Neuromuscular Disorders 33 (2023) 498–510

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Workshop report

253rd ENMC international workshop: Striated muscle laminopathies - natural history and clinical trial readiness. 24–26 June 2022, Hoofddorp, the Netherlands

Lorenzo Maggi[a,*], Susana Quijano-Roy[b,c], Carsten Bönnemann[d], Gisèle Bonne[e,*], 253rd ENMC Laminopathies workshop study group[#]

[a] Neuroimmunology and Neuromuscular Diseases Unit, Fondazione IRCCS Instituto Neurologico Carlo Besta, Milano, Italy
[b] APHP-Université Paris-Saclay, Neuromuscular Disorders Reference Center of Nord-Est-Île de France, FILNEMUS, ERN-Euro-NMD, Creteil, France
[c] Pediatric Neurology and ICU Department, DMU Santé Enfant Adolescent (SEA), Raymond Poincaré University Hospital, Garches, France
[d] Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, United States
[e] Sorbonne Université, INSERM, Institut de Myologie, Centre de Recherche en Myologie, Paris, France

ARTICLE INFO

Article history:
Received 20 April 2023

Keywords:
LMNA
Lamins
Myopathy
Emery-Dreifuss
Heart

1. Introduction and overview

Striated muscle laminopathies (SMLs) are a group of rare inherited neuromuscular and cardiac disorders due to mutations in the *LMNA* gene encoding A-type lamins [1]. They comprise *LMNA* related congenital muscular dystrophy (L-CMD), Emery-Dreifuss muscular dystrophy (EDMD), a form of limb-girdle muscular dystrophy (formerly LGMD1B), and isolated dilated cardiomyopathy with conduction defects (DCM-CD). In recent years, many efforts have been made to clarify clinical natural history, explore pathogenesis and develop therapeutic approaches for SMLs through international collaborations. Thus, the current knowledge in the field has greatly increased since the last ENMC workshop on Laminopathies back in 2006 [2]. However, there is currently a great need of a multidisciplinary approach, including clinical and basic research experts, to identify and define clinical outcome measures and biomarkers in SMLs. This would deeply impact on the comprehension of the disease natural history and the evaluation of the effect of experimental drugs for possible future clinical trials.

After two successive postponements due to the COVID-19 SARS2 worldwide pandemic, the 253rd ENMC international workshop on SML scheduled initially in March 2020 had been eventually held on June 24–26 2022 in Hoofddorp, The Netherlands. Between March 2020 and June 2022, the *SML Consortium* has been very active with numerous activities in order to progress in the preparative work necessary to reach the initial aims of the workshop which were: *1)* to share available data on natural history in adult and paediatric SMLs amongst experts and *2)* to create working groups focused on the identification of clinical outcome measures and biochemical, molecular and imaging biomarkers useful for natural history studies and future clinical trials. Three virtual conferences were held in 30–31 October 2020, 19–20 February 2021 and 12 December 2021; surveys circulated as well as a series of short virtual sessions every two months along 2021 were organized in small working groups. Thus, the *Striated Muscle Laminopathies Consortium* was extremely happy to eventually meet in June 2022 on a hybrid mode, gathering 31 participants, 21 in-person and 10 connected remotely, from Europe and America, including healthcare providers and researchers from Argentina, France, Germany, Italy, the Netherlands, Spain, UK and USA, as well as patients and representatives from four

* Corresponding authors.
E-mail addresses: lorenzo.Maggi@istituto-besta.it (L. Maggi), g.bonne@institut-myologie.org (G. Bonne).
[#] Listed at the end of this report.

https://doi.org/10.1016/j.nmd.2023.04.009
0960-8966

advocacy associations (Cure-CMD, Muscular Dystrophy UK (MD-UK), Associazione Italiana Distrofia Muscolare di Emery Dreifuss (AIMED) and LMNAcardiac.org). We also had two invited guest participants from industry, i.e. Sanofi and Nuevocor.

The workshop was introduced by Kate Adcock, member of the ENMC Executive Committee who welcomed all participants and introduced the ENMC commitments and objectives. Then, Gisèle Bonne (Paris, France) and Lorenzo Maggi (Milan, Italy) gave an overview of the main objectives of the workshop: *1)* to improve understanding and knowledge on SMLs natural history in paediatric and adult patients; *2)* to evaluate the state of patient registries; *3)* to identify clinically meaningful outcome measures for patient management and clinical trials.

2. Filling of gaps of our understanding using various preclinical models

Although numerous investigations have been reported focusing on the pathobiology of SMLs, to date only little is known about the underlying mechanisms involved in their development and progression and several gaps need to be filled. The current knowledge provided using preclinical models has been reviewed to identify the next direction where to focus basic research investigations to speed up the development of adapted therapies for patients with SMLs.

2.1. Fibrosis & contractures. Giovanna Lattanzi (ITA)

Giovanna Lattanzi summarized existing data, and resources available in different research teams. Fibrosis has long been considered a potential contributor to muscular dystrophies and it seems to play a major role in Duchenne muscular dystrophy, so patients treated with steroids have an efficient delay in muscle wasting and loss of ambulation. However, the relevance of fibrotic processes in the progression of SMLs is not yet clear. Published data and clinical and laboratory data shared by the workshop participants suggest a major role of fibrotic processes in SMLs. In fact, Cristina Cappelletti (Milan, Italy) determined a pro-fibrotic secretome in EDMD patient serum and cultured myoblasts [3,4]. Cardiac fibrosis has also been discussed as a common and early phenotype in different SMLs as determined by gadolinium-based imaging [5]. Consistent with these observations, therapeutic compassionate use of steroids in children affected by L-CMD avoided contractures and improved the overall disease course in children (see below §7). To systematically analyse the cellular and serum markers of fibrosis, workshop participants agreed to investigate cell cultures and sera available from the biobanks as well as SML mouse models. The SML consortium has access to a large variety of resources available for preclinical work *via* the French and the Italian networks of laminopathies, coordinated respectively by Gisèle Bonne/Rabah Ben Yaou and Giovanna Lattanzi and their links with biobanks (Myobank from Myology Institute, Paris, France, linked to EuroBiobank; BioLaM (Italy), CMDIR (Cure-CMD international registry, USA) and the LMNAcardiac.org network (The Netherlands) represented by Rogier Veltrop. These resources comprise tissues, skin fibroblasts, primary and immortalized myoblasts from patients and various preclinical models such as knock-in mouse models as well as patient-derived cells subjected to gene correction *via* CRISPR/Cas technology. Of note, Peter Meinke (Munich, Germany) suggested that investigating muscle fibroblasts or fibro-adipogenic progenitors may represent a good strategy to unravel early profibrotic stimuli in SML, taking advantage of the high number of available samples. At present, workshop participants consider that a more systematic investigation of fibrotic processes, both in preclinical models and in patient muscle, is needed to define the link between profibrotic conditions/molecules and muscle dysfunction in SMLs and identify a few biomarkers and therapeutic targets. In this respect, Cristina Cappelletti, in collaboration with members of the Italian Network for Laminopathies, reported that different secretome profiles might help predict may help predicting EDMD patients with cardiac involvement. At the same time, preliminary data obtained in L-CMD serum suggest a specific secretome profile of this disease. In this regard, the final aim of the SML consortium is to define the role of fibrosis in disease progression and possible therapeutic approaches based on currently available drugs and specific biological inhibitors. amongst possible approaches, those aimed at counteracting TGFβ 2 and/or related profibrotic pathways have been tested in preclinical models and warrant attention. On the other hand, molecular determinants of fibrosis directly triggered by mutated lamin A/C need to be determined as they could represent more efficient therapeutic targets.

