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REVIEW

Updated review of therapeutic strategies for Charcot-Marie-Tooth disease and related neuropathies

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ABSTRACT

Introduction: Charcot–Marie–Tooth disease (CMT) and related neuropathies represent the most prevalent inherited neuromuscular disorders. Nonetheless, there is still no pharmacological treatment available for any CMT type. However, the landscape is rapidly evolving and several novel approaches are providing encouraging results in preclinical studies and leading to clinical trials.

Areas covered: The authors review the most promising therapies under study and the ongoing/planned clinical trials. Several approaches to address PMP22 overexpression underlying CMT1A, the most frequent subtype, are being tested. Gene silencing, targeting PMP22, and gene therapy, to introduce specific genes or to substitute or modulate defective ones, are being experimented in animal models. Compounds acting on ER stress, unfolded protein response, neuregulin pathways, phosphoinositides metabolism, axonal transport and degeneration, inflammation, polyol pathway, deoxysphingolipid metabolism, purine nucleotide pool are potential therapeutic candidates for different forms of CMT and related neuropathies.

Expert opinion: We are getting closer to find effective therapies for CMT, but are far behind the exciting examples of other genetic neuromuscular disorders. The authors analyze the possible reasons for this gap and the way to fill it. Preclinical and clinical research is ongoing with coordinated efforts and they are confident that in the next few years we will see the first effective treatments.

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1. Introduction

Charcot–Marie–Tooth (CMT) disease represents a group of genetically heterogeneous peripheral neuropathies associated with mutations, or copy number variations, in approximately 100 different genes (Table 1), many of them recently uncovered thanks to next-generation sequencing (NGS) technology. It is the most common inherited neuromuscular disease with an overall prevalence estimated at 10–28/100,000. The usual phenotype is characterized by progressive wasting and weakness of distal limb muscles, loss of deep tendon reflexes, and distal sensory loss [1].

CMT classification is based on inheritance pattern – autosomal dominant (AD), autosomal recessive (AR), or X-linked – and nerve conduction studies, with demyelinating, axonal and intermediate types. Therefore, AD and AR demyelinating forms are designated CMT1 and CMT4, respectively, the axonal subgroups are AD-CMT2 and AR-CMT2, and the intermediate subtypes include the dominant DI-CMT and the recessive RI-CMT. There are 6 X-linked CMT subtypes, the most important being CMTX1 associated with *GJB1* gene mutations. Pure motor forms are labeled as distal Hereditary Motor Neuropathies (dHMN), whereas pure sensory or predominantly sensory types are named Hereditary Sensory (and Autonomic) Neuropathies (HSN or HSAN). Transient painless focal neuropathies are the key feature of Hereditary Neuropathy with liability to Pressure Palsies (HNPP). Further subdivision is based on the underlying genetic defect (Table 1). CMT1A associated with a duplication of the gene coding for the 22-kd Peripheral Myelin Protein (*PMP22*) is the most prevalent subtype and is characterized by overexpression of the PMP22 protein. Mutations in the Myelin Protein Zero (MPZ) cause either early-onset demyelinating/hypomyelinating neuropathies (CMT1B) or later-onset mainly axonal neuropathies (sometimes labeled as CMT2I/CMT2J). CMTX1 is the second most frequent form of CMT after CMT1A, is inherited as an X-linked dominant trait and is caused by mutations in *GJB1*, encoding connexin 32 (Cx32), which forms gap junctions in Schwann cells and oligodendrocytes. Most if not all *GJB1* mutations causing CMTX1 behave as loss-of-function mutations as the Cx32 gap-junctions are lacking or defective. Mutations in a protein localized in the mitochondrial membrane, Mitofusin-2, are the most common cause of axonal CMT (CMT2A) [2].

Despite the great advances in understanding the genetic and biological bases of inherited neuropathies, there is still no treatment available for any type of CMT. However, this is a rapidly evolving field and several novel approaches are providing encouraging results in preclinical studies and are leading to clinical trials.

Many different pathways are involved in the pathomechanisms of CMT and related neuropathies: myelin formation and

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Article highlights

- Although Charcot-Marie-Tooth disease (CMT) and related neuropathies represent the most prevalent inherited neuromuscular disorders, there is still no drug treatment for any CMT type, and rehabilitation therapy as well as surgical correction of skeletal deformities are the only effective options available.
- CMT1A associated with the duplication and overexpression of PMP22 is the most frequent and most studied form, and several approaches to downregulate PMP22 have been developed. PXT3003 (a mixture of baclofen, sorbitol, and naloxone) showed interesting results and a novel phase III trial is starting; *PMP22* gene silencing with ASO and siRNAs was effective in animal models and is under consideration for clinical trials.
- Promising approaches are represented by gene therapy to introduce neurotrophin-3 with a AAV1 vector for CMT1A and CMTX1, to replace defective gene via intrathecal delivery with lentiviral or AAV9 vectors for CMTX1 and CMT4C.
- Compounds modulating the unfolded protein response which is activated by several mutations in myelin genes are giving promising results in preclinical models and are candidate for clinical trials in CMT1A and CMT1B.
- Neuregulin molecules are determinant of myelin thickness and their modulation may be used to target both hypermyelinating neuropathies such as CMT4B and HNPP, and hypo- or dysmyelinating neuropathies, as confirmed in preclinical studies in rodents.
- Compounds preventing axonal degeneration or rescuing altered axonal transport have the potentiality to cure many hereditary neuropathies irrespective of the primary triggering defect: this is the case for the inhibitors of SARM1 and of HDAC6, an active field of research.
- Intervention on more specific metabolic pathways is also promising, such as for L-Serine to treat HSN1 by reducing neurotoxic deoxysphingolipids, or compounds acting on the PYKfive enzyme in the phosphoinositides metabolism in CMT4B, or S-adenosylmethionine (SAM) to supply purine nucleotides in CMTX5 and its allelic disorders.
- Mutations in sorbitol dehydrogenase has been very recently associated with a relatively common form of CMT2/dHMN and high levels of sorbitol. Inhibitors of aldose reductase, developed in the past for diabetic neuropathy, are good candidates for its treatment.

maintenance, transcription factors of myelin genes, gap junctions and channels, axonal transport (retrograde and anterograde, kinesins, dynein, dynactin), mitochondrial dynamics, and vesicle trafficking are the main ones. Therapies may be aimed at correcting the specific defect in selected CMT types or be addressed to treat common pathomechanisms in a more general approach valid for wider categories of CMT and possibly for other neuropathies (i.e. therapies targeting axonal degeneration and axonal transport).

The first trials with ascorbic acid for CMT1A were ineffective and highlighted the difficulties in translating preclinical positive results into successful clinical studies. The slow progression of CMT makes it difficult to demonstrate efficacy of any tested compound. Hence, research is also aimed at developing responsive outcome measures.

