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Workshop report

250th ENMC International Workshop: Clinical trial readiness in nemaline myopathy 6–8 September 2019, Hoofdorp, the Netherlands

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1. Introduction to nemaline myopathy, genetic basis and potential therapeutic approaches

On September 6–8, 2019, a group of 18 scientists and doctors, along with two patient advocates, from 11 different countries, convened in Hoofddorp, The Netherlands. The aim of the workshop was to plan for a thorough and reliable natural history and outcome measures study of congenital nemaline myopathy, as the basis for future therapy trials. After a welcome from Alexandra Breukel, Carsten Bönnemann and Laurent Servais, Alan Beggs and Carina Wallgren-Pettersson gave an overview of the genetics and clinical spectrum of nemaline myopathy (NM).

Alan Beggs (USA) discussed the genetic heterogeneity and highlighted specific details of the numerous genes associated with the clinical and histopathological phenotypes of NM. The currently known genes responsible for this condition include *NEB, ACTA1, TPM3, TPM2, LMOD3*, *KLHL40*, *KLHL41*, *CFL2*, *KBTBD13* and *MYPN* [1–3].

Carina Wallgren-Pettersson (Finland) discussed details of the genetics and clinical phentoypes of the more prevalent forms of NM related to the two genes most commonly identified as causing the disorder: the nebulin gene, *NEB* and the skeletal actin gene *ACTA1,* with their predominatly recessive (*NEB*) or predominantly *de novo* dominant (*ACTA1*) mode of causation [3,4].

NEB is a gigantic gene consisting of 183 exons, with four regions of alternative splicing and a triplicate region where eight exons are repeated three times. Complicating variant annotation within the *NEB* gene is the considerable and still incompletely known extent of normal variation. Most patients are compound heterozygouse for two different private disease-causing variants anywhere along the length of the gene, consistent with recessive inheritance. There are no hotspots for disease-causing variants, but one deletion, of exon 55, occurs with world-wide distribution, and the triplicate region commonly shows copy number variation interpreted to be pathogenic, i.e. two or more additional copies. Large pathogenic deletions and duplications are not uncommon and are best identified using a custom-made array. Very large deletions have been dominantly inherited in rare instances [3].

In contratst, *ACTA1* consists of only 7 exons and is highly conserved, so that most non-synonymous variants in this gene are disease-causing. Most of the disease-causing variants are *de novo* dominant, about 10% are recessive, while rare

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Table 1
Numbers describing several existing patient cohorts across Europe. Information gathered by Anna Sarkozy, Dubowitz Neuromuscular Centre London, UK.

Country	NEB	ACTA1	TPM3	TPM2	TNNT1	KLHL40	KLHL41	CFL2	KBTBD13	LMOD3	MYPN	MYO18B	Unknown	Total
Spain-Barcelona	9	7	–	2	1	3	–	–	–	–	–	–	4	21
Italy-Rome	5	4	–	3	–	–	–	1	–	1	–	–	–	14
Germany-Essen	2	10	–	–	–	–	–	1	1	–	–	–	–	14
Sweden-Goteborg	7	5	1	6	–	–	–	–	–	3	–	–	25	47
France	23	11	3	2	2	2	–	1	6	2	–	–	–	52
UK	54	24	6	6	4	7	–	2	–	4	–	1*	8	116
Finland	29	4	–	–	–	–	–	–	–	–	–	–	–	33
The Netherlands	7	8	2	3				2	38				6	68
Canada/USA	20	18		2									17	57
Total	163	113	12	24	7	12	0	7	45	10	0	1	69	471

families with mild disease may show dominant inheritance. Somatic and germline mosaicism has also been documented [3].

Both *NEB* and *ACTA1* encode proteins of the thin filament of the muscle sarcomere. The clinical phenotypes overlap, with both genes causing all categories of severity, although *NEB* most commonly causes the typical form of NM. Most cases of *ACTA1*-related NM are severe, but disease-causing variants in *NEB* are still a slightly more common cause than *ACTA1* of the severe form of NM [5].

