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Commentary

New perspectives for gene therapy of the X-linked form of Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disease is a genetically and clinically heterogeneous disorder characterized by distinct patterns of muscle weakness and sensory loss.[1] It is a disease with no effective treatment. In 1993,[2] the identification of *GJB1* gene mutations in patients with CMT established a rationale for molecular therapy of the X-linked form (CMT1X) of the disease, which accounts for 10–15% of all CMT cases, ranking it the second most common subtype. Despite numerous studies, mainly *in vitro*, that focused on the dysfunctionality of the connexin 32 protein (Cx32) encoded by the *GJB1* gene, the molecular pathogenesis of CMT1X still eludes. Some patients who lack the entire coding region of Cx32, or those expressing electrically conductive Cx32 channels, exhibited a severe clinical CMT1X phenotype. This suggests that most mutations cause loss of function, which led to gene replacement therapy testing in mutant mice. Such testing is possible provided that the fundamental role of Cx32 in the peripheral nervous system (PNS) remains unknown.[3]

The work by Dr. Kleopas Kleopa first demonstrated that a single lumbar intrathecal injection of a lentiviral vector carrying the *GJB1* gene can significantly improve the demyelinating neuropathy of Cx32-null mice when delivered at early and later stages of the disease.[4,5] His latest work,[6] recently published in *Molecular Therapy - Methods & Clinical Development*, culminates a decade of research on this therapeutic approach (Figure 1). To provide a mechanistic and clinically relevant proof of concept, the expression of wild-type (WT) Cx32 only targeted Schwann cells under the control of the myelin protein zero (Mpz) promoter. To examine its effectiveness in CMT1X patients expressing different Cx32 mutants, Dr. Kleopa treated transgenic mice expressing endoplasmic reticulum (ER)-retained (T55I) and Golgi-retained (R75W and N175D) Cx32 mutations in a Cx32-null background. In the first experiments,[7] only the T55I mutant showed improved motor and histological features, confirming that an interaction between the delivered WT and the endogenous mutant Cx32 could interfere with the correct targeting of the WT isoform.[8] To overcome low levels of lentiviral-derived expression of Cx32 in the PNS and the mutagenic risk of genome integration, a single lumbar intrathecal injection of an adeno-associated viral vector (AAV9) carrying WT Cx32 under the Mpz promoter was tested in Cx32-null mice.[9] Since these AAV vectors remain in episomal form, they evade regulation by the host's transcription machinery, resulting in higher expression levels. Both pre- and post-onset treatment lead to improved motor performance, sciatic nerve conduction velocities, and Schwann cell myelination, as well as reduced inflammation in the PNS. The latest work[6] applied the same approach to demonstrate the partial rescue of two previously studied Cx32 mutant mice (Golgi-retained R75W and N175D). The widespread expression of WT Cx32 correlated with improved functional and morphological outcomes that are characteristic of this demyelinating neuropathy. Furthermore, the rescue was effective in both R75W and N175D mutant lines, irrespective of whether the injection was administered before or after the neuropathy onset, with early intervention demonstrating greater efficacy. The work suggests that AAV-mediated expression of WT Cx32 can overcome possible interfering effects of Golgi-retained mutants previously observed with lentiviral expression. Interestingly, data show that the therapeutic effects of AAV9-mediated gene replacement in the PNS of CMT1X mutant mice can persist for up to 6 months following a single injection.

Overall, Kleopas's work has provided a proof of concept for a translatable gene therapy approach to treat CMT1X patients irrespective of their loss-of-function mutation. The gene replacement approach may be ineffective or even toxic with the gain-of-function mutations of CMT1X, which underscores the ongoing need for a deeper understanding of the disease's molecular pathophysiology.[3] In an era of precision medicine, the importance of stratifying CMT1X patients for the potential success of future clinical trials with AAV vectors is a critical issue. AAV vectors are currently favored in pre-clinical studies due to their tissue tropism, relatively low immunogenicity, and prolonged expression of the transduced gene.[10] Moreover, considering their status as the predominant viral gene delivery system in ongoing clinical trials, their use in pre-clinical studies holds significant relevance. In this instance, the addition of the Mpz promoter to restrict the expression of WT Cx32 to myelinating Schwann cells[6] may greatly improve the safety of the viral approach in the treatment of CMT1X.

