX-548 shows sub-nanomolar potency on Nav1.8 without state dependency

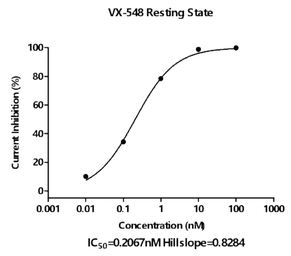


Figure 1. Dose response curve of VX-548 on hNav1.8 currents in resting state.

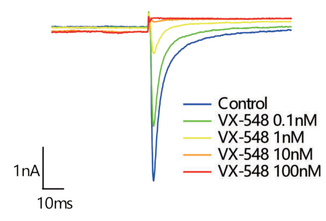


Figure 2. Representative traces of hNav1.8 currents, before and after VX-548 application at different concentrations in resting state.

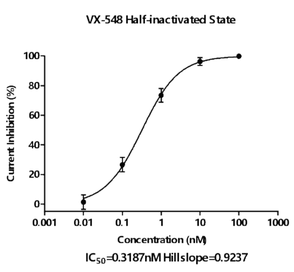


Figure 3. Dose response curve of VX-548 on hNav1.8 currents in half-inactivated state.

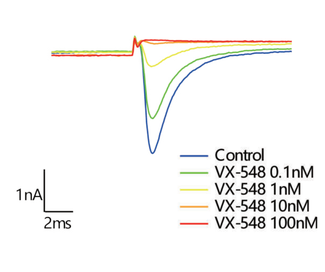


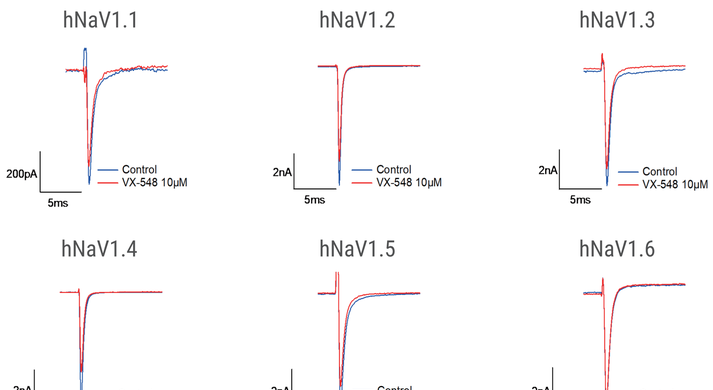
Figure 4. Representative traces of hNav1.8 currents, before and after VX-548 application at different concentrations in half-inactivated state.

Nav1.8	Resting state	Half-inactivated state
IC_{50} (nM)	0.2067	0.3187
HillSlope	0.8284	0.9237

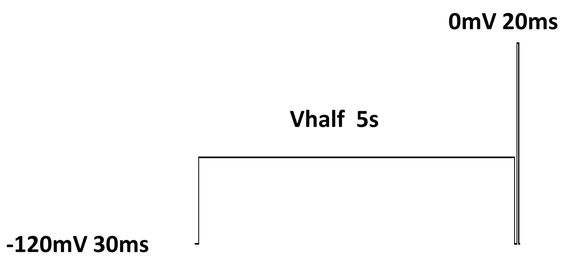
IPart-2: Isoform selectivity assessment

VX-548 has a more than 31000-fold selectivity ratio for Nav1.8

Channels	Inhibition%-10μM
Nav1.1	16.91%±1.87%
Nav1.2	17.71%±2.54%
Nav1.3	13.19%±0.92%
Nav1.4	32.79%±3.01%
Nav1.5	17.13%±1.36%
Nav1.6	6.32%±2.91%
Nav1.7	10.16%±1.72%

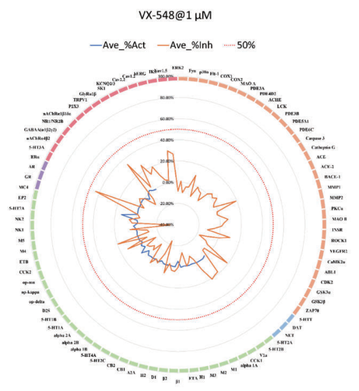


The recording protocol for hNaV1.1-1.7



In vitro safety screening

ICESTP 90 Safety Panel®



DRG assay panel : Mice, rat, dog, monkey and human

A.TTX-R current recording

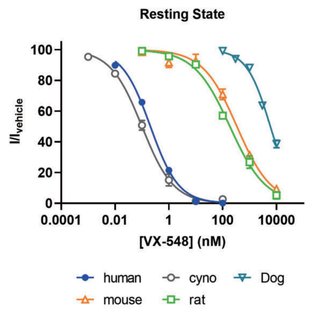


Figure 5. Concentration-dependent effect of VX-548 on human Nav1.8-CHO stable cell line, rat and mouse DRG neurons TTX-R currents in resting state.

