Allosteric Small Molecule Inhibitors of the NGF/TrkA Pathway
A New Approach to Treating Inflammatory Pain

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Associate Director of Drug Discovery
Challenges Faced with Discovery of New Drugs in the Pain Space

**Translation of pre-clinical results to clinical outcome**

**Efficacy**
- Poor translation from animal models to man and vice versa

**Safety**
- Mechanism specific toxicities are not always apparent until reaching the clinic
- Bar for safety for new pain drugs is very high

**Economic Considerations**
While existing SOC have deficiencies and liabilities, these treatments are quite cost effective and offer many convenient dosage forms

**Our approach: small molecule inhibitors for antibody validated pathways**

*AR-797 P38 inhibitor – targeting NSAID resistant pain (Phase II)*

*Trk inhibitors – targeting the NGF pathway (pre-clinical)*
Tanezumab- anti-NGF Antibody has Validated Clinical Efficacy

Similarly impressive efficacy reported in Chronic Low Back Pain\(^2\)

but concerns of joint findings in OA patients with chronic dosing, particularly with NSAIDs

Key Question- is there an alternative and perhaps safer approach to inhibit this pathway
Neurotrophin / Trk Signaling Mediates Peripheral Pain Response

**Local release**

**Peripheral Hypersensitization**

**Proinflammatory cell recruitment and degranulation**

**Central Hypersensitization**

*Neurotrophin Receptors- TrkA and B are critical signaling partners in the NGF pain cascade*
Mechanisms for Inhibiting Neurotrophin / Trk Signaling Cascade

Growth Factor Antibodies:
- NGF
- BDNF
- NT-4
- NT-3

Trk Receptor Antibodies:
- TrkA
- TrkB
- TrkC

Trk kinase domain inhibitors:
- 100% homology in ATP site

Our ATP site inhibitors selectively inhibit the pan-Trk axis, but not other kinases
Efficacy for Pain Relief?

- Added efficacy from the TrkB component?
- Differences related to mechanism of inhibition?
- Differences related to duration of inhibition?
  - Long term vs intermittent target knockdown?

Safety?

- Differences related to mechanism of inhibition?
- Differences related to duration of inhibition?
  - Long term vs intermittent target knockdown?
- Safety concerns for added TrkB and TrkC inhibition?

Our approach: *in vivo evaluation*

with selective small molecule pan-Trk inhibitors –
Approach to Finding Highly Selective Trk Chemical Matter

High Throughput Screen
- ARRAY diversity and kinase focused

Trk potency
- ~many compound hits in > 20 chemical series

Promising Kinase Selectivity and “drug-likeness”
- <50 compounds in 2 novel chemical series

~80 TrkA ATP site x-ray structures solved to date

DMPK Pharmacology Toxicology

Medicinal Chemistry Optimization
- >1500 designed compounds

Trk X-ray Crystallography

pan-Trk series (ATP site)
- Tuned for high kinase selectivity outside of Trk family
- equipotent for TrkA, TrkB and TrkC
## Select Properties of Pan-Trk Leads

<table>
<thead>
<tr>
<th>Program</th>
<th>Pan-Trk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARRY-470</td>
</tr>
<tr>
<td><strong>Lead</strong></td>
<td></td>
</tr>
<tr>
<td><em>hTrkA</em> cell IC$_{50}$</td>
<td>9.7 nM</td>
</tr>
<tr>
<td><em>hTrkA</em> free cell IC$_{50}$</td>
<td>23 nM</td>
</tr>
<tr>
<td><em>hTrkB / hTrkC</em> cell</td>
<td>24 nM</td>
</tr>
<tr>
<td><strong>230 member Kinase Panel</strong></td>
<td>Clean @ 1 µM</td>
</tr>
<tr>
<td><strong>Predicted hepatic CI</strong></td>
<td>10, 18 (med, low)</td>
</tr>
<tr>
<td><strong>Plasma protein binding</strong></td>
<td>68%, 82%,</td>
</tr>
<tr>
<td><strong>Solubility (ng/mL)</strong></td>
<td>&gt;1000, &gt;1000, &gt;1000</td>
</tr>
<tr>
<td><strong>Peripheral to CNS exposure</strong></td>
<td>16 to 1</td>
</tr>
</tbody>
</table>

