Poster #429

Preclinical Development of a Novel Gene Therapy for the Treatment of Peripheral Neuropathic Pain Through Zinc Finger Repressor-Mediated Repression of the *SCN9A* Gene

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Abstract

Peripheral neuropathy (PN) is a debilitating and painful condition affecting millions with a significant unmet need due to the ineffectiveness of present therapies. Loss of function mutations in human Nav1.7 voltage gated sodium channel, encoded by the *SCN9A* gene, have been shown to cause insensitivity to pain. We have developed a novel therapeutic for treatment of PN by epigenetically repressing the *SCN9A* gene using highly specific engineered zinc finger repressors (ZF-Rs) targeting mouse (*Scn9a*) or human/nonhuman primate (NHP) *SCN9A* genes in nociceptive neurons within the dorsal root ganglia (DRG). Mouse and human/NHP ZF-Rs were tested for efficacy in the gold-standard Spared Nerve Injury (SNI) mouse model of chronic PN, and safety in a 1-month dose range-finding (DRF) toxicology study in NHPs, respectively. ZF-Rs were administered intrathecally using the clinical recombinant adeno-associated viral vector (AAV) as the delivery system. In the SNI model, ZF-Rs led to significant repression of the *Scn9a* gene in DRG neurons at bulk and single cell levels, restored mechanical- and cold-induced pain responses to normal levels, and were well-tolerated (no neuroinflammation or neuronal loss). In the DRF study, ZF-Rs resulted in a well-tolerated 40-60% reduction of *SCN9A* gene expression in the DRG neurons with no dose-limiting toxicity. Anti-AAV neutralizing antibodies were detected in serum at all doses, and cerebrospinal fluid primarily at high doses, neither of which impacted pharmacology or safety parameters. Taken together our results describe successful design and selection of a novel AAV-ZF-R therapeutic for potential treatment of PN, and support advancement to IND-enabling studies.

Gene Regulation by Zinc Finger Proteins

Zinc Finger Proteins in cells regulate genes epigenetically.

- Naturally occurring
- Tunable
- Specific
- Easily packaged into AAV

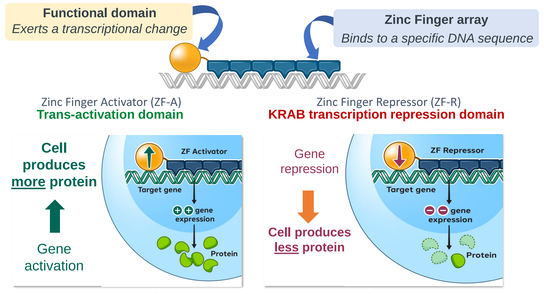


Figure 1. Epigenetic regulation by zinc finger activators and repressors. (Image made with Biorender.com)

Role of Nav 1.7 in Neuropathic Pain

Voltage gated sodium channel (Nav) 1.7 is encoded by SCN9A gene

- There are no selective inhibitor of Nav1.7
- Unmet need for treatment of Neuropathic pain
- Thus, reduction in *SCN9A* gene may be a potential treatment

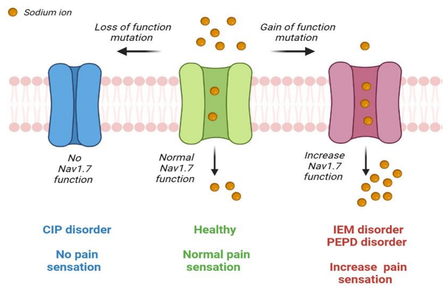


Figure 2. Mutations in *SCN9A* (Nav 1.7) gene leads to inherited disorders. IEM, Inherited Erythromelalgia; PEPD, Paroxysmal extreme pain disorder; CIP, Congenital insensitivity to pain. (Image made with Biorender.com)

Mouse (Surrogate) ZF-Rs *In Vitro* Specificity

Assessing ZF-R effect on *Scn9a* gene in mouse neurons

- ZF-Rs demonstrated dose-dependent repression of *Scn9a* gene
- ZF-Rs have exquisite selectivity with no off-targets in mouse neurons

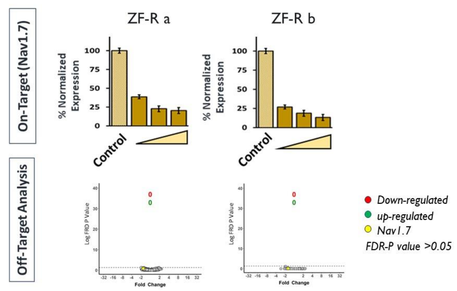


Figure 3. In vitro on- and off-target activity of mouse ZF-Rs in mouse neurons using RT-qPCR and microarray technology, respectively.