In this paper we will review the most promising treatment in the pipeline as well as the current or planned clinical trials for CMT and related neuropathies. There is a wide variety of possible therapies under investigation, including those aimed at lowering PMP22 overexpression in CMT1A, the gene therapy approaches to substitute, modulate or correct the defective genes, and compounds acting on the unfolded protein response (UPR) activated by misfolded protein accumulation, or working on myelination control mechanisms, or functioning specifically on metabolic pathways altered in certain CMT subtypes, or addressing common pathomechanisms underlying several CMT forms.

2. Therapeutics approaches for CMT and related disorders

2.1. Correction of PMP22 overexpression in CMT1A

Most of the ongoing research is aimed at treating CMT1A, caused by a 1.4 Mb duplication involving the *PMP22* gene and resulting in PMP22 protein overexpression. Even though the ultimate molecular mechanisms underlying CMT1A are still unclear, strategies to downregulate PMP22 expression have been a logical extension of research efforts (Figure 1, Table 2). Unfortunately, so far successful translation to human trials has been discouraging. Moreover, modulation of PMP22 protein levels has caveats: levels vary significantly over time in patients, and the absolute value at a given time point is not predictive of phenotype [3]. It might be that stabilizing excessive fluctuations of PMP22 levels rather than just downregulating the absolute value is necessary for disease modification

Table 1. List of CMT genes associated with the different types of CMT and related neuropathies.

Charcot-Marie-Tooth disease and related neuropathies													
Demyelinating CMT			Intermediate CMT			Axonal CMT				dHMN		HSAN	
AD	AR	X- linked	AD	AR	X- linked	AD		AR	X- linked	AD/AR/X-linked		AD/AR	
PMP22	*GDAP1*	*GJB1*	*DNM2*	*KARS*	*GJB1*	*MFN2*	*HARS*	*MFN2*	*AIFM1*	*HSPB8*	*ATP7A*	*SPTCL1*	*CLTCL1*
MPZ	*MTMR2*		*YARS*	*PLEKHG5*	*AIFM1*	*RAB7A*	*VCP*	*LMNA*	*PRPS1*	*HSPB1*	*DYNC1H1*	*SPTCL2*	*PRNP*
LITAF	*MTMR13*		*MPZ*	*COX6A1*	*DRP2*	*TRPV4*	*MORC2*	*MED25*	*PDK3*	*HSPB3*	*BICD2*	*ATL1*	*CCT5*
EGR2	*MTMR5*		*IFN2*			*GARS*	*NEFH*	*GDAP1*		*FBXO38*	*MYH14*	*DNMT1*	*FLVCR1*
NEFL	*SH3TC2*		*GNB4*			*NEFL*	*ATP1A1*	*MME*		*BSCL2*	*AARS*	*ATL3*	*ZFHX2*
PMP2	*NDRG1*		*NEFL*			*HSPB1*	*TFG*	*SPG11*		*GARS*	*HARS*	*WNK1*	
FBLN5	*EGR2*					*MPZ*	*BSCL2*	*MPV17*		*REEP1*	*HINT1*	*FAM134B*	
ARHGEF10	*PRX*					*GDAP1*	*NAGLU*	*HINT1*		*SLC5A7*	*VRK1*	*KIF1A*	
	HK1					*HSPB8*	*KIF5A*	*IGHMBP2*		*DCTN1*	*SETX*	*SCN9A*	
	FGD4					*DNM2*	*MTATP6*	*TRIM2*		*TRPV4*	*CHCHD10*	*IκBKAP*	
	FIG4					*AARS*	*DCAF8*	*DNAJB2*		*DNAJB2*	*SORD*	*NTRK1*	
	SURF1					*LRSAM1*	*TUBB3*	*C12orf65*		*IGHMBP2*	*TBX5*	*β-NGF*	
	CTDP1					*DHTKD1*	*DGAT2*	*SORD*		*PLEKHG5*		*DST*	
						MME		*SLC25A46*		*SIGMAR1*		*SCN11A*	
						MARS		*SACS*		*LAS1L*		*PRDM12*	

AD = autosomal dominant, AR = autosomal recessive, CMT = Charcot-Marie-Tooth disease, dHMN = distal hereditary motor neuropathy, HSAN = hereditary sensory and autonomic neuropathy

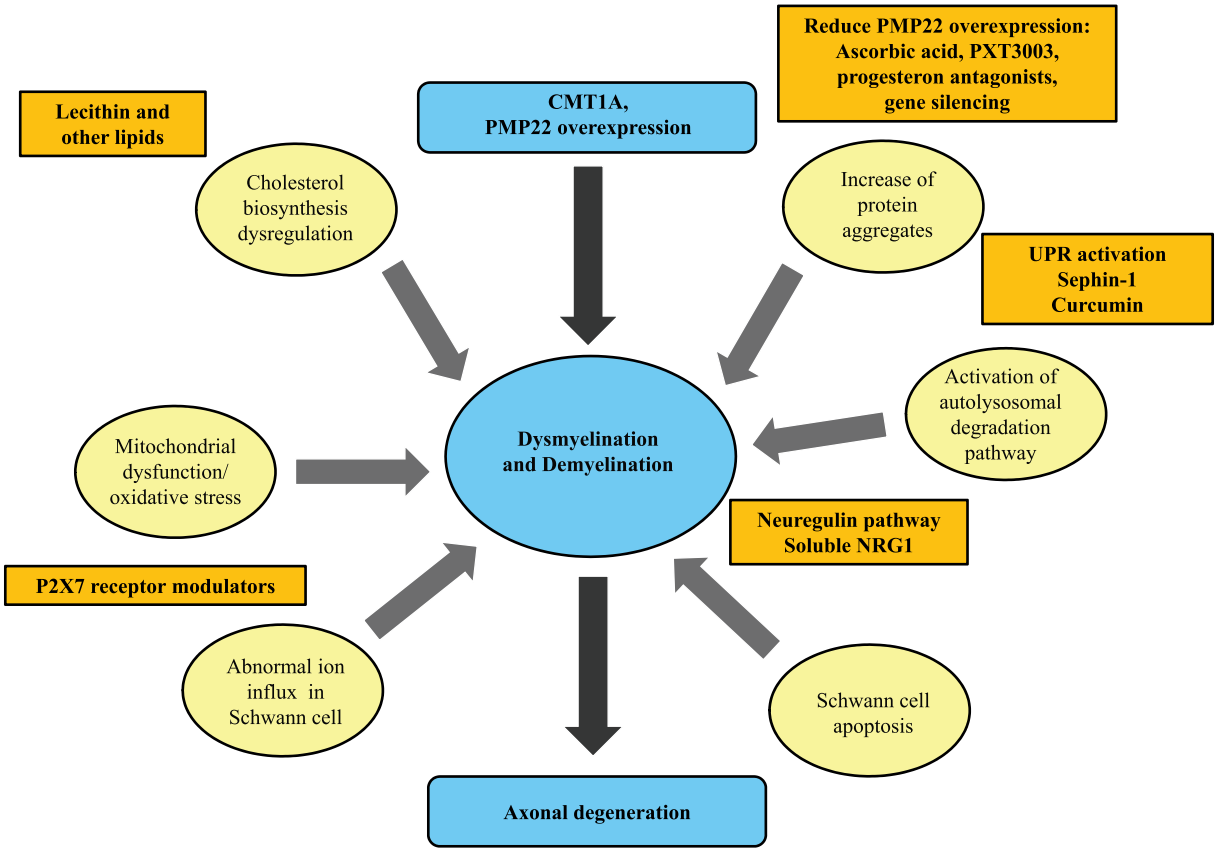

Figure 1. **CMT1A pathomechanisms and possible therapeutic approaches**. The mechanisms leading to dysmyelination and demyelination in CMT1A and the related treatments are depicted as circles (yellow, in the color figure version) and rectangles (orange, in the color figure version), respectively.

or that modulating PMP22 expression is only effective if initiated in an early, time limited window of the disease course. Notably, PMP22 haploinsufficiency causes HNPP, indicating that translation of this strategy to humans will require careful titration and monitoring.