2.2. Stem cells, muscle growth and regeneration. Ignacio Pérez de Castro (SPN) & Peter Meinke (GER)

Several studies have pointed out to a defective muscle stem cell (MuSC) function as the cause of SML development and/or progression [6]. In addition to intrinsic factors (i.e. *LMNA* mutation), MuSC function could also be impaired by extrinsic factors that provoke a chronic, hostile microenvironment. Altered composition of the ECM, chronic inflammation and fibrosis lead to defects in the MuSC niche and a subsequent malfunction of the muscular progenitors [7]. Differentiation defects have been shown in primary myoblasts isolated from patients with either *LMNA* or *EMD* mutations [8,9]. A large RNA-Seq study has been performed on differentiated myotubes from 10 EDMD-like patients carrying different mutations in 7 genes. Comparative pathway analysis to controls revealed that multiple genes involved in fibrosis, metabolism, myogenic signalling, and splicing were affected in all patients [10]. Knock-in mouse models or C2C12 cells expressing mutated lamin A could be interesting tools for studying the role of MuSC in SMLs [11,12]. Abnormal MuSC differentiation has been reported in EDMD [13]. A significant number of processes and molecular pathways have been associated with the normal and pathological function of MuSC [14]. amongst them are those included in the maintenance of quiescent status and the activation of MuSCs. A hypothesis worth exploring is that stem cell exhaustion as a cause of SML, like accelerated telomere shortening in muscle cells of mdx mice lacking the RNA component of telomerase (mdx/mTR) leading to severe muscular dystrophy that progressively worsens with age [15]. Finally, a recently published work [16] has shown that MuSC activation and the foetal gene program is maladaptive in chronic dystrophic skeletal muscle. This counter-intuitive result, which implies that depletion of MuSC improves muscular dystrophy phenotype, should be tested in future experimental approaches.

From a therapeutic point of view, improvement (or inhibition) of MuSC function is an attractive therapeutic strategy for the treatment of SMLs' MuSC population by transient p38 inhibition, increasing the regeneration potential of aged mice [17]. Transplantation of wild type MuSC or mesenchymal stem cells has shown benefits in different muscular dystrophies [18–20]. Unfortunately, no major information on using stem cell-based strategies as potential therapies for SMLs has been reported. Further studies must be carried out to explore the potential of MuSCs for the in vivo and *ex vivo* treatment of SMLs. In summary, besides the potential connections between muscular stem cells and SML, many questions are still awaiting answers. amongst the main needs of the field might be the status of muscle stem

cells in SMLs and the potential of muscle regeneration for their treatment.

2.3. Modifier variants. Eric Schirmer (UK) & Gisèle Bonne (FRA)

Modifier variants may account in part for clinical variability observed in SML, as even within the same family different members can have significantly different clinical presentations [21,22]. Several digenic cases have been reported for EDMD, where modifier variants are in genes linked to the same or other muscular dystrophies such as combinations of: *LMNA* with *EMD* [23,24]; *LMNA* with *SUN1* or *SUN2* [25]; *EMD* with *SUN1* [25]; *LMNA* with *DES* [23]. In each case the patient with two mutations has a worsened phenotype. Modifier loci have also been reported in a large *LMNA* mutated family presenting variable age at onset of their skeletal muscle symptoms [26]. The search for modifier variants is also complicated as 6 different genes *(EMD, LMNA, TMEM43, SYNE1, SYNE2, FHL1)* have been associated to EDMD with for *LMNA* both AD and rare AR inheritance (OMIM # EDMD1–7). As there are both AD and AR forms of *LMNA*-linked EDMD and loss of lamin A/C can yield less strong phenotype than a dominant mutation [27], it is also possible that modifier variants could reduce rather than exacerbate phenotype.

The question was posed how best to identify modifier variants and distinguish clinical variation due to modifier variants from that due to variation in allele penetrance. Gisèle Bonne presented the current ongoing project in her lab, exploring intra- and inter-familial clinical variability associated to *LMNA*, with *i)* whole genome sequencing of large mutated families and *ii)* RNA-Seq of biological material available from patients carrying recurrent *LMNA* mutations (i.e., p.Glu358Lys and p.Arg453Trp). Eric Schirmer suggested the generation of a primer library to use to sequence all patient samples that can be acquired, patterned after the one used in a recent study that used an iterative sequencing approach to identify new EDMD alleles [28]. A concerted effort to do more such family sequencing when possible across the SML would be valuable. Still, it should be associated with high-quality clinical information and thus linked to natural history efforts being undertaken with the patient registries. Combined with RNA-Seq of patient muscle cells, this could also help distinguish what clinical differences are due to allelic as opposed to modifier variation. What remains needed to do is to obtain funding for the sequencing and hiring a designated bioinformatician and to integrate this effort with the patient registries. It would make sense to centralize the sequencing, but this also requires establishing an MTA and IRB process. It would also benefit integrating this with biomarker design efforts to establish a uniform regimen to confirm candidate modifiers.

3. Cardiac involvement and stroke

Heart disease is the common and most life-threatening complication in SMLs, either by the occurrence of arrhythmias that may lead to sudden death or by end-stage heart failure. Therefore, cardiac follow-up and management is a major issue in SML and distinguishes these disorders from other myopathies with milder or no heart involvement.

3.1. Stroke in skeletal and cardiac muscle involvement, proposal for a study. Lorenzo Maggi (ITA)

Stroke frequency and related outcomes in SML have been raised amongst the main recurrent issues from a survey filled out by ENMC workshop participants on February 2021, including the patients' feedback. Poor data are available in literature: stroke frequency is relatively heterogeneous amongst the studies. A recent systemic literature review revealed that 56 thromboembolic events/strokes (5.48%) occurred in the total cohort of 1021 EDMD patients [29], including both *EMD* and *LMNA* mutated patients. Similarly, a survey completed by 103 patients or caregivers from the EDMD Facebook group moderated by Eleonora Cugudda and Salome Mist Kristjansdottir, reported stroke in 5.4% of the cases. According to the systematic review, the incidence rate of thromboembolic events ranged from 0.3 events/100 pts–year to 8.9 events/100 pts–year. Atrial fibrillation (AF) *per se* carries nine-fold increased odds for ischaemic stroke (odds ratio 9.2, 95%CI 1.1–74.7) [30], corresponding to a higher risk compared to that reported for conventional AF patients, either symptomatic or asymptomatic. In an Italian cohort of SML not included in the aforementioned review, 6/78 patients (7.7%) had a stroke likely of cardioembolic origin at an age ranging 41–59 years [31]. All of them had AF. Furthermore, 107 episodes of stroke in 68 patients were reported amongst the 753 *LMNA* mutated cases included in the French registry on laminopathies (OPALE) (updated to January 2022). Hence, *LMNA* mutations represent an underrated cause of stroke, particularly in young adults and paediatric populations. However, no study has been specifically set up to estimate stroke epidemiology in SML. Most of the available data mainly derived from cohorts including patients with cardiac involvement and usually mentioning incidence and related underlying cardiac impairment, without any further detail on clinical and radiological features of stroke or its neurological outcomes. Lorenzo Maggi proposed a retrospective international study on clinical and radiological features of stroke and related neurological outcomes in paediatric and adult patients affected by SMLs.

3.2. Cardiac recommendations: adult population. Karim Wahbi (FRA)

3.2.1. Diagnostic workup

At diagnosis and at least every year during follow-up, cardiac workup should include a visit with a cardiologist with a specific expertise in the field of cardiomyopathies, an electrocardiogram, an echocardiogram, NTproBNP assay, and 24-hour Holter ECG. Cardiac MRI should be performed at diagnosis and repeated every 3 to 5 years during follow-up. Long duration Holter ECG (7–14 days) or loop recorder implantation should be discussed in patients with unexplained palpitations, lightheadedness, or syncope.

3.2.2. Treatment

Heart failure (HF) management should follow the European Society of Cardiology, American Heart Association, and American College of Cardiology guidelines for the management of HF [32] with an early initiation and rapid up-titration of HF medications. Patients with severe left/right ventricular dysfunction and/or severe HF should rapidly refer for pre-transplantation assessments, due to the usually rapid progression of HF. In patients with terminal HF, left ventricular assist devices can be only scarcely implanted because right ventricular systolic function is frequently severely impaired. Right heart involvement associated with important tricuspid regurgitation represents an indication for cardiac transplantation rather than tricuspid valve surgery. The prevention of sudden death due to ventricular tachyarrhythmias (VTA) should rely on the prophylactic implantation of cardiac defibrillators in high risk patients. The implantation of subcutaneous cardiac defibrillators (ICD) should be avoided in most patients regarding the high risk for complete atrioventricular block requiring permanent pacing. The prophylactic implantation of an ICD should be considered in patients with *1)* two or more of the following risk factors: male sex, non-missense mutations, non-sustained VT, left ventricular ejection fraction <45% or *2)* a 5-year estimated risk for malignant

ventricular tachyarrhythmias of 10% or more (online calculator: https://lmna-risk-vta.fr/) [33]. The benefit of ablation procedures in patients with ventricular tachyarrhythmias appears to be low, with a high risk of recurrences, and patients with sustained ventricular tachyarrhythmias refractory to medical therapy should have a transplantation project discussed. In patients with persistent atrial arrhythmias, chemical or electrical cardioversion should be considered rather than a rate control strategy regarding the potential benefit of this approach for the preservation of left ventricular systolic function. Oral anticoagulation should be systematically used in patients with sustained supraventricular arrhythmias regardless to their CHADS VASC score [34,35] because the risk for thromboembolic complications is high in this population.

