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## REVIEW ARTICLE

# Treating *PMP22* gene duplication-related Charcot-Marie-Tooth disease: the past, the present and the future

SUZAN BOUTARY, ANDONI ECHANIZ-LAGUNA, DAVID ADAMS, JULIEN LOISEL-DUWATTEZ, MICHAEL SCHUMACHER, CHARBEL MASSAAD, and LILIANE MASSAAD-MASSADE

LE KREMLIN-BICÊTRE AND PARIS FRANCE

**Charcot-Marie-Tooth (CMT) disease is the most frequent inherited neuropathy, affecting 1/1500 to 1/10000. CMT1A represents 60%-70% of all CMT and is caused by a duplication on chromosome 17p11.2 leading to an overexpression of the Peripheral Myelin Protein 22 (*PMP22*). *PMP22* gene is under tight regulation and small changes in its expression influences myelination and affect motor and sensory functions. To date, CMT1A treatment is symptomatic and classic pharmacological options have been disappointing. Here, we review the past, present, and future treatment options for CMT1A, with a special emphasis on the highly promising potential of *PMP22*-targeted small interfering RNA and antisense oligonucleotides. (Translational Research 2020; 000:1 – 12)**

**Abbreviations:** Peripheral Nervous System = PNS; Charcot-Marie-Tooth = CMT; Human Peripheral Myelin Protein 22 gene (*PMP22*) protein = PMP22; Rodent Peripheral Myelin Protein 22 gene = *Pmp22*; Human Myelin Protein Zero = (*MPZ*); and Myelin Basic Protein = (*MBP*) genes; Guillain-Barré syndrome = GBS; Chronic Inflammatory Demyelinating Polyradiculo-Neuropathy = CIDP; Charcot-Marie-tooth 1A = CMT1A; Motor Nerve Conduction Velocity = MNCV; Compound Muscle Action Potential = CMAP; Hereditary Neuropathy with Liability to Pressure Palsies = HNPP; L1 cell adhesion molecule = (*L1CAM*); and nerve growth factor receptor = (*NGFR*); Neurotrophin-3 = NT-3; Adeno-Associated Viruses = AAV; Charcot-Marie-Tooth Neuropathy Score = CMTNS; Overall Neuropathy Limitations Scale = ONLS; RNA interference = RNAi; Small interfering RNA = siRNA; Hereditary Transthyretin Amyloidosis = hATTR; Transthyretin = TTR; Antisense Oligonucleotides = ASO; Heat Shock Proteins = HSP

## INTRODUCTION

Disorders of the peripheral nervous system (PNS) affect 2.5% of the population.<sup>1</sup> The glial cells of the PNS, known as Schwann cells, are responsible for

myelination. They were discovered in the mid-nineteenth century by Theodore Schwann.<sup>2</sup> Their main functions are to separate axons and to produce the myelin sheath that allows saltatory conduction of nerve impulses.<sup>3</sup> Several diseases affecting PNS myelin have

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been described, including (1) peripheral nerve trauma,<sup>4</sup> (2) autoimmune polyneuropathies such as Guillain-Barré Syndrome,<sup>5</sup> chronic inflammatory demyelinating polyradiculo-neuropathy,<sup>6</sup> and (3) inherited demyelinating sensory and motor neuropathies, including Charcot-Marie-Tooth disease (CMT).<sup>7</sup>

In this review, we will focus on CMT, the most frequent inherited diseases of the PNS with a prevalence of 1/1500 to 1/10000 worldwide.<sup>8-11</sup> CMT are progressive neuromuscular disorders characterized by degeneration of the peripheral nerves leading to muscle atrophy, weakness in the distal limbs, absence of deep tendon reflexes, and osteoarticular deformations such as *pescavus*. In certain cases, wheelchair is needed.<sup>12</sup> Although disease onset ranges from childhood to late adolescence, several forms of CMT may appear in adults and the severity of the symptoms differs between patients.<sup>9,10</sup> It is noteworthy, that phenotypic variations of CMT are observed both in members of the same and different families.<sup>13</sup>

CMT is classified into 5 types depending on the mode of inheritance, the pathophysiology and causative genes involved<sup>14</sup> (Table I). More than 90 genes comprising over 1000 rearrangements cause CMT<sup>9,15</sup> and contributed to the phenotypic heterogeneity. CMT1 is the autosomal dominant demyelinating form that accounts for 50%-80% of all CMT cases.<sup>14</sup> It is characterized by reduced motor nerve conduction velocity (MNCV) of less than 38 m/s, diminished muscle stretch reflexes and formation of onion bulbs.<sup>14,16</sup> CMT2, represents 10%-15% of CMT, is the autosomal dominant or recessive axonal form that shows normal MNCVs > 45m/s, decreased compound muscle action potential (CMAP) amplitude and reduced muscle

stretch reflexes.<sup>8,17</sup> CMT4, frequent in countries with high consanguinity, is the autosomal recessive demyelinating form and CMTX is the X-linked form that accounts for 10%-15% of all CMT cases.<sup>18,19</sup> Interestingly, it has been found that several genes responsible for CMT are also involved in other neurological and neuromuscular disorders such as myopathy, spastic paraplegia, and motor neuron disorders that share common pathophysiological pathways.<sup>20</sup> Recently, 4 genes were acknowledged as the main cause of the majority of the CMT cases: *PMP22*, myelin protein zero (*MPZ*), gap junction beta-1 protein (*GJB1*) and Mitofusin 2 (*MFN2*)<sup>8,14</sup> and further knowledge about these genes is needed to understand the pathology of each subtype and facilitate the discovery of new treatment strategies. Moreover, it has been speculated that some gene modifiers play an important role in the heterogeneity of the disease.<sup>21-24</sup>

