Experimental Therapeutics in Hereditary Neuropathies: The Past, the Present, and the Future

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Summary: Hereditary neuropathies represent approximately 40% of undiagnosed neuropathies in a tertiary clinic setting. The Charcot–Marie–Tooth neuropathies (CMT) are the most common. Mutations in more than 40 genes have been identified to date in CMT. Approximately 50% of CMT cases are accounted for by CMT type 1A, due to a duplication within the peripheral myelin protein 22 gene (*PMP22*). Mutations in the gap junction beta 1 gene (*GJB1*), the myelin protein zero gene (*MPZ*), and the mitofusin 2 gene (*MFN2*) account for a substantial proportion of other genetically definable CMT. Some 15% of demyelinating CMT

and 70% of axonal CMT await genetic clarification. Other hereditary neuropathies include the hereditary sensory and autonomic neuropathies, the familial amyloid polyneuropathies, and multisystem disorders (e.g., lipid storage diseases and inherited ataxias) that have peripheral neuropathy as a major or minor component. This review surveys the challenges of developing effective therapies for hereditary neuropathies in terms of past, present, and future experimental therapeutics in CMT. **Key Words:** Charcot–Marie–Tooth neuropathies, peripheral neuropathy, hereditary neuropathy, outcome measure, clinical trial design, therapy.

INTRODUCTION

Hereditary neuropathies represent approximately 40% of undiagnosed neuropathies in a tertiary clinic setting.¹ The most common hereditary neuropathies are the Charcot–Marie–Tooth neuropathies (CMT). Mutations in more than 40 genes have been identified to date in CMT (see, for example, <http://www.molgen.ua.ac.be/CMTMutations>). Among CMT, approximately 50% are accounted for by CMT type 1A (CMT1A), due to a duplication within the peripheral myelin protein 22 gene (*PMP22*).^{2,3} Mutations in the gap junction beta 1 gene (*GJB1*), the myelin protein zero gene (*MPZ*), and the mitofusin 2 gene (*MFN2*) account for a substantial proportion of other genetically-definable CMT.^{2,3} Some 15% of demyelinating CMT and 70% of axonal CMT await genetic clarification. Other hereditary neuropathies include the hereditary sensory and autonomic neuropathies, the familial amyloid polyneuropathies, and multisystem disorders (e.g., lipid storage diseases and inherited ataxias) that have peripheral neuropathy as a major or minor component. Major categories of hereditary neuropathies and any known associated gene defects are detailed in an online database resource

(<http://www.molgen.ua.ac.be/CMTMutations>). The present review illustrates the challenges of developing effective therapies for hereditary neuropathies, via a focus on past, present, and future experimental therapeutics in CMT.

REVIEW OF CURRENT TREATMENT OPTIONS FOR CMT AND PAST THERAPEUTIC TRIALS

Most forms of CMT are characterized by slowly progressive symmetric distal limb weakness, atrophy, foot deformity, and sensory loss.^{2–4} Particular genetic causes of CMT may also lead to hearing, respiratory, vocal cord, or autonomic impairment. The age of symptom onset and the degree of associated disability range widely, from a childhood requirement for a wheelchair to mild impairment of gait and balance in later life.

No disease-modifying therapies have yet been discovered for CMT. Supportive care includes exercise, physical and occupational therapy, and orthotics designed to address foot drop and stabilize gait.⁴ Surgery intended to maintain function of primarily the distal lower limbs is available. Analgesic agents are used for neuropathic and mechanical pain.⁴

What is the evidence that supports these interventions? A Cochrane Collaboration analysis of randomized trials of surgical, physical therapy, and orthotic interventions for foot drop in neuromuscular disorders found one study of

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CMT that met the review criteria.⁵ In this study of 29 CMT subjects, a 24-week muscle strengthening and exercise program resulted in improvement in a 6-meter timed walk, with trends favoring the treatment in other outcomes including quality of life (QOL), and stair climbing.⁵ A randomized, single-blind, cross-over study of nocturnal ankle splinting in CMT1A ($n = 14$) showed no effect on ankle range of motion or strength.⁶ Systematic studies on the effect of orthotics on gait and falls in CMT are relatively lacking, despite widespread use. Retrospective studies of arthrodesis surgery have reached divergent conclusions in CMT.^{7,8} In one study of 16 subjects with an average of 21 years follow-up, 14 of 30 arthrodesis sites had a poor outcome; the authors concluded that triple arthrodesis should be used only as salvage therapy for advanced CMT and severe rigid deformity.⁷ In contrast, in a study of 57 subjects (with 25- and 40-year follow-up), 95% of subjects were satisfied with their surgical interventions.⁸ Prospective studies comparing outcomes of conservative care *versus* early surgery for foot drop in CMT are lacking. There is also a paucity of controlled trials of pain management in CMT. Fatigue is also a significant symptom in CMT, but no randomized, double-blind controlled trials (RCT) of therapies for fatigue have been performed for CMT. In a single open-label study, four subjects receiving modafinil therapy reported improvements in disabling daytime fatigue.⁹