**Very potent for inhibiting neurotrophin driven TrkA, TrkB and TrkC signaling in cell**

**High kinase selectivity**

**Peripherally Selective:** only peripheral inhibition in efficacy studies
Kinase Selectivity of AR-470 compared to literature pan-Trk Kinase Inhibitors

**Amino pyrimidine**
ATP site

At 1 µM
Potent on Trks
Plus 83 off targets

**Diaryl urea**
Type II DFG out

At 0.5 µM
Potent on Trks
Plus ~7 off targets

**AR-470**
ATP site
Pan TRK inhibitor

at 1 µM
Potent on Trks
One weak off target

No activity at 10 µM against other pain targets:

Clean pharmacology for inhibiting TrkA, B and C
Summary of *in vivo* Efficacy Observed for Pan-Trk Leads

Excellent efficacy is observed in pre-clinical 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)
- Surgical Incision

**Chronic Pain**

- CIA Model of rheumatoid arthritis (pain and histological evaluation)
- MIA Model of osteoarthritis (pain)
- CFA paw model (mechanical allodynia)
ARRY-470 Broad Efficacy Observed in Multiple Pre-Clinical Models

Mantyh et al. Molecular Pain 6, art. no. 87

Mantyh et al. Bone 48 (2), pp. 389-398

* *p<0.05, **p<0.01 vs vehicle group (ANOVA).
ARRAY-470 is Superior to NSAIDs in the CFA Joint Model

Results shown 2-3 days after start of treatment

Guarding index

Dose (mg/kg p.o. twice daily)

Guarding index

Dose (µMol/kg p.o. twice daily)

Valdecoxib

Rofecoxib

Naproxen
Safety of Pan-Trk Inhibitors – Neuronal Safety

✓ No changes in functional observations in mice, rats, or monkeys at therapeutic doses / exposures

✓ No histological changes in peripheral neuronal density in brain, spinal cord, sciatic nerve or skin neurons to 300 mg/kg with 28 days of dosing

✓ No changes in normal pain response at therapeutic doses

Mantyh et al. Bone 48 (2), pp. 389-398
Safety of Pan-Trk Inhibitors – On Target Effects

Hyperphagia / weight gain
- Increased food consumption – *peripheral effect*
- Increase weight gain - even when food consumption is controlled
- Likely BDNF / TrkB effect – rodent specific?

Ataxia Scoring System
1 - "swimming" through litter, flattened or splayed on cage bottom
2 - head bobbing, jittery or hyperactive, head bobbing, nervous
3 - head rearing, disoriented, lethargy, agitated
4 - plus falling over when on hind legs, sleeping on back

Correlates with pan-Trk target coverage in the CNS

Potential narrow therapeutic window for broad clinical pain treatment
Efficacy

- Great efficacy across pre-clinical pain models
- Equivalent to historical anti-NGF in the same models
- Intermittent target knockdown is sufficient for efficacy
- Partial pathway knockdown is sufficient for efficacy
  - No apparent added effect for TrkB
  - (In the clinic- concerns of too much pain relief with anti-NGFs)

Safety

- No observed adverse effects on peripheral neuronal health or function
  - Hyperphagia and weight gain – rodent specific?
  - Reversible Ataxia when CNS target coverage is achieved. TI related to plasma to brain ratio

Would selective TrkA inhibition provide a broader TI?
Approach to Finding Highly Selective TrkA Chemical Matter

High Throughput Screen
ARRAY diversity and kinase focused

Trk potency
~many compound hits in > 20 chemical series

Promising Kinase Selectivity and “drug likeness”
<50 compounds in 2 novel chemical series

