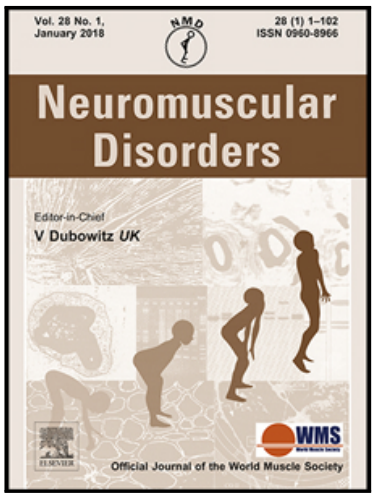

International Workshop Report Congenital muscular dystrophy 1A: the road to therapy

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Highlights:

- First workshop on the road to therapy for MDC1A
- Trial readiness for MDC1A/*LAMA2*-MD: clinical spectrum and natural history
- Novel therapeutic strategies for MDC1A/*LAMA2*-MD
- Majority of international experts participated
- Intense interaction with patients, relatives and caretakers

Title: International Workshop Report

Congenital muscular dystrophy 1A: the road to therapy

15-17 November 2019, Maastricht, the Netherlands

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None

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Keywords

- MDC1A/LAMA2 disease

- Natural history

- Clinical spectrum

- Therapeutic strategies

Introduction

On the 15-17 of November 2019, 20 experts (clinicians and scientists) from 9 countries gathered in Maastricht, The Netherlands, to discuss the natural history, trial readiness and potential treatment strategies for patients suffering from *LAMA2*-related muscular dystrophy (*LAMA2*-MD), the congenital form being known as merosin deficient congenital muscular dystrophy type 1A (MDC1A). The conference was an initiative of the foundation "Voor Sara', a Dutch patient organisation for MDC1A, and Maastricht University, who joint forces in 2018 in order to find a treatment for MDC1A. "Voor Sara" has become in a few years an international anchor point for MDC1A. The foundation has not only boosted MDC1A research financially, but has also inspired the researchers involved, with this successful conference, mainly funded by them, as one of the clear achievements. The full, second day of the conference was attended by affected individuals, their parents and other (health) carers and representatives from Cure CMD. The possibility for this group to listen to lectures and mix in an informal way with leading scientists and clinicians in the field was one of the clear and probably most rewarding

accomplishments of the conference. During the final break, the company Prothelia presented their work on a therapy under development.

MDC1A is a rare, congenital autosomal recessive muscular dystrophy, affecting 1 in 30,000-100,000 individuals and accounts for 24-37% of all congenital muscular dystrophies. It is caused by mutations in the *LAMA2* gene, which encodes laminin-alpha 2, a component of heterotrimeric laminin-211, also known as merosin. Laminin-211 which is expressed in basement membranes of striated muscle, placental trophoblasts, Schwann cells, neuromuscular synapses and brain cells. Complete loss of laminin-211 results in disruption of structural stability and signal transduction of the extracellular matrix. This manifests in failed regeneration, fibrosis, apoptosis, chronic inflammation and muscle wasting. Partial laminin-211 deficiency manifestations are much milder. To date, there is no effective treatment available for MDC1A patients and current treatment options focus on symptom relief.

Heterogeneity of clinical features and prognosis combined with a lack of natural history data implies that trial readiness is limited. Important are the identification of MDC1A patients worldwide and development of patient registry, selection of outcome measures and biomarkers that are sensitive to change, and consensus on the use of them, and natural history studies. This should be complemented with a rapid and open exchange on novel treatment strategies and therapeutic options. International exchange of knowledge leading to a broader understanding of the pathophysiology, clinical course and novel treatment options for MDC1A is therefore of great importance for both patients and their parents, caretakers, clinicians and researchers.