Herein, we address the most frequent type, CMT1A, from drug treatment to *PMP22* gene inhibition and highlight the most recent therapeutic innovations that open new avenues for the treatment of CMT1A patients.

## PERIPHERAL MYELIN PROTEIN: PHYSIOLOGICAL ROLE AND DISORDERS

*PMP22* is a 22 kDa glycoprotein of 160 amino acids produced by Schwann cells.<sup>25</sup> It was identified in Schwann cells and the fibroblasts.<sup>26,27</sup> Later, *PMP22* gene was reported to be also expressed in the intestines, lungs and heart.<sup>28,29</sup> The *PMP22* gene has 2 promoters that lead to translation of 2 transcripts containing

**Table I.** Classification of CMT disease. The classification is done according to the inheritance and pathophysiological trends and examples of causative genes

Type	Inheritance	Pathophysiology	Examples of causative genes
CMT1	Autosomal dominant	Demyelinating NCV <38m/s	<i>PMP22, PO, LITAF, EGR2, NEFL, FBLN5</i>
CMT2	Autosomal dominant	Axonal NCV >45 m/s	<i>MFN2, RAB7, TRPV4, PO, GDAP1, HSPB8, DNM2, LRSAM1, MT-ATP6, MARS, HARS</i>
	Autosomal recessive		<i>LMNA, MED 25, GDAP1, MFN2, NEFL, TRIM2</i>
CMT4	Autosomal recessive	Demyelinating NCV <38m/s	<i>GDAP1, MTMR2, MTMR13, EGR2, SBF1, NDRG1, FIG4, SURF1, PRX, HK1</i>
CMTX	X-Linked	Axonal NCV >45 m/s	<i>GJB1, PRPS1, PDK3</i>
RI-CMT (Recessive intermediate CMT)	Autosomal recessive	Intermediate 38 < NCV <45m/s	<i>GDAP1, KARS, COX6A1</i>
DI-CMT (Dominant intermediate CMT)	Autosomal dominant	Intermediate 38 < NCV <45m/s	<i>DNM2, YARS, MPZ, IFN2, GNBA</i>

different noncoding exons, 1A and 1B, which makes it tissue-specific and highly regulated.<sup>30,31</sup> Promoter one that generates exon 1A transcripts is active in myelinating Schwann while, promoter two and exon 1B transcripts are expressed in tissues that do not produce myelin.<sup>30</sup>

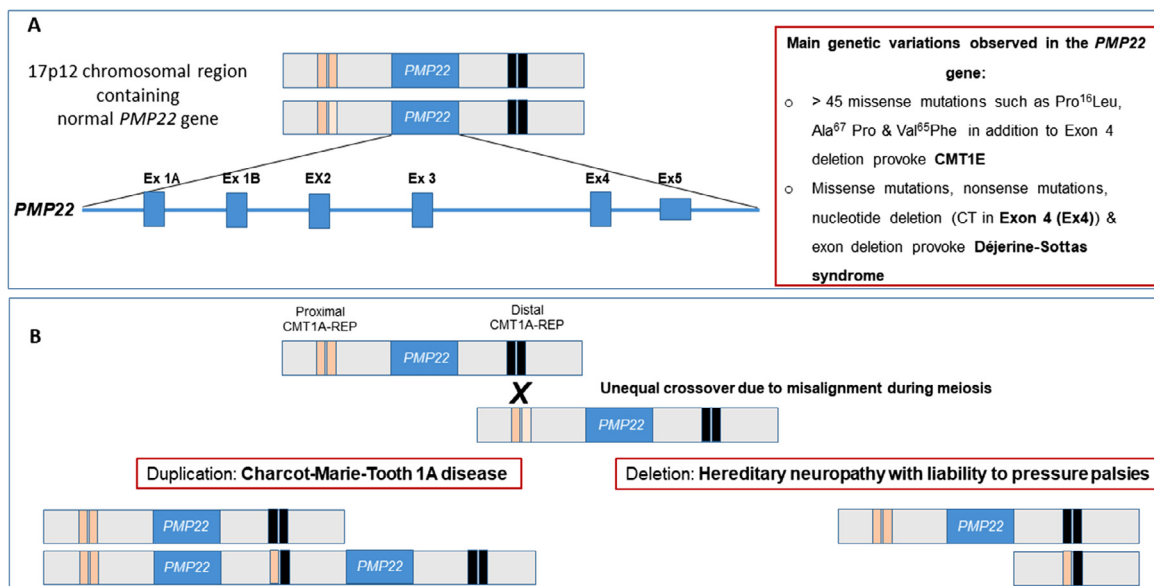
*PMP22* expression is regulated at the gene level as well as at the step of protein synthesis and translocation. In the peripheral nervous system, *PMP22* comprises 2%-5% of the total myelin proteins and is located in the compact region of the myelin sheath.<sup>25</sup> The majority of *PMP22* protein is degraded in the endoplasmic reticulum, while only few proteins are glycosylated and reach the Golgi apparatus.<sup>32</sup>