3.3. Cardiac recommendations: paediatric population, Sebastian Maldonado (ARG) & Georgia Brugada (SPN)

3.3.1. Diagnostic workup

At diagnosis, initial workup should include a visit with a cardiologist specialized in the field of cardiomyopathies, along with electrocardiogram, echocardiogram, NTproBNP assay, and 24-hour Holter ECG. Patients with abnormal echocardiogram should have a cardiac MRI done. Cardiac MRI should also be considered in patients with normal echocardiogram, when it can be carried out without anaesthesia or if the anaesthesia risk is low. During follow-up, cardiac workup including the aforementioned tests should be organized at least on an annual basis, and more frequently in patients with significant cardiac involvement. Long duration Holter ECG (7–14 days) has to be discussed for patients with cardiac symptoms and/or abnormal ECG and/or abnormal echocardiogram. According to expert opinion, the implantation of a loop recorder should be considered for patients with *1)* symptoms suggestive of paroxysmal arrhythmias and normal Holter ECG, *2)* non-sustained supraventricular or ventricular arrhythmias on Holter ECG in view of the high risk of thromboembolic complications and sudden death, or *3)* evidence of sudden death risk factors validated in adult *LMNA* populations (LVEF <45%, non-sustained ventricular tachycardia, atrioventricular block of any degree, and male sex). Cardiac MRI assessments should be repeated every three to five years.

3.3.2. Treatment

HF management in paediatric *LMNA* patients should follow the international guidelines for the management of HF in the general population [32]. The current classifications based on functional class are not applicable to this specific population. In paediatric patients with terminal HF, heart transplantation or permanent left ventricular assist device can be scarcely considered due to the severity of skeletal and respiratory muscles involvements and frequent presence of right ventricular systolic dysfunction. Prophylactic permanent pacing should be discussed for patients with marked conduction system disease on surface ECG (PR interval >240 ms and/or complete left bundle branch block) regarding the high risk of progression to complete atrio-ventricular block. In patients with an indication for permanent pacing, the ICD implantation should be discussed regarding the high risk for patients to have concomitant sustained ventricular tachyarrhythmias. In patients with an indication for permanent pacing and evidence of left ventricular systolic dysfunction, resynchronization therapy should be considered. The selection of patients for prophylactic ICD implantations for the prevention of sudden death due to ventricular tachyarrhythmias is extremely challenging in this population regarding the difficulties to estimate the benefit/risk ratio (no specific tool to estimate the VTA risk in paediatric patients and major difficulties to implant devices for anatomical reasons). There is currently no data supporting indications to implant ICDs prophylactically in patients with normal ejection fraction. In patients with atrial arrhythmias (fibrillation or flutter), curative anticoagulation should be initiated in view of the high risk for thromboembolic complications, even when ventricular systolic function is normal. The efficacy of atrial ablation procedures appears to be very low in *LMNA* patients, with a high risk for recurrences and procedural complications.

4. Skeletal muscle involvement

4.1. Deep phenotyping & classification of SMLs. Rabah Ben Yaou (FRA) & Soledad Monges (ARG)

Previous discussions during virtual meetings clearly suggested that the current classification of SML is still incomplete. In addition to the typical classical main pictures (L-CMD, EDMD, LGMD), classification should encompass further clinical aspects: *1)* The intermediate phenotypes, constituting a dark zone between L-CMD and EDMD. This raises questions related to the difficulties in differentiating them and the existence of a continuum between some paediatric cases (specifically in those patients with dropped head with late onset) and some EDMD patients with very progressive course and loss of cervical support. More generally, this brings issues on a more general continuum of the different subtypes. *2)* The involvement of specific muscle groups (cervical, paraspinal, lumbar) and the predominance of joint contractures versus weakness. *3)* The phenotype changes with a proactive care. *4)* The place of other features (cardiac, respiratory and metabolic) when associated to skeletal muscle involvement. *5)* The atypical cases in terms of age of onset, progression, overlap with other laminopathic traits (lipodystrophic, nerve, progeroid features) and the cardiac phenotypes with minimal muscle involvement. *6)* The significance of some specific pathological aspects, as dystrophic or inflammatory features. There was a consensus amongst participants on the need of a better definition of diagnostic criteria as: age of onset, initial signs, maximal motor function achieved, axial tone, presence of hyperlordosis, presence of early joint contractures, foot deformities aspect, weakness pattern, disease course and progression, including loss of ambulation, cardiac and respiratory compromise, imaging pattern and genetics.

An initial proposal has been suggested including severe and less severe L-CMDs, early, classical and progressive EDMD, and typical and atypical LGMD1Bs. Several avenues of future work were identified: *1)* to review the literature and databases to identify the main phenotypes and especially the atypical ones, consider outcomes for each phenotype; *2)* to share and discuss present clinical cases displaying the different phenotypes (typical/atypical, paediatric, adults), either from the literature or from personal cases; *3)* to finalize a survey/Delphi process amongst participants to reach consensus on skeletal muscle phenotype classification. A preliminary survey amongst clinical participants intending to pinpoint main clinical features that may be used for future classifications identified several trends: age of onset, motor milestones, cervical weakness, neck and spine stiffness, cardiac and respiratory compromise. Moreover, several typical and atypical cases were showed by Soledad Monges for paediatric forms and Rabah Ben Yaou for adult forms to illustrate the wide spectrum of SMLs phenotypes.

A further issue was the removal of the LGMD1B phenotype from the last release of LGMD classification [36]. Participants still consider this distinct LGMD form as relevant for diagnostic and

prognostic purposes. Although *LMNA*-related myopathies represent a continuum in the clinical spectrum, melting LGMD1B with EDMD as a unique entity is not appropriate considering the different clinical and radiological features. Actually, the LGMD1B is the first delineated autosomal dominant LGMD form and one of the most frequently reported as it was published in at least 192 patients (see UMD-LMNA database at www.umd.be/LMNA/). In fact, more exceptional forms of LGMDs were still considered as full LGMD forms in this new classification [36] even if dystrophic features were not the major pathological hallmark in some of them (i.e. *DNAJB6, TNPO3, HNRNPDL, Teletonin, POMT1, POMT2, POMGnT1, POMGNT2, α-dystroglycan, PLEC, TRAPPC11, GMPPB1, ISPD, PINCH, TOR1AIP1, POGLUT1* related). Participants asked whether melting LGMD1B with EDMD as a unique entity is legitimate or not, given the specific neuromuscular course of the disease. In practice, participant still consider that this phenotype is still relevant and had specific clinical and radiological features to make her different from EDMD. Finally, the participants agreed to continue discussions and brainstorming in the coming months to go further in the phenotypic and nosological characterization of SMLs.