1st section: Trial readiness for *LAMA2*-MD: clinical spectrum and natural history

LAMA2-MD patients with complete laminin-211 deficiency show hypotonia and muscle weakness at birth or within the first months of life, with delayed motor development. About 90% of these patients remain non-ambulant. Contractures develop at all major joints, especially in elbows, hips and knees, further restricting motor function. Weakness is most pronounced in appendicular and axial musculature, especially neck flexor muscles. Rigidity of the spine and hyperlordosis are also prevalent. Besides, facial weakness leads to malocclusion issues combined with macroglossia and ophthalmoplegia, predominantly affecting upward gaze. Many patients have a failure to thrive is also prevalent. Progressive restrictive respiratory dysfunction leads to the need for ventilation and secretion management. Additionally, defects in cardiac conduction have recently been reported. Brain imaging shows diffuse periventricular and subcortical T2 white matter changes on MRI. Some patients show cortical dysplasia, occipital polymicrogyria or pachygyria, whilst MRI of other patients show no sign of brain development abnormalities. Seizures occur in 10-30% patients and are usually focal but can also be generalised. The severity of the epilepsy ranges from febrile seizures to West syndrome to continuous spike slow waves during sleep (CSWS). Cognitive function of these patients is usually normal, but can be impaired [1].

Partial laminin-211 deficiency leads to milder phenotypes with large phenotypic variability. Symptoms of partial laminin-211 deficiency usually develop during adolescence or adulthood and include exercise

intolerance, fatigue, myalgia and having difficulties with walking, climbing stairs, cycling or running. Disease progression is usually slow in these patients [1].

Haluk Topaloglu reported on the discovery of MDC1A and its underlying genetic cause. He started with his report on young patients presenting with hypotonia, occasional joint contractures, elevated serum CK, dystrophic muscle biopsies and normal intelligence, published in 1989. Based on literature studies at the time, the specific disorder was named 'occidental type congenital muscular dystrophy' [2]. In 1994, a group of international scientists, including Haluk Topaloglu, Victor Dubowitz, Caroline Sewry, Fernando Tome, Michel Fardeau, Dominique Hillaire, and Pascal Guicheney, discovered causative variants in the LAMA2 gene in MDC1A patients.

Anna Sarkozy presented an overview of clinical and genetic features of congenital muscular dystrophies (CMD) and congenital myopathies (CM). *LAMA2* related muscular dystrophy and *RYR1* gene related myopathies are the most common forms of CMD and CM, respectively. A review of diagnostic outcomes at the Dubowitz Neuromuscular Centre in London, as well as literature reports, indicated that up to about 50% of patients with CMD and CM remain genetically undiagnosed despite increasing availability of next-generation sequencing [3]. A series of retrospective natural history reviews of cohorts of patients with CMD and CM, including SELENON myopathy, *NEB* and *RYR1* gene-related CM and *LAMA2*-related muscular dystrophies are in progress at the Dubowitz Neuromuscular Centre. Other similar efforts are now ongoing internationally to improve trial readiness for CMD and CM. Among others, a recent European Neuromuscular Centre workshop was organised to plan an international longitudinal natural history study for Nemaline myopathies and harmonise disease classification. In depth data of a retrospective, cross-sectional and longitudinal analysis of 46 patients with *LAMA2* related muscular

dystrophy seen at the Dubowitz Neuromuscular Centre, was presented. Median follow up period was 7.8 years with a median of 6 assessments/patients [4]. The presented data highlighted a linear progression in respiratory insufficiency, scoliosis and contractures that will be helpful for the planning of future translational studies. The results of this study also confirm a major intra- and inter-familial variability, highlighting the involvement of additional factors playing a role in the phenotypic expression as well as treatment response.

Andrea Klein described the different phenotypes and disease progression of *LAMA2*-MD. Natural history studies had thus far been limited to small retrospective studies and one recently published prospective study with 24 *LAMA2*-MD patients [5]. In these patients, motor function measured by MFM-32 showed a decrease of 2 points per year of the total score in non-ambulant patients. Strength measurement by myometry showed a significant decline in knee flexion, but not in other muscle groups. Forced Vital Capacity (FVC) did not significantly decline, but in a different study a decline of 1.5% was observed (unpublished data). Ambulation was retained by all participants. A long-term natural history study for *LAMA2*-MD with patients recruited from the Swiss Registry for Neuromuscular Disorders is currently being planned. The study will include all Swiss patients diagnosed with *LAMA2*-MD, established either by clear pathogenic mutations, or variants of unknown significance/no mutation, but presenting consistent biopsy, MRI finding and phenotype. Data on diagnostic findings, central involvement and longitudinal data on motor function, muscle strength, respiratory and cardiac involvement will be collected and retrospective data at baseline included. International collaboration is currently being sought to increase study sample sizes.