Following the discussion on underlying genetic causes of NM, Alan Beggs (USA) and Carsten Bönnemann (USA) presented the incidence and prevalence of NM along with a detailed discussion regarding the current state of potential therapeutic approaches for NM [6]. Attention was given to the following methods under consideration as approaches to address NM:

1. Myoblast transfer, or cell-replacement therapy, which would be a general approach for any primary myopathy, not only NM; however, unfortunately, major technical hurdles remain with this technique and body-wide delivery appears not to be feasible at this point.
2. Myostatin inhibition is a method that functions by promoting primary increases in muscle mass, but does not correct the underlying primary genetic defect. Myostatin inhibition may provide temporary symptomatic improvement by increasing the size and strength of muscle fibres. This could be a general therapy for a number of congenital myopathies, and there are a variety of myostatin inhibitors which are currently undergoing pre-clinical and early clinical trials.
3. Gene or protein replacement therapy is another therapeutic approach considered, and one that is the most disease- and gene-specific. This technique works best for loss-of-function (recessive) conditions. The technology and protocols for gene replacement in humans are just now becoming established, and translation of experimantal studies in animals into effective treatment for patients is moving ahead.
4. Genomic engineering involving the CRISPR/Cas9 system and its variations is another viable approach to consider, as this method may work for gain-of-function (dominant) conditions through allele-specific inactivation as well as correction of disease-causing variants.
5. The development of new targets for drugs for NM require a good understanding of basic biology/disease mechanisms or availability of appropriate model systems or organisms with appropriate readouts for the purpose of initiating high-throughput screens.

2. Patient cohorts and review of natural history studies

Ana Sarkozy (UK) reviewed natural history data in NM and the overall incidence and prevalence of several existing patient cohorts across Europe. She presented a review of the numbers from the workshop's participating centres of patients with a genetic diagnosis of NM across all genotypes.Tables 1

From the existing data, we can see that NM is mostly caused by disease-causing variants in the *NEB* (35%) and the *ACTA1* genes (24%). Disease-causing variants in *TPM2, TPM3* and *KLHL40* were also common in the paediatric populations, while a dominant founder variant in the *KBTBD13* gene causes the most common form of NM in the Dutch population. A single UK patient carrying two recessive *MYO18B* variants did not have pathological features of NM (Sarkozy, Longman et al.; unpublished data). Patients with *KLHL41*, *CFL2* and *MYPN* gene variants remain rare. This exercise was not able to provide prevalence and incidence figures for NM. However, given that until 2019 the DNC was the only centre offering genetic testing for NM genes in the UK, we believe the UK numbers constitute a good estimate of disease prevalence in this population.

In addition to genotypic categories, review of the above cohorts throughout Europe also served to gather and identify all available natural history data on patients with NM. Results of a natural history study on 125 patients with congenital myopathy were presented [5], as well as of a retrospective study on 51 patients with *NEB*-caused NM from the UK (Sarkozy et al., unpublished data). This work confirmed the typical form of NM is the most frequent (60%), with 90% of patients presenting proximal and axial weakness and 18% symptomatic foot drop as well. Respiratory and bulbar features were common, with 77% of patients having respiratory features and 56% needing PEG or continuous nasogastric or nasojejunal feeding (Sarkozy et al., unpublished

data). Longitudinal follow-up studies of Finnish patients were published in 1988 and 1989, establishing that the typical form is usually only slowly progressive and that respiratory insufficiency is the greatest health hazard in NM [7,8]. A retrospective longitudinal cohort analysis on 23 UK patients is currently ongoing to assess motor and respiratory trajectories over a median follow up time of 7.81 years (range 2.12–18.37 years). Analysis of the natural history data of 14 NM patients from Germany confirms high incidence of ventilatory support (9/14 patients) and feeding support (PEG or NG tube; 9/14 patients).

Despite the review of these European cohorts, very few data are available to date on the detailed natural history of NM, including data on the best possible outcome measures for clinical trials (motor, bulbar, paediatric and adult) as well as the social costs of this disease. Case series and reviews of individual patient data are helpful, phenotypically, but do not serve to give exact numbers of prevalence of NM in the global population. Additional complications arise, depending on healthcare systems and resources established in various parts of the world, with varying ability to identify and diagnose NM, as access to genetic testing varies widely from country to country.

To illustrate a potential solution to overcome these issues, Laurent Servais (UK & Belgium) presented the experience and the insight gained in two industry-funded prospective natural history studies in spinal muscular atrophy [9] and myotubular myopathy [10]. In these two examples, working with pharmaceutical companies in natural history studies (NHS) dramatically increased the level of exigence in data collection and studies conducted in comparison with pure academic studies. Multicentric studies present a certain number of pros in comparison with single-site studies in which patients have to travel, in terms of patients' burden and risk of fatigue interference on the assessment. However, guaranteeing the reliability of assessments in a multi-site setting can also be much more challenging. Employing a dedicated physiotherapist to travel to the various sites, to ensure consistency of assessments, either by performing, and re-performing, on a regular basis, or by training local physiotherapists, may help to amend this.

