Comparison of Analgesic Effects of an Allosteric Inhibitor of TrkA to that of an ATP Site Inhibitor of the pan-Trk Axis in a Rodent Model of Inflammatory Pain

Karyn S. Bouhana, Anna M. Gomez, Renata Impastato, Robyn Hamor, Yutong Jiang, Timothy S. Kercher, Maralee McVean, Lisa Pieti Opie, Jeongbeob Seo, Ross D. Wallace, and Steven W. Andrews

Array BioPharma Inc., Boulder, CO

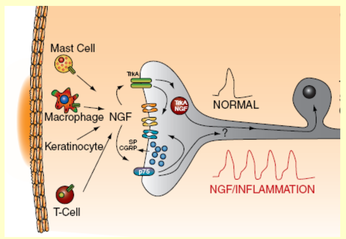
Abstract

Nerve Growth Factor (NGF) has been implicated as playing a significant role in the generation and maintenance of the nociceptive pain associated with a variety of human diseases. NGF is thought to signal primarily through binding to tropomyosin receptor kinase A (TrkA) which triggers subsequent downstream upregulation of various cellular processes implicated in neuronal hypersensitization, including upregulation of Brain Derived Neurotrophic Factor (BDNF) and its cognate receptor tropomyosin receptor kinase B (TrkB) in subpopulations of neurons in the DRG and their terminals in the spinal dorsal horn.

Despite extensive study, the relative contributions of the NGF/TrkA axis and the BDNF/TrkB axis to various peripheral pain states remains poorly understood. To address this, we have developed potent, highly selective small molecules that inhibit TrkA kinase by allosteric modulation or the entire TrkA / TrkB / TrkC kinase axis in concert by binding to the ATP site. Moreover, these inhibitors demonstrate good oral exposure in rodents and maintain high peripheral distribution relative to CNS making them useful tools for separating peripheral vs CNS contributions of the NGF/TrkA and BDNF/TrkB axis.

Herein the effects of these two distinct classes of Trk inhibitors in a rodent model of peripheral pain are discussed. In the model tested, inhibition of the TrkA receptor via allosteric modulation was sufficient to demonstrate significant and sustained relief of nociceptive pain. There was no observed benefit to simultaneously inhibiting the BDNF/TrkB axis. Furthermore these studies suggest that potent, selective small molecule inhibitors of the TrkA receptor may provide a novel therapeutic approach to pain management.

Putative Role of NGF/TrkA and BDNF/TrkB in Peripheral Pain

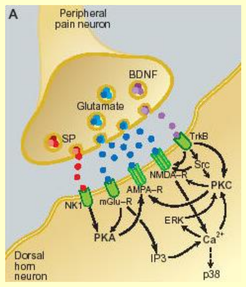


NGF / TrkA

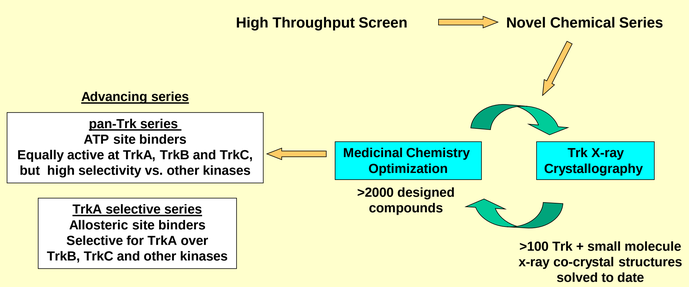
- NGF mediates peripheral pain in response to **injury and inflammatory**
- Recruits pro-inflammatory cells to the injury site
- Directly binding to neurons and elicits pain
- Painful diseases may involve defect in the autocrine loop of NGF signaling
- Stimulates release of BDNF

BDNF / TrkB

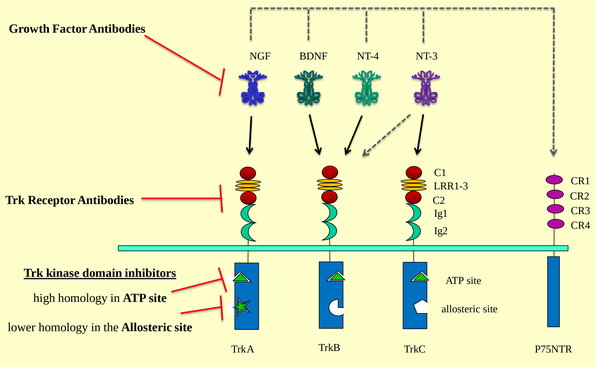
- BDNF sensitizes nerves, exacerbating the pain response
- Binding of BDNF to TrkB results in activation of signaling through other neuronal pain pathways (NMDA, AMPA, mGlu and NK1 pathways)
- Prolonged signaling of this pathway, defects in down regulation, or neuronal damage can lead to chronic **neuronal hypersensitization**