Before the availability of contemporary genetic approaches, few trials aimed to modify the biology of CMT. Intramuscular Cronassial (a ganglioside mixture) (Fidia Pharmaceutical Corporation, Washington, DC) was ineffective in 30 CMT subjects treated in a RCT.¹⁰ A small RCT of linoleic and γ -linoleic essential fatty acids, which were added to a placebo containing vitamin E, was conducted in CMT1 ($n = 20$) and controls. No effect of essential fatty acids was noted at 12 months; however, after a lead-in period of 3 months of vitamin E containing placebo, improvements in neurologic disability measures were seen, which were sustained for 1 year.¹¹ It was unclear whether this improvement reflected a sustained placebo effect, or therapeutic benefit of vitamin E.¹¹ A single, double-blind, placebo-controlled cross-over study of low-dose coenzyme Q10 (CoQ10) (100 mg/day) enrolled patients with several forms of muscular dystrophy, and also clinically defined CMT; benefit was reported among the three CMT subjects included.¹²

DEVELOPMENT OF NOVEL THERAPIES FOR CMT: PATHOGENETIC CONSIDERATIONS

Mechanisms of peripheral nerve damage in CMT are uncertain, but unraveling of the genetic causes of CMT has led to novel therapeutic targets. CMT1A stems from a duplication of a 1.5-Mb domain of the *PMP22* gene, with PMP22 overexpression in peripheral nerves.²⁻⁴

PMP22 overexpressing transgenic mice develop a demyelinating neuropathy, and the animal phenotype depends on the degree of PMP22 expression.¹³ A toxic gain of function mediated by PMP22 overexpression is inferred in CMT1A. PMP22 is important in Schwann cell (SC) differentiation and regulation and in the integrity of compact myelin.²⁻⁴ It influences axon–glial cell interactions and axonal integrity, loss of which is thought to be critical in the progression of CMT1A.²⁻⁴ PMP22 overexpression produces ubiquitinated PMP22 aggregates, impaired proteosomal function, and autophagic profiles in rodent SC cytoplasm.¹⁴ Correction of PMP22 levels in animals leads to improvement of the neuropathy, and is a rational strategy for CMT1A.¹⁵

Hereditary neuropathy with liability to pressure palsies (HNPP) is usually due to a *PMP22* gene deletion and haploinsufficiency.²⁻⁴ Therapeutic downregulation of PMP22 in CMT1A, if excessive, could provoke a HNPP phenotype, whereas upregulation may be effective for HNPP.³ An alternative approach for CMT1A, which requires preclinical testing, would be one that targets PMP22 aggregates, on the hypothesis that the aggregates cause proteosomal impairment and SC toxicity.^{3,14} Trophic factor deficits (e.g., ciliary neurotrophic factor) have been found in rodent models and CMT1A nerves, suggesting that trophic factor replacement may be efficacious.¹⁶ Animal models of CMT1A suggest that inflammatory responses influence demyelination. Small subsets of patients with CMT1A develop acute worsening, with T-cell infiltrates in nerve biopsies, and respond to immunotherapy.¹⁷⁻¹⁹ It has not been shown, however, that anti-inflammatory therapies influence animal models or the course of most patients with CMT1A.²⁰

CMTX is usually due to mutations of the *GJB1* gene, which encodes the connexin 32 (Cx32) gap junction protein, located in noncompact myelin, and accounts for 15% of CMT.^{2-4,20-22} More than 300 pathogenic *GJB1* mutations have been described (<http://www.molgen.ua.ac.be/CMTMutations>). Inheritance is X-linked dominant. Most males develop CMT with intermediate range conduction velocity slowing on nerve conduction studies, whereas females have normal or minimally reduced conduction velocities consistent with axonal CMT. Pathologic findings vary, but are primarily axonal in type.^{2-4,20-22} Cx32 has multiple functions, including serving as a conduit for diffusion of ions and molecules between the perinuclear and adaxonal SC cytoplasm, regulating SC gene expression, and playing a role in early axon regeneration.^{3,21,22}

It is disputed whether a relationship exists between the *GJB1* genotype and the human phenotype.^{21,22} For example, a mutation in which the mutant Cx32 failed to reach the SC membrane was found to impair axon regeneration in human sural nerve xenografts and to be nonfunctional *in vitro*. This mutation produced a more severe human phenotype than did a mutation that in-

served within the SC membrane and showed only subtle abnormalities in channel function.²¹ In contrast, a study assessing 73 patients with 28 *GJB1* mutations revealed no mutations that were associated with a phenotype more severe than missense mutations, suggesting a loss of function mechanism.²²

Animal transfection with a *GJB1* gene that expresses in myelinating SCs ameliorates the neuropathy, indicating that replacement of deficient wild-type Cx32 channels may be effective for the human disease.²² An inflammatory component is suspected in Cx32 null mice. Occasional CMTX patients with accelerated weakness have inflammation on biopsy, suggesting superimposed immune neuropathy.¹⁸ Segmental demyelination is not a feature of most descriptions of CMTX, and thus the contribution of inflammation to the human condition is unclear.