Hemant Sawnani reported on pulmonary morbidity in *LAMA2*-MD patients, which is often characterised by pulmonary restriction. A recent natural history study examining pulmonary function test data from international participating centres (Belgium, Germany, United Kingdom, United States) had explored whether mean Forced Vital Capacity (FVC) and trended FVC respiratory measures recapitulated functional phenotypes, and whether trended FVC declined annually and thus, could be used as a marker of disease progression and as an outcome measure. It was observed that non-ambulatory patients experienced greater annual FVC declines than ambulatory patients; non-ambulatory patients without ventilator support declined faster than those on ventilator support. (Manuscript under preparation).

Corry Erasmus and **Nicol Voermans** presented an overview of the clinical symptoms of LAMA2-MD patients in The Netherlands. Identification of patients, data on natural history and the selection of outcome measures are prerequisites of executing clinical trials for rare inherited myopathies. Starting in 2020, a natural history study is carried out to warrant these essentials in both LAMA2-MD. The protocol includes a number of questionnaires on physical activity and quality of life, neurological history and examination, functional tests. Ancillary tests will include whole muscle MRI (≥10 years), muscle ultrasound, spinal x-ray, ECG and cardiac ultrasound. At the time of the workshop, 19 LAMA2-MD patients were registered at the Radboud University Medical Centre (age range 0 – 63 years; 8 males and 11 females). Clinical data of 12 patients had been analysed: (age range 5 – 63 years; 8 males and 11 females; 6 males and 6 females (manuscript under preparation). Five of them had (current or previous) epilepsy, four of them had a severe scoliosis and six of them were wheelchair dependent. One patient was non-invasively ventilated and two had a PEG. This data will be extended with the start of the natural history study.

Rene de Coo presented a series of four Dutch patients with late-onset *LAMA2*-MD (age range 32-50 years; two males, two females). All patients had mild, slowly progressive muscle weakness in limbs and shoulder girdle (Medical Research Council scale 4) and epilepsy. There were no signs of cardiomyopathy or arrhythmia. One patient was operated for scoliosis. CK levels were slightly elevated at around 700U/L. Both women had experienced uncomplicated pregnancies and deliveries and there were no reported signs of peripheral neuropathy. Brain MR imaging of all four patients showed white matter involvement and a cortical migration disorder varying from heterotopia to polymicrogyria and pachygyria. Onset of seizures was in first, second or fourth decade (age 7, 15, 31 and 35). Cognitive function was normal. Detailed neuropsychological had not been performed. This data can contribute to the design of a late onset natural course cohort study [6].

Gustavo Dziewczapolski represented Cure CMD (https://www.curecmd.org/), a patient advocacy organization founded in 2008 with the mission to advance research for treatments and cures for the CMDs and, through engagement and support of the community, to improve the lives of CMD patients. Cure CMD has helped many of the research developments in *LAMA2*-MD, contributing with patients' tissue and cells stored at its biobank and tissue repository. To date, a total of 310 *LAMA2*-MD patients from more than 35 countries are registered in its international registry, the CMDIR (https://www.cmdir.org). Patient recruitment for the omigapil clinical trial and other clinical studies was achieved through the CMDIR. Other activities to achieve clinical trial readiness for *LAMA2*-MD include an annual research grants program, organization of scientific and family conferences, and advocacy and support for the patients, family, and caregivers. Cure CMD acts, in numerous instances as "the patients' voice", representing them at meetings, workshops [7], and diverse committees like the Muscular

Dystrophy Coordinating Committee (MDCC) at the NIH in the United States (https://www.mdcc.nih.gov/). The patients' perspective is now being considered, more than ever, by both regulators and payers. Cure CMD aims to identify the most important symptoms of the disease for patients and what meaningful improvement would be for them. This set of tools and activities are planned and executed with the goal of achieving and improving the clinical trial readiness for *LAMA2*-MD and CMDs in general.