Ascorbic acid was among the first therapies tested for CMT1A, after it proved promising in a mouse model. High-dose ascorbic acid (vitamin C) decreased PMP22 levels and symptoms in mice with CMT1A, so that they were able to stay on a rotating rod longer, cross a beam more rapidly, and grip for longer than untreated mice [4]. Several studies have been performed in humans with CMT1A, testing different doses of vitamin C (1 to 4 g/day) for up to 2 years, in adults or children [5,6]. Unfortunately, all studies failed to meet their primary endpoints and did not show a significant effect on phenotype.

Steroid hormones are epigenetic regulators of gene expression and progesterone can stimulate PMP22 expression in cultured Schwann cells. Onapristone, a progesterone antagonist, was able to decrease PMP22 expression in a rat model of CMT1A, improving the phenotype, and reducing axonal loss [7]. Unfortunately, onapristone has unacceptable side effects. Efforts to develop bioequivalent compounds with a better safety profile are ongoing. A trial with another progesterone antagonist, ulapristil, started in France, but it has not been completed and results have not been published (ClinicalTrials.gov NCT02600286, Table 2).

$GABA_B$ receptor isoforms are specifically expressed in Schwann cells of the rat sciatic nerve, and exposure of cultured Schwann cells to selective $GABA_B$ agonist (i.e. baclofen) inhibits their proliferation and reduces the synthesis of specific myelin proteins, providing evidence for a physiological role of $GABA_B$ receptors in peripheral nerves [8] and for new useful therapeutic approaches with GABA-mimetic drugs in inherited demyelinating neuropathies.

Interestingly, PXT3003, a combination of low doses of already marketed medications – baclofen (a GABA agonist), sorbitol (a disaccharide) and naltrexone (an opioid antagonist) – was predicted by computer-based analyses to act synergistically in down-regulating PMP22 and experiments in the transgenic CMT1A rat model seemed to confirm reduction in PMP22 mRNA expression and improvement in some disease parameters [9,10]. PXT3003 has been shown to be safe in a phase II trial in 80 CMT1A patients with a significant difference of the mean percentage improvement of the ONLS (Overall Neuropathy Limitations Scale) score between the high-dose group and the placebo arm [11]. A first Phase III trial confirmed better results in the Overall Neuropathy Limitations Scale (ONLS) and the 10 m timed walking in the treated groups (ClinicalTrials.gov NCT03023540). However, the high-dose compound crystallized in vials leading to unblinding in almost one half of patients in this group and the FDA required a second Phase III trial which will start soon in the US and Europe. Although this is currently the treatment for

Table 2. Summary of the main therapeutic approaches for Charcot-Marie-Tooth disease and related neuropathies.

Compound	Route of Administration	CMT type	Mechanism	Clinical trials
Ascorbic acid	Oral (humans: 1 to 4 g/day, for up to 2 years)	CMT1A	Reduction of *PMP22* expression by reducing cAMP levels	Phase III studies concluded; all failed to meet their primary endpoints and did not show a significant effect
Progesterone antagonists/ modulators: onapristone, ulapristal	Oral (humans)	CMT1A	Reduction of PMP22 synthesis	Onapristone: unacceptable side effects. Ulapristal: phase II trial conducted (n = 23 out of 45 planned). Results?
PXT3003 (mix of low doses of baclofen, sorbitol and naltrexone)	Oral (humans)	CMT1A	Inhibition of Schwann cells proliferation and reduction of the synthesis of PMP22; baclofen, $GABA_B$ receptor modulator	PXT3003: phase II (n = 80) concluded. Phase III (n = 323) unpublished. New Phase III requested by FDA and starting soon
Gene silencing (ASOs, siRNAs, shRNAs, CRISPR-Cas9)	Subcutaneous ASO/siRNA or intranerve shRNA/ CRISPR-Cas9 editing (rodents)	CMT1A, CMT1E	Partial silencing of overexpressed (CMT1A) or mutated (CMT1E) *PMP22*	Under consideration
Gene therapy (e.g. AAV1-NT-3)	AAV1-NT-3 intramuscular in both legs (humans)	CMT1A, CMTX1	Neurotrophic action	NT-3: open trial (n = 3) planned for CMT1A
Dietary lipid supplementation	Oral (rats)	CMT1A, other CMT type?	Dietary correction of defective myelin lipid biosynthesis	Trial with oral lecithin supplementation planned in Germany
P2X7 receptor modulators (e.g. A438079)	Intraperitoneal injection (rats)	CMT1A	Reduction of abnormal calcium influx into Schwann cells	P2X7 antagonist acceptable safety and tolerability in a previous phase II trial in rheumatoid arthritis
Curcumin, sephin-1	Oral (rodents)	CMT1A, CMT1E, CMT1B	UPR inhibition by attenuation of the IRE1 branch	Possible clinical trial in CMT1A/ CMT1B
Sodium channel blockers	Oral (mice)	CMT1B, other dysmyelinating CMT?	Blocking of Na_v 1.8 channel	Lamotrigine could be a candidate compound
Neuregulin pathways (particularly Neuregulin-1 III)	Intraperitoneal injection/ exogenous overexpression/soluble (rodents)	CMT1A, CMT1B, CMT4B, HNPP	Regulation of myelin thickness	Niacin-niaspan candidate for CMT4B and HNPP?
PIKfyve enzyme inhibitors	Intraperitoneal injection (mice)	CMT4B1, CMT4B2	Inhibition of PIKfyve and decrease of PI3,5P2 levels	To be considered for CMT4B1 and CMT4B2
SARM1 inhibitors	Intrathecal virus injection (mice)	All CMT and related neuropathies	Prevention of axonal degeneration	–
HDAC6 inhibitors	Oral (mice)	CMT2F, dHMN 2A	Reduction of microtubules acetylation, action axonal transport	Under consideration
ACE-083	Intramuscular (humans)	CMT1, CMTX1	Action on myostatin pathway	Phase I+II (n = 62 overall) trial did not produce significant clinical improvement
CSF1R inhibitors	Oral (mice)	CMT1A, CMT1B, CMTX1	Decline in nerve macrophages	–
FLX-787	Oral (humans: 30 mg three times a day)	CMT	Activation of TRPA1 and TRPV1 channels, for cramps	Phase II (n = 120) stopped for oral intolerability in a subset of patients
Aldose reductase inhibitors	To be defined	CMT and dHMN associated with biallelic *SORD* mutations	Inhibition of aldose reductase, the enzyme converting glucose into sorbitol	Under consideration
L-Serine	Oral (mice and humans: high doses)	HSN1	Reduction of neurotoxic deoxysphingolipids	Phase II trial (n = 18) performed, primary endpoint not reached, but underpowered trial
S-adenosylmethionine (SAM)	Oral (Arts syndrome patients)	CMTX5, Arts syndrome and the X-linked non syndromic sensorineural deafness DFN2	Purine nucleotides supply	Anectodal report