Mutations in *MPZ* occur in 5% of CMT. The gene product *MPZ*, a member of the immunoglobulin superfamily, is located in compact myelin and accounts for 50% of PNS myelin protein.²⁻⁴ *MPZ* facilitates myelin compaction. More than 120 disease-causing mutations, located primarily in the coding portion of the gene, have been identified (<http://www.molgen.ua.ac.be/CMTMutations/>). *MPZ* mutations can manifest as several phenotypes: an autosomal dominant CMT, with an early onset severe (congenital hypomyelinating [CHN] or Dejerine Sottas [DSS]) phenotype, a childhood-onset demyelinating phenotype, or a milder adult onset phenotype that shows intra- and interfamilial variability in its severity and its degree of nerve conduction velocity slowing.²³ Phenotype-genotype correlations suggest that a severe early-onset phenotype may be produced by mutations adding a charged amino acid, by mutations that alter a cysteine residue in the extracellular domain, or by mutations truncating the cytoplasmic domain.²⁴

Another important determinant of severity is the system of nonsense-mediated decay of mutant *MPZ* mRNA.²³ Upstream premature stop codon mutations (e.g., nonsense or frameshift mutations) result in degradation of mutant mRNA via nonsense-mediated decay, with a typically mild *MPZ* haploinsufficiency phenotype.²⁴ Downstream mutations predict a truncated protein that escapes nonsense-mediated decay. This results in translation of a mutant *MPZ* protein, which generally produces severe disease, likely via a toxic gain of function. In this situation, mutant *MPZ* protein is retained in the endoplasmic reticulum as aggregates of misfolded protein.²⁴ This may trigger an unfolded protein response, which is a chaperone system that mediates proteasome degradation of aggregates. *MPZ* protein aggregates have in preclinical studies been associated with apoptosis.²⁴ Therapies that target misfolding or aggregation of protein mutants in severe *MPZ* neuropathies may ameliorate the phenotype. Although augmentation of the unfolded protein response-proteasome pathway could be considered as a therapeutic

target, overactivation of this pathway can lead to apoptosis.

Axonal CMT account for approximately 30% of autosomal dominant CMT.²⁻⁴ Of these, 20% are due to mutations in the guanosine triphosphatase outer mitochondrial membrane fusion protein, mitofusin-2 (*MFN2*).^{25,26} More than 50 mutations in *MFN2* have been associated with CMT2A. Some kindreds with hereditary motor sensory neuropathy 5 (HMSN 5) (axonal CMT with pyramidal tract signs) and HMSN6 (axonal CMT and optic atrophy) harbor a mitofusin mutation (<http://www.molgen.ua.ac.be/CMTMutations/>). Cyclic fusion and fission is vital to the maintenance of mitochondria.

Studies suggest diverse mechanisms whereby *MFN2* mutations contribute to axonal degeneration. Dorsal root ganglion cells transfected with each of six *MFN2* mutants demonstrated a marked defect in mitochondrial transport, with abnormal clustering of fragmented mitochondria in the soma and proximal axon.²⁶ In this study, ATP production, oxidative enzyme activity, and mitochondrial membrane potential were normal.²⁶ It was hypothesized that a paucity of mitochondria in distal axons resulted in local energy failure and thus in and dying-back degeneration.²⁶ A further study analyzed cultured skin fibroblasts in four subjects with CMT-2A, and three novel *MFN2* mutations.²⁵ These mutations impaired oxidative phosphorylation, but mitochondrial morphology, enzyme activity, and DNA content remained normal.²⁵ Additional proteins associated with mitochondria include kinesin, which is important in mitochondrial transport, and GDAP-1, a glutathione transferase protein, which has been associated with recessive demyelinating CMT4A and recessive axonal CMT2.⁴ Therapies that address mitochondrial dysfunction may be viable for CMT2A, and for additional rare forms of CMT.