Dutch siblings and *LAMA2*-MD patients **DJ**. and **H. Stelwagen** shared personal experiences and insights in late-onset *LAMA2*-MD. Interestingly, disease progression was different for each sibling. Both present varying symptoms of differing severity, highlighting the heterogeneity of clinical features of MDC1A, even amongst family members with the identical underlying genetic defect. Both mentioned yearly visits to cardiologists and neurologists as part of their monitoring, and physiotherapy being particularly helpful to manage muscle contractures. Pulmonologists with expertise in MDs remain challenging to find.

Section 2: Therapeutic strategies

Madeleine Durbeej reported transcriptomic and proteomic profiling data as well as functional analyses that demonstrated significant metabolic impairment (with reduced mitochondrial respiration and a compensatory upregulation of glycolysis) in skeletal muscle from MDC1A patients and mice models [8–10]. She hypothesized that skeletal muscle metabolism may be a promising pharmacological target to improve muscle function in MDC1A. It was demonstrated that administration of metformin significantly

increased weight and energy capacity, enhanced muscle function and improved skeletal muscle morphology in female dy^{2J}/dy^{2J} MDC1A mice (and to a lesser extent in males) [11]. A more recent study investigated the involvement of oxidative stress in the pathogenesis of MDC1A and analysed the effects of two antioxidant molecules, N-acetyl-L-cysteine (NAC) and vitamin E, respectively, on disease progression in the dy^{2J}/dy^{2J} mouse model [12]. ROS levels were shown to be increased in MDC1A mice and patient skeletal muscle. NAC treatment enhanced grip strength and reduced central nucleation, apoptosis, inflammation, fibrosis and oxidative stress in dy^{2J}/dy^{2J} muscle. In addition, vitamin E improved morphological features and reduced inflammation and ROS levels in dy^{2J}/dy^{2J} skeletal muscle. These findings suggest the potential of metformin, NAC and vitamin E as future supportive treatments for MDC1A patients as they have shown to improve numerous pathological hallmarks of MDC1A. These agents are also already approved for use in humans and more specifically in children, facilitating usage in MDC1A patients.

Sweta Girgenrath reported on combinatorial therapy to treat MDC1A. Loss of laminin-211 results in a significant disruption of the structural stability and signal transduction of the extracellular matrix, which manifests in failed regeneration, fibrosis, apoptosis, chronic inflammation, and muscle wasting. This creates a devastating domino effect, as all of these secondary pathologies become disease drivers in their own right. Targeting major drivers of MDC1A pathology does indeed lead to measurable improvement in the mouse models of MDC1A, with a caveat that each of these individual treatments results only in partial amelioration of the pathology. Thus, a treatment approach that targets more than one disease driver has the potential to achieve a level of pathology-amelioration beyond what can be attained when targeting one driver alone. Published data confirmed that Insulin like growth factor-1 (IGF-1) overexpression/ or administration of recombinant IGF-1/ growth hormone in the context of

either inhibition of apoptosis (via Bax inhibition) or prevention of inflammation/ fibrosis (treatment with Losartan) led to a marked improvement in the muscle phenotype and locomotor functions of dy^w/dy^w MDC1A mic*e*. It has been shown that combining anti-apoptotic therapy with an expression of mini-agrin (an intervention that is proximal to the loss of laminin-211) results in an additive effect on regenerative and force producing capacities in dy^w/dy^w mice. Recent breakthroughs with strategies such as AAV-mediated expression of mini-Agrin ± αLNNd, CRISPR/CAS9-mediated overexpression of laminin-alpha 1, or treatment with recombinant laminin-111 would be logical choices to compensate for the missing laminin-alpha 2. However, the maximal translational impact of gene correction therapy might still require addressing fibrosis in MDC1A patients as well, since it is a very early signature of the disease pathology [13].