2 TrkA vs TrkB selective hits

DMPK Pharmacology Toxicology

Medicinal Chemistry Optimization
>2000 designed compounds

Induced Fit

Trk X-ray Crystallography

>120 TrkA allosteric + small molecule x-ray structures solved to date

TrkA Selective Series (allosteric site)
- high kinase selectivity outside of Trk family
- high selectivity for TrkA over TrkB and C
Challenges of Drug Design in an Induced Fit Site

Allosteric site optimization is enabled by X-ray crystallography

existing pocket
ATP site

ATP site
Potency is easy
Selectivity is hard

induced fit
allosteric site

Allosteric site
Potency is hard
Selectivity is easy

Potency is hard
Selectivity is easy
TrkA Selective Inhibitors - Identifying the Allosteric Site

>100 TrkA Constructs Cloned

Triaged by Expression, Purification, and Binding

Thousands of Crystal Screens

Several Crystal Forms

>120 TrkA/Inhibitor Structures

>120 Selective TrkA Inhibitor Structures
Median Resolution: 2.8 Å
Range: 2.3 Å – 3.3 Å
Mechanism for Inhibiting Neurotrophin / Trk Signaling Cascade

Trk kinase domain inhibitors
- high homology in ATP site
- low homology in the allosteric site

Trk Receptor Antibodies
- Allosteric site inhibitors selectively inhibit TrkA, but not TrkB, TrkC, or other kinases

Growth Factor Antibodies
- NGF, BDNF, NT-4, NT-3

TrkA, TrkB, TrkC
- ATP site
- Allosteric site
## Select Properties of Pan-Trk and TrkA Selective Leads

<table>
<thead>
<tr>
<th>Program</th>
<th>TrkA Selective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>AR-786</td>
</tr>
<tr>
<td><strong>hTrkA cell IC$_{50}$</strong></td>
<td>0.6 nM</td>
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<tr>
<td><strong>hTrkA free cell IC$_{50}$</strong></td>
<td>12 nM</td>
</tr>
<tr>
<td><strong>hTrkB / hTrkC cell</strong></td>
<td>&gt;1000 nM</td>
</tr>
<tr>
<td>230 member Kinase Panel</td>
<td>Clean @ 10 µM</td>
</tr>
<tr>
<td>Predicted hepatic Cl</td>
<td>10, 38 (med, med)</td>
</tr>
<tr>
<td>Human, Rat</td>
<td>750</td>
</tr>
<tr>
<td>Solubility (ng/mL) pH 1.2 / 6.5 / 7.4</td>
<td>60</td>
</tr>
<tr>
<td>peripheral to CNS exposure</td>
<td>10 to 1</td>
</tr>
</tbody>
</table>

### Notes

- Very potent for inhibiting NGF driven TrkA signaling in cell
- High selectivity over TrkB / C
- High kinase selectivity
- Peripherally Selective: Only peripheral inhibition in efficacy studies
Kinase Selectivity of Array pan-Trk and TrkA Selective Inhibitors

**Amino pyrimidine**
ATP site
- At 1 µM
  - Potent on Trks
  - Plus 83 off targets

**Diaryl urea**
Type II DFG out
- at 0.5 µM
  - Potent on Trks
  - Plus ~7 off targets

**AR-470**
ATP site
Pan
- At 1 µM
  - Potent on Trks
  - One weak off target

**AR-786**
Allosteric
Selective
TRKA inhibitor
- at 10 µM
  - Potent on TrkA
  - weak on TrkB/C

No activity at 10 µM: against other pain targets

Clean TrkA pharmacology
Key Scientific Questions- Small Molecule pan-Trk vs TrkA Selective

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

- 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*?
TrkA Selective and pan-Trk Inhibitors are Equivalent in the Rat CFA Paw Model