MECHANISM-BASED THERAPIES CURRENTLY UNDER STUDY IN HUMAN CMT TRIALS

Ascorbic acid (CMT1A)

Ascorbic acid (AA) is essential for myelination in Schwann cell-dorsal root ganglion (SC-DRG) cultures, where it is necessary for SC basal lamina formation.²⁷ Passage et al.²⁸ studied AA versus placebo in a C22 mouse model of CMT1A. After 1 month of therapy, all AA-treated mice had arrest of motor decline, or improvement that was sustained while on treatment. The AA-treated mice showed a marked survival benefit and partial correction of the myelination defect.²⁸ Ascorbic acid appeared to exert its effect by downregulation of PMP22 mRNA expression.²⁸ Subsequent studies have suggested that high-dose AA reduces PMP22 mRNA expression in a dose-dependent manner, via decreasing adenylate cy-

class activity and intracellular cAMP levels, thereby inhibiting cAMP stimulation of PMP22 expression.²⁹

Although these preclinical data have yet to be replicated, trials of AA in CMT1A have been initiated. A study in North America aims to enroll 120 subjects (age 13 to 70 years) in a futility design protocol (see below) of 24 months of AA (2 g orally, twice daily) or placebo to determine the impact of AA on progression of CMT1A compared with historical controls and a cohort of placebo-treated subjects (NCT00484510 at <http://www.clinicaltrials.gov>). A secondary aim is to assess whether AA administration affects PMP22 mRNA levels of myelinated nerve fibers in the skin. The primary outcome is a composite clinical and nerve conduction study (NCS) measure, the CMT Neuropathy Score (CMTNS). The neuropathy impairment score (NIS), upper extremity NCS, PMP22 mRNA expression in forearm skin biopsies, and the 36-item Short Form Quality of Life questionnaire (SF36) scale are secondary outcomes.

Another multicenter, parallel-group design RCT in CMT1A of 1.5 g of AA or placebo daily for 2 years is being conducted in Italy.³⁰ This study uses the CMTNS as a primary outcome, and maximal voluntary isometric contraction, overall neuropathy limitations scale, timed 10-meter walk test, nine-hole peg test, visual analog scale for pain and fatigue, SF-36, NCS, and changes in skin biopsy PMP-22 expression as secondary outcomes.³⁰ The study design, with 220 subjects, assumes that placebo-treated subjects will worsen by an average of 1 point on the CMTNS over 2 years and that AA-treated subjects will improve by 0.5 points, to achieve 80% power to detect an effect of AA.³⁰

Coenzyme Q10

Coenzyme Q10 is an electron acceptor in the mitochondrial electron transport chain with antioxidant properties; it has shown a possible beneficial effect in early studies in Parkinson's disease and Huntington's disease. A single small study of low-dose CoQ10 reported benefit among three clinically defined CMT subjects.¹² A double-blind, randomized, placebo-controlled trial of CoQ10 300 mg orally twice daily administered for 60 weeks is currently enrolling, with a target of 46 CMT subjects (NCT00541164 at <http://www.clinicaltrials.gov>). However, this study does not focus enrollment on those forms of CMT in which mitochondrial dysfunction appears to be a fundamental mechanism, nor did the prior small pilot study.

Neurotrophin-3

Studies have suggested a deficit of neurotrophin-3 (NT-3) in CMT1A.³¹ Human mutant SCs harboring PMP-22 duplications fail to support axonal regeneration of nude mice axons in an injury model. Addition of NT-3, but not brain-derived neurotrophic factor (BDNF) partially corrects the defect in axonal regeneration in a human CMT1A xenograft in nude mice.³¹ NT-3 is

known to be important for SC survival and axonal regeneration. A pilot, double-blind, RCT of 150 μ g/kg of r-metHuNT-3 three times a week for 24 weeks or placebo in CMT1A, showed encouraging results, with significant improvement in sensory and reflex subsets (but not motor subsets) of the NIS, and increased density of regenerative clusters and single myelinated nerve fibers on pre- and post-treatment nerve biopsies, compared with placebo-treated subjects.³¹ NT-3 was well tolerated.

THERAPIES IN PRECLINICAL TESTING FOR FORMS OF CMT

Curcumin, a widely available turmeric extract, has shown antioxidant, antiproliferative, anticancer, anti-inflammatory protein folding–misfolding modulatory effects *in vitro* and in animal studies.^{24,32} Curcumin appears well tolerated in humans in doses as high as 12 g/day, and protects against apoptosis in cell cultures expressing *MPZ* or *PMP22* mutations that ordinarily lead to intracellular aggregates and apoptosis.^{24,32} Newborn *Trembler-J* mice with missense *Pmp22* mutations that are associated with severe human CMT1 show marked improvement in their clinical and pathologic phenotype on curcumin therapy.³² Studies in this model indicate that curcumin does not affect PMP22 levels, but rather likely alleviates mutant PMP22-induced SC apoptosis.³² Precisely how curcumin is active in these models is unclear; its effects may variously occur via release of mutant protein from the endoplasmic reticulum, by alleviating sequestration of endoplasmic reticulum chaperones, through reversal of the impairment in cellular protein synthesis and trafficking associated with an unregulated unfolded protein response, or by correction of misfolding of mutant proteins.^{24,32} Curcumin is thus a potential therapy for forms of CMT that are associated with endoplasmic reticulum aggregate formation and protein misfolding.