Another insight from these studies was the need to reduce the number of assessments to decrease the burden on patients, but also to account for interference of fatigue and motivation on the assessment. Quality-of-life scales consistently show surprisingly high scoring for the patient's own perceived quality of their life, even in very disabled patients (the "rare disease paradox"), and should thus be used in their pure motor or function subscale rather than in their general quality versions, as these cannot distinguish severely disabled patients from the overall population.

Natural history studies have also shown that patients' trajectories vary a great deal from one individual to another. Patients may achieve their top performance at different ages, and this should be carefully taken into account in the overall analysis to avoid matching patients improving with those deteriorating, thus incorrectly creating a false statistical impression of stability.

Ulrike Schara (Germany) and Laurent Servais (UK & Belgium) discussed their proposed design for a Natural History Study in NM in Europe. The discussion centred around the need to develop two parallel studies, one based in Europe and another in the USA/Canada, both sharing an essential core data set with the same patient assessments to allow for direct comparisons between the two groups. Recommended NHS length is a total of three years (one year for patient inclusion and two years of follow up). The study will also plan to allow for flexibility in including both retrospective and prospective data, as both paediatric and adult NM patients are diagnosed at various stages of life, and this structure allows the NHS to include early data for older patients.

It was also discussed that it will be important to consider early inclusion of non-exclusive industry sponsorship, if possible, to increase the reach and rigour and assist in the scope of NHS reach across various countries and to ensure that the design is in line with the need in clinical development. Creating a multi-site design for rare disease NHS is key to both reduce patient travel burden and to increase patient participation.

Regarding inclusion criteria for the NHS, much discussion was dedicated to the consideration of which genetic subtypes of NM to include. It was determined, after much debate, that all genotypes resulting in histologically confirmed NM or with a compatible clinical presentation should ideally be included in the NHS. This was felt to be important despite the fact that the two main genes for NM, *NEB* and *ACTA1,* account for the majority of cases. This will allow for the most inclusive clinical trial designs in the setting of potential therapeutic treatment trials in the future which may include precision approaches to even ultrarare genotypes.

Reliable confirmation of the diagnosis of NM was deemed to be essential for inclusion in the NHS. A confirmed genetic diagnosis with disease-causing variants in one of the recognized NM-associated genes in a patient with a compatible phenotype was deemd to be acceptable even in the absence of a confirmed histological diagnosis of NM. Given the earlier use of next-generation sequencing, a genetic diagnosis is currently often achieved in early-onset patients prior to the use of histological diagnosis. Muscle biopsy would be considered an additional benefit, if collected retrospectively, but would not be deemed mandatory. Given the challenge of diagnosis with certain disease-causing variants being questionable as causes of NM, it was also determined that a panel of experts (or Adjudication Committee) would review the indeterminate cases. However, patients with a confirmed histological diagnosis in the setting of a compatible phenotype but insufficient genetic testing will still be included with the primary goal of establishing a genetic diagnosis in known or new genes.

Subsequent to the review of the NHS design in the European countries, Carsten Bönnemann (USA) and Alan

Beggs (USA) discussed a similar NHS design, along with clinical trial perspectives, and a proposed model for the USA and Canada. Using the concept of a network of national multidisciplinary clinics supported by the Muscular Dystrophy Association (MDA) care centre network in the USA and the Canadian paediatric Neuromuscular Group (CPNG) in Canada, these sites plan to use this network across North America to serve as the central architecture for the identification, diagnosis and recruitment of patients with NM into NHS. The overarching goal is to establish a clinical research network for NM and to use this network to define disease natural history and clinical outcome measures, and to thus enable clinical trial readiness. Inclusion criteria for these sites will be consistent, allowing for any patient with a confirmed diagnosis of NM, including all genotypes, to participate. Participants will be required to have either a muscle biopsy consistent with NM, or (a) confirmed disease-causing variant(s) in one of the established genes.

Detailed outcome measures for the NHS, as agreed upon by the committee, including motor, bulbar, respiratory, patient-reported, and non-clinical outcome measures, will be detailed in the final section.