*PMP22* has an essential role in myelination in the PNS but its biological functions are not clear yet. Proper folding and regulation of *PMP22* is required for the normal function of myelinating Schwann cells. Evidence was shown that *PMP22* promote the organization of membrane ultrastructure in compact myelin.<sup>33</sup> Moreover, it was documented that *PMP22* plays a key role in the maintenance of cholesterol homeostasis in Schwann cells. *PMP22* interacts with the cholesterol efflux regulatory protein ABCA1 to regulate lipid metabolism and cholesterol trafficking.<sup>34</sup> Other than its role in the formation of myelin, studies have shown that *PMP22* is involved in different cellular functions such as adhesion and cell proliferation since it was first

discovered to be upregulated in growth arrested fibroblasts.<sup>35,36</sup> It should be noticed that *PMP22* protein expression was shown to be elevated in the proliferative phase of human endometriosis.<sup>37</sup> Moreover, *PMP22* plays an important role in proliferation and invasion of some types of cancers such as osteosarcoma<sup>38,39</sup> and breast cancer.<sup>40</sup>

*PMP22* gene is under tight regulation and small changes in its expression can drastically influence myelination and, by extension, affect motor and sensory functions.<sup>41,42</sup> Genetic abnormalities in *PMP22* gene accounts for more than 50% of the inherited peripheral neuropathies including CMT1A, hereditary neuropathy with liability to pressure palsies (HNPP), Déjerine-Sottas syndrome and CMT1E<sup>43</sup> (Fig 1). Overexpression of *PMP22* levels resulting from the trisomy of *PMP22* gene lead to CMT1A.

HNPP is an autosomal dominant inherited neuropathy that causes episodes of numbness and weakness. It is due to deletion of one copy of *PMP22* gene on chromosome 17 p11.2. Its symptom onset is at the second and third decade of life although some symptoms start at early age. Similar to CMT patients, HNPP may be asymptomatic and shows intrafamilial phenotypic variability. Electrophysiological studies demonstrated increased distal motor latencies and reduced motor and sensory NCV. The pathological characteristic of HNPP is the presence of. tomaculas, which are massive



**Fig. 1.** Pathologies related to *PMP22* gene. **A**, Representation of chromosome 17p11.2 region; the normal *PMP22* gene that carry 5 exons (Ex) is highlighted in between two lines. The principal genetic variations described in the literature that lead to Charcot Marie Tooth 1E (CMT1E) and Déjerine-Sottas syndrome (DSS) are emphasized in the red box. **B**, Shows an unequal crossover in ch17p11.2 region due to misalignment during meiosis leading to either 1.5 Mb duplication or deletion responsible for the CMT1A or Hereditary neuropathy with liability to pressure palsy (HNLP) diseases. Unequal crossover is mediated by CMT1A-REPs which are conserved 27 kb pair sequences flanking the 1.5Mb region of ch 17p11.2.

folding of layers of variable thickness in the myelin sheath.<sup>44</sup> That's why it is also known as tomacular neuropathy.

CMT1E is referred to the CMT1 subtype resulting from *PMP22* gene point mutations. Mutations alters the protein amino acid sequence and can lead to gain of new function of the gene or loss of gene function due to premature termination of translation similar to HNPP pathology.<sup>45</sup> One of the severe heterozygote point mutations on *PMP22* gene mice is the Leu<sup>16</sup>Pro. This point mutation is also expressed in the transgenic trembler J mouse model. It leads to abnormal Schwann cell-axon interaction, demyelination and axonal loss. The severity of the phenotype in the *PMP22* point mutation is mainly due to loss of intact *PMP22* at the plasma membrane and cellular stress induced by misfolded *PMP22*.<sup>46</sup> Point mutations disrupt *PMP22* plasma membrane trafficking resulting in its misfolding. Misfolded *PMP22* is directly targeted by the endoplasmic reticulum (ER) associated degradation for clearance. However, the clearance by ER is not 100 % efficient thus causing cellular aggregation of *PMP22* resulting in cellular stress.<sup>47</sup> Indeed, this excess in *PMP22* aggregates was detected in sural nerve biopsies of CMT1E patients harboring the *PMP22* mutation but not in sural nerve biopsies of CMT1A patients.<sup>48</sup> This highlights the difference between CMT1E and CMT1A neuropathies.