AAV1-NT-3 = adeno-associated virus–mediated neurotrophin-3, ASO = antisense oligonucleotide, cAMP = cyclic adenosine monophosphate, CMT = Charcot-Marie-Tooth disease, CSF1R = colony stimulating factor 1 receptor, FDA = Food and Drug Administration, $GABA_B$ = gamma-aminobutyric acid B receptor, HDAC6 = histone deacetylase 6, dHMN = distal hereditary motor neuropathy, HNPP = hereditary neuropathy with liability to pressure palsies, HSN = hereditary sensory neuropathy, IRE1 = inositol-requiring enzyme 1, PIKfyve = phosphatidylinositol 3-phosphate 5-kinase, SARM1 = sterile alpha and TIR motif containing 1, shRNA = small hairpin RNA, siRNA = small interfering RNA, TRPA1 = transient receptor potential cation channel, subfamily A, member 1, TRPV1 = transient receptor potential cation channel subfamily V member 1, UPR = unfolded protein response

CMT1A placed in the most advanced phase, there is caution among researchers in interpretation of results, particularly as the ONLS did not prove to be a responsive measure in the ascorbic acid trials [12].

2.2. Gene silencing and gene therapy

Therapy aimed at correcting the gene defect with different approaches is becoming a reality for many previously incurable neuromuscular disorders. *PMP22* gene silencing in

Schwann cells (hence addressing the overexpression of PMP22) is a strategy already tested to treat CMT1A. Recently, Zhao et al. [13] treated two rodent models of CMT1A with subcutaneous administration of PMP22-targeting antisense oligonucleotides (ASOs) and showed a 35% reduction in PMP22 mRNA. Such treatment was not only adequate to slow disease progression, but also improved the neuropathy phenotypes in both models. Normalization of PMP22 levels and improvement in locomotor, electrophysiological and pathological parameters have been recently obtained in two transgenic CMT1A mouse models with a small interfering RNA (siRNA) conjugated to squalene nanoparticles [14]. The CRISPR-Cas9 approach has been successfully tested to down-regulate *PMP22* expression by targeting the TATA-box of its Schwann cell-specific P1 promoter in C22 mice, a model of CMT1A: intraneural injection at P6 or at P21 in the sciatic nerve of single guide RNA (sgRNA) encapsulated with liposome aimed at deleting the TATA-Box resulted in reduced PMP22 expression, prevention (P6) or partial rescue (P21) of abnormalities in CMAP amplitude, nerve conduction velocities, and histopatology [15]. Intranerve injection of small hairpin inhibitory RNA (shRNA) targeting PMP22, by using an adeno-associated viral serotype 9 (AAV2/9) vector, in rodents and primates was effective in inducing transduction in resident Schwann cells relatively distant from injection sites and when employed to treat CMT1A rats' sciatic nerves resulted in improved myelination and prevention of clinical and electrophysiological abnormalities [16].

Clinical trials to partially silence PMP22 expression in CMT1A patients are therefore desirable, although there are issues to be considered in the cost-benefit ratio for long-lasting therapies which also need a precise down-regulation to avoid the risk of developing HNPP features.

Selective gene silencing of the mutant allele by allele-specific siRNAs has also been attempted with success in animal models. Lee at al. designed allele-specific siRNAs for the Tr-J mice, harboring the L16P missense mutation in *PMP22*. The treatment reduced L16P-*PMP22* mRNA in Schwann cells and improved the mouse phenotype [17].

Gene therapy can be employed also to introduce specific genes into the target tissue. The growth factor neurotrophin 3 (NT-3) promotes either nerve regeneration after injury and Schwann cells survival. Accordingly, injection of recombinant NT-3 improved regeneration and remyelination in animal models [18–20]. A pilot study in eight patients with CMT1A resulted in an increase in myelinated fiber density, a reduction in the neurologic impairment score, and improved sensory modalities as compared with placebo controls, calling for a larger trial [18]. However, long-term treatment was not feasible due to its extremely short serum half-life. An additional study provided preclinical data demonstrating efficacy of adeno-associated virus (AAV)–mediated NT-3 (AAV1-NT-3) gene therapy in a mouse model of CMT1A [19,20] and very recently also in Cx32 knockout (KO) mice [21]. Therefore, an open-label study has been designed to deliver ascending doses of the AAV1-NT-3 gene (scAAV1.tMCK.NTF3) intramuscularly in both legs (in medial and lateral heads of triceps and in tibialis anterior muscles) in 3 young CMT1A subjects (ClinicalTrials.gov NCT03520751).

Gene therapy to replace the defective gene is being studied for different types of CMT characterized by loss of function mutations, such as CMTX1 and the CMT recessive forms. A major issue is how to reach the target tissue, which is particularly challenging for Schwann cells. Intrathecal *GJB1* delivery has been successfully performed by Kleopa and colleagues using lentivirus or AAV9 vectors and the myelin-specific *MPZ* promoter, in *GJB1*-knocked-out mice lacking Cx32 expression [22–24]. Gene delivery appeared to result in stable Cx32 expression in Schwann cells and peripheral nerves as well as in clinical improvement. Whether this approach can be beneficial for *GJB1* mutants resulting in protein production remains to be determined, as counterproductive interactions between the delivered wild-type Cx32 and mutant Cx32 forms may occur. Thus, screening for mutant-wild-type interaction will be an important caveat to gene replacement therapy strategies in CMTX1 as well as for other CMT types. Kleopa et al. successfully used the same intrathecal gene delivery for the Sh3tc2-/- mouse model of CMT4C, and observed improvement of motor function, electrophysiological and morphological abnormalities, and reduction of abnormally elevated levels of blood neurofilament light protein (NFl), a biomarker of axonal damage [25].