Peter Yurchenco reported on linker protein-mediated repair of laminin polymerization in *LAMA2*-MD. Normally, laminin-211, the principal laminin of muscle and peripheral nerve, becomes anchored to the cell surface receptors -dystroglycan and integrin-71 and polymerizes. This initial matrix binds to the proteoglycans agrin and perlecan, to nidogens and to type IV collagen, forming a mature basement membrane (BM). In *LAMA2*-MD, selective laminin polymerization loss, or loss of both polymerization and anchorage, occurs with disruption of BM molecular architecture. An understanding of BM assembly provides a foundation for protein repair strategies. In particular, the identification of BM protein binding domains and binding targets has enabled the engineering of novel proteins that link BM components together such that their functions are enhanced. This approach led to the development of two laminin-binding linker proteins to repair dystrophic BMs, i.e., αLNNd (enables laminin polymerization) and mini-agrin (enables strong receptor binding). The protein αLNNd, alone or in combination with mini-agrin, was used to ameliorate mouse models of ambulatory and non-ambulatory dystrophy respectively [14].

Collectively, these studies pave the way for the development of somatic gene delivery of repair proteins for treatment of *LAMA2*-MD.

Markus Ruegg reported on the development of a gene therapy for *LAMA2*-MD. Laminin-alpha 2 assembles with laminin-beta 1 and laminin-gamma 1 to form the heterotrimeric laminin-211. Laminin-211 can form large polymers to stabilize the basement membrane and bind to specific receptors expressed by muscle fibers. Hence, lack of laminin-211 leaves the muscle prone to damage during contraction, causing muscle fiber degeneration and wasting. Gene therapy using *LAMA2* is not possible as the gene is too large to fit into AAV vectors. It has previously been shown that loss of laminin-211 causes persistent expression of developmental laminin-411 (alpha-4, beta-1, gamma-1). However, the functions of laminin-411 differ from those of laminin-211. In particular, laminin-411 does not form large polymers and does not bind to the muscle fiber receptors of laminin-211. However, it has been shown that the expression of two linker proteins, mini-agrin and αLNNd, strongly improve disease phenotype in several *LAMA2*-MD mouse model [14,15]. As both linker proteins can be expressed by AAV, we initiated pre-clinical experiments in *LAMA2*-MD mice. Preliminary results show that AAV-mediated delivery of mini-agrin and αLNNd to skeletal muscle is possible and that AAV-treated *LAMA2*-MD mice gain significantly more weight than vehicle-injected littermates. These results indicate that AAV-based delivery of mini-agrin and αLNNd has a high potential to ameliorate the dystrophy in *LAMA2*-MD patients.

Stefano Previtali reported on how cell and gene therapy can be combined to treat *LAMA2*-MD CMDs. It was investigated whether cell therapy with mesoangioblasts may constitute a feasible tool to treat MDC1A. Mesoangioblasts are perivascular myogenic progenitors that, unlike satellite cells, are able to

cross the vessel wall and thus can be delivered systemically through the arterial tree to reach a widespread distribution in downstream muscles. Preliminary in vitro and in vivo studies showed that mesoangioblasts could synthetize and secrete laminin-411 and -511 but only negligible amounts of laminin-211. Thus, simple delivery of mesoangioblasts would not constitute a valuable treatment option. For this reason, by means of lentiviral vector, mesoangioblasts were engineered to deliver mini-agrin in MDC1A mouse models. Treated mice showed engraftment of engineered mesoangioblasts and their release of mini-agrin. These same mice showed amelioration in muscle histology (including increased fiber size and reduced fibrosis), increased expression of laminin-211 receptors in skeletal muscle, and attenuated deterioration of motor performances [16]. As mesoangioblasts were delivered by intramuscular injections, there were no observed engineered cells in peripheral nerves and, accordingly, the treatment did not ameliorate the peripheral neuropathy. This study demonstrates the potential efficacy of combining cell with gene therapy to treat MDC1A. Future studies combining linker molecules delivery and different cell delivery strategies may constitute a valuable alternative for multiorgan treatment of MDC1A.

