Targeting muscle to treat Charcot-Marie-Tooth disease

David Villarroel-Campos, James N. Sleigh*

Charcot-Marie-Tooth disease (CMT) is a hereditary peripheral neuropathy causing muscle weakness/wasting and sensory dysfunction predominantly in limb extremities. CMT patients display gait abnormalities, foot deformities, loss of sensation, and decreased/absent deep tendon reflexes, with motor symptoms usually being more prominent than sensory. Resulting from > 1500 different mutations across > 100 diverse genes, CMT affects 1 in <2500 people and is inherited in an autosomal recessive, autosomal dominant or X-linked fashion. Based on assessment of nerve conduction velocity, CMT is divided into Type 1/demyelinating CMT, in which perturbed Schwann cell homeostasis affects saltatory conduction and reduces nerve conduction velocity, and Type 2/axonal CMT, where motor and sensory axons are lost without affecting nerve conduction velocity. There are also intermediate forms of CMT that show features of demyelinating and axonal neuropathies, including intermediate nerve conduction velocity values.

CMT usually manifests during adolescence without curtailing lifespan, thus, the disease causes lifelong disability and a significant economic burden. It is therefore critical that the current lack of approved CMT treatments is rapidly improved upon. Fortunately, a plethora of promising therapeutics, including many gene therapies that address the genetic causes of CMT, have proven effective in preclinical CMT models (Bolino and D’Antonio, 2023). Given that Schwann cells and peripheral nerves are the primary sites of pathology in CMT, the focus of many preclinical treatments is designed to selectively localize to or target disease pathways in these cell types. Recently, however, several encouraging preclinical approaches for CMT have been directed to the third major component of the neuromuscular system: muscle. Here, we briefly review these therapeutics (Figure 1), highlighting the value of using muscle as a source for systemic neurotrophic factors, touching upon non-cell autonomous pathomechanisms, and discussing the opportunity for combinatorial approaches to treat CMT.

Neurotrophins are a family of secreted neurotrophic factors that support neuronal development, differentiation, and survival by selectively interacting with different tropomyosin receptor kinase (Trk) receptors: nerve growth factor (NGF) signals through TrkA, brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) via TrkB, and NT-3 through TrkC. Given its role in Schwann cell maintenance and peripheral nerve regeneration, NT-3 was identified by Zarife Sahenk and colleagues as a potential therapeutic protein for several different CMT subtypes, beginning with the most common form of genetic peripheral neuropathy, CMT1A. CMT1A is caused by duplications in the gene encoding the essential myelin protein, peripheral myelin protein 22 (PMP22) (PMP22). Initially, repeated subcutaneous injections (three times per week for eight weeks) of recombinant NT-3, but not BDNF, were shown to enhance nerve regeneration and myelination in immunodeficient mice hosting CMT1A patient sural nerve xenografts. A similar positive effect of NT-3 treatment was also shown post-nurse crush in Trembler mice, which carry a point mutation in endogenous Pmp22 (Sahenk et al., 2005). A double-blind, randomized, pilot clinical trial of subcutaneous NT-3 injections (three times per week for 24 weeks) was then performed in eight CMT1A patients (four receiving placebo and four receiving NT-3). Significant improvements in sural nerve regeneration, Neuropathy Impairment Score and sensory function were detected in patients treated with NT-3, but not with placebo.

To circumvent issues of short serum half-life and the need for repeated injections, NT-3 cDNA was packaged into the muscle-tropic adeno-associated virus (AAV) serotype 1 under the control of either the constitutive CMV promoter (AAV1.CMV.NT3) or the muscle-specific tMCK promoter (AAV1.tMCK.NT3) (Sahenk et al., 2014). NT-3 is usually expressed by Schwann cells and acts in an autocrine fashion; however, targeting AAV to Schwann cells is difficult due to the lack of appropriate capsids, the requirement of crossing the blood-brain barrier and the need to transduce many myelinating cells. Consequently, muscle was chosen as the AAV target, as it can act as a secretory organ for systemic and long-lasting NT-3 distribution, which was confirmed in plasma for both AAV1.CMV.NT3 and AAV1.tMCK.NT3 vectors. The gene therapies were then individually and unilaterally injected into the gastrocnemius or quadriceps muscles of Trembler mice 3 weeks prior to sciatic nerve crush. NT-3 treatment improved myelinated nerve and Schwann cell density, neurofilament cytoskeleton integrity, neurophysiological parameters, and motor function (grip strength and rotarod), with greater effects observed on hindlimb independent function (40 > 20 weeks). In a separate study not involving nerve crush, both AAV1.CMV.NT3 and AAV1.tMCK.NT3 were also shown to restore muscle fiber cross-sectional area and fiber type proportions in gastrocnemius muscles of Trembler mice 16 weeks post-treatment (Yalvac et al., 2018); this suggests that in addition to its systemic impact, NT-3 may also directly affect muscles. The CMV and tMCK promoters drive similar levels of transgene expression and equally affect nerve regeneration (Sahenk et al., 2014); thus, the use of CMV is now being prioritized to reduce off-target NT-3 expression. Indeed, a Phase 1/2a clinical trial of multiple, single AAV1.tMCK.NT3 injections into lower leg muscles of CMT1A patients, with safety as the primary outcome, is planned; however, the trial is currently suspended due to vector production issues (clinical trial identifier: NCT03520751).