Despite significant progress, particularly in the last decade for conditions like spinal muscular atrophy (SMA) and hemophilia A and B, challenges persist in the use of AAV vectors for human gene therapy. A clinical trial for the X-linked myotubular myopathy (XLMTM) stopped when two of its participants, who received the higher dose of an AAV8 vector, suffered from progressive liver disease resulting in fatal consequences.[11] In

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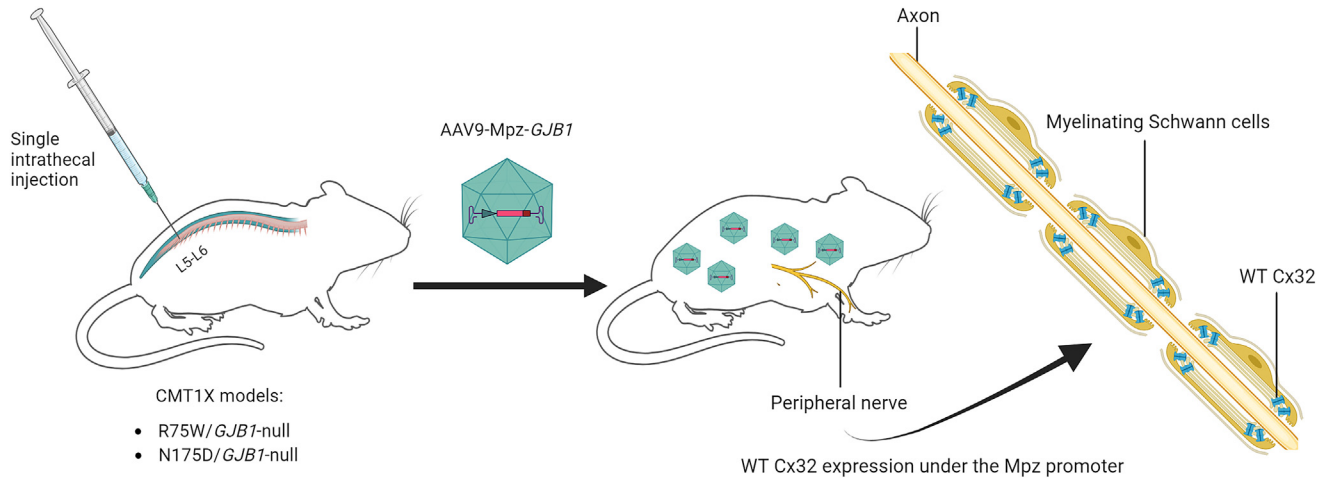

Figure 1. Expression of WT Cx32 in myelinating Schwann cells of selected CMT1X mouse models through a single intrathecal injection of an AAV9 vector
Images were created with BioRender.com.

another trial employing an AAV9 vector for the treatment of SMA, about a third of the recipients experienced liver damage associated with inflammation.[12] However, all of them recovered after receiving treatment. Assessing the toxicity of AAV vectors is crucial and should take into consideration specific diseases, delivery methods, target tissues, and the transgene of interest. In this context, new recombinant AAVs (rAAVs) hold promise to overcome current limitations.