Dwi Kemaladewi presented two CRISPR/Cas9-mediated strategies in the dy^{2J}/dy^{2J} mouse model of *LAMA2*-MD [17–19]. In the first strategy, she aimed at correcting a splice site mutation in *LAMA2* gene in the mice. This approach uses *S. aureus* Cas9 and two guide RNAs to excise an intronic region from the exon border to a putative splice donor site downstream in the intron, leading to the creation of a functional, alternative splice donor site and restoration of full-length *LAMA2* protein. CRISPR/Cas9 components were systemically delivered using AAV9 to neonatal dy^{2J}/dy^{2J} mice leading to amelioration of disease phenotypes, including muscle fibrosis and hind limb paralysis. This strategy established the first direct correction of the primary genetic defect that causes *LAMA2*-MD in an *in vivo* model. The

heterogeneity of mutations reported in MDC1A patients poses significant yet realistic challenges for the development of a therapeutic strategy based on mutation correction. An alternative approach is to upregulate *LAMA1,* which can compensate for the lack of *LAMA2,* using CRISPR activation technology. Here, the dy^{2J}/dy^{2J} mice were treated with AAV9 carrying deactivated Cas9 (dCas9), VP64 transcriptional activators and sgRNAs targeting L*AMA1* promoter. Robust upregulation of *LAMA1* was shown in skeletal muscles and peripheral nerves, leading to improved muscle fibrosis and paralysis. Ongoing work involving a preclinical study in the severely affected mouse model dy^{w}/dy^{w} and patient-derived cells with diverse genetic backgrounds will determine a multitude of therapeutic outcomes, including survival, neuromuscular and respiratory functions, mitochondrial metabolism, laminin-dependent migration and others.

Giulio Cossu reported work aiming at the development of a safe and efficacious clinical protocol based upon intra-arterial delivery of mesoangioblasts to muscles of patients affected by Duchenne muscular dystrophy (DMD) and other recessive forms of MDs, such as *LAMA2*-MD. Fifteen years of pre-clinical work in three mice models and one dog model, had demonstrated the safety and efficacy of this protocol. This prompted the execution of a first-in-man trial based upon repeated intra-arterial administrations of HLA-matched donor mesoangioblasts in five DMD patients [20]. The trial showed safety but minimal efficacy due to low engraftment. To compensate for the poor engraftment of donor cells, a mechanism for using genetically corrected autologous mesoangioblasts is currently being developed. The main aim of this strategy is cell-mediated exon skipping: DMD mesoangioblasts are transduced with a lentivector expressing a snRNA, engineered to skip exon 51 of dystrophin. Since snRNA is produced by a donor nucleus, assembles in the cytoplasm and then enters all the neighbouring nuclei, this mechanism should amplify several-fold the production of dystrophin. Presently, safety and

efficacy of genetically corrected autologous mesoangioblasts by intramuscular injection in a foot muscle of 5 non-ambulant DMD patients is being tested. In case of dystrophin production ≥ 10% of a healthy muscle, cells will also be injected in the thumb muscle, whose increased force of contraction would ameliorate the quality of patients' life. The Wellcome Trust HICF funded this first-in-man clinical trial that was expected to start in Manchester in the first half of 2020 but has been delayed. The overall outcome of this work will allow for the execution of a novel phase II clinical trial, based upon intra-arterial systemic delivery of autologous, genetically corrected mesoangioblasts in very young DMD patients with an optimized protocol, which may be later applied to all recessive muscular dystrophies.

Florence van Tienen presented the use of an autologous wild-type mesoangioblast treatment in the framework of mitochondrial myopathies due to an mtDNA mutation, which could also yield valuable insights regarding mesoangioblast use for other myopathies, including *LAMA2*-MD. In the majority of mitochondrial myopathy patients, the mtDNA mutation is heteroplasmic (mixture of wild-type and mutant mtDNA copies) with varying mutation loads between tissues of an individual. Preclinical data has demonstrated that in half of the mtDNA mutation carriers, their mesoangioblasts are nearly mutation free and can be used without interventions as a source for autologous cell therapy to combat myopathy [21]. Mesoangioblasts with little or no mutation load (<10%) displayed normal mitochondrial function, proliferative capacity and myogenic differentiation capacity. After such promising preclinical data, a phase I/II clinical study to be carried out in 2020 will assess the safety and preliminary efficacy of autologous mesoangioblast therapy in patients. To this end, a GMP-compliant production protocol to generate sufficient mesoangioblasts for clinical application has been developed and validated, in addition to a protocol to label the mesoangioblasts to assess migration capacity to the muscles and stability of the medicinal product. Approval from the Dutch Central Committee on Research Involving

Human Subjects has already been obtained and this phase I/II clinical study will provide insights into the therapeutic potential of autologous mesoangioblasts. If successful, this approach could be extended to treat myopathies caused by nuclear gene defects, such as *LAMA2*-MD or myotonic dystrophy type 1.