There are potential barriers to use of curcumin, including concern about bioavailability. Studies in *Trembler-J* mice suggest, however, that (at least in this model) curcumin crosses the blood–brain barrier, and, although measurable plasma levels are not achieved, it is taken up by peripheral nerve.³² Pharmacokinetic modifications of curcumin are being sought to develop a form that maximizes its therapeutic effects while optimizing its bioavailability. Another concern is that, in the *Trembler-J* model, curcumin ameliorates the CMT phenotype only in newborn mice, but not when treatment is initiated in adulthood.³² Explanations for the difference include that higher concentrations of curcumin are achieved in peripheral nerve in the newborn mice, and that curcumin may be most active during brisk myelination and prior to axon loss.³² If the early treatment effect applies to human CMT, it would limit the therapeutic potential of curcumin.

Progesterone is produced by SCs and neurons, and influences myelin gene expression (*PMP22*, *MPZ*, and their transcription factors).^{33,34} Studies in an animal model of CMT1A (5-day-old transgenic mice) have shown that progesterone worsens the clinical and pathologic phenotype and increases sciatic nerve *PMP22* mRNA by approximately 30%.³³ Wild-type mice also show a 25% increase in sciatic nerve *PMP22* mRNA expression. Treatment of 5-day-old CMT1A mice for 7 weeks with the pure progesterone receptor antagonist onapristone improved the clinical and pathological phenotype, and reduced sciatic nerve *PMP22* mRNA levels by 15%.³³ In a subsequent study, adult CMT1A-type mice treated with onapristone showed improvement in the clinical, pathological, and electrophysiological phenotype, with a 20% reduction in *PMP22* mRNA expression in sciatic nerve and skin biopsies.³⁴ Onapristone mediated its effects in these studies via an increase in axon density, although there was no effect upon myelin pathology.³⁴

These studies provide proof of principle for consideration of progesterone antagonists in CMT1A, and also indicate that the effects of SC *PMP22* overexpression on axon integrity are divorced (at least in part) from myelinopathy.³⁴ The results are important, because progesterone antagonists address axon loss, which correlates with disability in CMT1A. There are barriers to use of progesterone antagonists in human CMT1A clinical trials. Onapristone is unsafe in humans (liver toxicity). Mifepristone (RU486), an oral progesterone antagonist and partial agonist, has a better long-term safety record in both males and females, and has been used in meningiomas and leiomyomas for its antiprogesterone effects and has been in trial for Alzheimer's disease. There are no reports on RU486 in animal models of CMT1A, but this may be worthy of investigation. It is unclear, however, whether partial agonists will abrogate any beneficial effects of progesterone antagonism. Additionally, assuming that lifelong therapy would be required, effects of RU486 on endometrial hyperplasia and possibly endometrial carcinogenesis may be limiting. A role for progesterone antagonists in CMT1A awaits development of safer preparations, and then human clinical trials.

Progesterone agonists, for which several safe preparations are in wide human use, may conversely have therapeutic potential for some forms of hereditary neuropathies. In HNPP, with its *PMP22* haplotype insufficiency, and potentially also for *MPZ* mutations associated with a haplotype insufficiency mechanism (in which the mutant *MPZ* allele undergoes mRNA nonsense-mediated decay), upregulation of the normal allele could conceptually improve haploinsufficiency.³³ To date, preclinical studies are lacking to provide proof of concept for consideration of progestins in these neuropathies.

FUTURE THERAPEUTIC STRATEGIES FOR CMT: RNA AND GENE-BASED THERAPIES

Several evolving gene-based technologies hold therapeutic promise in hereditary neuropathies and require study in preclinical CMT models. RNA interference (RNAi) is a naturally occurring process in which small, double-stranded RNA strands (short interfering RNA) target undesired homologous mRNA sequences for destruction, or prevent their translation.^{35,36} This regulates gene and protein expression, and has led to use of exogenous viral-based and non-viral-based RNAi to silence genes in cell lines and in animal models. Potential therapeutic applications of RNAi include silencing of mutant alleles, which lead to a toxic gain of function, or of alternatively spliced m-RNA to regulate expression of protein isoforms.³⁵

Antisense oligonucleotides (ASO) are short, single-stranded RNA or DNA sequences that bind to mRNA via hydrogen bonds and inhibit translation or cause degradation of target mRNA.^{35–37} ASOs can also be used to modify pre-mRNAs in several ways: through blocking of cryptic pre-mRNA splice sites; via blockade of normal splice sites to induce specific exon skipping; or through targeting of splicing inhibitors, to facilitate inclusion of specific exons.³⁵