We are witnessing an exciting era in the field of gene therapy. AAV and lentiviral vectors, together with chimeric antigen receptor T cell (CAR-T immunotherapy)- and CRISPR/Cas9-based strategies, paint a promising future. However, a substantial amount of work still lies ahead. The significant increase in clinical holds[13] emphasizes the need for diverse enhancements, with a particular focus on dose refinement, among others. Finally, in the case of CMT1X, a non-life-threatening disease, a risk-benefit assessment should guide the application of this kind of precision medicine treatment.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Bolino, A., and D'Antonio, M. (2023). Recent advances in the treatment of Charcot-Marie-Tooth neuropathies. J. Peripher. Nerv. Syst. *28*, 134–149.
2. Bergoffen, J., Scherer, S.S., Wang, S., Scott, M.O., Bone, L.J., Paul, D.L., Chen, K., Lensch, M.W., Chance, P.F., and Fischbeck, K.H. (1993). Connexin mutations in X-linked Charcot-Marie-Tooth disease. Science *262*, 2039–2042.
3. Bortolozzi, M. (2018). What's the Function of Connexin 32 in the Peripheral Nervous System? Front. Mol. Neurosci. *11*, 227.
4. Kagiava, A., Richter, J., Tryfonos, C., Karaiskos, C., Heslegrave, A.J., Sargiannidou, I., Rossor, A.M., Zetterberg, H., Reilly, M.M., Christodoulou, C., and Kleopa, K.A. (2019). Gene replacement therapy after neuropathy onset provides therapeutic benefit in a model of CMT1X. Hum. Mol. Genet. *28*, 3528–3542.
5. Kagiava, A., Sargiannidou, I., Theophilidis, G., Karaiskos, C., Richter, J., Bashiardes, S., Schiza, N., Nearchou, M., Christodoulou, C., Scherer, S.S., and Kleopa, K.A. (2016). Intrathecal gene therapy rescues a model of demyelinating peripheral neuropathy. Proc. Natl. Acad. Sci. USA *113*, E2421–E2429.
6. Kagiava, A., Karaiskos, C., Lapathitis, G., Heslegrave, A., Sargiannidou, I., Zetterberg, H., Bosch, A., and Kleopa, K.A. (2023). Gene replacement therapy in two Golgi-retained CMT1X mutants before and after the onset of demyelinating neuropathy. Molecular therapy. Mol. Ther. Methods Clin. Dev. *30*, 377–393.
7. Kagiava, A., Karaiskos, C., Richter, J., Tryfonos, C., Lapathitis, G., Sargiannidou, I., Christodoulou, C., and Kleopa, K.A. (2018). Intrathecal gene therapy in mouse models expressing CMT1X mutations. Hum. Mol. Genet. *27*, 1460–1473.
8. Kyriakoudi, S., Sargiannidou, I., Kagiava, A., Olympiou, M., and Kleopa, K.A. (2017). Golgi-retained Cx32 mutants interfere with gene addition therapy for CMT1X. Hum. Mol. Genet. *26*, 1622–1633.
9. Kagiava, A., Karaiskos, C., Richter, J., Tryfonos, C., Jennings, M.J., Heslegrave, A.J., Sargiannidou, I., Stavrou, M., Zetterberg, H., Reilly, M.M., et al. (2021). AAV9-mediated Schwann cell-targeted gene therapy rescues a model of demyelinating neuropathy. Gene Ther. *28*, 659–675.
10. Pupo, A., Fernández, A., Low, S.H., François, A., Suárez-Amarán, L., and Samulski, R.J. (2022). AAV vectors: The Rubik's cube of human gene therapy. Mol. Ther. *30*, 3515–3541.
11. Mendell, J.R., Al-Zaidy, S.A., Rodino-Klapac, L.R., Goodspeed, K., Gray, S.J., Kay, C.N., Boye, S.L., Boye, S.E., George, L.A., Salabarria, S., et al. (2021). Current Clinical Applications of In Vivo Gene Therapy with AAVs. Mol. Ther. *29*, 464–488.
12. Ertl, H.C.J. (2022). Immunogenicity and toxicity of AAV gene therapy. Front. Immunol. *13*, 975803.
13. Wills, C.A., Drago, D., and Pietrusko, R.G. (2023). Clinical holds for cell and gene therapy trials: Risks, impact, and lessons learned. Mol. Ther. Methods Clin. Dev. *31*, 101125.