Equivalent and Compelling efficacy for TrkA Selective and Pan-Trk in a model of inflammatory pain

*p<0.05 by One-Way ANOVA with Bonferroni's correction compared to vehicle
Duration of Action of a TrkA Selective Inhibitor in the Rat CFA Paw Model

30 mg/kg AR786

Mean Difference in Print Area (%) ±SEM

-10 0 10 20 30 40 50 60 70 80

1HR 2HR 4HR 8HR

Vehicle 30 mg/kg AR-786

Promising onset and duration of action
TrkA Selective and pan-Trk in a Surgical Incision Model

Equivalent and compelling efficacy for TrkA Selective and Pan-Trk in a model of surgical pain
TrkA Selective and pan-Trk in the Rat CIA Model of Polyarthritis

Equivalent and compelling efficacy for TrkA Selective and Pan-Trk in a model of rheumatoid arthritis
TrkA Selective Inhibitors are Equivalent to Celecoxib in the Rat MIA Model

Algos

Compelling efficacy for TrkA selective in a model of Osteoarthritis
Neurotrophin / Trk Signaling Mediates Peripheral Pain Response

**Local release**

**Peripheral Hypersensitization**

**Proinflammatory cell recruitment and degranulation**

**Central Hypersensitization**

**Blocking TrkA at the allosteric site is sufficient to block the NGF pain cascade**
Do TrkA selective (allosteric) inhibitors have a similar safety profile to Pan-Trk (ATP site) inhibitors?

- Hyperphagia and Weight gain?
- Ataxia with CNS target coverage?
TrkA Selective Inhibitors Do Not Cause Hyperphagia or Ataxia in Rat

Hyperphagia / weight gain
- No increased food consumption
- No weight gain / hyperphagia
- Supports pan-Trk effect was BDNF / TrkB driven

Ataxia Scoring System
1 - "swimming" through litter, flattened or splayed on cage bottom
2 - head bobbing, jittery or hyperactive, head bobbing, nervous
3 - head rearing, disoriented, lethargy, agitated
4 - all of above plus falling over when on hind legs, sleeping on back

<table>
<thead>
<tr>
<th>Pan-Trk Treated Females</th>
<th>Ataxia Score (max)</th>
<th>Incidence</th>
<th>TrkA Selective Treated Females</th>
<th>Ataxia Score (max)</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR523</td>
<td>1</td>
<td>1 of 3</td>
<td>10 mg/kg AR256</td>
<td>0</td>
<td>0 of 3</td>
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<tr>
<td>AR523</td>
<td>1</td>
<td>3 of 3</td>
<td>50 mg/kg AR256</td>
<td>0</td>
<td>0 of 3</td>
</tr>
<tr>
<td>AR523</td>
<td>2</td>
<td>3 of 3</td>
<td>300 mg/kg AR256</td>
<td>0</td>
<td>0 of 3</td>
</tr>
</tbody>
</table>
Mechanistic Difference - Small Molecule, Oral TrkA kinase vs anti-NGF m-ABs

Intermittent target knockdown is sufficient for efficacy, required levels seem to vary by model

Potential Clinically Advantages

1. Ability to adjust inhibition by varying dose and schedule
2. Ability withdraw drug
3. Allows flexible titration to address different pain indications
Small Molecule TrkA Selective Inhibitors: What we know so far

**Efficacy**

- Great efficacy across pre-clinical pain models: similar to pan-Trk
  - Looking for Collaborators – new models for mechanistic comparison
- Intermittent target inhibition is sufficient for efficacy
- Similar efficacy to that reported for anti-NGF antibodies

**Safety**

- No observed adverse effects on peripheral neuronal health
- No hyperphagia or weight gain to 28 days
- No ataxia even when CNS target coverage is achieved for 28 days

*TrkA Selective Kinase Inhibition at the Allosteric Site Represents a Promising New Mechanism for Modulating the NGF / Trk Pain Pathway*
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