CLINICAL INVESTIGATION

COMMENTARY

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An update on clinical trials in Charcot-Marie-Tooth Disease

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How far away is an effective therapy for Charcot-Marie-Tooth disease (CMT), the most frequent inherited neuropathy affecting approximately one individual in every 2500 around the world [1]? The next few years will be fundamental to giving the answer to this key question about a still untreatable disorder, for which rehabilitation therapy and surgical correction of skeletal deformities still represent the only available approaches. Different lines of research are rapidly providing encouraging data on possible effective treatments in cellular and animal models, and clinical studies are progressively filling the gaps to be fully ready for clinical trials, creating great hope in patients and researchers alike. However, CMT poses a series of difficulties in the pathway to the cure and in running clinical trials, as testified to by the failure of recent randomized-controlled trials (RCT) with ascorbic acid.

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Difficulties in the pathway to treatment

The clinical presentation of CMT (consisting of a slowly progressive length-dependent polyneuropathy with distal muscle wasting and weakness, distal sensory loss, foot deformities and reduced-to-absent deep tendon reflexes) is the result of a primary axonopathy or myelinopathy causing a chronic axonal degeneration [1].

A first difficulty arises from CMT and related neuropathies being highly heterogeneous disorders with about 70 causative genes identified so far, encoding proteins localized in different cellular compartments and involved in several functional pathways, the most frequent of which are: myelin formation and maintenance, mitochondrial dynamics, cytoskeleton organization, axonal transport, membrane and vesicular trafficking [1]. It is unlikely that a single therapeutic approach will be effective for CMT as a whole, unless we succeed in halting the final common pathway of chronic axonal degeneration. Should we, therefore, seek out selective therapies targeted to specific pathomechanisms, mutated genes, mutation types or rather aim directly at halting axonal loss?

A second issue is that most CMT types run a very slowly progressive course, which, while less cumbersome for the patients, makes it difficult to detect disease progression over time and to assess intervention efficacy, and consequently requires development of sensitive-to-change outcome measures.

The diverse motor, sensory and skeletal involvement typical of CMT constitutes a further obstacle to the design of appropriate and comprehensive outcome measures assessing impairment, activity limitations and handicaps.

Ascorbic acid trials

All these problems became evident when we entered the clinical trial era in CMT, following the observation that ascorbic acid was beneficial to transgenic mice

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overexpressing PMP22 and reproducing features of the most common CMT type, namely CMT1A associated with the *PMP22* gene duplication [2]. Several trials were carried out all over the world, in adults and children, lasting 1 or 2 years and employing different drug dosages (1–4 g daily); they partially shared outcome measures, after researchers reached agreements about trial conduction in an international workshop [3–5]. Unfortunately, all these trials were negative and the conclusion we can draw is that ascorbic acid is not an effective treatment for CMT1A. However, the trials were incredibly useful to gain information about CMT1A disease course and the use of outcome measures. Overall, more than 700 patients were recruited and we learnt a lot about how to conduct a trial in CMT. It became clear that current measures were not responsive enough and that we needed further natural history studies. Moreover, we realized that future trials should be adequately powered (and consequently multicenter), of sufficient duration, and employing reliable and more responsive outcome measures, including paraclinical measures and biomarkers [3].

Current approaches under investigation

CMT1A is the ideal candidate for treatment research as it is the most common form, accounting for almost half of all CMT cases, has good animal models, and can be theoretically cured by lowering the PMP22 overexpression (an objective potentially easier than targeting a point mutation in any CMT gene). Indeed, the STAR program, funded by the US CMT Association (CMTA) advocacy group, uses high-throughput screening technology to systematically screen for compounds able to downregulate PMP22 in CMT1A cell cultures, to be confirmed in animal models and hopefully soon in human clinical trials [6].

We already know that progesterone antagonists lower PMP22 overexpression and improve clinical, electrophysiological and histological abnormalities in the CMT1A rat model; however, currently available drugs are still too toxic to be tested in the human disease [7]. Lonaprisan, the best candidate, is not hepatotoxic but still has a hormonal effect unacceptable for premenopausal females.