Dejerine-Sottas syndrome is an early onset demyelinating hereditary neuropathy that occurs in the first two years of life. It is characterized by a very low MNCV of less than 12 m/s, thin myelin sheath and onion bulb formation. It shows common symptom with CMT as muscle wasting, weakness and foot deformity. It is caused by *PMP22* or *MPZ* genes mutations.<sup>45</sup>

**CMT1A: the most frequent CMT subtype.** CMT1A represents 60%-70% of all CMT.<sup>49</sup> It is caused by duplication of a 1.4 mega base (Mb) region on chromosome 17p11.2 that lead to overexpression of *PMP22* gene<sup>8,49</sup> due to unequal crossover during meiosis.<sup>50</sup> An increase of *PMP22* levels has been reported in biopsies from sural nerve of CMT1A patients using immunological electron microscopy.<sup>51</sup> However, using skin biopsy techniques it has been shown that the *PMP22* mRNA expression and protein levels are variable in CMT1A patients.<sup>52</sup> It has been speculated that the *PMP22* levels fluctuate over time.<sup>53</sup> At a certain time point, *PMP22* level will be either elevated or normal.<sup>54</sup> This variability was also detected in a study to define Schwann cell biomarkers from skin biopsies using RNA-seq technique and a digital gene expression platform by nanostring technology. Using this approach, Svaren et al, were able to detect an increase in *PMP22* expression by 1.5-2 folds in skin biopsies from CMT1A patients

when normalized to Schwann cells specific genes.<sup>55</sup> This method can be helpful for CMT1A clinical trials to detect the changes of *PMP22* and other CMT1A related genes such as L1 cell adhesion molecule (*LICAM*) and nerve growth factor receptor (*NGFR*).<sup>55</sup>

Moreover, to better understand CMT1A pathology and find new targeted therapy, several sensitive biomarkers reflecting CMT1A impairment and progression have been studied recently. These biomarker can be helpful in the diagnosis and the detection of the severity of CMT1A. Measurements of intramuscular fat fraction of calf muscle that was found to be increased in CMT1A patient could be one of them.<sup>56</sup> Another biomarker found to be elevated in CMT1A plasma sample is the neurofilament L protein that was correlated with the increase in the severity of CMTN scores in CMT1A patient.<sup>57</sup> A new specific biomarker for CMT1A was recently identified by Wang et al.<sup>58</sup> The transmembrane protease serine 5 (TMPRSS5) protein was found to be elevated by around 2-folds in the plasma of CMT1A patients.<sup>58</sup> Moreover, several genes were found to be altered in *PMP22* transgenic mouse model (TgN248) that could be targeted for future therapy.<sup>59</sup>

Since the discovery of the main cause of CMT1A, that is *PMP22* overexpression resulting from the trisomy of *PMP22* gene in addition to neurological dysfunctions which are due to loss of large-caliber motor and sensory axons, several animal models and therapeutic strategies were developed.

Rat CMT1A model and TgN248, C22, C61 or C3 mouse models were generated by random insertion of a fragment of mouse or human *PMP22* cDNA into their genome. These mice showed an increase of *PMP22* level paralleled with slow conduction velocity and dysmyelination<sup>60-67</sup> (Table II). However, some of these rodent models (TgN248 and C22) partially failed to mimic the human CMT1A as they showed very high levels of *PMP22* in Schwann cell due to the high number of *PMP22* copies inserted in each strain. These models are quite severe from early onset and they don't recapitulate the histopathological and clinical phenotype of CMT1A disease. They are artificial disease-models that were used to understand the disease process for translation medicine. Indeed, the duplication in human CMT1A is 1,4 Mb DNA segment which make it hard to generate a humanized rodent model of CMT1A and the rodent model failed to reflect the altered genome structure and did not show the variability in the *PMP22* level found in CMT1A patients.<sup>53</sup> This may explain why several drug strategies failed to give positive results when transferred from rodent models to human CMT1A patients.

**Table II.** CMT1A animal models. The most frequent animal models used to test drugs

CMT1A transgenic animal models	Origin of the <i>PMP22</i> insertion	Number of <i>PMP22</i> extra copies	Phenotype	References
C61 mouse	Human <i>PMP22</i> YAC	4	Mild demyelination MNCV 25m/s	Huxley et al, 1998 <sup>60</sup>
C3-PMP mouse	Human <i>PMP22</i> YAC	4	Mild neuromuscular impairment	Verhammeet al, 2011 <sup>66</sup>
C22 mouse	Human <i>PMP22</i> YAC	7	Severe demyelination	Huxley et al, 1996 <sup>60</sup>
TgN248 mouse	Mouse <i>Pmp22</i> cosmid PTCF-6.1	16	Very severe demyelination leading to age-related paralysis	Magyar et al, 1996 <sup>61</sup>
JP18/JY13 tetracycline conditional transgenic mouse	Mouse <i>Pmp22</i> plasmid pJP7m Human <i>PMP22</i> YAC	2	Moderate demyelination at 16 weeks of age	Pereaet al, 2001 <sup>65</sup> Robertson et al, 2002 <sup>64</sup>
<i>PMP22</i> transgenic rat	Mouse <i>Pmp22</i> cosmid	3	Mild demyelination	Seredaet al, 1996 <sup>62</sup> Fledrichet al, 2012 <sup>67</sup>

Interestingly, the tetracycline conditional model has not been used until now for treatment testing.<sup>65</sup> This transgenic model was made to overexpress *PMP22* under specific control where in the absence of tetracycline since birth, the *PMP22* gene is overexpressed, thus leading to dysmyelination in the embryonic life followed by demyelination in about 26 % of myelinated nerve fibers in adult mice.<sup>64</sup> These observations are in parallel with the finding in human CMT1A patient. Dysmyelination was detected in young patients with CMT1A without affecting the NCV<sup>68</sup> whereas, demyelination was detected in adults CMT1A patients.<sup>69</sup> Normalization of *PMP22* expression by tetracycline administration led to a rapid remyelination.<sup>65</sup> Therefore, this model could be useful to introduce a personalized medicine based on normalization of *PMP22* expression to reverse the CMT1A neuropathy.