Reghan Foley presented the recent CALLISTO clinical trial of the anti-apoptotic compound omigapil [N-(dibenz(b,f)oxepin-10-ylmethyl)-N-methyl-N-prop-2-ynylamine maleate]. Preclinical studies of omigapil had demonstrated inhibition of GAPDH-Siah1-mediated apoptosis in muscle in the dy^{w}/dy^{w} and *the* dy^{2J}/dy^{2J} *LAMA2*-RD mouse models with concomitant improvement in weight and locomotor activity in the dy^{w}/dy^{w} mouse, with unpublished work demonstrating decreased apoptosis, in particular of the diaphragm muscle, in the Col6a1$^{-/-}$ COL6-RD mouse model [22,23] This study was a phase I open-label, sequential group, cohort study in patients 5-16 years of age with LAMA2-MD or COL6-RD, and it established the PK profile, safety and tolerability of omigapil at a range of doses, using novel adaptive algorithms. A total of 20 patients were randomly assigned to one of three dosing cohorts, with each patient receiving four weeks of vehicle run-in and 12 weeks on study drug. Slightly greater than dose proportional increases in systemic exposure to omigapil were seen at doses 0.02 – 0.08 mg/kg/day. The dose which achieved patient exposure within the pre-established target of AUC0-24h range was 0.06 mg/kg/day. In general, omigapil was safe and well tolerated. No consistent changes were seen in the disease-relevant clinical assessments, including assessments of motor function, respiratory function and muscle strength; however, their interpretation is limited due to the short duration of the study. The omigapil study set a precedent that successful recruitment and running of a trial for *LAMA2*-MD is possible. The CALLISTO trial highlighted that clinical trial readiness depends on clinical care being optimized, as many patients in the trial were not using nocturnal non-invasive ventilation (NIV) at the time of enrolment despite the clinical evidence of respiratory insufficiency, and were thus started on

NIV during the trial. Additionally, viable outcome measures which correlate with muscle function and that are sensitive to change over time are needed. These will likely combine motor function scales, muscle imaging and assessments of respiratory function. As the neuromuscular field aims to improve clinical trial readiness for patients with *LAMA2*-MD, early natural history should be studied, given the hope that future clinical trials will be designed to potentially treat patients with *LAMA2*-MD from infancy. Disease-relevant biomarkers which could adequately reflect potential therapeutic changes are also needed, and exploratory work in identifying serum and/or urine biomarkers in *LAMA2*-MD is ongoing.

Hemant Sawnani reported on ongoing investigations on the effectiveness of daily passive stretch of the chest wall to combat the pulmonary complications of *LAMA2*-MD (Manuscript under preparation). In a relevant randomized control study, subjects in the intervention group demonstrated varying gains in lung volume. However, treatment adherence was found to be about 50% by multimodal assessment, similar to other paediatric chronic diseases. Nevertheless, the majority of families had difficulty identifying barriers to adherence. In discussing pulmonary related endpoints and trial readiness, trials need to consider the known natural history of the individual patient and inter-patient variability. A clear understanding of the trajectory of a pulmonary test variable of interest is needed. Respiratory muscle strength should be measured by oesophageal manometry if muscle strength improvement is the desired outcome. Improvements in respiratory muscle strength may not yield a measurable change in vital capacity if thoracic restrictions cannot be overcome as the latter often tends to become fixed and less likely reversible. This leaves the need for qualitative improvements despite improved muscle performance.

