PMP22 Gene Repression Mediated by Zinc Finger Repressors (ZFRs) as a Therapeutic Approach for CMT1A

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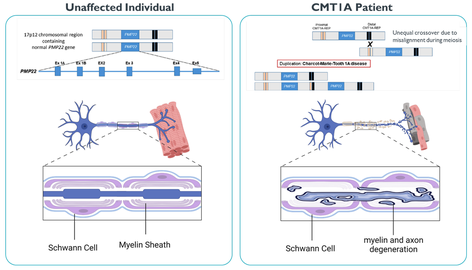
Introduction

- Charcot-Marie-Tooth Type 1A (CMT1A) is a disease of the peripheral nerves involved in muscle control.
- CMT1A is a subtype of CMT and accounts for 66% of all patients with CMT (cmtusa.org).
- The prevalence is estimated to be 24,000-80,000 patients in the United States.
- CMT1A has an early onset, with symptoms appearing in children and young adults.
- CMT1A is characterized by:
  - slowly progressing muscle weakness and atrophy
  - loss of reflexes, sensations
  - reduced nerve conduction velocity

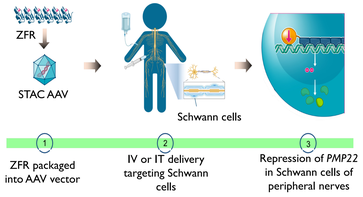
Duplication of Peripheral Myelin Protein 22 (PMP22) causes demyelination of the peripheral nerves in CMT1A



PMP22 in an integral myelin protein expressed in Schwann cells within the peripheral nervous system (Snipes et al. 1992). Schwann cells function to myelinate axonal projections in developing and mature peripheral nerves. Overexpression of PMP22 results in CMT1A pathology due to the heterozygous duplication of chromosome 17p11.2-12 (Roa et al. 1993; Patel et al. 1992). Increased levels of PMP22 results in demyelination and neurodegeneration of peripheral nerves driving the clinical features of CMT1A.

ZFR + AAV as a potential approach for treating CMT1A

ZFR technology, packaged in AAV, could potentially be used to reduce *PMP22* expression in Schwann cells.



PMP22 ZFRs exhibit on-target activity in a rat Schwann cell line

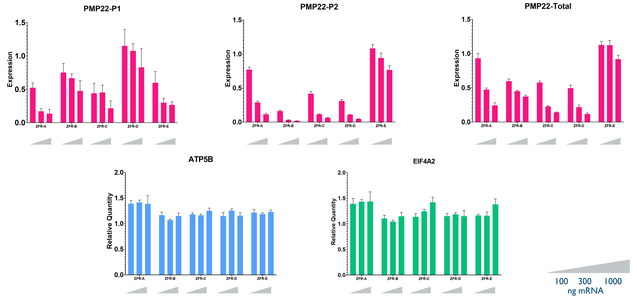


Figure 1: Screening of ZFRs in S16 cells.
A subset of conserved *PMP22*-targeting ZFRs were screened in rat primary Schwann cells, (S16) and assessed ~20 hours after nucleofection. These experiments successfully identified ZFRs that repressed the expression of P1, P2, or total *Pmp22* transcripts. Housekeeping genes (*Atp5b* and *Eif42a*) were used as an internal control for cellular health and viability.

PMP22 ZFRs repress critical PMP22 transcripts in human CMT1A patient-derived fibroblasts

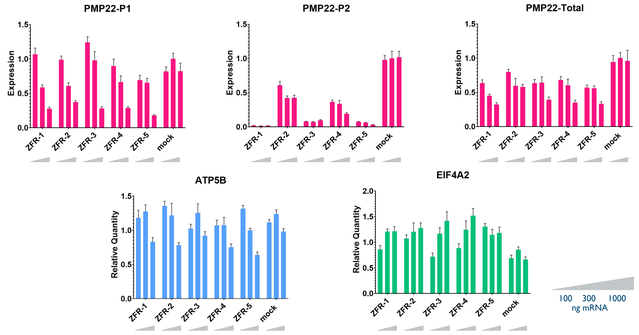


Figure 2: Screening of a subset of PMP22 ZFRs in CMT1A human patient fibroblasts
CMT1A human patient fibroblasts nucleofected with *PMP22*-targeting ZFRs were assessed ~20 hours after nucleofection. Select ZFRs demonstrate greater than 50% repression of total *PMP22* transcript including two critical transcripts (P1 and P2) expressed in myelinating Schwann cells within peripheral nerves. Housekeeping genes (*ATP5B* and *EIF4A2*) were used an internal control for cellular health and viability.

AAV vector-mediated targeting of Sox10+ Schwann Cells *in vivo*

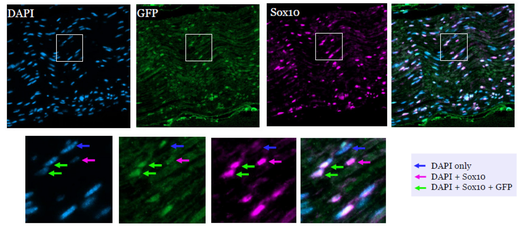


Figure 3: Proof of concept for AAV-mediated transgene delivery to Schwann cells. Adult mice were dosed with intravenous administration of PHP.B serotype vector containing a CAG promoter and expressing an EGFP transgene. 15 days post-administration immunofluorescent staining was performed in PNS tissue to identify GFP and the Schwann cell marker Sox10.

Conclusions and Next Steps

- We have identified robust human *PMP22*-targeting ZFRs and confirmed the ability to target Schwann cells with a BBB-penetrant AAV capsid.
- For next steps, we seek to identify Schwann cell-specific regulatory elements to drive high levels of ZFR in the target cell type.
- In addition, we plan to package select ZFRs into AAV vector and determine "off-target" differential gene expression in top constructs from a patient-derived cell line screen.
- Finally, in a separate study we hope to determine the tropism profile of internally-developed, novel AAV capsids using single-cell analysis of Schwann cells.

References

Boutary S, Echaniz-Laguna A, Adams D, et al. Treating PMP22 gene duplication-related Charcot-Marie-Tooth disease: the past, the present and the future. Transl Res. 2021;227:100-111. doi:10.1016/j.trsl.2020.07.006

Patel PI, Roa BB, Welcher AA, et al. The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. Nat Genet. 1992;1(3):159-165. doi:10.1038/ng0692-159

Roa BB, Garcia CA, Suter U, et al. Charcot-Marie-Tooth disease type 1A. Association with a spontaneous point mutation in the PMP22 gene. N Engl J Med. 1993;329(2):96-101. doi:10.1056/NEJM199307083290205

Snipes GJ, Suter U, Welcher AA, Shooter EM. Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). J Cell Biol. 1992;117(1):225-238. doi:10.1083/jcb.117.1.225

Acknowledgments

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Disclosures

All listed Sangamo authors are current or former employees of Sangamo Therapeutics.

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