Until now, there is no effective CMT1A treatment despite worldwide efforts of several research groups and the existence of representative animal models of this disease<sup>60-67</sup> (Table II). At this time, physical therapy and surgical corrections are the only available treatments.<sup>12,70,71</sup>

At the beginning of the 21st century, efforts were made to modulate the activity of signaling proteins acting upstream of the *PMP22* gene such as adenylatecyclase using ascorbic acid or the progesterone receptors using the selective antagonist onapristone. Nowadays, molecules acting on adenylatecyclase activity, tyrosine kinase receptor or on pain are tested to reduce the progression of CMT1A.

The future treatment options for CMT1A may involve specific targeting of the *PMP22* gene. All the mentioned molecules and their clinical outcomes are further discussed. Table III and Fig 2 summarize the

past, recent, and future treatments as well as the signaling pathway targeting *PMP22* expression.

**Ascorbic acid and onapristone.** Adenylatecyclase use ATP as a substrate to increase the intracellular levels of cyclic adenosine monophosphate (cAMP). The first compound tested targeting cAMP was ascorbic acid also known as Vitamin C.<sup>72,73</sup> In fact, ascorbic acid acts as a competitive inhibitor of adenylatecyclase activity and thus decrease the intracellular levels of cAMP.<sup>74</sup> Ascorbic acid is a water-soluble compound composed of 6 carbons with 2 ionizable hydroxyl groups. It is an essential nutrient in the human diet that is required for the synthesis of collagen in fibrous tissues, bones, teeth, capillaries, and skin. It is a reducing agent and a powerful antioxidant. It gained interest for its two roles as a neuroprotector and neuromodulator.<sup>75</sup> Noteworthy, it was tested as a treatment of CMT1A because (1) it is easily synthesized and commercially available, (2) vitamin C deficiency leads to PNS dysfunction and, (3) it promotes myelination of Schwann cells in vitro.<sup>76</sup>

Passage et al,<sup>76</sup> have shown that weekly treatment, with 1.2 mg of ascorbic acid for a 20 g CMT1A mice (C22) carrying 7 extra copies of human *PMP22* gene, improved locomotion, myelination and the life span and decreased *PMP22* mRNA levels in sciatic nerves.<sup>60</sup> Based on this study, several clinical trials have been performed. A study conducted by Shy et al on CMT1A patients showed that daily administration of 1 g of ascorbic acid for one year had no significant effect on the motor and sensory activities.<sup>77</sup> Micallef et al observed the same results even after increasing the dose to 3 g.<sup>78</sup> The same year, another clinical trial was conducted on young CMT1A patients' age below 25 years, and no significant improvement in myelination was recorded. In this study myelination was



**Table 3.** Main drugs tested for CMT1A disease. The targeted receptors, animal models used, administration route and type of research done are shown

Drug nomenclature	Receptor or transporter and pathway	Animal models	Number of <i>PMP22</i> gene copies and age at start of treatment	Administration route & treatment duration	Investigation type
<b>Onapristone</b>	Progesterone receptor 1 antagonist	CMT1A Rat	3 Copies P5	Subcutaneous Six weeks	Research
<b>Ascorbic acid</b>	Sodium-Dependent Vitamin C Transporter 2. Reduce Adenylate Cyclase Activity cAMP pathway	C22 mice	7 Copies P5	Force feeding Once per week till death	Research, Preclinical and Clinical studies
<b>Neurotrophin-3 SCAAV1.iMCK. NTF3</b>	Nerve growth factor, tyrosine Kinase receptor	Trembler J mice & xenografts from CMT1A patients	Point mutation 6-8 weeks	Intramuscular 20 and 40 weeks	Research, preclinical and clinical study recruiting
<b>PXT3003</b>	i) GABA <sub>B</sub> receptor agonist, ii) narcotic antagonist and iii) muscarinic receptor antagonist	CMT1A Rat	3 copies 4 weeks	Oral gavage daily For 4 months	Research, Preclinical and Clinical (Phase II and Phase III ongoing trials)
<b>siRNA Leu<sup>16</sup>Pro</b>	Leu <sup>16</sup> <i>PMP22</i> point mutation siRNA machinery	Trembler J mice	Point mutation P5 or P6	Intraperitoneal 2 weeks	Research
<b>ASO directed against <i>PMP22</i></b>	<i>PMP22</i> mRNA expression Watson-Crick Base pairing	C22 mice CMT1A Rat	7 copies/ 5 weeks 3 copies/ 6 weeks	Subcutaneous 9 weeks for C22 12 weeks for rats	Research