4.2. Contractures. Proposal for retrospective & prospective longitudinal studies. Andrés Nascimento (SPN) & Laura Carrera (SPN)

Early development of contractures in different joints represents a main feature of SMLs. Several studies tried to describe the distribution of contractures, the chronology of their onset and progression, but there is no study that delves into their impact on patient mobility. Furthermore, the development of contractures does not always correlate with the pattern and degree of muscle weakness in SMLs. In this regard, there is a clear difference in the progression and distribution of contractures when comparing natural history studies in classic EDMD and L-CMD [37]. In the latter, the contractures are characterized by early onset, faster progression and the distribution could be different according to the maximum level of motor function reached (no sitters, sitters, or walkers-dropped head syndrome). In a retrospective study in a group of 10 cases with dropped head syndrome (L-CMD-Dhs), all patients presented before 20 months of age, started walking between 16 and 20 months and were followed-up at the neuromuscular unit of the Hospital San Jose de Deu- Barcelona. Moreover, patients were divided into two groups according to the presence of ongoing treatment with corticosteroids (prednisone 1-0.75 mg/kg/day daily dose). Patients with Dhs phenotype without corticosteroid treatment ($n = 5$, mean age at review: 21 years (range: 20–24)), presented in 100% of the cases contractures before the age of 10 years in all the joints studied, as follows in chronological order (mean age in years; range): Achilles (3.4; 1.5–5), hip (6.1; 5–8), elbows (6.3; 2.5–8), knee (6; 4.5–8), mean age of gait loss was 7.4 years (range: 5–11). Similar data were described in previous studies of natural history in L-CMD [37,38], but without any data of hip contractures, which are particularly relevant due to their impact on the gait pattern. In the group receiving corticosteroids ($n = 5$; mean age 9 years; range 8–10), contractures were observed in the following chronological order (mean age of onset in years; range): ankle (3.8;1–7), hip (5.3;4–7), elbows (6.3;4–7), knee (7.6;7–9); none of the patients had lost autonomous ambulation at last examination included in the study and 2 patients did not develop retraction of knees or hips. When comparing the two subgroups, a significant ($p<0.05$) difference in walking ability was observed in favour of the patients who received corticosteroids. These preliminary data suggested that larger cohorts of patients should be investigated, even including different phenotypes and adult patients; furthermore, additional information on management (physiotherapy, orthosis, surgery) and related outcome should be collected, aiming to improve the standard of care. A 2-year prospective natural history study has been proposed to quantify the degree of contractures and their progression (standardizing the methodology for their measurement) and to establish the correlation with the motor function scales, scoliosis, respiratory function, and cardiology status.

4.3. Gastrointestinal & nutritional, respiratory & spinal problems. Adele D'Amico (ITA) & Susana Quijano-Roy (FRA)

Gastrointestinal and respiratory involvement represent relevant issues in SMLs, more often observed in the more severe phenotypes. No specific standard of care or clinical guidelines have been published for SMLs, although pulmonary, gastrointestinal and nutritional care for L-CMD were substantially addressed in the 2010 standard of care consensus together with other congenital muscular dystrophies [39]. In addition, while EDMD management recommendations are included in recent reviews [40], little discussion about respiratory management and no reference to gastrointestinal or nutritional complications are reported.

In SMLs the restrictive respiratory disease is the result of respiratory muscles weakness together with the progressive chest rigidity and the spinal deformity which develop in the first decades of life and may further impair the pulmonary function (scoliosis with thoracic lordosis) or lead to swallowing disorder (hyperextended stiff neck, dropped head). The type and frequency of respiratory assessments must be adapted according to age of patients and disease severity and due to the slow and silent progression of respiratory failure, regular surveillance should be maintained (every 6–12 months). Pulmonary function tests should include Spirometry - to test Forced vital capacity (FVC) and if available peak of flow (PCF) and maximal inspiratory pressure (MIP). Diurnal or waking capillary blood gases samples and sleep oxy-capnography studies are of major importance at least annually to rule out signs of hypoventilation, in particular increased partial pressure of carbon dioxide [41]. The primary goals for pulmonary treatments include clearance of secretions through the use of assisted coughing techniques and noninvasive positive-pressure ventilation in case of symptomatic daytime hypercapnea, symptomatic nocturnal hypoventilation, non-symptomatic nocturnal hypercapnia or hypopneas, failure to thrive, recurrent chest infections [39].

The main gastrointestinal and nutrition issues for SMLs are failure to thrive, feeding difficulties (anorexia, poor accepted volume of meals) and slow gastrointestinal motility (constipation). In congenital and severe phenotypes, the most encountered problem is undernutrition and poor weight gain. Clinical assessment should include *1)* a serial anthropometric recording of weight and height to calculate body mass index (BMI), *2)* a dietary recall and food diary. Treatment and management of nutritional problems should focus on safe and adequate intake, but this may be difficult to define due to the lack of standardized growth curves in SMLs. To this purpose, the reduced muscle bulk means that normal weight for a L-CMD child may not be a “normal” weight for the age. It is nevertheless important that curves are progressive and no stagnation is present. A multidisciplinary management is needed and dietary recommendations should include*: 1)* adapt positioning and seating as well of utensils to facilitate safe swallowing and supports for self-feeding, *2)* advocate thickened feeds, adequate caloric and micro and macronutrient intake privileging slow sugars, *3)* frequent meals during the day (avoid fast of 4 or more hours during the day), *4)* treat effectively chronic constipation, ensuring good hydration and prescribing macromolecules rather than laxatives, *5)* consider the placement of nasogastric tube feeding or, in case of long-

term treatment, gastrostomy if conservative management is insufficient.

5. Patients' perspective, registries and clinical recommendations

5.1. Patients' expectations & needs. Christine Graskamp (GER), Eleonora Cugudda (ITA) & Danielle Baumont (NL)

Three patient representatives participated in the workshop. They highlighted the need of a consensus of care for patients and doctors with no experience in dealing with SML to ensure a homogeneous multidisciplinary management and follow-up of patients. They stressed the importance to get available guidelines of management in particular of the cardiac (indications of ICD, anticoagulants) respiratory and global periodic check-ups. They proposed to create a shared international platform with easy access for everyone gathering updated information on clinics, genetics, research as well as a reference to specialists (for adults and children).

They also manifest the need of an international combined patient registry (European or global) for *LMNA* mutations which would provide real-world data and which would allow linking research to patients. Finally, they remarked that patient groups want to support researchers to spread the research. Cure-CMD, MDUK and LMNAcardiac.org proposed to join patient representatives to help to start up shared platform, but also to organize fund and grant applications.

5.2. Patients' registries, their present & future. Gisèle Bonne (FRA) & Gustavo Dziewczapolski (Cure-CMD, USA)

Gisèle Bonne presented *OPALE*, the French Observatory of Patients affected with Laminopathies and Emerinopathies (NCT#03,058,185). This national registry includes currently 98 clinicians from 40 departments and 10 diagnostic laboratories. To date, 753 patients have been enroled (from whom 451 have SML). The registry enrols at least 38 new patients per year. The main perspectives are *1)* to upgrade *OPALE* towards a European (international) patient registry by creating a multicentre web-based registry (ethical and regulatory procedures are already in progress in close interaction with European Reference Network (ERNE) Euro-NMD and ERNE Guard-Heart), *2)* to establish a scientific committee and *3)* to identify how to connect this new registry with the worldwide CMD patient registry (*CMDIR*) dataset. The patient advocacy organization, Cure-CMD (www.curecmd.org), through its Scientific Director, Gustavo Dziewczapolski, presented the new platform of the Congenital Muscular Disorders International Registry, CMDIR (www.cmdir.org). The CMDIR is the largest collection of patient-reported and curated clinical data of CMD patients worldwide (established in 2009). The CMDIR platform has been upgraded in 2021 to allow flexibility to share data with other registries and avoid duplication of individuals registered in different national/international registries. Registrants have "Opt-In" options to share data with other registries and generate a Globally Unique Identifier, GUID. The CMDIR has been used to recruit patients and inform various clinical studies/trials. Regarding LMNA-related CMD (L-CMD), patients from the CMDIR were included in the recent retrospective natural history study [38]. Currently, the CMDIR counts 100 *LMNA* mutated individuals from 21 different countries, 94 living (males/females: 53/41) and 6 deceased.

Gustavo Dziewczapolski highlighted the role of Cure-CMD and the CMDIR as the hub to collect the patient's voice which nowadays is considered, more than ever, by Industry, Regulators, Payers, and granting bodies. At the time of the workshop, Cure-CMD was organizing a Patient-led Focused Drug Development Meeting (PFDD Meeting) with the U.S Food & Drug Administration (FDA). As an example of the efforts of Cure-CMD to carry the voice of most patients with L-CMD, results of a survey of 32 affected patients/families, were presented. One significant outcome of the survey was that the 3 issues which resulted to have the most significant impact on the affected individual's quality of life were mobility limitations (28%), joint contractures (15%) and heart-related issues (10%).