Other CMT forms can be targeted with gene therapy either addressed at substituting the defective gene, as for CMT4J, or with CRISPR-Cas9 approach to correct the defective gene, or with a different gene to overcome or modulate the mutated gene. For instance, Mitofusin-1 (MFN1) could be employed to restore mitofusin function in CMT2A associated with mitofusin-2 (*MFN2*) gene mutations: MFN1 and MFN2 can form homo- and hetero-dimers and peripheral nerve is particularly poor in MFN1 (which could be a possible explanation for the neuropathic phenotype of CMT2A). Zhou and colleagues showed that an increase in MFN1 levels in vivo through a transgenic approach rescued the phenotype in mutant $MFN2^{R94Q}$-expressing mice [26]. An alternative, promising approach to CMT2A treatment involves the use of mitofusin agonists, small molecule mimicking the MFN2 peptide–peptide interface, which improved mitochondrial dysfunction in neuron cultures expressing MFN2 mutants and in sciatic nerves of $MFN2^{T105M}$ transgenic mice [27].

2.3. Lipid biosynthesis

Another potential therapeutic path, particularly for CMT1A, involves modification of lipid metabolism. Transcription of genes for myelin lipid biosynthesis was found to be reduced in myelinating Schwann cells in the CMT1A rat model and lipid incorporation into myelin was therefore reduced. Dietary lipid supplementation

with phosphatidylcholine and phosphatidylethanolamine modulated the myelination deficit of affected Schwann cells in cultures and resulted in improved myelin biosynthesis and reduced neuropathic symptoms in the CMT1A rat model [28]. Since dietary phospholipids are devoid of significant side effects, clinical translation is easy and a clinical trial with oral lecithin supplementation is planned.

2.4. P2X7 purinoreceptors and calcium influx

An abnormally high, potentially toxic, intracellular Ca^{2+} concentration was observed in Schwann cells from CMT1A rats, and there is evidence that this is at least partly caused by the PMP22-mediated overexpression of the P2X7 purinoceptor, which acts as a calcium channel and is involved in apoptosis, regulation of receptor trafficking, and inflammation [29].

A pharmacological inhibitor of P2X7 receptors, A438079, was tested in CMT1A rats and ameliorated their strength and histological parameters (increased number of tibial nerve myelinated axons, decrease in hypermyelinated small fibers, and increase in large diameter myelinated fibers) [30]. The translation to clinical trials in CMT1A is facilitated by previous Phase II clinical trials in rheumatoid arthritis with a P2X7 antagonist which showed acceptable safety and tolerability [31].

2.5. Endoplasmic Reticulum (ER) stress and Unfolded Protein Response (UPR) activation

CMT1B is caused by mutations of MPZ, which is the major protein constituent of peripheral myelin. Mutations in MPZ can prevent the mutant protein from reaching the membrane and cause its retention and accumulation in the ER inducing apoptosis and subsequent neuropathy. Retention of the mutant proteins in the ER leads to activation of the UPR, an adaptive and protective process to relieve stress from misfolded proteins (Figure 2). However, when cells are in chronic stress, such as in CMT1B, the UPR becomes inadequate, and it activates apoptotic pathways resulting in cell death or abnormal signaling. Many MPZ mutations were shown to activate the UPR and cause CMT1B, and these mutations may be susceptible to future therapies targeting the UPR [32]. The first studies showed a potential benefit from curcumin, which is able to modulate the UPR among its many activities [33,34]. More recently, Sephin-1 (IFB-088) – an inhibitor of the dephosphorylation of eIF2a, a kinase belonging to the PERK arm of the UPR – that prolongs protein translation attenuation in response to stress, was shown to be beneficial in the S63del and R98C MPZ mouse models, prolonging the response and preventing the molecular, morphological and motor defects of the neuropathy [35,36]. ER accumulation of misfolded proteins and UPR activation have been demonstrated also in rodents with *PMP22* point mutations (CMT1E) [37,38], and PMP22 molecule aggregates have been detected in CMT1A models

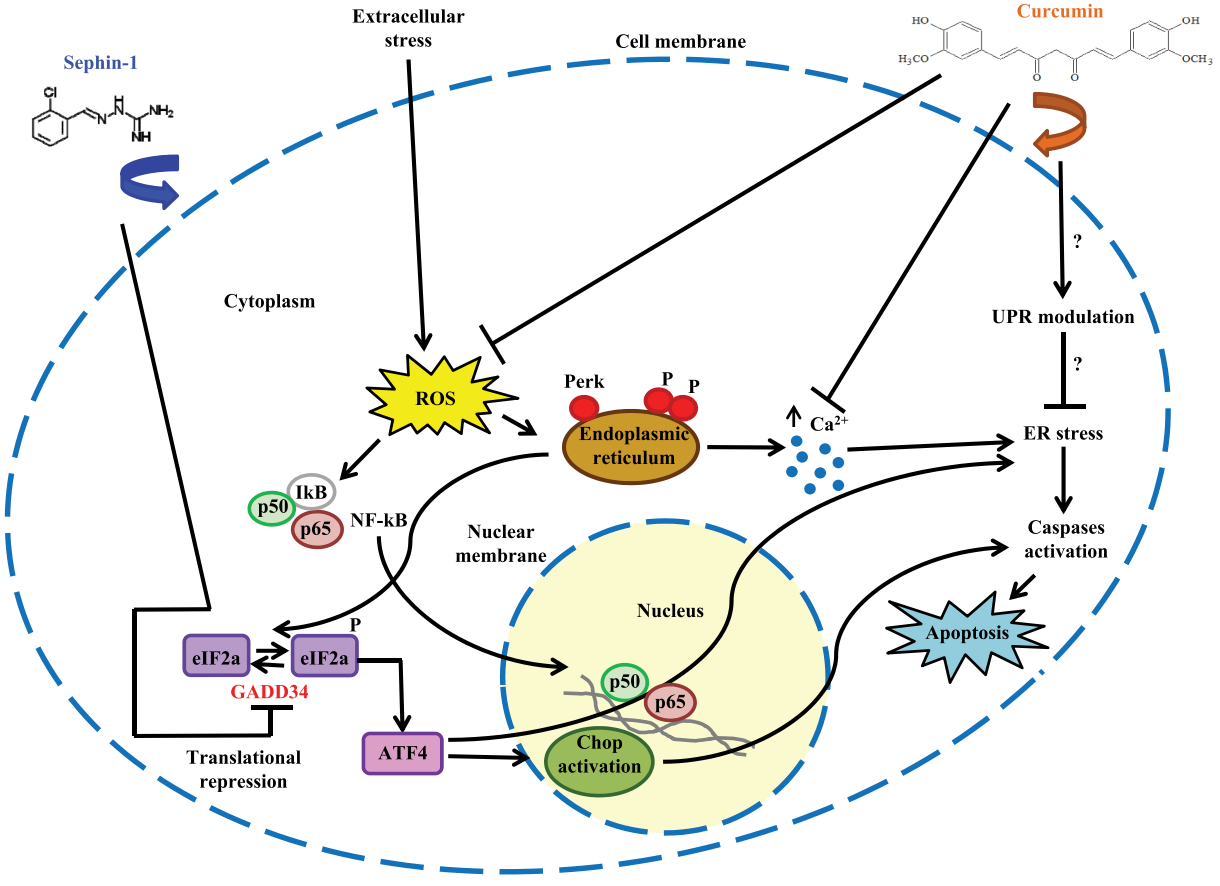

Figure 2. **Sephin-1 and curcumin mechanisms of action on unfolded protein response (UPR) in mammals.** Chop = C/EBP homologous protein, ER = endoplasmic reticulum, GADD34 = growth arrest and DNA-damage-inducible protein 34, ROS = reactive oxygen species, UPR = unfolded protein response.