Array's Approach to Finding Trk Selective Chemical Matter



Mechanisms for Approaches to Inhibition of the Trk / Neurotrophin Axis



Array ATP site inhibitors selectively inhibit the pan-Trk axis, but not other kinases

Array allosteric site inhibitors selectively inhibit TrkA, but not TrkB, TrkC, or other kinases

Select Properties of Lead Molecules

Program	Pan-Trk		TrkA Selective
Lead	AR470	AR772	AR786
Human TrkA binding IC_{50}	8.6 nM	1.6 nM	1.2 nM
Human Trk A cell IC_{50}	9.7 nM	1.6 nM	0.6 nM
TrkB cell	24 nM	1.6 nM	>1000 nM
230 member Kinase Panel	Clean @ 1 µM (TNBK-2)	Clean @ 1 µM (TNBK-2)	Clean @ 10 µM
Predicted hepatic Cl Human, Rat	10, 18 (med, low)	13, 32 (med, med)	10, 38 (med, med)
Plasma protein binding Human, Rat	68%, 82%,	79%, 79%,	95%, 91%
Solubility (ng/mL) pH 1.2 / 6.5 / 7.4	>1000, >1000, >1000	>1000, 780, 820	750 / 60.0 / 1
in vivo brain exposure	~ 6% of plasma at relevant doses	~ 4% of plasma at relevant doses	~ 11% of plasma at relevant doses

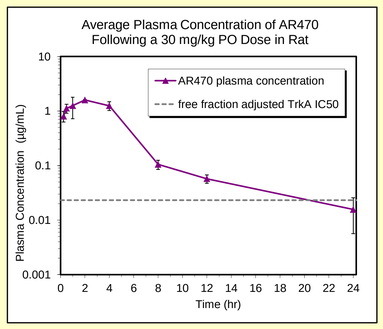
High potency for inhibiting NGF driven TrkA signaling

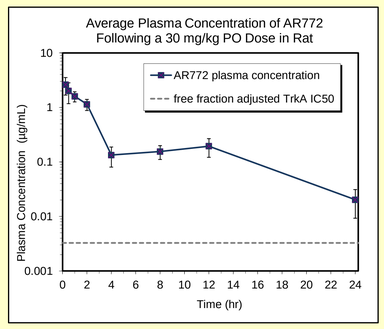
Pan-Trk equipotent on TrkB
TrkA selective not potent on TrkB

High off-target kinase selectivity

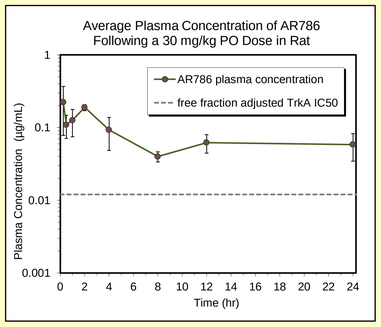
ADME properties to provide good oral exposure and selective peripheral distribution

Oral Exposure in Rats of Pan-Trk Leads Relative to the TrkA Cell IC_{50}





Oral Exposure in Rats of TrkA Selective Lead Relative to the TrkA Cell IC_{50}



Summary of *in vivo* efficacy and safety observed for Pan-Trk Leads (previously reported)

Excellent efficacy in multiple models of inflammatory pain

Acute Pain

- ✓ UV burn model (thermal hyperalgesia)
- ✓ CFA paw model (thermal hyperalgesia, gait analysis)
- ✓ CFA joint model (gait analysis)
- ✓ Fracture pain (flinching and guarding)
- ✓ Bone cancer pain (flinching, guarding and nerve budding)

Chronic Pain

- ✓ CIA Model of RA (Thermal hyperalgesia and histological damage of bone)
- ✓ MIA Model of OA
- ✓ CFA paw model (mechanical allodynia)

Peripheral Neuronal Safety

- ✓ No changes in functional observations in mice, rats, or monkeys at therapeutic doses / exposures
- ✓ No changes in normal pain response in rats at therapeutic doses
- ✓ No histological changes in peripheral neuronal density or morphology even at high doses for 28 days
- ✓ No histological changes in brain, spinal cord, sciatic nerve or skin neurons even at high doses for 28 days

Key Scientific Questions

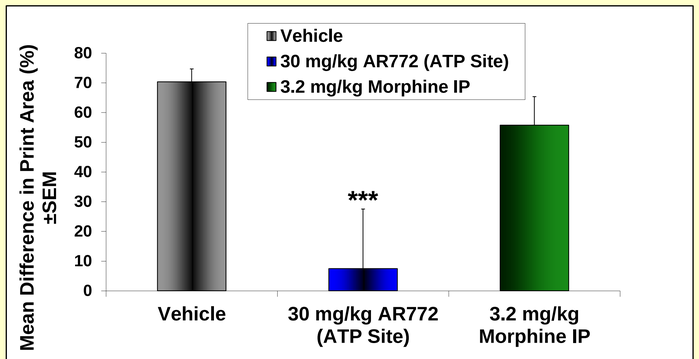
Do TrkA selective (allosteric) inhibitors show similar pain efficacy to Pan-Trk (ATP site) inhibitors?