5.3. Recommendations for SML diagnosis & management. Susana Quijano-Roy (FRA) & Lorenzo Maggi (ITA)

Considering the rarity of the SMLs, their clinical heterogeneity and the relatively poorly defined natural history, optimization and consensus on the standard of care represents a complex task and requires multicentre international collaboration in expert centres. However, specific international consensus on diagnosis and management of SMLs is still lacking. The fact that these rare diseases show often life-threatening complications and most affected individuals reach adult life in variable disabled situation makes this a priority.

The collaborative international network established over the years by clinical and research groups represented at this workshop has provided updated fundamental insights in the field. Heart involvement being a major issue, the diagnosis and follow-up of cardiac issues produced a quantity of real-life data, with relative homogenous collection in all the referent centres present at the workshop. The assessment of other aspects, such as respiratory function, gastrointestinal disturbances or orthopaedic deformities (scoliosis, joint contractures) has been less systematic and homogeneous amongst the different centres, providing less evidence for guidelines.

In the care consensus on CMDs published in 2010 [39], 82 international multidisciplinary experts and 33 patients or parents suggested that L-CMD shares similar care recommendations to other CMDs with respect to pulmonary, orthopaedic or gastrointestinal aspects (explore and treat restrictive respiratory insufficiency, joint contractures and scoliosis, respectively). In contrast, cardiac involvement was found to be a specificity in *LMNA* patients compared with the rest of CMDs. In this regard, cardiologists defined consensual guidelines in the diagnosis and management of the rhythm and heart function disturbances of children affected with L-CMD (see above). Conversely, no recommendation or consensus on diagnosis and management of EDMD or LGMD1B have been published to date, other than global cardiac care [33].

Since 2010, a few studies have been reported on large cohort of patients or in small series focusing on specific disease features, as the dropped head phenotype in L-CMD [42], the end stage cardiac complications including right ventricle failure [43], or the effect of long-term oral steroids [31,36,44]. A recent international collaboration study investigated clinical and genetic data in a large cohort of paediatric patients [37]. This study which includes more than 150 children allowed describing the most frequent mutations, raised questions about different potential pathogenicity of variants (e.g., severe phenotype in the most common p.Arg249Trp *LMNA* mutation), established preliminary clinical endpoints and identified specific clinical subgroups of particular interest for preventive interventions and clinical trials. Moreover, several publications have been focused on cardiological issues [5,43] with important advances in the early identification of risk of fatal events and their management [41,45].

As a conclusion of this session, high or moderate level of evidence appeared from the existing literature to warrant

the publication of recommendations/guidelines on cardiac management in SML in the next future, in particular in adults, according to the methodology proposed by the European Academy of Neurology guidance for developing and reporting clinical practice guidelines on rare neurological diseases [46]. On the other hand, the level of evidence from literature for the management of other clinical aspects (respiratory, orthopaedic, gastrointestinal), still remains poor, hence, no recommendations/ guidelines can be yet produced, but the participants agreed that a consensus amongst experts on this topic may be achieved through a Delphi process.

6. Outcomes measures & e-CRF

6.1. Prospective muscle evaluations. Rabah Ben Yaou (FRA) & Valérie Decostre (FRA)

Rabah Ben Yaou began with a short reminder on the French OPALE registry main goals: improving knowledge on the clinical spectrum and natural history of laminopathies and emerinopathies, support research and readiness for future drug trials. OPALE is authorized by the French regulatory authorities, starting in 2013 in four pilot centres in the Parisian area, then progressively extended to all French territories. Currently almost 500 patients affected with SMLs are followed in 40 French centres. The last amendment to the protocol was performed in 2018 to allow prospective muscle function assessments in those patients previously included in the OPALE registry. Valérie Decostre then detailed the systematic prospective muscle assessments that are currently being performed in the *Neuromuscular Physiology & Evaluation Laboratory at the Institute of Myology*, Paris in adult patients with SMLs. Skeletal muscle symptoms in SML such as LGMD1B and EDMD are well known, but not quantified in the literature. However, clinical trials require prior natural history studies of the disease to identify the outcome measures most sensitive to disease evolution. Within the frame of OPALE registry, Valérie Decostre has started a 36-month follow-up of skeletal muscle strength and function scores in *LMNA* mutated adults with or without skeletal muscle involvement (DCM-CD, familial partial lipodystrophy (FPLD)), in order to rule out subclinical muscle weakness in the later. Skeletal muscles are assessed at 3 time points for each patient: at inclusion, 18 months, and 36 months. Strength, function and goniometry (to assess joint contractures) measurements are performed. Results of knee extension/flexion strength, 6-minute walk test (6MWT), and Muscle Function Measure 32 (MFM32) were presented for the first 17 adult patients assessed, 3 of whom had already completed their 18-month follow-up visit. Several patients, usually with DCM-CD or FPLD, showed normal 6MWT distance for age, sex and height, associated with either normal or impaired knee extension/flexion strength. Most patients with muscle weakness (LGMD1B and EDMD) and interestingly also several with motor preserved function (DCM-CD or FPLD) showed impaired knee extension/flexion strength and 6MWT distance covered. Concerning the motor function scores (MFM32), global scores were impaired in all weak patients (LGMD1B and EDMD). Interestingly, this impairment was mostly due to the decrease in the standing and transfer motor function (D1 sub-score), while the sub-scores measuring axial-proximal (D2) or distal motor skills (D3) were much better preserved. As a conclusion of this session, quantification of skeletal muscle strength deficit and its functional impact in laminopathies is ongoing and it should allow the identification of the outcome measures most sensitive to disease progression in adult laminopathies in order to assess the benefit of future therapies.

6.2. Proposal for structuring e-CRF. Lorenzo Maggi (ITA) & Susana Quijano Roy (FRA)

An eCRF (electronic case report form) is a digital questionnaire that is used to collect data about a clinical study and research participants. The data collected in eCRFs is what biostatisticians analyse to draw a conclusion from a study, hence proper definition of eCRF is fundamental for the good outcome of any clinical trial or registry. Susana Quijano-Roy revisited the French registry (OPALE) to focus on key items for the historical data to be included in the electronic case report form (eCRF) in view of a prospective study. General items, including referral centre, body measurements and phenotype definition, should be mandatory in any eCRF focused on SML. Other than cardiac, items related to motor and respiratory function, spine, contractures and their progression over the time should be included.

Regarding the phenotype characteristics (usually described by age at onset, maximal motor milestones achieved and symptoms as contractures, stiffness, pattern of weakness and cardiac involvement) an interesting discussion took place during the session, suggesting that the order of onset of contractures (particularly elbows versus lower limbs and ankles) might be a marker to distinguish different phenotypes within the paediatric laminopathies with different clinical motor outcomes (e.g., L-CMD outcomes are more severe than those in early EDMD). From the motor function point of view, all participants agreed that *1)* highest motor milestone achieved, *2)* motor function at last visit, and *3)* use of scales adapted to the patient's age (CHOP INTEND and HINE2 for infants; MFM20 for children from 2 to 5 years. and MFM32 for children older than 5 years and adults) were important data in order to capture the progression of the muscle disease. It was also suggested to add RULM score for evaluating the upper limb function in more severe non-ambulant patients. In contrast, the use of timed scales could be interesting to add in the more performant patients. In adults, the loss of walking ability is not frequent and North Star Ambulatory Assessment is currently used by several groups.

Considering the impact of joint contractures and spinal deformity on motor and respiratory function, respectively, it seems very important to capture the age of onset, localization and course of limb contractures, presence of scoliosis (annual spinal X-rays to measure Cobb angle) pulmonary function (FVC), and the presence of hypoventilation (blood gazes, sleeping studies). Similarly, capturing data on orthopaedic and respiratory treatments (preventive, symptomatic or definitive) is strongly advised due to the worse disease progression in their absence. Finally, concomitant treatments and other complementary tests in case of cardiac, metabolic, gastrointestinal and nutritional complications may give important additional information and should be collected retrospectively and prospectively.