3. Additional considerations for clinical trial readiness

Melanie Annoussamy (France) discussed the regulatory aspects of international non-therapeutic studies and shared the experience of the Institute of Myology in Paris, who had the opportunity to work with external sponsors for different European NHS in rare diseases: centronuclear myopathy (X-linked myotubular myopathy) [10], spinal muscular atrophy [9,11,12] and Duchenne muscular dystrophy [13,14]. These sponsored situations were discussed with the group and their (positive) experiences were shared. Overall, it was concluded that having a single sponsor appears to be the most effective option, allowing for effective and efficient coordination across countries with the use of a homogenous, well-detailed protocol, institution of common procedures, synchronized training and identical data quality control between centres.

Teresinha Evangelista (UK) presented the role of registries as a complementary approach to NHS, specifically in the case of rare disease research. She also discussed how to use the Joint Research Centre for rare diseases (JRC RD) platform and a brief overview of the TREAT-NMD model.

Rare disease, with its small, highly heterogeneous populations and scarce, at times incomplete data, must innovate to overcome the challenges faced during drug development. Therapeutic development requires a comprehensive understanding of the disease mechanisms and natural progression. NHS are, by definition, focused on the understanding of the disease phases, from pre-symptomatic through subsequent clinical stages. NHS support the development of multiple therapeutic approaches and are most useful when completed before clinical development starts. Unlike registries, NHS capture information in a very granular way and concern smaller populations.

In contrast, registries are organised systems that use non-interventional methods for collecting, storing, analysing and disseminating information on a disease population. Registries can be of use across all drug development stages and may have broad purposes, such as collection of disease information, recruitment of patients for clinical trials, monitoring of patient care and outcomes, to advance research hypotheses, observe patient behaviour patterns, establish disease-specific standards of care, or to support the regulatory evaluation of drugs.

Although there are several types of registry designs, they all should aim at following the FAIR principles of data collection (data should be findable, accessible, interoperable, and reusable). Interoperability is key when developing a registry. Registries should ensure an exhaustive enrolment of patients and avoid selection bias. Definitions should be standardised across different registries. There should be an accurate recording of dates of important events. It is strongly advised that a list of a core data set is used across registries for the same disease, together with the use of common ontologies for clinical terms and genetic annotation.

The JRC RD has put in place a series of tools to help the field of rare diseases (RD) to cope with the enormous fragmentation of RD data contained in patient registries across Europe. These are tools to allow interoperability for RDs data exchanges and they promote EU-level standards for data collection (https://eu-rd-platform.jrc.ec.europa.eu/erdri-description). Some useful tools to set up a registry can also be found on the TREAT-NMD website (https://treat-nmd.org/).

At present, there is a tendency to move from the traditional registries towards developing longitudinal, observational, prospective registries. These registries make use of automated data extraction from electronic health records and have some advantages over traditional registries: they are less labour-intensive for data entry, the cost of the registry maintenance is lower, and they require less manpower for maintenance and organisation. In the neuromuscular field, there is an example of such a registry in the SMArtCARE platform, that collects real-life outcome data of patients with spinal muscular atrophy [15].

4. Proposed study in nemaline myopathy

Eva Michael (Sweden) discussed the planning of a Swedish prospective, double-blind, randomised, placebo-controlled interventional study using salbutamol in patients with congenital myopathy. The trial, known as COMPIS, (Congenital Myopathy Intervention Study), proposes the hypothesis that daily oral salbutamol can increase muscle function and strength in patients with congenital myopathy. Due to the high costs of this optimal clinical trial design, as well as the limited patient population with specific subsets of various congenital myopathies in Sweden, the group does not currently have an adequate number of patients to perform sub-analysis on various subtypes of congenital myopathy, therefore the group presented their protocol design to the

ENMC committee in order to receive feedback, generate a discussion about the study and see if there is possibility for international collaboration. Feedback from the discussion generated about this protocol determined that a smaller, more effective clinical trial design utilising only Swedish patients would be recommended. An alternate efficacious design to consider would be an open-label, randomised, single-blinded, 6-month cross-over study. This trial design would facilitate lower overhead costs, initially, and would enable pilot data that could potentially lead to a larger, more expansive study on the efficacy of salbutamol for congenital myopathies in the future.

5. Clinical outcome measures

Participants of the workshop then discussed a variety of outcome measures to be considered for the nemaline myopathies.