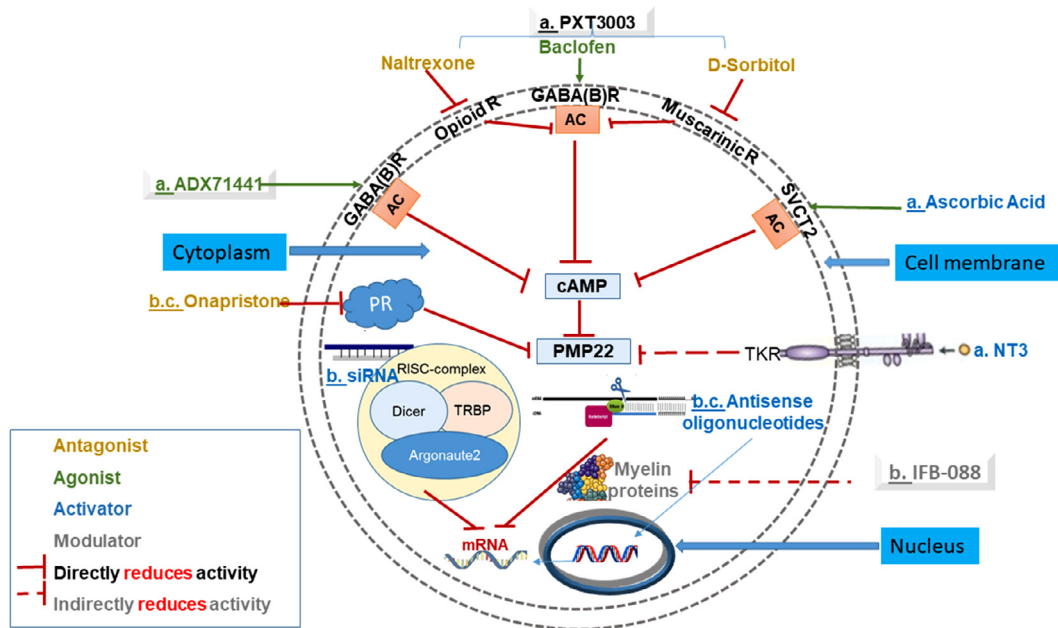
assessed by measuring the MNCV as a primary outcome while the secondary outcomes were CMAP, muscle strength, sensory functions and Charcot Marie tooth neuropathy score (CMTNS).<sup>79</sup> To assess the efficacy and safety of ascorbic acid in CMT1A patients a multi-center 2-year study was conducted in United Kingdom and Italy, and found that ascorbic acid treatment had no significant adverse events but also no effect on CMTNS.<sup>80</sup>

It is well-known that ascorbic acid is up taken by Schwann cells through the sodium-dependent vitamin C transporter 2,<sup>81,82</sup> and acts as a competitive inhibitor of adenylate cyclase.<sup>83</sup> Knowing that cAMP is a cross-link for different signaling pathways, the effect of ascorbic acid on *PMP22* gene expression could be reduced. In addition, the use of a severe demyelinating mouse model (C22) that may not reflect the CMT1A neuropathy could explain the absence of response to Vitamin C in CMT1A patients.

In the same period, Sereda *et al.*, speculated that inhibiting the action of progesterone at its receptor may be a treatment for CMT1A.<sup>84</sup> Progesterone stimulates promoter 1 of *PMP22* gene through the

progesterone receptor which is expressed in Schwann cells.<sup>85,86</sup> Onapristone, a progesterone receptor antagonist, is known to prevent progesterone receptor dimerization which is required for its transcriptional activity.<sup>87,88</sup> Administration of onapristone improved CMT1A phenotype and decreased *PMP22* mRNA expression in rats.<sup>84</sup> Subsequently, a long-term study of 5 months duration was conducted on *PMP22* over-expressing rats and confirmed the beneficial effects of onapristone.<sup>84,89</sup> However, in clinical trials, liver toxicity was found to be a major limitation for the use of onapristone (NCT02600286).<sup>8,90</sup>

**Drugs in development for the treatment of CMT1A.** Neurotrophin-3 (NT-3) plays an important role in the development, functioning and plasticity of the nervous system.<sup>91-93</sup> It binds to 3 Tyrosine kinase receptors (Trk) with a great affinity for TrkC.<sup>94</sup> NT3 has been studied for development of treatment for CMT pathology in both the trembler-J mice which have a demyelinating phenotype and xenograft of CMT1A patients. In 2005, NT-3 treatment was shown to improve axonal regeneration and to significantly increase the number of solitary myelin fiber as well as their diameter in



**Fig. 2.** Drugs and Pathways. Representative diagram showing some of the drugs tested for CMT1A treatments and the pathways involved. **a.** Several membrane receptors were targeted by one molecule (ascorbic acid, ADX71441, NT3) or a combination of 3 molecules (PXT3003) to inhibit *PMP22* expression directly or indirectly through regulation of adenylatecyclase (AC) activity (cAMP) or tyrosine kinase receptor (TKR). **b.** progesterone receptor (PR) antagonist (onapristone) and nucleic acids such as siRNA and antisense oligonucleotides (ASO) are used to inhibit directly the *PMP22* expression or to indirectly modulate its expression through molecules acting on myelin proteins (IFB-088). **c.** PR and ASO can have a double localization cytoplasmic and nuclear.