7. Clinical trial readiness

7.1. Clinical trial readiness - focus on skeletal muscle involvement. Carsten Bönnemann (USA)

The first priority in terms of clinical trial readiness is to create a clinical trial network that includes clinical centres, aiming to collect data prospectively; data should be collectable in all patients and across all sites. This network should engage even patient advocacy, authorities and pharmaceutical partners, aiming to capture all groups involved with SML patients. The second priority is the creation and maintenance of dedicated disease registries to collect data longitudinally and establishing a database. Current registries including data on SML are the OPALE registry for French patients, the Muscular Dystrophy Association

(MDA) registry for US patients and the Congenital Muscle Disease International Registry (CMDIR), without any national restriction. Outcome measures to be considered in registries and clinical networks should be classified according to the following domains *1)* motor and function; *2)* cardiac; *3)* pulmonary and *4)* quality of life. Moreover, outcome measures should be adjusted for functional abilities and age categories. No outcome measure has been specifically validated for SML, hence outcome measures validated in other conditions close to SML, including patients' reported outcome measures, should be considered. amongst them, outcome measures that could function as a "core data" set must be prioritized being: important, reliably collected, feasible in the most patients and collected properly across all the centres. Additional, but conventional set of outcomes, as those focused on nutrition and bone health, may be included. Notably, the priority is to collect single patient data longitudinally which could then be used as run-in data. Exploratory outcome measures such as device-based monitoring tools, skeletal and cardiac muscle imaging and biomarkers should be considered, although centre-dependant and expertise-dependant. When considering outcome measures, minimal clinically important difference should be calculate using the clinical global impression of change and cross-validation amongst outcome measures is strongly recommended. Biomarker selection should be based even on the link to outcome measures and correlated to deep genotyping. Lastly, there is need to create groups of trial candidates which should not be too tightly defined in order to allow sufficient enrolment.

7.2. Clinical trial readiness- focus on cardiac muscle involvement. Karim Wahbi (FRA)

Karim Wahbi emphasized that cardiac involvement in paediatric- and adult-onset SMLs is highly prevalent and has a major impact on both quality of life and life expectancy. Conventional cardiac management has greatly improved patient prognosis over the past decades, but up to 15% of *LMNA* patients still develop terminal heart failure, which remains a lethal complication for many paediatric or adult patients who are not eligible for transplantation due to severe skeletal muscle and respiratory involvements. Therefore, there are great expectations regarding the development of innovative therapies targeting heart disease in this population to address these current unmet needs.

Several endpoints have been suggested by the French group for further phase 2 trials to estimate the effect of innovative therapies in studies including only a limited number of patients, with short follow-up durations (few months to 2 years). Unfortunately, these suggestions rely on experts' clinical experience and on data from a limited number of natural history studies (particularly in the paediatric groups [43]), which are unfortunately almost exclusively retrospective with a limited number of cardiac assessments. These endpoints are all surrogates, since clinical events such as hospitalisation for heart failure, embolic stroke, ventricular tachyarrhythmias can hardly be used in such phase 2 studies. We classified these endpoints in categories corresponding to the main types of manifestations of *LMNA* cardiomyopathy. We specified which ones have a validated prognostic value for the prediction of major cardiovascular events in dilated cardiomyopathy (DCM) of any cause or specifically caused by *LMNA* variants (see Table 1).

7.3. Radiological biomarkers in future clinical trials. Robert Y Carlier (FRA), David Gomez-Andrés (SPN) & Susana Quijano-Roy (FRA)

In the last decade muscle MRI has become an important diagnostic tool in hereditary myopathies [47]. Non-quantitative techniques were the first to be used in clinics and allow a good contrast between conserved muscle tissue and fibroadipous replacement (T1-TSE weighted sequences) or detection of oedema or inflammatory lesions (STIR or T2 fat saturated sequences). More recently, quantitative techniques have been developed and are increasingly available in expert centres. The most known technique is Dixon, which allows, in addition to quantification, the acquisition of images with similar interpretation to the classic sequences and therefore also useful in the clinical setting. Robert-Y Carlier presented the Dixon T2 protocol (IDEAL T2) which is a "two in one exploration", faster than the classic T1-STIR protocol because only one acquisition is required, but both types of images are produced to be able to assess both the degree of fibroadipous replacement (FAT images equivalent to T1-TSE weighted sequences) and the presence of inflammation or oedema in the muscles (WATER images, equivalent to STIR sequences) [48].

Representation of semi-quantitative scores for signal and volume changes using heatmaps has been described in series of different myopathies including children and adult SMLs [49,50]. These representations, when performed in a series of patients affected with a genetic myopathy may be expressed as hierarchical or regional graphics with whom clinicians obtain respectively, either the fingerprint of a myopathy useful for pattern recognition or identify groups of muscles with different signification: 1) muscles initially selectively affected (positive pattern) 2) end-stage spared muscles (negative pattern) 3) muscles that are affected progressively as the disease evolves. The ability to perform tissue signal quantification by 2 or 3-point Dixon techniques of a region of interest (ROI) opens the possibility of using this technique as a radiological biomarker in natural history studies or therapeutic trials. In the case of paediatric laminopathies, David Gomez-Andrés has identified pertinent muscles in the thighs by using heatmap analysis artificial intelligence analysis [49,51]. This is an interesting starting point for future natural history studies which should investigate the pertinence of the ROI and MRI changes over time. Robert-Y Carlier remarked that quantification studies, although feasible, are at present limited to the research settings because they are very time consuming and require important resources due to high cost of post-processing.

Susana Quijano-Roy showed the recent results of muscle Whole-Body MRIs in several infants with L-CMD using the IDEAL T2 protocol. A marked bright signal in Water sequences, suggestive of oedema or inflammatory lesions, were detected in all the cases in thigh, leg and shoulder muscles, with possible implications for the monitoring of therapeutic effect of corticosteroids or other future therapies. The series consisted of children younger than 4 years. Further studies during follow-up and in older patients will be necessary to understand the course of these changes and the presence at later ages. Previous studies using STIR sequences in older children and adults did not detected these changes but Dixon images show better contrast and sensibility to the identification of increase of water content in the muscle tissue.

7.4. Non-clinical biomarkers in future clinical trials. Eric Schirmer (UK)

The definition of a "non-clinical" biomarker can take several forms and change as technological and knowledge innovations transform a non-clinical biomarker into a clinical biomarker. A central aspect is that it does not necessarily give a readout that would provide immediate benefit for diagnosis or treatment simply because more research is needed before it can become a clinical biomarker. Nonetheless, assaying patients and/or patient material using such tests can yield a critical understanding of the pathomechanisms of a disorder and eventual correlations made between clinical presentation and data obtained can frequently lead to improvements in prognostic grading and treatment. Data from a RNA-Seq and miRNA-Seq study indicated a common set

Table 1
Main types of manifestations of LMNA cardiomyopathy and their biomarkers.

Test/tissue	*Measured Biomarker*	*Comments*	*Validation*
Myocardial involvement / heart failure			
Blood	BNP, NTproBNP	Correlated to high left ventricular filling pressures and HF outcomes in cardiomyopathies	not validated in *LMNA* populations
Echocardiogram or MRI	Left ventricular ejection fraction	Robust prognostic endpoint, correlated to clinical HF outcomes	validated in *LMNA* populations
Echocardiogram or MRI	Left ventricular systolic strain	High sensitivity for the detection of contractility impairment	validated in DCM but not in *LMNA* populations
MRI	Late gadolinium enhancement burden	Correlated to HF outcomes in DCM	not validated in *LMNA* populations
MRI	Global T1, ECV	Accurate quantitative assessment of myocardial fibrosis burden	
Ventricular arrhythmias			
24-hour Holter ECG, long duration Holter ECG, implantable loop recorders	Ventricular premature contractions burden or Non-sustained ventricular tachycardia	Independent predictor of malignant ventricular tachyarrhythmias in *LMNA* populations	
Conduction defects			
ECG	PR interval duration	Independent predictor of malignant ventricular tachyarrhythmias in *LMNA* populations	
Supraventricular arrhythmias			
24-hour Holter ECG, long duration Holter ECG, implantable loop recorders	Supraventricular premature contractions burden or sustained atrial fibrillation	Evidence of association of AF with stroke in *LMNA* populations	

BNP: B-type natriuretic peptide; NTproBNP: N-terminal pro-BNP; MRI: magnetic resonance imaging; HF: heart failure; ECV: extracellular volume fraction; DCM: dilated cardiomyopathy; AF: atrial fibrillation.

of functional pathways (metabolic, cell cycle licensing/myogenic fusion, ECM/fibrosis, and splicing resulting in loss of muscle-specific splice variants) altered in 10 EDMD-like patients with mutations in 7 different genes [10]. This study also found that the set of patients broke down into 3 separate groups depending on the specific genes representing these pathways that, at least from data available, would suggest they may similarly segregate according to disease severity. Thus, if confirmed with better correlations from more patients and a more complete clinical picture, this could be used prognostically to identify particular metabolic or cytokine pathways that could be therapeutically targeted, even with FDA/EMA approved drugs. Again, integration with the patient registries being built in several countries patterned after the nice OPALE started in France and a coordinated and funded sequencing effort are both necessary to advance this. Finally, several outstanding pathomechanistic issues that need to be resolved were noted such as the need to distinguish for heart involvement arising directly from the mutations, direct from mechanical stress, or indirect from fibrosis or fat accumulation and whether this breaks down according to mutation or modifier variants. From the standpoint of metabolism questions such as where energy is drawn from and the real effect of fat accumulation in muscle need to be answered. Whether different cytokines/collagens change direct different patient outcomes or contraction severity was also noted as an important question to address in the search for non-clinical biomarkers.