A Phase II RCT employing a combined pleotropic multitherapy approach with small doses of three compounds (RS-baclofen, naltrexone and D-sorbitol), seemingly able to downregulate PMP22 *in vitro*, has been recently concluded; whether this approach deserves further investigations will become clear when definite results are published [8].

GABA-B receptor modulators also appear to downregulate PMP22 and current experiments in the CMT1A rat seem encouraging [Sereda M, Pers. Comm.].

Another interesting line of research has shown that there is an excessive intracellular calcium concentration in CMT1A cell cultures and nerve tissue, and that blocking the P2X7 receptor, a cell surface receptor allowing calcium entry into the cell, reduces calcium concentration and partially reverts the phenotype [9]. A trial of a P2X7 receptor inhibitor in the rat CMT1A model is ongoing as a further step [Schenone A, Sereda M, Pers. Comm.].

A completely different approach has been used for CMT related to mutations in MPZ, the most important protein component of peripheral myelin, causing either early-onset demyelinating neuropathy (CMT1B) or late-onset axonal CMT (CMT2I/J). Different *MPZ* point mutations prevent correct positioning in the cell membrane of the mutated protein, which is then retained in the endoplasmic reticulum (ER), thereby causing ER stress and activating the unfolded protein response (UPR), a pathway of clearance of misfolded proteins. Notably, partial block of the UPR in CMT1B mice improved the neuropathy. Compounds able to partially prevent activation of one or more of the three arms of UPR are a concrete possibility for CMT associated with mutations in MPZ (and perhaps other myelin proteins) causing ER retention. Curcumin is one such compound and proved effective in cellular and animal models of *MPZ* and *PMP22* point mutations causing ER stress and UPR activation [10]. Salubrinal and guanabenz also showed promising results *in vivo* [11]. A trial with curcumin in MPZ-related CMT appears to be the closest to being initiated.

Another promising therapeutic approach for ER stress and protein misfolding disorders involves the enhancement of chaperone levels such as the small heat shock proteins (HSPs) HSP27 (notably, mutations in *HSPB1,* the gene encoding HSP27, are associated with some CMT subtypes), HSP40 and HSP70. Inhibition of HSP90 results in chaperone level increase and HSP90 inhibitors, such as geldanamycin and its derivatives, are under study [12].

Other researches approached autophagy. Rapamycin, an autopaghy-enhancer agent, was effective in scavenging PMP22 aggregates and improving myelination in explant cultures from neuropathic mice [13]. Interestingly, intermittent fasting, which is known to activate chaperones involved in the stress response as well as autophagy, proved beneficial in a CMT1A animal model. These results support the potential use of autophagy-enhancing compounds as therapeutic agents for PMP22-associated demyelinating neuropathies.

Myelin thickness is tightly regulated and the pathway of neuregulin-1 III (NRG1 III) appears to be particularly important for this task. NRG1 type III activity is

regulated by BACE1 secretase, which increases myelination. Conversely, the TACE secretase, which also cleaves NRG1 III, inhibits myelination. This system can be approached with already commercially available drugs and might be an incredibly valuable approach both for hypermyelinating neuropathies, such as hereditary neuropathy with liability to pressure palsies, and hypomyelinating CMT forms [14].

Another very important line of research has shown that mutations of *HSPB1* lead to pure motor or sensorymotor forms of CMT by altering the axonal transport and the system of microtubules, which are excessively acetylated. Remarkably, treatment of transgenic mice carrying *HSPB1* mutations with HDAC6 inhibitors decreased acetylation of microtubules, improved axonal transport and rescued the CMT mice phenotype. This is a very important finding as HDAC6 inhibitors might be theoretically useful not only to treat CMT forms depending on HSP mutations, but also for the much wider group of neuropathies resulting from altered axonal transport [15].

NT-3 is a neurotrophic factor contrasting axonal degeneration and has been tested, with some possible benefit, in CMT1A patients by subcutaneous delivery in a small pilot trial. Experimental NT-3 gene therapy by intramuscular delivery of rAAV1.NT-3 was beneficial in a *PMP22* point mutation mouse model, and seems promising for inducing regeneration in different neuropathy types [16].