AAV1.tMCK.NT3 has also proven effective in mouse models of two additional CMT subtypes: CMTX1 and CMTX2. CMTX1 is an X-linked form of neuropathy, is the second most prevalent genetic lesion. CMTX2 is caused by duplications in the gene encoding the housekeeping enzyme glycyl-tRNA synthetase (GlyRS) that charges the glycyl-tRNA for several different CMT subtypes, and thus a promising strategy for the treatment of neuropathy across major CMT subtypes, and is the second most prevalent CMT and is caused by deleterious mutations in the gene encoding Gap Junction Protein Beta-1/Connexin32 (GJB1/Cx32). GJB1 is a membrane-spanning protein that forms channels to enable ion and small molecule transfer between cells, including through the myelin sheath. CMT2D results from dominantly inherited mutations in GARS, which encodes the housekeeping enzyme glycyl-tRNA synthetase (GlyRS) that charges the amino acid glycine to its cognate tRNA for protein synthesis. Following a similar paradigm of single, unilateral injections into the gastrocnemius at 3 months of age, AAV1.1MCK.NT3 was shown to preserve neurophysiology measurements, axon number and morphology, myelination, muscle fiber size and motor function of homozygous Gars knockout mice (Ozes et al., 2021). In a slight variation in approach, Gars and Gars mice modeling CMT2D received bilateral injections of AAV1.1MCK.NT3 at 4–6 and 8–10 weeks, respectively (Ozes et al., 2021). 12 weeks post-treatment, NT-3 improved neurophysiological parameters, neuromuscular junction innervation, myelin thickness, muscle fiber health and rotarod performance in Gars mice, while myelin thickness and myofibers were also improved in Gars mice, albeit to a lesser extent. Together, studies from the Sahenk Laboratory indicate that muscle-specific NT-3 gene therapy can improve neuropathy across major CMT subtypes, and is thus a promising strategy for the treatment of CMT independently from the disease-causing genetic lesion.

A similar strategy of using muscle as an injection site for systemic therapy delivery has been employed for CMT2A, which is caused by dominantly inherited mutations in mitofusin 2 (MFN2) and is the most common form of axonal CMT found in the outer mitochondria. MFN2 is crucial for the fusion of mitochondria and thus for their dynamics, distribution and

Figure 1 Pre-clinical gene therapies targeting muscle in CMT.
Perhaps surprisingly, NT-3 and VEGF CMT2D muscles and showed that this acutely impaired neurotrophic signaling endosomes in CMT2D axonal transport of neurotrophic signaling endosomes, contributing to neurotrophic impairment (He et al., 2015). GlyRS is secreted from several different cell types, including muscles, suggesting that antagonisation of the VEGF
neuropilin-1 interaction is non-cell autonomous in origin. To counteract this, VEGF
was overexpressed bilaterally in a model of muscle-specific, newborn Gars mice via injection of lentiviral vectors (LV). Treatment with LV-VEGF
resulted in improved neurotrophic function as shown by an inclined plane test at 4 weeks, as well as rotarod and gait analysis at 7 weeks. Gars mice were then subjected to bilateral LV-VEGF injections versus LV-GFP control, followed by a leg extension test at 5 weeks. Muscle weakness was more prominent in the control-injected leg, suggesting that VEGF
functions locally in the injected muscles rather than having a systemic impact like NT-3.

We recently determined that a third neurotrophic factor, BDNF, is also a promising therapeutic for CMT2D mice and not neurons in the contralateral sciatic nerve. It remains to be determined whether AAV-
BDNF corrects additional features of CMT2D and neuromuscular junction dysfunction and morphology.

Conclusions: While most of the muscle-directed treatment strategies discussed above do not address the genetic cause of neuropathy, they can perhaps serve as complementary therapeutics that are delivered as part of a combinatorial approach; this has proven beneficial in preclinical models of other neuromuscular diseases and is currently being trialed in spinal muscular atrophy (e.g., NCT03921528). Moreover, mutation-independent therapeutics have the considerable benefit that they may treat several different CMT subtypes, as has already been shown for NT-3. It is therefore not surprising that similar growth factor-related strategies have been tested in clinical trials. For instance, Engensis (VM202) is a hepatocyte growth factor-expressing DNA plasmid that was repeatedly injected into lower limb muscles of CMT1A patients in a Phase 1/2a trial in 2021 (NCT04613031; outcome unreported), and CLZ-2002 is an allogeneic mesenchymal stem cell treatment in which Schwann cell-like cells capable of increasing neurotrophic factor availability are being injected into muscles of CMT1A patients in a Phase 1 trial (NCT05947578; results due in late 2024). Alternatively, it is possible to direct gene therapies to muscle for introducing viral vectors to select central and peripheral nerves and/or to muscle (Tosolini and Sleigh, 2020), as are muscle-protective strategies, such as inhibiting negative regulators of skeletal muscle, which has been shown to enhance total muscle volume when injected into the tibialis anterior of CMT1 and CMTX patients (Thomas et al., 2022). In summary, treating muscle in CMT appears to be a viable strategy to preserve neurotrophic function in several different subtypes of the disease – either by creating a secretory hub for systemic therapy delivery or by directly targeting for local benefits on the neuromuscular system.

This work was supported by the funding from the Medical Research Council (MR/S006990/1), the Wellcome Trust (101319/A/13/2), the Rosetrees Trust (M806) and the UCL Neurogenetic Therapies Programme funded by The Sigrid Rausing Trust.

JNS is a named inventor on patent GB2303495.2 (patent applicant, UCL Business Ltd., status pending), which describes AAV-BDNF technology for treatment of CMT.

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