8. Development of new therapies

8.1. Corticoid experience in children. Susana Quijano Roy (SPN), Soledad Monges (ARG) & Andres Nascimento (SPN)

The L-CMD was first described in 2008 [52] and since then a number of patients have been identified worldwide, allowing a better understanding of the clinical features and course of this rare and severe disease. L-CMD patients show variable severity depending on age of onset, ranging from an early form in very hypotonic infants with absent head or trunk motor support to a later phenotype with typical development of neck weakness after acquisition of sitting or walking ability (Dhs). In any case, course is constantly towards a loss of motor, respiratory and cardiac function. Outcomes are poor, with frequent pulmonary and cardiac life-threatening complications before adulthood. As a matter of fact, Susana Quijano-Roy reported that only one third of the 15 patients included in the ancillary L-CMD article 15 years ago [52] remain still alive. Most of them died suddenly or in the context of severe arrythmias, and a few in the context of end-stage respiratory insufficiency or cardiomyopathy.

The interest of oral steroid treatment in SMLs raised in the last decade due to the marked inflammatory changes observed in muscle biopsies of many *LMNA* patients, in particular in children [53]. Susana Quijano-Roy stressed that no response was observed in a 2-year-old girl from the ancillary study in 2008 who initially was diagnosed with an inflammatory myopathy due to the muscle biopsy features. However, striking improvement of motor and respiratory course was observed in 3 boys treated with oral steroids for several years, one due to an initial suspicion of Duchenne muscular dystrophy and the remaining 2 due to inflammatory changes in muscle biopsies. Interestingly, all 3 patients remained ambulant through the years and did not required nocturnal ventilation. Interestingly, they displayed a different phenotype to that expected in a classic L-CMD, characterized by the absence of major limb contractures and spinal stiffness but a marked thoracolumbar hyperlordosis. Based on these observations, 11 L-CMD children were treated for at least 2 years with corticosteroids in a preliminary study in 3 expert paediatric groups at Buenos Aires, Barcelona and Garches, respectively [45]. The cohort was heterogeneous, including 2 early severe L-CMDs with no motor milestones achieved, 6 Dhs L-CMD, and 3 early EDMD. No progressive motor impairment was observed in the L-CMD patients after onset of steroid treatment (prednisone or prednisolone 0.75–1 mg/kg/d): the 2 early severe infants acquired unsupported walking in the weeks or months after introducing oral steroids and this achievement was maintained through the years. A Dhs sitter girl remained stable; four ambulant Dhs boys remained ambulant until the last follow-up (range 2–5 years) without developing respiratory insufficiency or requiring nocturnal ventilation. The most striking results were observed in a Dhs boy who had loss walking ability at the age of 3, was treated at 3.5 yo and recovered unsupported walking 2 years

later, also showing a significant improvement of joint contractures, except for the ankles, which required Achilles tendon surgery. He is still ambulant indoors at the age 10 years. More recently, an additional case showed a remarkable response to steroids: a boy with Dhs, who developed the progressive loss of axial and limb weakness by one year of age after acquiring sitting support, walked one year after starting steroids and does not require night ventilation. A recent Chinese publication reports similar results in four L-CMD children, in particular boys [36]. Andrés Nascimento reported the results of a follow-up study in several older L-CMD patients with Dhs who were treated after age 5 years and at a more advanced stage than those mentioned before, reporting no improvement of motor function, but showing a milder contracture phenotype in lower limbs compared to historical controls in the centerd. In contrast with L-CMD, oral steroid treatment was not clearly effective in EDMD children. Soledad Monges reported severe metabolic complications (diabetes, hypertension, vertebral collapse) in a 8-year-old boy and Susana Quijano-Roy described discontinuation of the treatment in another preadolescent EDMD boy due to the loss of motor strength and increase of weight.

In conclusion, the collected data and literature suggest that there may be a "responding" *LMNA* profile and a temporal therapeutic window, with responders being rather L-CMD males in a progressive period, under the age of 6 years. No formal recommendation can be made in older patients, especially in a stable period or in EDMD older patients. Further studies are required to confirm these results, provide a better understanding of the underlying physiopathology and determine the indication of oral steroids in paediatric SMLs.

8.2. Ongoing development of MEK inhibitors for LMNA *cardiomyopathy*

Uday Khire from AlloMek Therapeutics (USA) presented the application of MEK inhibitors for the potential treatment of *LMNA* related cardiomyopathy and *LMNA*-related congenital muscular dystrophy (L-CMD). He presented a mechanistic rationale for the use of MEK inhibition in these diseases based on *1)* role in slowing or halting progression of cardiac fibrosis and *2)* aids in preserving sarcomere structure. He presented a literature-based case study where MEK inhibition led to improvement in heart function of a paediatric patient with hypertrophic cardiomyopathy with Noonan syndrome [54]. He then showed data of selumetinib, a MEK inhibitor approved for paediatric patients with NF1 related plexiform neurofibromas. Finally, he presented summary of CIP-137,401, AlloMek's MEK inhibitor including in vivo efficacy of CIP- in *Lmna*$^{H222P/H222P}$ mouse model and excellent safety profile in toxicology studies which altogether pave the way to further clinical development of this molecule.

8.3. Current status of gene therapies development in laminopathies. Ignacio Perez de Castro (SPN), Anne Bertrand (FRA) & Colin L Stewart (Nuevocor, Singapore)

Genetic disorders such as SML are amendable for gene therapy aiming at correcting the mutation at the DNA level using CRISPR-based strategies, presented by Ignacio Perez de Castro and tested in his lab. amongst the different options, HITI (homology independent targeted insertion) using a minigene comprising exons 3 to 12 has been tested in patient myoblasts and in the knock-in mice, all with the *LMNA* p.Arg249Trp mutation. While HITI edits a low percentage of cells in vitro, all the HITI-positive clones revert their malignant phenotype. A second approach tested consists of the use of mutation-specific guides. In cultured cells, c.745C>T-sgRNAs have shown a high efficiency against the mutant allele and show low activity for the wild type one. Importantly, for both approaches, AAV-mediated gene therapy studies show significant survival improvement of the early lethality associated with the *Lmna*^{R249W} mice.

Gene therapy aiming at the correction of the mutation at the RNA level have also been tested in the Bonne lab as presented by Anne Bertrand. amongst them, the spliceosome-mediated RNA trans-splicing technology, based on the preferential incorporation of WT *LMNA* exons over the endogenous mutated ones at the step of intron exclusions from pre-messenger RNA has shown presence of corrected RNA and protein with reversion of cell's phenotype [55]. Efficient correction has also been observed following another gene therapy based on a combination of allele-specific RNA knock-down by shRNA and WT lamin A overexpression. However, here again, both approaches tested in vivo via AAV-mediated systemic delivery have shown only limited correction at the molecular level leading to absence or significant but limited improvement in terms of survival of the extremely severe *Lmna*^{dK32del} mouse model. While other gene therapy approaches are still under investigation, mitigated results obtained in vivo highlighted the urgent need for the development of an efficient delivery system for striated muscles as for other muscular dystrophies.