Nicole Voermans (The Netherlands) presented her experiences of NM in adult patients. First, she showed that congenital myopathies are not only a paediatric topic [16,17]. A recent study in 44 adult patients with congenital myopathies showed that 26 of them had an onset in adulthood (median age of onset 47 years). Furthermore, 23 of the 44 patients had previously received other diagnoses, most commonly non-neurological disorders [16]. NM type 6 (NEM6) is the most prevalent myopathy in the Netherlands, with a founder disease causing variant in *KBTBD13* (c.1222C > *T* (p.Arg408Cys) causing an autosomal dominant NM with a peculiar slowness of muscles. The onset is mostly in childhood, but patients generally do not seek medical attention until adolescence or adulthood if the diagnosis is not known in the family yet [18–21]. A subset of NEM6 patients develop a cardiomyopathy (Voermans, manuscript in preparation). Currently, Voermans and Doorduin are investigating the clinical features and in particular respiratory function in all types of NM. Patients with reduced respiratory function are invited for respiratory muscle training.

Dr. Voermans subsequently discussed selected outcome measures that could be used in adults in addition to the outcome measures intended for paediatric populations. Transcranial magnetic stimulation (TMS) of the motor cortex can induce muscle relaxation by abruptly inhibiting corticospinal drive. Thus, the maximal rate of muscle relaxation can be assessed to detect delayed muscle relaxation, which is experienced as muscle slowness in NEM6 patients [22]. Next, she discussed results of some of the studies on experienced fatigue in adults with various neuromuscular disorders. Fatigue is not directly caused by muscle weakness, but related to the reduction of physical activity, and to pain and sleep disturbances [23]. Questionnaires on fatigue (such as Checklist individual strength – subscale fatigue) should therefore preferably be used in combination with assessment of physical activity (by actimeter) and quality of life (e.g. RAND 36) [24]. Finally, a questionnaire on work and social participation should be included for adult patients, e.g. USER-P [25].

To enable measurement of changes in the target tissue muscle in the setting of a clinical trial, two techniques were presented. First, MRI- or ultrasound-guided biopsy allows choice of biopsy site during the procedure, which may reduce sampling error in patients with non-homogenous involvement of the biopsied muscle [26]. Secondly, muscle ultrasound (US) and quantitative MRI are important biomarkers in other neuromuscular disorders [9,14]. A recent cross-sectional study in facio-scapulo-humeral dystrophy has shown that quantitative muscle MRI and US are both promising imaging biomarkers for differentiating between degrees of structural muscle changes. US is more sensitive to detect subtle structural changes, whereas MRI is more accurate in end-stage muscles and detecting oedema. As such, these techniques are complementary and the choice of a particular technique should be based on trial design [27].

Sarah Neuhaus (USA) presented on clinical motor outcomes in early onset paediatric neuromuscular disease and shared the experience of the clinical trials from the National Institutes of Health (NIH), with respect to motor outcomes in patients of various age groups, more specifically the paediatric populations 0–5 years and then greater than 5 years of age. Direct strength measurement methods were discussed, such as quantitative muscular assessment, handheld dynamometry and manual muscle testing, however these methods are not preferred in the paediatric population. Functional motor assessments are preferred and can be utilized depending on the age of the patient as well as the degree of motor impairment. The following scales were discussed and reviewed, including the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), Bayley Scales of Infant Development-III, Peabody Developmental Motor Scales 2, Motor Function Measure (MFM20 and MFM32), Hammersmith Functional Motor Scale, North Star Ambulatory Assessment, and the 6 Min. vs. 2 Min. Walk Test. Several graded and timed functional tests were also reviewed, including the stand from supine and the 10 m. run tests. The concept of integrating wearable devices or inertial movement sensors was also briefly reviewed, as these would be ideal devices, once validated, for non-ambulant patients, and also for very young paediatric patients, under 2–3 years of age, who developmentally cannot follow specific instructions for functional motor testing.

The experience from the NIH over the last several years of collecting natural history data on young patients with neuromuscular disease would agree that the following assessments are recommended in the paediatric population for various age groups: in the 0–2-year-old group, the CHOP-INTEND is recommended; however, if the CHOP-INTEND has a score greater than 50, it is recommended to use the Bayley Scales of Infant Development III - Motor sub-section. For children aged 2–5 years of age, it is recommended to use the MFM20, and for children 5–18 years of age, it is recommended to use the MFM32. Lastly, the NIH experience has also shown that integration of pulmonary function testing (PFTs) performed in both an upright and supine position is a testing modality that is incredibly informative. PFTs

performed in both positions has allowed the clinical team to identify diaphragmatic weakness that is, perhaps, out of proportion to a patient's appendicular weakness. This finding of diaphragmatic weakness is a particularly relevant and clinically actionable finding, often leading to the initiation of respiratory support by non-invasive ventilation.