[39] and in dermal fibroblast from CMT1A patients [40]. Sephin-1 is also being tested in CMT1A rodent models [41] and this molecule is therefore a promising candidate to be tested in clinical trials for both CMT1A and CMT1B; theoretically the approach can be valuable also for other mutants retained in the ER as occurs for some *GJB1* mutations associated with CMTX1.

2.6. Sodium channel blockers

Dysmyelination in MPZ knock-out mice is associated with an ectopic expression of the sensory neuron-specific sodium channel isoform $Na_V1.8$ on motor axons. Rosberg and colleagues showed that the progressive impairment of motor performance in MPZ deficient mice was reversed by $Na_V1.8$ blocker treatment [42]. These findings provide a rationale for the potential use of oral subtype-selective $Na_V1.8$ blockers to treat motor dysfunction in severe forms of demyelinating CMT1B and possibly other dysmyelinating CMT types [43].

2.7. Neuregulin pathway

Neuregulin1 type III (Nrg1-III) is produced by axons and is a fundamental signal for myelination by acting on Schwan cell membrane ErbB2/B3 receptors and activating the PI3K–Akt signaling pathway. The amount of Nrg1-III determines the myelin thickness and its activity is regulated by the secretases BACE1 (activity enhancer) and TACE (inhibitory effect), by means of different extracellular cleavage [44]. Reduction of Nrg1-III activity may constitute a therapeutic strategy to treat hypermyelination neuropathies; indeed, increasing TACE activity with the commercially available niacin-Niaspan proved of benefit in two different animal models of hypermyelinating neuropathies, CMT4B1, a severe recessive neuropathy characterized by the widespread presence of hypermyelination abnormalities (myeling outfoldings) and HNPP where hypermyelination changes lead to tomacula formation [45]. It is also reasonable that modulating Nrg1-III activity may constitute a general therapeutic strategy to treat CMT that are characterized by de/dysmyelination. Recently genetic overexpression of Nrg1-III has been shown to ameliorate the neurophysiological and morphological parameters in a mouse model of CMT1B and stimulating Nrg1-III signaling by pharmacological suppression of the Nrg1-III inhibitor TACE also ameliorated the neuropathy [46]. The importance of the neuregulin pathway is confirmed by the notable finding that soluble Neuregulin1 type I promoted myelin repair after nerve injury and its administration to CMT1A rats improved their motor performance, increased the number of myelinated axons in sciatic nerves, and restored the reduced axonal caliber [47].

2.8. CMT4B: other approaches

CMT4B1 and CMT4B2 are caused by loss-of-function mutations in the myotubularin-related 2 (MTMR2, CMT4B1) and MTMR13 (also known as SBF2, SET binding factor 2, CMT4B2) genes, involved in the phosphoinositides (PIs) metabolism [48]. Decrease phosphatase activity of MTMR2 results in deleterious accumulation of PI3,5P2 which can be counteracted by inhibiting the PIKfyve enzyme in the previous step in PIs metabolism. There are compounds under study able to inhibit PIKfyve and lower the PI3,5P2 levels and theoretically able to correct the defect underlying CMT4B1/B2 [49].

2.9. Addressing axonal degeneration and axonal transport

Axonal degeneration is the final common pathway of all CMT types, irrespective of whether they represent primary myelinopathies with secondary axonal damage or they are primary axonopathies, and axonal transport appears to be altered in many inherited neuropathies. Thus, any therapeutic intervention aimed at preventing axonal degeneration and/or improving axonal transport is of utmost importance as potentially useful for all CMT types.

SARM1 (sterile alpha and toll/interleukin 1 receptor motif-containing 1) is a main actor of the axonal degenerative program and SARM1 loss prevents axonal degeneration and improves functional outcome after nerve injury as well as in different toxic-metabolic neuropathy models [50]. SARM1 inhibitors are being developed and are of great interest as potential treatment of all CMT and related neuropathies.

Acetylation of microtubules is important for axonal transport. Defect in axonal transport occurs in many neuropathy models including mice carrying mutations in the *HSPB1* gene associated with dHMN type 2B and CMT2F. Inhibition of Histon De-ACetylase type 6 (HDAC6), by increasing acetylated alpha-tubulin, has been shown to correct axonal transport defects and rescue the phenotype of mutant HSPB1 mice [51]. To translate these results into clinical application, potent and selective HDAC6 inhibitors are being explored. Furthermore, HDAC6 inhibition has been shown to partially restore nerve conduction and motor behavior in mutant *Gars* mice (model of CMT2D/dHMN type VA) with reduced levels of acetylated alpha-tubulin [52]. Hdac6 deletion prevented motor and sensory dysfunction in the $MFN2^{R94Q}$ mouse model of CMT2A [53]. All these findings suggest that decreased acetylated alpha-tubulin may represent a common pathomechanism of many axonal neuropathies and HDAC6 inhibitors may have therapeutic potential for all of them.

2.10. Muscle hypertrophy

A more general approach aimed at increasing muscle volume has been explored by using ACE-083, a locally acting follistatin-based molecule that has been shown to increase skeletal muscle mass and force through inhibition of myostatin and other muscle regulators [54]. After a phase I dose escalating trial, in the phase II study up to 250 mg ACE-083 was injected bilaterally in Tibialis Anterior muscles every 3 weeks for up to 9 doses. Overall 62 patients with CMT1 and CMTX1 were treated, but the program was stopped before the planned phase III trial because of unsatisfactory results: an increase in muscle volume was obtained, but it failed to translate into significant improvements in functional or quality-of-life measures, compared to placebo (ClinicalTrials.gov NCT03124459).

2.11. Inflammation

The role of inflammation in CMT is matter for debate and investigation. Low-grade inflammation from phagocytizing macrophages contributes to the neuropathic phenotype in mouse models for CMT1A, CMT1B and CMTX1. An important macrophage activator is colony-stimulating factor 1 (CSF1). It has been shown that inhibition of CSF1 receptor (CSF1R) leads to a decline in nerve macrophages. In CMTX1 and CMT1B mice, CSF1R inhibition was a safe and effective treatment option resulting in improvement of neuropathological and clinical findings [55].

2.12. Cramps

Cramps may be disabling symptoms in CMT. A randomized, double-blind, placebo-controlled, parallel group phase II study for cramps in CMT have been conducted using oral FLX-787, 30 mg TID, a small molecule activating TRPA1 and TRPV1 channels. Though the molecule was promising, the trial was halted for oral intolerability in a subset of patients (ClinicalTrials.gov NCT03254199).