Maurilio Sampaolesi discussed different types of adult stem cells available for treatment. The ability to incorporate mononucleated myoblasts into the multinucleated fibres makes the skeletal muscle an ideal target for stem cell therapy, as it has an inherent regenerative capacity. In fact, when a genetically corrected stem cell fuses with the existing myotube, this feature will be transferred within the long multinucleated syncytial tissue. Upon injury, the satellite cells, muscle stem cells that reside under the basal lamina of the myofibres, start to differentiate through the sequential expression of Myf5, MyoD and Myogenin in order to reconstitute the myofibres while maintaining the initial stem cell pool. In recent years, it has become more and more evident that epigenetic mechanisms such as histone modifications, DNA methylations and microRNA modulations play a pivotal role in this differentiation process. Mesodermal progenitors derived from induced pluripotent stem cells (MiPs) can regenerate both striated muscle types simultaneously in mice. Importantly, MiP myogenic propensity is influenced by somatic lineage retention. Human MiPs can also successfully engraft into the skeletal muscle and hearts of dystrophic immunodeficient mice. Finally, combining RNA-seq and miRNA-seq data, we define miRNA cocktails that promote the myogenic potential of human MiPs. By understanding the mechanisms behind myogenesis, we will be able to use this knowledge to enhance the differentiation and engraftment potential of different muscle stem cells. Besides manipulation on an epigenetic level, recent advances in the field of genome-engineering allow site-specific modifications in the genome of these stem cells. Combining epigenetic control of the stem cell fate with the ability to site-specifically correct mutations or add genes for further cell control, can increase the use of stem cells as treatment of muscular dystrophies drastically.

Heterogeneity of clinical features and prognosis combined with a lack of natural history data implies that trial readiness is limited. Important are the identification of MDC1A patients

worldwide and development of patient registry, selection of outcome measures and biomarkers that are sensitive to change, and consensus on the use of them, and natural history studies. This should be complemented with a rapid and open exchange on novel treatment strategies and therapeutic options. International exchange of knowledge leading to a broader understanding of the pathophysiology, clinical course and novel treatment options for MDC1A is therefore of great importance for both patients and their parents, caretakers, clinicians and researchers.

Conclusions

The road to therapy for MDC1A is built on two pillars. Important to achieve trial readiness are the identification of MDC1A patients worldwide and development of patient registry, the selection of outcome measures and biomarkers that are sensitive to change, and consensus on the use of them, and natural history studies. The conference created the momentum to make further steps in this process. Following an inventory of the current knowledge and ongoing clinical studies, a framework was defined for joint follow-up studies in defining both retrospectively and prospectively the clinical spectrum and natural history of *LAMA2*-MD. International collaboration is key in very rare disorders as LAMA2-MD to achieve statistically reliable data and agreement on the outcome measures to be used. The involvement of patients is also fundamental in this respect to define these measures correctly, as any treatment should eventually improve their health and quality of life. It was also recognized that while maintaining quality of life as high as possibility was essential to increase the possible success of any future therapy, the standards of care were often not known among patients and clinicians due the rarity of the disorder.

It is the objective of CureCMD and the "Stichting Voor Sara" to increase the awareness of these standards, keeping them up-to-date and translating them in as many languages as possible. For example, it was advocated that preventing pulmonary restriction and complication is key for this and should be more central in the treatment of patients.

The second pillar of the conference was an inventory of current therapeutic strategies and their progress in preclinical and clinical applications. Many therapeutic possibilities were being explored and it became clear that one single therapy might eventually not be able to cure *LAMA2*-MD fully, but that a combination of therapies would be required to combat both the primary defect and the downstream pathologies. Technical expertise developed, for example, for *in vivo* therapy could also be used *ex vivo* in stem cells. The participants agreed on sharing openly their expertise to be able to incorporate it across the different approaches used, which will accelerate the process of finding a cure for *LAMA2*-MD considerably. In follow-up meetings, this collaboration will be further established, and progress monitored to make the road to therapy as short as possible. So far, due to COVID19 restrictions, an online meeting has been organized with a more limited focus.

Finally, it is hard to underestimate the important role patient organizations, like the foundation "Voor Sara" or CureCMD, have in moving the field forward in MDC1A. These foundations generate financial resources for research, conferences and information campaigns. They provide information to patients and their relatives and (health) carers. Due to the rareness of the MDC1A, patients and their parents often feel lost, with nobody in a similar situation to talk to and without specialised care nearby. The foundations fill in a crucial gap, connecting nationally and internationally and providing a home for everybody affected by or involved in MDC1A. Their connection to researchers and clinicians provides

creates a strong bond between those affected by MDC1A and those working on care and therapies. As demonstrated during this conference, both groups appreciated this as highly motivating and rewarding.

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