Loss-of-function mutations in *PRPS1* alter purine metabolism and are associated with three partially overlapping allelic disorders: CMTX5, Arts syndrome and the X-linked nonsyndromic sensorineural deafness DFN2. *S*-adenosylmethionine supplementation in two Arts syndrome patients seems to have improved their condition by replenishing purine nucleotides [17].

Hereditary sensory and autonomic neuropathy type 1 is caused by missense mutations in the genes encoding serine palmitoyltransferase subunits 1 and 2 (*SPTCL1* and *SPTCL2*). The mutant forms of the enzyme show a shift in substrate specificity from L-serine to L-alanine, increasing formation of neurotoxic deoxysphingolipids (dSLs). In *SPTCL1* mice, L-serine supplementation reduced dSL levels and improved measures of motor and sensory performance. In a pilot study with 14 hereditary sensory and autonomic neuropathy type 1 patients, L-serine supplementation similarly reduced dSL levels, supporting the hypothesis that L-serine supplementation may be an effective treatment [18].

This long list of possible therapeutic targets and related compounds for CMT and similar inherited neuropathies is encouraging and promising, although some drugs still carry unacceptable risks to be tested in CMT patients.

Clinical trial readiness

Therefore, it is very important to work on clinical trial readiness, with the aim of starting RCT in the best conditions. As a matter of fact, either we develop a very effective therapy able to rapidly revert the phenotype, with effects which can be easily seen even with an unsophisticated trial design, or we risk missing a beneficial effect of any drug which produces less striking though important results, such as a halt in the disease progression, if the trial is not adequately planned. The target population is a first important issue and theoretically children are the best population to recruit, as axonal loss is still initial and a drug has more chances to be effective than when axonal loss is advanced and the phenotype may be less reversible, if at all.

The development of simple, reliable, valid and responsive outcome measures is another fundamental requirement. The CMT Neuropathy Score (CMTNS) is the only CMT-specific scale thus far developed. It is a nine-item composite scale assessing in a length-dependent manner the severity of sensory and motor symptoms (disability) and the sensory and motor signs (impairment) in upper and lower limbs, integrated by two electrophysiological items related to amplitude of one motor and one sensory nerve response in the upper limbs. It has demonstrated good reliability but relatively poor sensitivity to change in the ascorbic acid trials, so a second version was developed to make it more responsive (CMTNSv2) [19] and as a further step, different weight will be given to the scores to accomplish with Rasch analysis approach, to obtain a more linear scale [20].

Burns and colleagues, following the Rasch analysis method, developed a composite scale specific for CMT children, the CMTPedS, which showed very good validity and reliability; its responsiveness is under investigation, with encouraging preliminary results [21]; one study showed that transition is possible from the CMTPedS at pediatric age to the CMTNSv2 for adults [22]. A composite scale for infancy is under development [23] and CMT-specific disability and quality of life scales are also under study.

Other lines of research are investigating paraclinical outcome measures and biomarkers to assess disease progression and intervention efficacy in CMT. Computerized gait analysis proved to be a reliable way of studying locomotion in CMT patients, of detecting early changes in CMT1A children, and of following the evolution of CMT gait patterns (from the early changes in toe and heel walking in CMT1A children, to the development of foot dorsiflexion defects in the swing phase of gait, to the impairment of the push-off phase due to plantar-flexion defects in the advanced

disease stages) [24]. Muscle MRI can detect signal changes due to fat substitution secondary to denervation; ongoing studies are showing that this process can be reliably quantified and used to measure disease progression and might be the most responsive outcome measure available to date [25]. Eventually, glabrous skin biopsy provides easy and repeatable access to myelinated fibers and is currently used in the search for adequate biomarkers. Unfortunately, mRNA *PMP22* levels did not prove to be a suitable biomarker in CMT1A [26], but other molecules might have a better fate as biomarker in skin biopsies and blood [27].

In conclusion, we are now moving between a great hope raised by several approaches and promising compounds under study, and a necessary caution in carrying out Phase III clinical trials to avoid easy illusions.

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