Efficacy Study in Spared Nerve Injury (SNI) Mouse Model

SNI model and Pain Assessment

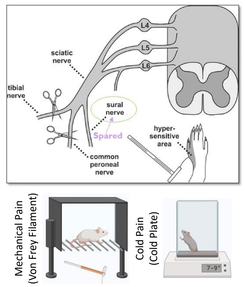


Figure 5. SNI model generation and assessment of pain using Von Frey filament or cold plate set at 7-9°C.

Study Design and Group Assignments

Group	Treatment	Total Dose (vg/mouse)	Dose mg/kg	No. of Mice
1	Sham Operated*	0	-	8M/8F
2	Vehicle Control (Buffer for ZF-R)	0	-	8M/8F
3	Gabapentin (Gaba)	-	50**	8M/8F
4	ZF-R a	8.12E+11	-	8M/8F
5	ZF-R b	7.99E+11	-	8M/8F

* Animals had mock surgery and did not receive ZF-TF or Vehicle
** 50 mg/kg given one hour before assessment on each assessment day
Note: SNI model was generated in C57BL/6 mice

Human ZF-R *In Vitro* Specificity

Assessing ZF-R effect on SCN9A gene in Human primary neurons

- High specificity to Nav1.7 and no effect on other channels
- No off-target demonstrating selectivity

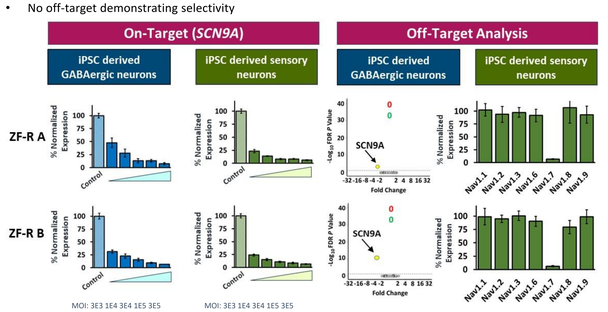


Figure 4. In vitro on- and off-target activity of human ZF-Rs in human IPSC derived GABAergic and sensory neurons. ZF-Rs repressed SCN9A gene specifically with no effect on other Nav channels.

ZF-Rs Repressed Scn9a in DRGs

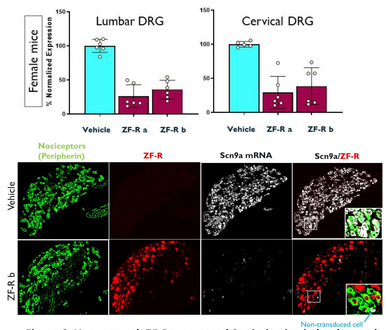


Figure 6. Upper panel: ZF-Rs repressed Scn9a in vivo in lumbar and Cervical DRGs (RT-qPCR) by 60-70%; Lower panel: Single cell analysis of lumbar DRGs for ZF-R b.

ZF-Rs Rescued Pain Phenotype

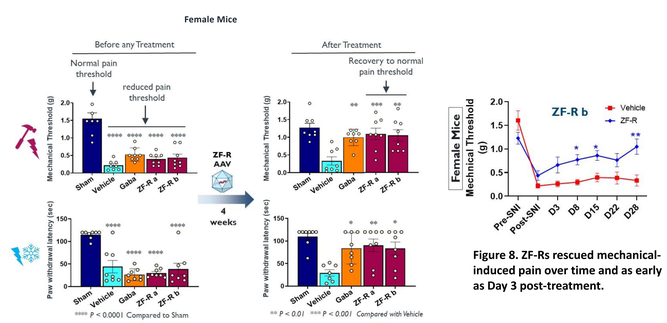
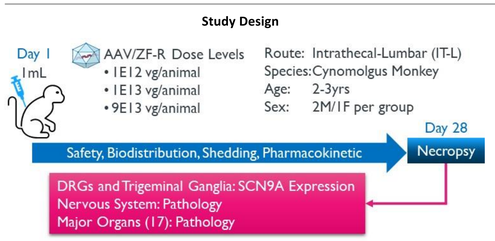


Figure 7. ZF-Rs rescued mechanical-induced (upper panel) and cold-induced pain (Lower panel) to the normal levels after one month.