In addition to traditional motor outcome measures, Laurent Servais (UK & Belgium) recapitulated some innovative outcome measures which were recently developed at the Institute of Myology. Grip and pinch strength can be reliably measured in Duchenne muscular dystrophy [28], in spinal muscular atrophy [9] and myotubular myopathy [10] and has been demonstrated to be sensitive to change in spinal muscular atrophy [12] and in Duchenne muscular dystrophy [13,14]. Recently, the European Medical Agency qualified as a valid secondary outcome in Duchenne muscular dystrophy the 95th centile stride velocity [29,30], as measured with a wearable magneto-inertial sensor [31,32]. This new outcome measure has the potential to shorten clinical trials and to considerably decrease the number of patients required, in comparison with other more traditional outcome measures. Laurent Servais presented ample data to illustrate the sensitivity to change in non-ambulant patients in several conditions, such as spinal muscular atrophy and myotubular myopathy. In these patients, the sensors are worn on the ankle and the wheelchair, and aims to quantify every single active upper limb movement to extract a single meaningful, robust and sensitive-to-change outcome. For this aim, the proper choice and calibration of the sensors is of outstanding importance.

Following this discussion, Francina Munell (Spain) reviewed patient-reported outcome measures, known as PROMs. It is noted that improved design of clinical trials, including PROMs, can help ensure high-quality data. Questionnaires utilized in the clinical trial or natural history setting should take care to reflect patient and career perception about the efficacy of the specific intervention, considering that quality-of-life measures *do not* always correlate with changes in disease progression. PROMs should include quality-of-life questionnaires and specific evaluation of symptoms that are not properly reflected in clinician-reported outcome measures.

Considering that NM is a rare disease with wide clinical heterogeneity (clinical signs, age of presentation, evolution, severity), outcome measures should be selected according to the type of intervention (e.g. generic or specific approaches, such as fatigue or dysphagia). Patient-reported quality of life, fatigue, and pain questionnaires are considered to be relevant and important to the patient population as well.

A NHS should include health-related quality-of-life (HRQoL) measures. The paediatric Quality of Life Inventory (Generic and Neuromuscular modules) has been validated in other neuromuscular diseases such as spinal muscular atrophy (Iannacone) and translated into several languages, specifically, the PedsQLTM (Neuromuscular Module) integrated generic and disease specific measurements. The PedsQL could be a valid instrument to measure PROMs in the paediatric population of the NHS [33]. For adult patients, there are several validated scales [34-36].

Cristiane Moreno (Brazil) then reviewed the clinical bulbar outcome measures most feasible to consider in the assessment of children and adults with NM. As has been established, NM has clinical features which are very heterogeneous. Clinical presentations range from severe neonatal forms to a mild childhood-onset phenotype [37]. Most of the patients have the "typical" form characterized by early onset muscle weakness, delayed milestones and dysmorphic facial features. The patients usually develop with motor gain, however, scoliosis, respiratory and bulbar involvement commonly appear in the first decade [3]. Bulbar weakness is found ubiquitously throughout all disease spectrums; however, the precise prevalence is underestimated due to the lack of standardized evaluation tools, especially in children [38]. A previous NHS found that 52% of the *ACTA1* and 57% of the *NEB* patients presented bulbar weakness [5], highlighting how frequent and important this feature is across this patient population.

The formal evaluation of bulbar weakness is challenging. Early signs such as weak cough, failure to thrive, difficulty to gain weight and long meal duration are often discreet and can be masked by other symptoms [38]. There is no validated clinical assessment for evaluating bulbar weakness in congenital myopathies, but tests validated for other neuromuscular diseases like the SLURP test 39], the Sidney Swallowing Questionnaire (SSQ), [40] and the Neuromuscular Disease Swallowing Status Scale (NdSSS) [41] may be helpful in assessing patients with NM as well. The videofluoroscopic swallow study (VFSS) is considered the gold standard test for the detection of dysphagia; however, the use of radiation, the risk of aspiration and lack of standard protocols are limiting factors for its use in young children [38,42].