Morpholino phosphorodiamidate ASOs (morpholinos), which substitute a morpholine for the ribose in the oligonucleotide backbone, and phosphorodiamidate to link nucleotides instead of phosphodiester, hold particular promise because they are quite resistant to nucleases.^{36,37} Morpholinos injected into the tibialis anterior muscle, and directed toward Cl-c1 exon 7a, ameliorated the chloride channelopathy and myotonia in that muscle in a myotonic dystrophy mouse model, and, in a proof-of-concept study, injection of an ASO (PR0051) into the tibialis anterior muscle of four boys with Duchenne muscular dystrophy did safely and successfully produce skipping of exon 51 and reading frame restoration, allowing for translation of a partially functional dystrophin protein.^{38–40} Fomivirsin, a CMV retinitis therapy, is the first ASO drug to receive FDA approval; others are in various stages of clinical evaluation in oncology and virology studies.

Deoxyribozymes (catalytic DNA, or DNAzymes) are deoxynucleotide sequences that target mRNA via Watson–Crick base-pair binding, and catalyze breakdown of that mRNA.³⁶ DNAzymes targeting vascular endothelial growth factor (VEGF) mRNA have been used in animal models of cancer, and to inhibit neovascularization in models of macular degeneration. They can be synthesized more economically than some other gene-silencing tools, and can be modified to be relatively resistant to degradation. DNAzymes could be considered for silencing mutant RNA for CMT genes that lead to toxic gain of function.³⁶

The technique of therapeutic RNA trans-splicing allows for repair of defective m-RNA.⁴¹ RNA-trans-splic-

ing is a rare naturally occurring splicing process, which occurs between two separately transcribed pre-mRNAs producing a composite m-RNA.^{35,41} Spliceosome- or ribozyme-based therapeutic trans-splicing approaches can be used to remove a mutant sequence in pre-mRNA and replace it with an exogenously constructed sequence to permit production of corrected mature mRNA, and translation of a normal protein.^{35,41}

A further therapeutic approach to dominant negative effects in PMP22 overexpression disorders includes downregulation of the promoter sequences for PMP22. The *PMP22* gene has two promoters, P1 and P2. P1 is of particular interest, because it exerts its effect mainly in myelinating SCs.⁴² Downregulation of the activity of P1 may therefore have therapeutic value with specificity for PMP22 overexpression. Development of sequence triplex-forming oligonucleotides that bind competitively to the P1 promoter in a sequence-specific manner has been achieved.⁴² The triplex-forming oligonucleotides exert their effects in the nucleus. This may be an advantage, in that they are less subject to nucleases than are strategies targeting mature mRNA (e.g., conventional ASOs, RNAi), and also if multiple copies of the mRNA exist.⁴²

In summary, knockdown of mutant alleles using RNAi, ASOs, or DNazymes may be effective for dominant forms of CMT associated with a toxic gain of function.^{3,4} ASO therapy may also have a role for hereditary neuropathies with abnormal pre-mRNA splicing.⁴³ Repair of mutant pre-mRNA using therapeutic trans-splicing could be applied to address various mechanisms in hereditary neuropathies including haploinsufficiency and dominant negative disorders. Traditional gene replacement or stem cell approaches could also be considered for haploinsufficiency in hereditary neuropathies.⁴ These strategies have varying advantages and disadvantages in terms of cost, barriers to effective delivery to target tissue, stability, and potential for off-target effects, but hold future therapeutic potential for various forms of CMT.³⁵ Although major challenges lie ahead in harnessing these technologies for therapeutic advantage in CMT, *in vitro* and animal model work for hereditary neuropathies should now proceed. Gene and RNA-based therapies would usher in an era of therapy customized to the mutations of individuals and families, raising also the vexing questions of how to manage the attendant costs, clinical testing, and regulatory approval.⁴⁰

FUTURE THERAPEUTIC STRATEGIES FOR CMT: TARGETING OF CYTOPLASMIC AGGREGATES OF MUTANT OR MISFOLDED PROTEIN

Although curcumin shows preclinical promise for forms of CMT mediated via cytoplasmic aggregates of mutant protein, other approaches that break down protein aggregates,

or correct protein misfolding, could be valuable in CMTs to convert the phenotype from a more severe toxic gain of function to a milder haploinsufficiency. Protein aggregation and misfolding is believed to be a key event in neurodegenerative disorders, including Alzheimer's disease, prionopathies, Huntington's disease, and Parkinson's disease.⁴⁴ A number of strategies are being considered in these disorders, including targeting proteases important in aggregate formation, and disruption of aggregates with antibodies and other compounds, such as Congo red analogs.⁴⁵ A better understanding of mechanisms of protein aggregation and misfolding in particular forms of CMT is required to develop such therapies.³