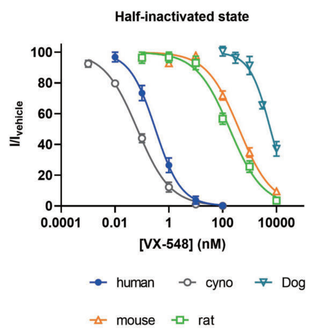
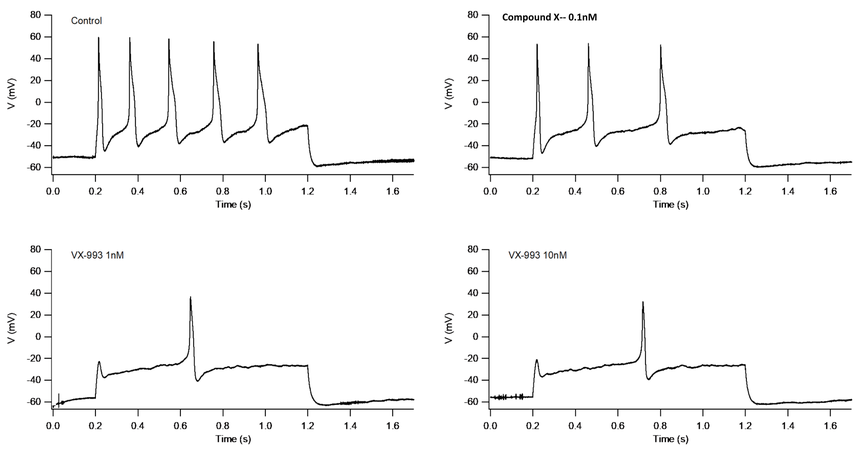


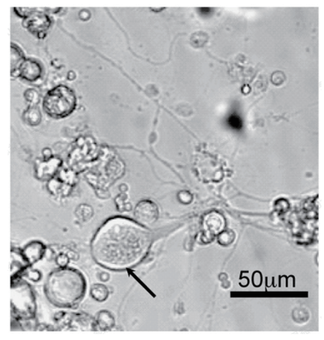
Figure 7. Concentration-dependent effect of VX-548 on human Nav1.8-CHO stable cell line, rat and mouse DRG neurons TTX-R currents in half-inactivated state.

	TP1-IC50	TP2-IC50
human	0.2067nM	0.3187nM
Cyno monkey	0.1005nM	0.0674nM
Rat	186.4nM	185.2nM
Dog	5.955μM	5.949μM
Mouse	339.0nM	407.7nM

B.Action potential recording

Compound X showed Inhibitory effect of pain signaling in cyno monkey DRG

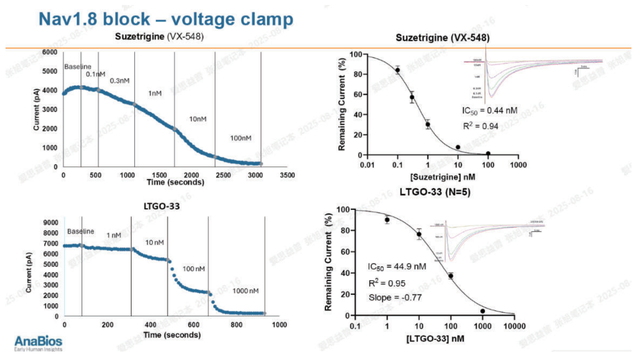


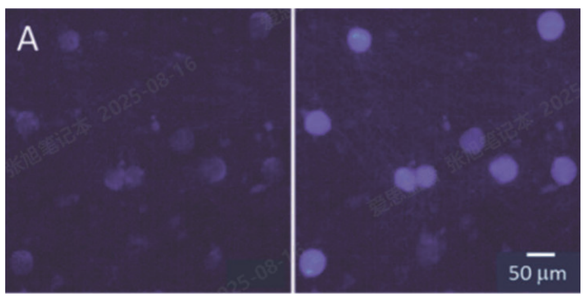


Cynomolgus DRG neurons (arrow) in culture

C.Human DRG assay

Now，We are partnering with Anabios for human DRG assay

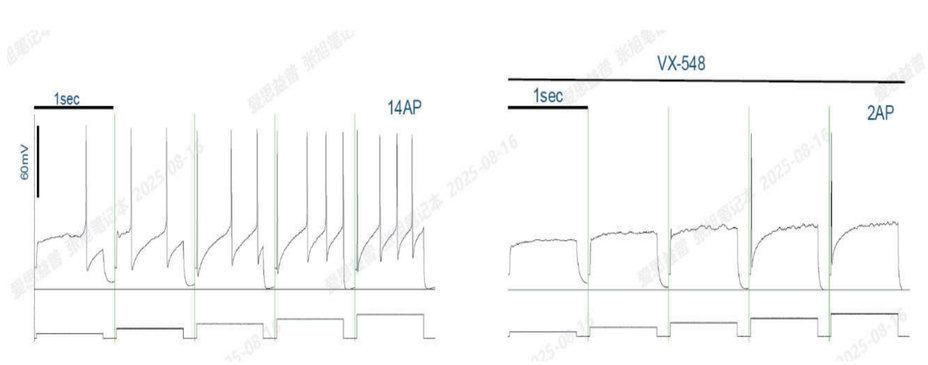




Calcium imaging assay in human DRG

Inhibition of repetitive AP firing by VX-548

1 second injection protocol at multiples ofrheobase



Summary

This approach facilitates the streamlined discovery and refinement of sodium channel modulators featuring enhanced specificity, potency, and safety attributes. By tackling key hurdles like subtype selectivity and unintended off-target interactions, it accelerates the advancement of cutting-edge treatments for ion channel targets and increase the translational value.