Jonne Doorduin (The Netherlands) presented on the clinical respiratory outcome measures most applicable for the assessment of patients with NM. Respiratory muscle weakness is a clinically important aspect of NM [43]; therefore, outcome measures that quantify respiratory muscle function are essential to report in an NHS of NM. Pulmonary function tests, like (slow) vital capacity (seated and supine), forced vital capacity, forced expiratory volume (in 1 s) and peak cough flow can be used to assess the physiological impact of respiratory muscle weakness. However, respiratory muscles have large reserves of strength that are seldom required, so considerable weakness may occur before it is reflected in decreased pulmonary function [44]. Measurement of static maximal inspiratory (P_{Imax}) and expiratory pressures (P_{Emax}) at the mouth and sniff nasal inspiratory pressure (SNIP) can be used to evaluate global respiratory muscle function [45]. Pulmonary function tests, P_{Imax} and P_{Emax} require good patient cooperation and are not reliable in patients aged < 6 years. SNIP has been demonstrated to be useful for children > 2 years [46]. Diaphragm ultrasound

is a simple, non-invasive and accurate technique to evaluate specific diaphragm function [47]. It can be used to measure thickness, thickening and displacement of the diaphragm. Measurement of twitch-mouth or transdiaphragmatic pressure following magnetic phrenic nerve stimulation is the gold standard to quantify the pressure generating capacity of the diaphragm but is a rather invasive technique. Both diaphragm ultrasound and magnetic phrenic nerve stimulation require specific expertise and equipment that are not always readily available in every clinical setting and thus less suitable for an international NHS effort.

6. Biomarker and imaging outcome measures

Biomarkers in NM were next discussed and presented by Andreas Roos (Germany). The identification and definition of biomarkers is an important aspect of translational biomedical research and has become a valuable tool in the stratification of patients with a range of diseases, including neuromuscular disorders. In a recent study involving patients with NM, muscle biopsy specimens derived from eleven patients were biochemically studied by proteomic profiling and Coherent anti-Stokes Raman microscopy to identify marker proteins, enabling better stratification of patients with NM caused by dominant disease-causing variants in *ACTA1*.

Given that *ACTA1*-related NM encompasses a wide range of severity, the patients included in this study were categorized into three clinical subgroups according to their current ventilation status. After careful re-evaluation of the progression of the disorder (clinical follow-up to 15 years), those subgroups were defined as “mild” (no ventilation required), “moderate” (8–10 h of ventilation per day by full-face masque) and “severe” (24 h ventilation by tracheostomy).

Proteomic studies enabled the identification of potential marker proteins allowing the stratification of patients belonging to the “mild” and “severe” clinical groups based on changes in abundances of proteins involved in cytoskeleton maintenance as well as in the control of cellular stress responses. Immunological studies of paradigmatic proteins in patient-derived biopsies confirmed the proteomic findings. The CARS microscopic findings focused on characterisation of the nemaline bodies; the results of these studies revealed that nemaline bodies consist not only of accumulated proteins, but also of lipids in varying ratios.

An additional discussion regarding the use of muscle imaging in NM was presented by Giorgio Tasca (Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy). He presented on the use of muscle MRI as a diagnostic tool, i.e. as an imaging modality able to identify patterns of involvement unique to NM, as well as potential applications for MRI in NHS, namely as a possible imaging technique to provide outcome measures related to progression in NM.

Early MRI studies in patients with NM [48] showed a different pattern of involvement in *ACTA1*- compared with *NEB*-mutated patients; the involvement is noted to be more proximal in *ACTA1*, in particular at the level of the thigh (sartorius) and pelvis (gluteus maximus) [2], while involvement is more distal in *NEB*-mutated patients. Involvement of tibialis anterior, together with tongue and lateral pterygoid muscles, as shown by whole-body imaging, is common in both genetic forms of the disorder, whilst soleus is often affected in *NEB* cases and gastrocnemii are consistently spared in *ACTA1* [49]. In addition, in *NEB*-mutated patients, the pattern seems consistent irrespective of phenotype (mild, severe, purely distal presentation) and histopathology (rods, cores and rods, absence of rods). Interestingly, both patterns are different from what has been described in other NM, with particular regard to *TPM2* [50,51].

However, not enough data is yet available to ascertain whether MRI can be helpful to derive valid outcome measures or biomarkers in these disorders. The possible parameters quantitatively assessed by MRI could include muscle volume, which would be of particular interest if the interventional drug is supposed to have an anabolic effect, fat fraction and water T2. However, despite scattered evidence of increased fat fraction and mildly increased water T2 in congenital myopathies compared with controls [49], systematic cross-sectional and longitudinal studies are currently missing and would be advisable to address this issue.

7. Patient perspectives

The patient advocates Charles Park and Stephanie Colquhoun outlined and shared the views of patient advocacy groups and individual patients with NM, encouraging clinicians and scientists to strike an important balance between the quest for scientific enquiry and the emotional needs of the patients. They reiterated the importance of considering both the physical, as well as the mental, emotional and social aspects of patients with NM, and reminded the committee to keep at the forefront, when designing NHS and other clinical trials moving forward, the importance of precise and early genetic diagnoses, the progressive disease course and cumulative disease burden over time, as well as the definition of clinically meaningful change.