CHALLENGES TO DEVELOPING THERAPIES IN INHERITED NEUROPATHIES

The translation of the expanding range of therapeutic targets into therapies for CMT will be a major challenge. Apart from diabetic neuropathy and neuropathic pain, the pharmaceutical industry has devoted relatively limited effort toward therapies for neuropathies. Limited resources and funding sources are available to clinical and research scientists and academic institutions for independent development of therapies for hereditary neuropathies, and collaborations across institutions and continents and among clinician investigators interested in CMT have been few. Patient advocacy in hereditary neuropathies has not been of the scale seen with other neurodegenerative disorders. The initiation of clinical trials of AA in CMT1A in North America and Europe has led to improved infrastructure for conduct of treatment trials. Nonetheless, partnerships among the pharmaceutical industry, clinical investigators, patient advocacy groups, and private foundations—in addition to enhanced levels of governmental funding of therapeutic trials in peripheral neuropathies—will be necessary for successful therapy development for hereditary neuropathies.

Specifically considering CMT, given that CMT1A accounts for 50% of all cases, achieving effective therapy for PMP gene dosage disorders would represent a major milestone. Traditional experimental therapeutic models appear feasible in CMT1A, because all patients harbor the same mutation. For other forms of CMT (e.g., Cx32 or MPZ disorders), in which hundreds of mutations exist, individualizing therapies and assessing efficacy for mutations that affect only a handful of kindreds worldwide will be especially challenging. This underscores the need to focus therapy development in such forms of CMT to be effective (where possible) for a variety of mutations. For example, therapies for *MPZ* mutations with toxic accumulation of mutant proteins might ideally target a common mechanism in the accumulation of diverse *MPZ* mutants.

Beyond the hurdle of developing approaches for multiple mutations, there is wide intra- and interfamilial variability in the age of onset, phenotype, and severity of CMT, such that a unitary approach may not be appropriate, not even for two members of the same family. For some patients with very mild, late-onset phenotypes, intervention with costly, designer therapies may not be justifiable. Because patients with CMT demonstrate disability primarily in relation to axon loss,⁴ early therapy before significant axon loss has occurred may be more effective. Once axonal loss has taken place, reinnervation of fibrotic muscle or skin that has lost its SCs may be more difficult to achieve. Research that identifies predictors of a severe course for a given mutation will be critical for selection of patients who need disease-modifying therapy, and for timing of interventions.

EXPERIMENTAL THERAPEUTICS IN CMT WILL REQUIRE USE OF NOVEL OUTCOME MEASURES AND TRIAL DESIGNS

For CMT1A, there are sufficient numbers of patients to permit multicenter, parallel-group RCTs to assess efficacy.⁴⁶ There are, however, only scarce data on optimal outcome measures for such studies.^{46–48} Current outcome measures include the NIS, a quantified neurological examination, or the CMTNS, a composite measure consisting of nine elements (sensory symptoms, motor symptoms [lower limb, upper limb], strength [lower limb, upper limb], vibration and pin sensation, ulnar or median sensory nerve action potential amplitude, and an ulnar or median compound muscle action potential amplitude). Limited natural history data for CMT1A suggest that there is slight, yet measurable worsening of a mean of 1.37 NIS points per year, or 0.69 points per year on the CMTNS.⁴⁸ Given the available natural history data, at least 280 subjects would be required to demonstrate a 50% slowing of progression of CMT1A relative to placebo in a parallel-group design over a 2-year period.⁴⁸ Because CMT is a lifelong progressive disorder, even more modest effects on progression may translate, over decades, into a clinically meaningful effect. Lengthy treatment trials to identify small effects would be difficult to achieve for more than a handful of prospective therapies in CMT1A.

One approach to streamlining screening of potential therapies in CMT1A is the futility study design. It is considered futile to proceed with further larger efficacy trials if the treatment group does not worsen by at least a prespecified amount less than expected based on natural history data. This approach reduces the required sample size by comparing changes in the outcome in study subjects to changes expected based on natural history data, and also uses single-tailed hypothesis testing. Futility studies are nonetheless a major undertaking in CMT1A, because they require the same length of trial as

a traditional efficacy study. This study design also depends on reliable natural history data and on selection of an appropriate prespecified amount of slowing of progression relative to natural history.

Development of surrogate measures for hereditary neuropathies that rapidly and reliably predict a beneficial clinical effect is therefore critical. One promising biomarker involves analysis of myelinated dermal nerve bundles in punch skin biopsies.^{43,49} Skin biopsies can be obtained to assess SCs and myelinated and unmyelinated innervation, and to track expression of myelin components at the mRNA and protein levels. These biopsies have been used to reveal differences in PMP22 expression and localization among controls, CMT1A, and HNPP, and to explore molecular mechanisms of novel CMT mutations.⁴⁹ Skin biopsies are under pilot trial as a secondary outcome in two ongoing trials of AA in CMT1A.^{4,30} In these studies, PMP22 mRNA levels are being followed, with the hypothesis that downregulation of PMP22 mRNA levels will predict clinical slowing of progression or improvement of CMT1A, and will provide a suitable surrogate measure for future studies in CMT.^{4,30} Skin biopsies may also be useful to monitor mutant mRNA or protein levels, or aggregate formation in other forms of CMT. If skin biopsies are to be used in this manner, the reliability and reproducibility of these assessments will need to be established.