Colin L Stewart presented an alternative gene therapy approach under development, which does not target the *LMNA* primary defect but its downstream effects. Indeed, the *Lmna* mutations result in elevated levels of the LINC complex protein SUN1 located in the nuclear membranes. Mice in which *Lmna* gene is deleted in cardiomyocytes rapidly die within four weeks due to DCM. Similarly, mice carrying *Lmna* point mutations, e.g., p.Asn195Lys, also die from DCM. However, when these mutations are present in mice lacking SUN1, survival is extended for more than one year, with seemingly normal heart function. This finding identifies a potential therapeutic route to treating DCM by interfering with or removing SUN1 to prevent heart failure progression. Colin Stewart team developed a dominant-negative SUN1 minigene that, upon introduction into DCM hearts using AAV, suppresses many *Lmna*-induced cardiac muscle pathologies with a significantly extended lifespan [56]. They are developing this approach in their spin-out company Nuevocor, for eventual clinical use, as it is a potential route in using one reagent to suppress DCM caused by a wide range of different *LMNA* mutations.

9. Conclusions and workshop deliverables

The workshop allowed substantial and productive discussions and ended with propositions for both further developments of preclinical research including deeper understanding of pathological mechanisms at play and development of therapeutic approaches as well as starting prospective natural history studies, all being necessary for complete clinical trial readiness for SML.

To progress further towards clinical readiness, it was agreed to: *1)* prospectively collect clinical and imaging data as well as biological material (i.e. blood serum) along the routine follow-up of patients; *2)* retrospectively review available clinical data to clarify disease natural history, with specific focus on deep phenotyping, contractures, stroke, respiratory, spinal and gastrointestinal involvement; *3)* share between clinical centres eCRF and standard protocols to explore imaging data and to collect and store blood serum; *4)* organize grant(s) proposal taking in account complementarity expertise of the different research teams to develop further preclinical research including identification and validation of biomarkers and development of therapeutic approaches; *5)* write reviews of the current status of pathomechanisms knowledge of SML.

Moreover, the importance of collaborations with patient organisations was stressed as this would increase the impact of research; in this regard, together with patients' needs, it was

agreed to: *1)* prepare care guidelines with support from the European Reference Network Euro-NMD for cardiac involvement in patients with skeletal muscle laminopathies; *2)* produce consensus for management of non-cardiac features of SML for both adult and paediatric patients; *3)* produce lay recommendations/guidelines towards the families; *4)* set up, with the help of representatives from the advocacy associations, a shared international platform gathering updated information with easy access for every patients and families; *5)* upgrade the French patient registry OPALE towards an European/International patient Registry.

Finally coming together after a two-year delay, the preparative work done in the meantime and the workshop itself have provided excellent opportunities to update and deepen our knowledge of natural history of striated muscle laminopathies, promoting the international research and collaborative studies to better characterize, follow and care for SML patients.

List of participants

- Kate Adcock, Patient representative, Muscular Dystrophy UK, London and ENMC representative, UK
- Lindsey Armstrong, Patient Representative, UK
- Danielle Beaumont, Patient representative, The Netherlands
- Rabah Ben Yaou, Sorbonne University, Inserm, Institut de myologie, Paris, France
- Anne T Bertrand, Sorbonne University, Inserm, Institut de Myologie, Paris, France
- Silvia Bonanno, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
- Gisèle Bonne, Sorbonne University, Inserm, Institut de Myologie, Paris, France
- Carsten Bönnemann, NIH, Bethesda, MD, USA
- Cristina Cappelletti, IRCCS Istituto Carlo Besta, Milan, Italy
- Robert-Yves Carlier, APHP, R Poincare Hospital, Garches, France
- Laura Carrera, San Joan de Deu Hospital, Barcelona, Spain
- Eleonora Cugudda, Patient representative, AIMED, Italy
- Adele D'Amico, Bambino Gesu Children's Hospital IRCCS, Roma, Italy
- Valérie Decostre, Institut de Myologie, Paris, France
- Gustavo Dziewczapolski, Patient representative, Cure CMD, USA
- David Gòmez Andrés, Hospital Universitari Vall d'Hebron, Barcelona, Spain
- Marta Gómez-Garcia de la Banda, APHP, R Poincare Hospital, Garches, France
- Christine Graskamp, Patient representative, Germany
- Uday Khire, Allomek Therapeutics, USA
- Giovanna Lattanzi, ITOI-CNR Unit of Bologna, Bologna, Italy
- Agnieszka Madej-Pilarczyk, Children's Memorial Health Institute, Warszawa, Poland
- Lorenzo Maggi, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
- Sebastian Maldonado, Garrahan Buenos Argentina, Argentina
- Peter Meinke, University Hospital, Ludwig-Maximilians-Universität München, Munich, Germany
- Soledad Monges, Hospital de Pediatría J.P. Garrahan, Buenos Aires, Argentina
- Andres Nascimiento Osorio, San Joan de Deu Hospital, CIBERER, Barcelona, Spain
- Ignacio Perez de Castro, Institute of Rare Diseases Research, ISCIII, Madrid, Spain
- Susana Quijano-Roy, APHP, R Poincare Hospital, Garches, France
- Georgia Sarquella-Brugada, Sant Joan de Deu Hospital, Barcelona, Spain
- Eric Schirmer, Edinburgh University, Edinburgh, UK
- Rogier Veltrop, Maastricht University, LMNAcardiac.org, Maastricht, The Netherlands
- Karim Wahbi, APHP, Cardiology Department, Cochin Hospital, Paris, France
- Luisa Politano, Luigi Vanvitelli Campania University, Naples, Italy

Invited guests

- Elena Gargaun, Sanofi
- Colin Stewart, A*STAR and Nuevocor, Singapore

Declaration of Competing Interest

Dr. Maggi has received honoraria for speaking, advisory boards and compensation for congress participations from Sanofi Genzyme, Roche and Biogen, Amicus Therapeutics, Alexion, Janssen, Lupin, outside the submitted work. Dr. D'Amico has received honoraria as consultant in scientific advisory boards and for sponsored lectures form PTC therapeutics Roche, Avexis, Sanofi-Genzyme and Biogen outside the submitted work. Dr. Bonanno has received honoraria for speaking, advisory boards and compensation for congress participations from Biogen, Roche, Alexion and Sanofi Genzyme. Dr Andrés Nascimento has received honoraria for speaking, advisory boards and compensation for congress participations from Sanofi Genzyme, Roche, Sarepta, PTC therapeutics, Biogen, SRK, and Novartis.

Acknowledgments

The workshop and early-career programme are made possible thanks to the financial support of the European Neuromuscular Centre (ENMC) and its Full Partners: Association Française contre les Myopathies (France), Deutsche Gesellschaft für Muskelkranke (Germany), Muscular Dystrophy Campaign (UK), Muskelsvindfonden (Denmark), Prinses Beatrix Spierfonds (The Netherlands), Schweizerische Stiftung für die Erforschung der Muskelkrankheiten (Switzerland), Spierziekten Nederland (The Netherlands), Telethon Foundation (Italy). In addition, we would like to thank the Associated Partners: Finnish Neuromuscular Association (Finland), Österreichische Muskelforschung (Austria), SMA Europe and World Duchenne Organisation, and the members of the ENMC Company Forum: Amicus Therapeutics, Astellas, Biogen, Ionis Pharmaceuticals, Lupin Neuroscience, Novartis, PerkinElmer, Roche, Sanofi, and Sarepta. The organizers warmly thank the staff of ENMC, as well as participants of the preparative online sessions along 2020–2021: Reghan Foley, NIH, USA; Greta Gladney, patient representative, USA; Alexandre Méjat, Patient representative, AFM-Telethon, France; Luisa Politano, University of Campania Luigi Vanvitelli, Naples, Italy; Benedikt Schoser, Ludwig-Maximilians University, Munich, Germany; Anneke van der Koo, AMC, Amsterdam, The Netherlands; Howard J. Worman, College of Physicians & Surgeons of Columbia University, NY, USA. Several authors of this publication are members of the European Reference Network for rare neuromuscular diseases (EURO–NMD). This work was partially supported by the Italian Ministry of Health (RRC) and by the AFM-Telethon, the Institut National de la Santé et de la Recherche Médicale (INSERM) and Sorbonne Université.

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