Trembler-J mice and in animal models having xenografts from CMT1A patients.<sup>95,96</sup> However, the short plasmatic half-life of NT-3 hindered its use as an effective long-term treatment for CMT1A patients. Thus, NT-3-encoding AAV1 vectors were delivered intramuscularly to Trembler-J mice carrying Leu<sup>16</sup>Pro point mutation in the putative first transmembrane region of the *PMP22* polypeptide.<sup>97</sup> In Trembler-J mice, AAV1-NT-3 increased the serum levels of NT3, improved the sensory and motor functions<sup>95</sup> and promoted axonal regeneration after peripheral nerve injury.<sup>98,99</sup> Recently, a “CMT Gene Therapy Program” was launched with hope to use of AAV1-NT3 for future CMT1A clinical trials (NCT03520751).

Chumakov et al, combined 3 commercially available drugs targeting several pathways involved in myelination and acting together to increase directly or indirectly the intracellular levels of cAMP.<sup>100</sup> This combination therapy named PXT3003 comprises: (1) (RS)-baclofen, a selective GABA<sub>B</sub> receptor agonist and a skeletal muscle relaxant, (2) naltrexone, a noroxymorphone derivative with competitive opioid and narcotic antagonistic activities, and (3) D-sorbitol, a muscarinic receptor antagonist and laxative. It was

tested on the CMT1A transgenic rat model carrying three extra copies of mouse *PMP22* gene with 1.6-fold increase in *PMP22* expression.<sup>49,100</sup> In rats, PXT3003 treatment down-regulated *PMP22* gene expression and improved nerve conduction velocity and CMT1A clinical phenotype.<sup>100</sup> Moreover, in newborn CMT1A rats, PXT3003 decreased *PMP22* mRNA expression and ameliorated the motor activity. Furthermore, it regulated the misbalanced downstream the PI3K-AKT/MEK-ERK signaling pathway.<sup>101</sup>

A Phase II clinical study of PXT3003 conducted in randomized, double-blind and placebo-controlled groups of CMT1A patients confirmed its safety and tolerability. However, an efficacy of the treatment was only observed in the high-dose group, with significant improvement in the Overall Neuropathy Limitation Scale.<sup>102</sup> To compare the clinical outcomes of PXT3003 and ascorbic acid that both target cAMP, a meta-analysis of randomized double-blind clinical trials was conducted. PXT3003 showed enhancement of CMTNS and Overall Neuropathy Limitation Scale in comparison with ascorbic acid patients, who were stable but not significantly different from the placebo group.<sup>103</sup> This encouraged pursuing, and a phase III PXT3003 clinical trial (NCT02579759) is ongoing. In



case of success, this treatment could open a therapeutic approach for CMT1A patients.

**IFB-088** or sephin1 is a new molecule, designated as an orphan drug by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA). Sephin1 is involved in controlling the misfolding of myelin proteins that occurs during mechanical, thermal and oxidative stress.<sup>104</sup> The development of this drug candidate (preclinical and Phase I trial) is supported by AFM-Telethon (NCT03610334). Preclinical studies have shown that the administration of sephin1 in mouse models of CMT1A or CMT1B, which is caused by mutation in *MPZ*, restores motor functions.<sup>105</sup> In May 2018, approval to start a Phase I trial of IFB-088 in 72 healthy volunteers was announced. If the results are positive, a Phase II trial will be conducted in patients with CMT1A or CMT1B.

ADX71441 and FLX-787 are pain-modulating drugs. **ADX71441** is a modulator of GABAB receptor.<sup>106</sup> It was tested in rodent models and showed efficiency for the treatment of anxiety, pain and spasticity. Activating GABAB receptor reduces *PMP22* gene expression.<sup>107</sup> ADX71441 has received regulatory approval to start a Phase I trial (not listed in the Clinical Trials website) and is being investigated for its therapeutic use in CMT1A. **FLX-787** is a small molecule that acts as antispastic and muscle relaxant and was tested on CMT1A patients. Phase II COMMIT trial (NCT03254199) was stopped due to low tolerance.

**What is the future of CMT1A treatment?**  
**Oligonucleotides: a golden opportunity for CMT1A treatment.** Small interfering RNA (siRNA) was recently recognized as an extraordinary breakthrough reflected by the increase in clinical studies over the last few years. In the PNS, the success story using siRNA for treatment of Hereditary Transthyretin Amyloidosis (hATTR), a lethal disease caused by transthyretin (TTR) systemic accumulation, gave a boost to siRNA research in the PNS disorders field. Preclinical data showed that inhibition of hepatic *TTR* gene by siRNA reduced systematic levels of *TTR*, thus stabilizing TTR deposits in several tissues.<sup>108</sup> Then, clinical phases were conducted, the latest is the Apollo phase III clinical study, where Patisiran, the siRNA against *TTR* was shown to improve clinical disease manifestations.<sup>109,110</sup> Patisiran received approval from the FDA and EMA and is now used in clinical practice. This raises hope for other siRNAs to reach clinical trials.