For rare forms of CMT, in which particular mutations occur in only a few kindreds worldwide, conduct of traditional efficacy trials will not be possible. Here, single-patient (N-of-1) double-blind, randomized, placebo controlled, crossover trial designs could be considered.^{50–51} In N-of-1 trials, an active therapy and placebo are administered to an individual patient in a multiple cross-over manner, and the outcomes for each treatment period are compared to assess for a treatment effect. Results of multiple N-of-1 clinical trials can be combined to draw population inferences regarding treatment effects.⁵¹ N-of-1 clinical trials are inherently most suitable for assessing therapies for chronic disorders such as neuropathic pain, diabetes, and hypertension. In diseases such as these, independent measurement of the effects of interventions can be determined during successive periods, because they do not cause contamination between periods, nor require lengthy washout periods.

For CMT, the N-of-1 design is problematic. Any effective treatment might take a long period of time to show a clinical effect (e.g., axonal regeneration may be required before a clinical effect can be measured). Likewise, it may take considerable time for washout of therapeutic effects. Treatment periods may thus need to be lengthy. This approach may be viable if sensitive surrogate measures can be developed. For example, if a hypothetical RNAi therapy was developed for a toxic gain-of-function *MPZ* mutation, the effects of blinded cross-over

application of the RNAi *versus* sham RNAi could be assessed within a short time interval by measuring expression levels of the mutant mRNA or protein in skin biopsies. Conventional clinical and electrophysiological outcomes might be expected to respond too slowly to an intervention to make them suitable for N-of-1 applications in CMT. Careful consideration of the optimum manner in which to assess efficacy of designer therapies in rare hereditary neuropathies is therefore required. Proper dosing of prospective therapies and measurements to verify that desired tissue concentrations and half-life are achieved in the human subject are critical elements that have often received only limited attention in prior failed therapeutic trials in neuromuscular disorders.

ECONOMIC IMPLICATIONS OF DESIGNER THERAPIES FOR RARE FORMS OF CMT

Several of the therapeutic strategies discussed would likely require chronic or intermittent administration. For some agents (e.g., AA), compounds are widely available and inexpensive. For RNAi and various gene-modifying approaches, costs of therapy development could be considerable. This is exemplified by the development of enzyme replacement therapy for Pompe's disease. Enzyme replacement therapy for this disorder requires life-long biweekly infusions, with a potential cost of approximately \$200,000 to \$300,000 per year. Many of the effective therapies of the future will likely need to be customized for a diversity of genetic causes of hereditary neuropathies, and so society will need to confront the challenge of how to provide costly designer therapies to patients with rare disorders, for lengthy periods of time.

ETHICAL CONSIDERATIONS IN DEVELOPMENT OF FUTURE THERAPIES FOR INHERITED NEUROPATHIES

Early genetic characterization of CMT will be desirable for timely therapeutic intervention. Preimplantation genetic diagnosis (PGD), with a goal of avoiding transmission of hereditary disorders, is an evolving discipline. PGD has been used in at-risk families to select embryos without a *PMP22* duplication for *in vitro* fertilization.⁴ Although PGD will become increasingly available, it probably will not initially be universally accessible or accepted by families for prevention of hereditary neuropathies. Severe congenital or early-onset CMT implies a need for prenatal diagnosis for optimum timing of future therapies. Those at risk for later onset CMT will similarly require presymptomatic genetic screening.

Ethical, social, psychological and economic considerations surrounding presymptomatic genetic screening have been confronted in other neurodegenerative disorders, including some with devastating consequences

(e.g., Huntington's disease). The Huntington's disease experience will be of value in prenatal or presymptomatic diagnosis in CMT, although the highly variable phenotype of CMT raises different ethical questions, among them the genetic labeling of individuals who may or may not develop a severe disorder years or decades later. Predictors of which individuals are at risk for severe hereditary neuropathies phenotypes will be critical for informing the decisions of patients and families regarding presymptomatic screening and therapy.

SUMMARY COMMENTS

Rapid molecular and genetic advances have enabled genetic characterization of many hereditary neuropathies syndromes. Isolation of causative genes for CMT over the past decade has led to emerging therapeutic targets, and advances in gene and mechanism-based strategies provide hope that therapies will emerge to slow or partly reverse several forms of hereditary neuropathies. The advent of designer strategies for hereditary neuropathies will challenge stakeholders in health care in the 21st century to develop ways to make novel therapies available to those who need them.

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