ZF-TF mediated epigenetic repression of SCN9A gene as a therapeutic approach for painful peripheral neuropathies


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Disclosure

I am a full-time employee of Sangamo Therapeutics
Neuropathic pain is one of the most difficult pain syndromes to manage.

Damage or alterations to sensory neurons

Usually associated with diabetes, stroke, or infection

Large number of patients are affected globally

Manifests as burning and stabbing feeling in the feet and hands

Many patients are refractory to common pain medications

Given the high unmet need and lack of effective treatments, there is an urgent need to develop novel therapeutics for the treatment of chronic neuropathic pain.
Mutations in the SCN9A gene (Nav1.7) are linked to inherited pain disorders

• Nav1.7 is a voltage gated sodium channel expressed in the Dorsal Root Ganglion (DRG)
• Alterations in Nav1.7 activity directly regulate pain levels in several genetic disorders, validating Nav1.7 as a therapeutic target for pain
• Lowering Nav1.7 is expected to reduce pain without adversely affecting other sensory functions
• High structural similarities among Nav channels has made it challenging to develop Nav1.7 selective inhibitors
Zinc Finger based epigenetic regulation

- Require binding just two DNA targets per cell to regulate RNA and protein levels
- Highly potent, specific and optimizable
- Derived from naturally occurring human genes
- No modifications or edits to the genome
Blocking pain transmission to the brain has the potential to treat multiple pain indications

- Blocking Nav1.7 in the DRG is expected to prevent the transmission of nociceptive pain signals to the brain.
- This allows us to target multiple neuropathic pain indications, regardless of the cause of the pain.
- Reducing pain by inhibiting Nav1.7 is not predicted to be associated with any CNS adverse effects.
Zinc finger-mediated repression of Nav1.7 as a potent and specific therapeutic avenue for neuropathic pain
Developmental path to identify mouse and human ZF-repressors targeting the Nav1.7 gene

**MOUSE ZF-Repressors (ZF-R)**

- **Mouse Nav1.7 gene (Scn9a)**
- **500+ 6-finger ZF-Rs designed & screened in neurons**
- **Efficacy in pain mouse model**
- **In vivo proof of concept**

**HUMAN ZF-Repressors (ZF-R)**

- **Human/NHP Nav1.7 gene (SCN9A)**
- **600+ 6-finger ZF-Rs designed & screened in neurons**
- **On-target engagement & safety in nonhuman primates (NHPs)**
- **Clinical candidate**
The efficacy of ZF-Rs was evaluated in the Spared Nerve Injury (SNI) neuropathic pain model

- SNI is the most validated mouse neuropathic pain model (“Gold standard”)
- Surgically induced hypersensitivity to pain

**Surgical Procedure**
- SNI surgery
- Pain measurement (baseline)
- Pain measurement

**Flowchart**
- Day -7
- Day 1
- Day 28

**Data Collection**
- Collect DRGs

**Pain Measurement**
- Mechanical and cold induced pain were measured before (baseline) and 4 weeks after ZF-R treatment
- Scn9a repression in DRG was evaluated at the bulk and single-cell (nociceptor) level
- Gabapentin was used as a positive control and administered one hour before the pain measurement on day 28
Mouse specific ZF-Rs induced up to 70% bulk repression of Scn9a in DRGs

- No changes were observed in the DRG for molecular markers of neuroinflammation or neuronal loss

**Scn9a (Nav1.7) expression (RT-qPCR)**

**Lumbar DRG**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>ZF-R a</th>
<th>ZF-R b</th>
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<tbody>
<tr>
<td>% Normalized expression</td>
<td>100</td>
<td>50</td>
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**Thoracic DRG**

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**Cervical DRG**

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<tbody>
<tr>
<td>% Normalized expression</td>
<td>100</td>
<td>50</td>
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**P < 0.01  *** P < 0.001  ****P <0.0001**

Compared with Vehicle
One-way ANOVA
Potent repression of Scn9a mRNA in nociceptors of mouse lumbar DRG
**In vivo** repression of mouse *Scn9A* reverses pain hypersensitivity in a mouse model of neuropathic pain

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**Mechanical Pain**

- **Before any Treatment**
  - Normal pain threshold
  - Reduced pain threshold

- **4 weeks**
  - **ZF-R**
  - **AAV**

- **Mechanical Threshold (g)**
  - **Sham**
  - **Vehicle**
  - **Gaba**
  - **ZF-R a**
  - **ZF-R b**

- **Post SNI surgery**

- **Recovery to normal pain threshold**

- **Gabapentin** was administered one hour before the measurement

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**Paw withdrawal latency (sec)**

- **Before any Treatment**
  - Normal pain threshold

- **4 weeks**

- **Paw withdrawal latency (sec)**
  - **Sham**
  - **Vehicle**
  - **Gaba**
  - **ZF-R a**
  - **ZF-R b**

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**Mechanical induced pain**

**Cold induced pain**

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**One-way ANOVA**

- **** $P < 0.0001$ Compared to Sham
- **P** $< 0.01$
- ***** P** $< 0.001$
- ****** P** $< 0.0001$ Compared to Sham
ZF-Rs repressed SCN9A by more than 90% over a wide dose range with no detectable off-target activity

- SCN9A was the only significantly regulated transcript out of >20,000 genes analyzed
- No repression of any other Nav channel was observed
The potency and safety of the human ZF-R candidates were evaluated in nonhuman primates.

AAV/ZF-Rs

- $1 \times 10^{12}$ vg/animal (2M/1F)
- $1 \times 10^{13}$ vg/animal (2M/1F)
- $9 \times 10^{13}$ vg/animal (2M/1F)

Vehicle control (1M/1F)

4 weeks

Necropsy

Cynomolgus Monkey

AAV/ZF-Rs injection

IT injection

SCN9A expression in DRGs

Safety
- General toxicity
- Histopathology

M: Male
F: Female

Images made with Biorender.com
ZF-Rs repressed the expression of SCN9A in a dose-dependent manner in multiple DRG levels.

Clinical candidate ZF-Rs repressed SCN9A by up to 40-60% across a 100-fold dose range one month after intrathecal-lumbar treatment.

- Comparable data were obtained in sacral DRG
- Multiple DRGs were evaluated for each level per animal
ZF-Rs were well tolerated in nonhuman primates

These results support the initiation of IND-enabling GLP Toxicology study

ZF-Rs were well tolerated at all doses
No clinical signs of toxicity
No adverse macroscopic or microscopic findings were observed
Conclusion

• Mouse ZF-Rs potently repressed Scn9a at the bulk and single-cell level in mouse DRG
• IT-L administered ZF-Rs reversed pain hypersensitivity in the SNI model of neuropathic pain

• Human ZF-Rs potently repressed SCN9A >90% in human iPSC-derived neurons
• ZF-Rs were highly specific with no off-target activity detected, including no repression of any other Nav channels

• Human ZF-Rs repressed SCN9A by up to 40-60% at all DRG levels in NHP
• ZF-Rs were well tolerated at all doses tested with no adverse findings related to treatment
• These results support the continued progression to IND-enabling nonhuman primate study
Thank you

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