2.13. Sorbitol and the polyol pathway

Biallelic mutations in the sorbitol dehydrogenase (*SORD*) gene were recently identified as a frequent cause of autosomal recessive CMT2/dHMN. Sorbitol dehydrogenase catalyzes the oxidation of sorbitol into fructose in the polyol pathway. Patients carrying *SORD* mutations show very high levels of blood sorbitol, which, as shown in previous studies on diabetic neuropathy, may be toxic for the peripheral nerves. Inhibitors of aldose reductase, the enzyme converting glucose into sorbitol, were developed in attempts to treat diabetic neuropathy and they are being considered as candidates for therapeutic intervention to decrease sorbitol levels [56].

2.14. Serine in hereditary sensory neuropathy type I

Hereditary Sensory Neuropathy type I (HSN1) is associated with mutations in the *SPTLC1* and *SPTLC2* genes, encoding subunits of the serine palmitoyltransferase (SPT) enzyme catalyzing the first step of sphingolipids synthesis by conjugating palmitoyl-CoA and L-serine. HSN1 mutations reduce the affinity of SPT for L-serine and increase its affinity for alanine and glycine, leading to formation of neurotoxic 1-deoxysphingolipids (1-deoxySLs). The levels of 1-deoxySL can be reduced in mice and humans by treatment with high doses of L-serine, the normal substrate of SPT. Prolonged dietary supplementation with L-serine in transgenic mice resulted in significant clinical improvement, including improved motor performance and increased unmyelinated sciatic nerve fibers. A randomized, double-blinded, placebo-controlled, 2-year trial in 18 adults with HSN1 showed that L-serine treatment was well-tolerated and led to significant reduction in 1-deoxySL levels. The primary outcome did not differ between the L-serine and placebo groups [57]. However, participants taking L-serine did demonstrate a significant quantitative improvement in CMT neuropathy score as compared to those taking placebo during the first year of the study, suggesting that L-serine may offer clinical benefit in HSN1 and that trial failure was related to the too small sample size, due to the rarity of the disease, with insufficient statistical power. Therefore, further research is needed to confirm L-serine efficacy [58].

2.15. CMTX5

Loss-of-function mutations in phosphoribosylpyrophosphate synthetase 1 (PRPS1), an enzyme involved in purine metabolism, are associated with three partially overlapping allelic disorders: X-linked Charcot-Marie-Tooth disease-5 (CMTX5), Arts syndrome and the X-linked nonsyndromic sensorineural deafness DFN2. S-adenosylmethionine (SAM) supplementation in two Arts syndrome patients seems to have improved their condition by replenishing purine nucleotides pool [59] and therefore SAM is a candidate for CMTX5 as well.

2.16. CMTX6

The p.R158H mutation in the gene encoding pyruvate dehydrogenase kinase isoenzyme 3 (PDK3), one of the four isoenzymes regulating the activity of the pyruvate dehydrogenase complex (PDC), has been described in two unrelated X-linked CMT families (CMTX6). The mutation by determining PDK3 hyperactivity results in reduced PDC activity and impaired ATP production. Genetic editing of the mutation or pharmacological correction of the metabolic defect with the PDK inhibitor dichloroacetic acid (DCA) in patients' iPSCs-derived motoneurons reversed the functional defects, providing a proof of concept for screening of therapeutic molecules [60].

3. Expert opinion

From the review of potential treatment currently under investigation, we can conclude that we are getting closer to the target of finding effective therapies for CMT, but, at the same time, that we are far behind the exciting examples of Spinal Muscle Atrophy (SMA), Duchenne Muscular Dystrophy and TTR-related hereditary amyloidosis (ATTRv), where effective treatments now exist and have greatly improved the prognosis of these devastating diseases. Indeed, we are still without any drug treatment for CMT and there is no major impact on real world coming from ongoing research, yet.

Which are the reasons for the gap with the other neuromuscular disorders? There are several contributing factors. The peripheral nerves are intrinsically more difficult to study as compared to the muscles and even to the motor neurons: it is problematic to obtain nerve tissue to be studied and characterized, Schwann cells and myelination studies are particularly challenging, and it is currently extremely hard if not impossible to obtain reliable Schwann cells from iPSCs. It is also arduous to target drugs and gene therapies to the peripheral nerves, particularly to correct Schwann cell defects. Other reasons are constituted by: the great genetic heterogeneity of this group of diseases, with about 100 genes and loci involved in CMT and related neuropathies; the great variability of disease expression, with different phenotype severity

occurring for the same gene mutation (particularly for the CMT1A duplication), sometimes even within the same family; the slow disease progression in many CMT cases, which is good for the patients but makes it difficult to measure worsening in natural history studies and assess benefit from tested treatments; and the rarity of the majority of CMT types, a challenge for studies on pathomechanisms and clinical trials. Therefore, there is still a lot of work to do with preclinical and clinical studies before effective treatments become available for many CMT forms.

From the preclinical standpoint, it is important to work on reliable animal models reproducing the disease as close as possible. There are many novel available models, particularly rodents, but flies and zebrafishes are also gaining popularity because they allow cheaper and more rapid studies with greater animal numbers; the use of patient-specific iPSCs to derive neural cells constitutes a major advance to understand pathogenic mechanisms of disease and to test potential therapeutic compounds [61]. There are also important technical advances in several research areas. For instance, skin biopsies allow obtaining nerve tissue in a minimally invasive and repeatable manner, and beyond immune-histochemical studies it is now possible to reliably measure mRNA expression of several key genes from skin nerves with the nanostring technique [62]. Manipulation of DNA is becoming easier and easier, with CRISPR-Cas9 and more recent genome editing techniques, and more efficient and safer vectors and delivery systems, thus constituting perhaps the major hope for CMT treatment through genetic therapy.

From the clinical point of view, first of all we must not forget to offer to CMT patients currently available management possibilities, that include symptomatic treatment for pain (which can be osteo-artho-muscular or neuropathic in origin) [63], rehabilitation therapy [64,65], and surgery for skeletal deformities [66]. Definition of standard of care is important and is ongoing.

Secondly, it is essential to deliver a precise genetic diagnosis to CMT patients whenever possible and feasible, not only for proper genetic counseling and prognostic advice, but also because future therapies will likely be at least in part specific to CMT subtype and even to mutation type (e.g. for UPR activating mutations). Moreover, it is crucial to collect large patient series for performing natural history studies and preparing cohorts of patients for clinical trials. As more potential treatments will enter the clinical phase, there is the risk to have different clinical trials competing and consequent difficulties in patients' recruitment.