Figure 8. ZF-Rs rescued mechanical-induced pain over time and as early as Day 3 post-treatment.

A One-Month Dose Range-Finding Toxicology Study in Cynomolgus Monkeys

Study Design



Safety Assessment

- All toxicological parameters (clinical observations, clinical pathology, body weight, organ weight, and necropsy observations) appeared to be normal for all groups
- Only pathology findings were noted

Pathology Assessment

Affected Tissues	Findings & Score
All evaluated tissues were normal except for the following tissues: • DRGs (S, L, T, C) • Spinal cord (L, T, C) • Sciatic nerve • Trigeminal ganglia	•Minimal mononuclear cell infiltration oDue to inflammatory response and recruitment of lymphocytes and monocytes into the tissue •Minimal axonal degeneration •Minimal single neuronal degeneration/necrosis

Scoring System

Grade	Percent of Tissue Affected
Normal	0
Minimal	<5%
Mild	5-20%
Moderate	20-40%
Marked	>50%

S = Sacral; L= Lumbar; T = Thoracic; C = Cervical; DRGs = Dorsal Root Ganglia

ZF-R A Repressed SCN9A in DRGs

- Clinical Candidate ZFR repressed SCN9A by 40-60%
- Similar data was obtained in Sacral DRGs and Trigeminal Ganglia
- Multiple DRGs were evaluated per level per animal

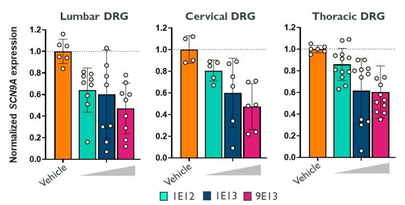


Figure 8. ZF-R A (clinical Candidate) reduced SCN9A in lumbar cervical and thoracic DRGs one month post-treatment.

Anti-AAV Antibody Measurements

- Anti-AAV antibody formation in CSF and plasma had no impact on safety or repression of SCN9A

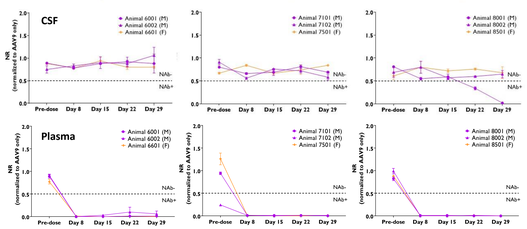


Figure 9. Anti-AAV antibody formation in CSF (upper panel) and Plasma (lower panel).

Overall Conclusion

***In Vitro* Studies**

- Mouse Surrogate and Human ZF-Rs repressed gene encoding Nav 1.7 sodium channel selectively with no off-target activity
- Human ZF-Rs specifically repressed the gene encoding Nav 1.7 with no impact on other Nav Channels

Efficacy Study in SNI Mouse Model

- Mouse ZF-Rs were well tolerated in the SNI model
- Mouse ZF-Rs repressed *Scn9a* (Nav1.7) gene by 60-70% in lumbar and cervical DRGs. Lower repression was observed in thoracic DRGs
- At single cell level, mouse ZF-Rs are demonstrated to significantly reduce *Scn9a* (Nav1.7) gene
- Mouse ZF-Rs rescued pain phenotype in SNI mouse model (gold standard) back to normal threshold for both mechanical and cold induced pain as early as Day 3 post-treatment

Clinical Candidate ZF-R (Human ZF-R) in a One-Month Dose Range Finding Study in Monkeys

- The clinical candidate was well tolerated at doses up to 9E13 vg/animal
- There were no adverse findings in body weight, clinical observations, clinical pathology, necropsy, or organ weights
- No adverse pathology findings were found in 17 peripheral tissues, and in brain and olfactory bulb
- Pathology findings of minimal severity were noted in DRGs, spinal cord, and trigeminal ganglia but these findings were not dose-limiting
- The clinical candidate reduced *SN9A* (Nav1.7) gene expression in DRGS of the monkeys by 40-60% and similar findings were noted in trigeminal ganglia (data not shown)
- Anti-AAV antibody formation in CSF and plasma of the treated animals did not have any impact on safety or repression of *SCN9A* gene
- Based on this data, a definitive toxicology study initiated to support the IND
- Planning to file IND in 2024

Disclosures

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