8. Conclusion: towards an international natural history study

Laurent Servais, Ulrike Schara, Carsten Bönnemann and Alan Beggs once again reviewed the specific criteria agreed upon by the committee for the design of a NHS for both paediatric and adult patients with NM across various sites in Europe, as well as Brazil, the USA and Canada.

After rigorous discussion on inclusion/ exclusion criteria, upon conclusion, it was deemed important to include all genetic disease-causing variants known to cause clinical and/or histopathological NM, in addition to the main two genes: *NEB* and *ACTA1*. Although it is perhaps pragmatic to limit a large-scale NHS effort to a more specific subset of this rare genetic disease, it was felt that reducing the

Table 2
Proposed outcomes for the different age and functional groups.

Age	Ambulant	Non-Ambulant	Frequency of Assessment
0–2y	PT/Motor: CHOP-INTEND If CHOP>50 = Bayley Motor HINE2	PT/Motor: CHOP-INTEND If CHOP>50 = Bayley Motor HINE2	Every 6 months
2–5y	PT/Motor: MFM20 Respiratory: SNIP Time on/off vent # respiratory infections Hospital stay # (URI)	PT/Motor: CHOP-INTEND MFM20 Respiratory: SNIP Time on/off vent # respiratory infections Hospital stay # (URI)	Every 6 months
5–18y	PT/Motor: MFM32 2MWT Timed Gowers Timed 10 m walk Timed stair climb Myo-grip Myo-pinch Respiratory: Peak cough flow Spirometry seat and supine (FVC, FEV1, PEF), MIP/MEP SNIP Time on/off vent	PT/Motor: MFM32 Myo-grip Myo-pinch Respiratory: Peak cough flow Spirometry seat and supineMIP/MEP SNIP Time on/off vent	Every 6 months
18+*y*	PT/Motor: 5MWT Timed Gowers Timed 10 m walk Timed stair climb Myo-grip Myo-pinch Respiratory: Peak cough flow Spirometry seat and supineMIP/MEP SNIP Time on/off vent	PT/Motor: Myo-grip Myo-pinch Respiratory: Peak cough flow Spirometry seat and supineMIP/MEP SNIP Time on/off vent	Every 6 months

pool of eligible patients would disadvantage those with rare genetic disease-causing variants, inadvertently excluding them from future treatment trials. It is possible that new therapeutic developments could occur in the next few years, perhaps even techniques that are not feasible today, and for this reason, it is important that the design of the NHS maintain an inclusive and diverse patient cohort, while simultaneously remaining mindful of the patient burden, in relation to visit frequency and volume of assessments.

Ulrike Schara discussed various funding mechanisms that could be utilized to support a NHS for NM. It was determined that optimal funding would be sought across different European sites on an individual country basis, rather than across multiple sites. Funding options include government grant funding, private foundation grant funding, patient advocacy group funding or partnering with an industry sponsor.

Regarding the specifics of the trial design, the committee came to a consensus regarding the following aspects for the structure of a NHS between the North American, South American and European sites. The data collection period would proceed for 3 years; however, the patients will be required to participate for a minimum of one year. Table 2

Issues that remain in discussion include specific biomarker processing (specific sample types to include, location of processing), database management, data monitoring and quality assurance. It was also concluded by the group that this NHS would require a lead physiotherapist who is able to travel across all sites and train other physiotherapists at alternate locations to perform assessments using a standardised technique to best harmonise the results obtained.

It is the expectation of this consortium that collecting this natural history and outcome measures data starting as soon as possible will allow future clinical trials in any of the NM subtypes with much greater ease and effectiveness. Its availability will also be helpful in enticing therapeutic developments to advance to the clinic.

The following key deliverables were achieved:

1. Constitution of an integrated network for clinical readiness for NM, comprising all the participants of this meeting and extended to other investigators who could not be present.
2. Development of an international registry to collect all data on a large cohort of patient with NM and by this, to address trial readiness.
3. Establishment of a natural history working group and outcome measures working group. These working groups will define which type of outcome measures can be adopted for evaluation in clinical trials and how to proceed to establish adequate outcome measures for various paediatric and adult patient populations.
4. Constitution of a group working on biochemical outcomes, including blood and urine biomarkers.

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