For CMT1 neuropathy, Lee et al, designed 19 siRNAs specific for the mutation Leu<sup>16</sup>Pro responsible for the development of CMT1E.<sup>111</sup> First, they tested their efficiency on cultured Schwann cells, and then identified one siRNA able to counteract the effects of the Leu<sup>16</sup>Pro mutation. After that, the siRNA Leu<sup>16</sup>Pro

was administered to Trembler-J mice by a nonviral delivery system *via* intraperitoneal injection at the postnatal age of 6 days. Interestingly, improvement in motor function and muscle volume, paralleled with an increase in motor nerve conduction velocity and CMAP, was observed. In addition, myelination was increased in the sciatic nerve with a simultaneous increase in the expression of the myelin genes Myelin Basic Protein (*MBP*) and Myelin Protein Zero (*MPZ*). They concluded that suppression of the *PMP22* mutant allele by nonviral delivery of siRNA could reconstitute CMT1 neuropathic phenotype.<sup>111</sup> This was a proof of concept study showing that siRNA could reach the peripheral nervous system and suggest that siRNA administration at postnatal day 6 may raise questions toward clinical translation of CMT disorders with primary Schwann cell genetic defects.

Another oligonucleotide approach is to inhibit gene expression using synthetic antisense oligonucleotides (ASO). ASO are 12-30 base pairs in length that use the RNase H1 nuclease to induce RNA degradation.<sup>112,113</sup> In 2018, ASO TTR (inotersen) obtained marketing authorization for hATTR by FDA and EMA and is now also used in clinical practice.<sup>114</sup>

Concerning CMT1A, Zhao et al designed two ASOs against *PMP22* gene expression, both of them improving severe and moderate forms of rodent CMT1A models but at high dose level (100 mg/kg).<sup>115</sup> The use of high-dose of ASO or siRNA could dysregulate other genes, named the “off-target effect” and impedes their use as a treatment. Knowing that CMT1A is a genetic disease requiring a lifelong treatment, the dose of ASO and siRNA should be carefully designed to prevent toxic adverse events (thrombocytopenia, nephrotoxicity, hepatotoxicity, proinflammatory effects).<sup>116</sup>

It should also be noted that both siRNA and ASO are unstable *in vivo* and require modifications to be effective. Nanotechnology development allows to overcome this barrier and to carry siRNA to their targets. This is attested by the recent success of Patisiran, based on a lipid nanoparticle (LNP) system for vectorizing siRNA.<sup>117</sup> For ASO, phosphothioate modification is the first and most common modification used to date. ASO bearing phosphothioate-modified DNA and 2'-sugar modifications are water soluble, and show increased stability and uptake by cells.<sup>115,118</sup>

Although studies by Lee et al<sup>111</sup> using siRNA and Zhao et al<sup>115</sup> using ASO against *PMP22* gave hope to patients with CMT1A, further investigation is required to define the optimal conditions and sequence of siRNA and ASO. Safety and pharmacokinetics studies must be completed to insure the tolerability of the siRNA and to achieve tight regulation of *PMP22* gene expression, as its inhibition below normal levels can

lead to HNPP.<sup>119</sup> Overall, the RNAi approach should be investigated since it opens a new hope for CMT1A disease as well as for other hereditary neuropathies.

**Targeting macrophages and heat shock proteins may help preventing CMT1A progression.** CMT1A is characterized by an increase in PNS macrophages and accumulation of *PMP22* aggregates in Schwann cells.<sup>120</sup> Therefore, targeting monocyte chemoattractant protein-1 (MCP-1/CCL2) and enhancement of Heat Shock Proteins (HSPs) would represent new therapeutic approaches for CMT1A. To this end, Kohl *et al*, showed that reduced levels of MCP-1/CCL2 are paralleled by a reduction in the number of macrophages in the CMT1A mouse strain C61 carrying four extra copies of human *PMP22*.<sup>60,121</sup> To counteract the buildup of misfolded *Pmp22*, Chittoor-Vinod *et al*, enhanced the HSP70 expression and improved proteasome activity thus preventing aggregation of *PMP22*.<sup>122</sup> It should be noticed that ethoxyquin, an antioxidant used to protect against neurotoxicity of chemotherapeutic agents and known to be a modulator of HSP90<sup>123</sup> could become effective as a treatment for CMT1A patients.<sup>124</sup>

## CONCLUSION

Since the discovery of CMT1A in the 19th century, several drugs have been tested. However, most of them did not provide significant clinical benefits. Today, CMT is considered an economic burden with an annual cost of around 10,000 € per patient.<sup>125</sup>

The untreated CMT1A disease may become “treatable” in the next few years. The polytherapeutic approach PXT3003 is still evaluated, with the design of a new phase III clinical study. Another approach, which is making its way toward clinical trials, is the use of the RNAi machinery. Until now, most of the studies focused on reducing cAMP activity upstream of *PMP22* instead of targeting directly *PMP22* gene expression. Regulation of *PMP22* gene expression using siRNA can be beneficial once proven safe and tolerable in CMT1A patients. This genetic approach paves the way for treatment of other inherited neuropathies.

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