- Is there an independent role for neuronal hypersensitization by BDNF / TrkB?
- Is blocking TrkA upstream of BDNF / TrkB sufficient to alleviate various modalities of pain / hypersensitization?
- Is ATP site and allosteric site inhibition functionally equivalent *in vivo*?

CFA Inflammatory Paw Model - Gait Analysis

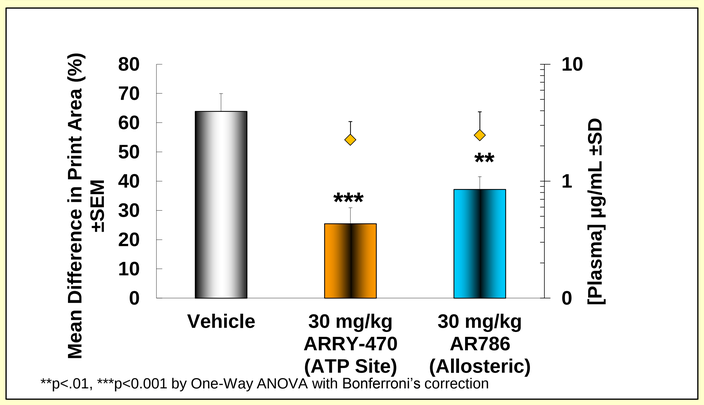
- Animals are trained to walk on the glass walkway for 2 days prior to beginning experiment
- Prophylactic dosing of Trk inhibitors 1 hour prior to Complete Freund's Adjuvant injection in the left hind foot pad
- Pain is expressed as a difference in paw print area between injected and contralateral paw

A Pan-Trk Inhibitor Is Superior to a Standard Dose of Morphine In the Rat CFA Model



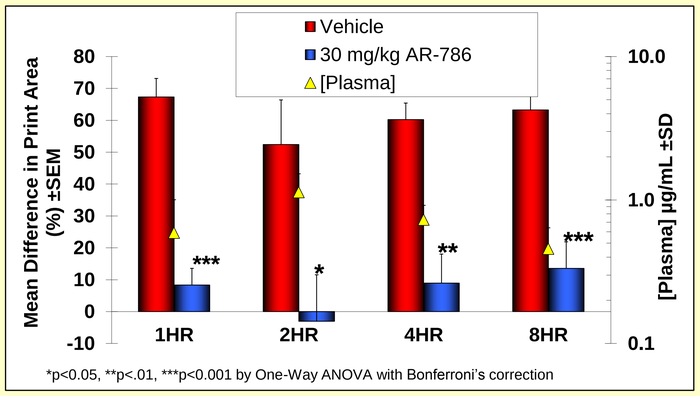
***p<0.001 by One-Way ANOVA with Bonferroni's correction

A TrkA Selective Inhibitor Is Equivalent to a Pan-Trk Inhibitor In the Rat CFA Model



**p<.01, ***p<0.001 by One-Way ANOVA with Bonferroni's correction

A TrkA Selective Inhibitor Is Equivalent to a Pan-Trk Inhibitor In the Rat CFA Model



*p<0.05, **p<.01, ***p<0.001 by One-Way ANOVA with Bonferroni's correction

Conclusions

- Potent Pan-Trk (ATP site) and TrkA selective (allosteric site) inhibitors with high selectivity over off-target kinases have been developed using an iterative medicinal chemistry approach guided by molecular modeling
- Lead compounds from the Pan-Trk and TrkA selective series are orally bioavailable, demonstrate good target coverage when dosed orally in rat and have peripherally selective distribution
- In the CFA gait model, pan-Trk (ATP site) and TrkA selective (allosteric site) inhibitors show similar efficacy for inhibition pain and are superior to IP morphine
- Evaluation of these distinct Trk inhibitor profiles for inhibition modalities of pain in other preclinical models is in progress
- These experiments suggest inhibition of TrkA by allosteric kinase inhibition may represent a novel mechanism for the treatment of chronic and acute pain

Contact information sandrews@arraybiopharma.com