To overcome the difficulties in the pathway to the cure for CMT there are helpful steps that the CMT world stakeholders can undertake together. Indeed, it is fundamental to create extensive networks bringing together researchers working in the field, from basic scientists to clinicians, to facilitate collaborative studies and exchange of expertise. There are already consortia working with these aims, like the international Inherited Neuropathy Consortium (INC) of the Rare Disease Clinical Research Network (RDCRN) (https://www.rarediseasesnetwork.org/cms/inc), collecting 20 centers in the US, UK, Italy, Australia, which is more and more interacting with national networks in Italy, France, Germany, Spain, and with the Asian-Oceanic Inherited Neuropathy Consortium, AOINC, as well as with the different national and international Patients Advocacy Groups, under the umbrella of the CMTR Consortium, a Special Interest Group of the international Peripheral Nerve Society (https://www.pnsociety.com/i4a/pages/index.cfm?pageID=3322&pageid=3370). The interaction of these consortia with the companies is also important to properly drive the preclinical and clinical trials.

Another area of active research, often through extensive collaborative studies, is aimed at clinical trial readiness [67]. Large natural history studies have been performed, or are under way or planned, and they are important to better define the behavior of the different CMT forms and mutations in order to design appropriate clinical trials [68–70]. Huge databases (the one alimented by the INC includes thousands of patients) and several national registries have been developed and are important for epidemiological reasons and to facilitate recruitment in clinical trials. A big effort has been addressed at developing responsive outcome measures, both clinical and paraclinical. The first CMT-specific clinical scale has been refined (CMTNSv2) [71] and a Rasch version is available [72]; the CMTPedS, the first specific scale for children, which is normalized toward age-matched controls as the performances improve with growth, demonstrated good responsiveness in longitudinal studies [70,73]. The CMT-FOM is the correspondent scale for adults, derived from the CMTPedS, showed high reliability and internal consistency, and is currently tested for responsiveness [74,75]. The CMT-HI is a novel patient-reported OM aimed at measuring disease burden in CMT adults and is becoming available in languages other than English [76,77]. The CMT-PQoL is a quality-of-life scale for children [78]. Clinical scales have still limitations in responsiveness, and therefore efforts are concentrated also on paraclinical biomarkers. Currently, the most sensitive-to-change measure is the quantitative MRI of thigh and leg muscles with 3-point Dixon sequences (qMRI). By means of qMRI it is possible to reliably measure the muscle fat fraction (FF), which reflects the percentage of muscle substituted by fat as a consequence of axonal loss, increases in a detectable and significant manner after one year in CMT1A and HSN1, and correlates with clinical outcome measures [58,79]. Blood and skin nerve biopsy biomarkers are also promising. As examples, neurofilament light-chain blood levels are increased in CMT as a consequence of axonal damage [80] and, as mentioned above, measure of several genes' expression at the mRNA level is possible in skin nerve biopsy by means of nanostring technique [62].

Preclinical and clinical studies are costly and progress toward therapy requires adequate funding. This is a major issue as the CMT neuropathy group is considered marginal with respect to other neurological disorders with greater impact on health and economics. However, among the rare disease, CMT is one of the most frequent, with a prevalence ranging between 10 and 28/100,000 [1], corresponding to up to 200,000 cases in Europe and up to almost 100,000 in the US, and affects the individual for her/his entire life. Therefore, an effective therapy for CMT would have a relevant impact also

from the socio-economic and health system points of view [81]. Although there is still a long way before CMT can be cured or prevented, there are great potentialities in current research and novel approaches are continuously being explored. Looking at close areas like ATTRv and SMA, where rapid developments and achievements occurred in a few years [82–84], it is likely that we will face incredible advances in CMT treatment in the near future.

Ideally the aim is to treat the disease before it causes significant axonal degeneration and fiber loss, which are difficult to revert. As the vast majority of CMT types has early onset, the aim is to treat children as soon as they are diagnosed with the disease. Thus far, most clinical studies targeted adult patients for practical reasons and regulatory aspects, but future studies should be addressed mainly to children, although another arising problem is the difficulty to measure treatment efficacy when impairment and disability are limited as in the first disease phases.

Which are the treatments under study closer to success and possible translation into clinical practice? This is difficult to say. PXT3003 for CMT1A is the drug in the most advance phase and it will be of utmost importance to see the results of the novel Phase III trial. There has been some criticism to the rationale of treatment (initially based on computer analyses of interactions of the three compounds with PMP22 and on the concept of pleotropic therapy aiming at a synergistic effect of very low doses of the drugs) and to the selected outcome measures (with the poorly responsive ONLS as primary endpoint). The rationale for using anti-progesterone drugs for CMT1A is strong but the hormonal effects prevent their use in fertile women. The compounds acting on UPR and Neuregulin pathways are very interesting and promising, but they need to complete the preclinical phases and to verify their efficacy in patients. Drugs and intervention aimed at axonal degeneration prevention and reversion as well as at axonal transport restoration have great potentialities of impacting on all neuropathies, which would be an outstanding achievement. It is also possible to envisage to combine different treatments once they will be available. However, it is important to remember that any drug needs to be administered all life long, with problems of side effects, compliance, and costs. This is also true for repeat gene silencing approaches, like those with ASOs and siRNAs, as for ATTRv and SMA, which however are lethal diseases as compared to most CMT forms which do not shorten the life span. A clinical trial with ASO or siRNAs for partial *PMP22* gene silencing is certainly reasonable to consider and has all the premises for being successful. Indeed, gene manipulation appears to be the most promising approach for the future for the different CMT types and the hope is to be able to correct the genetic defect in one shot (as with the novel gene therapy for SMA) [85], but several issues still need to be solved. The main ones are related on how to deliver the gene therapy to the target cells, difficult for motor and sensory neurons and even more complicated for Schwann cells, which are widely dispersed in all the body nerves. While ASO could be administered subcutaneously as for other diseases, it is more problematic to deliver gene therapy to the target cells, and safe and effective vectors are needed. Recently employed AAV9 seem to be safer and more efficient [24]. Direct intranerve injection may carry risk of lesions and was not effective enough in previous attempts for the CMTX mouse model [22], but is being thoroughly investigated by the group of Nicolas Tricaud with encouraging results in terms of percentage of targeted Schwann cells and of length of diffusion along injected nerve [16,86]. Intrathecal delivery showed greater than expected vector diffusion with high levels of new gene expression in roots and nerves at distance from the subarachnoid spaces. Further investigations are needed before attempting translation to human trials and there is need to continue research to find more selective vectors and delivery routes. Use of nanotechnologies may be the turning point in this field.

The next five years will be fundamental in the pathway to therapy. There are so many potentially effective treatments under investigation that it is difficult to predict what will happen in this time frame, but some treatments will reach the clinical trial phase, other trials will be completed; there will be advances to study the peripheral nerves and to make gene delivery and editing feasible in CMT patients. L-Serine for HSN1, ASO and siRNAs for CMT1A, and drugs targeting UPR and neuregulin pathways are the most promising in the short term, while there is hope for effective gene manipulation in the medium term. A review written in 2026 will deal many trials completed, ongoing, or planned, either successfully or very promising and a cautious optimism in the field is reasonable that a therapy for different CMT forms will be available within the end of the current decade.

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