Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 25 (2015) 932–935

www.elsevier.com/locate/nmd

Personal Point of View

Challenges of clinical trial design for DMD

Valeria Ricotti [a,*], Francesco Muntoni [a], Thomas Voit [b]

[a] Dubowitz Neuromuscular Centre, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK
[b] NIHR Biomedical Research Centre, UCL Institute of Child Health and Great Ormond Street Hospital, London, UK

Received 30 September 2015; accepted 14 October 2015

1. The changing natural history of DMD

The natural history of Duchenne Muscular Dystrophy (DMD) is undoubtedly a shifting target, as shown by numerous recent studies [1–3]. And by "natural history" it is intended DMD-with-all-the-intervention-available-to-date, which are summarised and elucidated in the standard of care documents published by Bushby and colleagues [4,5]. The natural history of this disorder goes hand in hand with the on-going evolution of therapies; hence this phenomenon needs to be respected and factored into the design of clinical trials. Applying metrics based on old knowledge of the condition without taking into account new interventions, risks making clinical trial design poorly adapted or obsolete, and recruitment into studies is very challenging. For example, most ambulant patients are now on glucocorticoid therapy, and the age of starting steroids is indeed shifting towards a younger age as shown in the UK clinical practice [6]; hence, designing studies with steroid therapy as an exclusion criterion in the young ambulant population risks making recruitment virtually impossible in most specialised centres. This could in turn skew recruitment and introduce recruitment biases. Furthermore, cardiac medications used prophylactically (i.e. beta blockers and ACE-inhibitors) are increasingly supported by evidence that their use is beneficial for DMD patients [7–9], although a consensus on what is the optimal age for commencing therapy is still lacking. An on-going 5-year clinical trial funded by the British Heart Foundation testing ACE-inhibitor (perindopril) combined with beta-blocker therapy (bisoprolol) is targeting DMD boys 7–12 years of age who have not developed signs of cardiomyopathy (EudraCT number: 2007-005932-10). This study will likely strengthen the body of literature in support of early intervention [10] and indeed prevention; hence, clinical trial design will have to become permissive towards heart medication given prophylactically. However, at least in animal models, medications targeting angiotensin not only impact on cardiac load, but appear also beneficial for skeletal muscle [11] making it difficult to assess if such medications introduce a bias into skeletal muscle-based trial outcome. A similar scenario will apply to anti-oxidative treatment such as ibedenone; with the recently published study [12] supporting the beneficial effect on respiratory function it is expected that soon enough many patients will be treated with ibedenone alongside steroids, although the study results were obtained on steroid-naïve patients and it is unknown to what degree the two drugs are complementary.

2. Targeting the multisystem nature of DMD

While current efforts are mainly focused on improving the skeletal muscle function, and some also cardiac function, it is becoming increasingly clear that, the evolving therapeutic interventions for DMD should ideally also reflect the multisystem nature of this disorder. Whilst the most prominent symptomatology was on the forefront for early treatment (for example glucocorticoids for delaying loss of ambulation), it is now more evident that therapies will have to address all other components of the disease, such as the heart and the brain. This requires a shift in thinking: the target of experimental therapies should therefore not be solely the muscle but should ideally include other tissues and organs, such as smooth muscle, and the brain, also affected in this disorder. Such interventions are indeed under development, for example with novel chemistries such as the modified antisense oligonucleotides peptide conjugated morpholinos (PPMO) and the tricyclo-DNA, not only target efficiently skeletal and cardiac muscle but also the brain in animal models [13,14]. If proven to be beneficial in patients, these therapies have the potential of prolonging life span even further, as currently the most important cause of premature mortality in DMD is cardiomyopathy. Furthermore it is conceivable that therapies could also ameliorate the emotional/behavioural problems associated with disrupted dystrophin protein products in the brain. Indeed systemic

* Corresponding author. Dubowitz Neuromuscular Centre, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK. Tel.: +44 07565490633; fax: +44 02079052832.
E-mail address: v.ricotti@ucl.ac.uk (V. Ricotti).

http://dx.doi.org/10.1016/j.nmd.2015.10.007

administration of the tricyclo-DNA chemistry showed complete correction of behavioural features in the treated mdx mouse, yielding promising perspectives for future intervention also in humans; some of the most recently developed PPMOs also appear to be effective in crossing the blood brain barrier (M. Wood, F. Muntoni, M. Gait, personal observation). If proven to be beneficial in patients, these novel class therapies have the potential of not only prolonging life span even further, as currently a very important cause of premature mortality in DMD is cardiomyopathy, but could potentially also address some of the issues related to deficiency of dystrophin in the CNS.

3. The spectrum of phenotype within DMD

The current clinical trials and natural history studies have also brought to surface further heterogeneity in the DMD population. For example, when plotting a functional scale score such as the NorthStar Ambulatory Assessment versus age it becomes evident that the motor function varies even in a cohort of patients assessed and treated with uniform protocols [6,15,16]. A wide phenotypical spectrum is also a feature in other multisystem manifestations of DMD, such as the neuro-cognitive profile and is strongly influenced by the genotype [17–20] Similarly, response to treatment may vary from one subject to the other, including response to corticosteroid therapy [2]. With this in mind, in-depth zoom on the inter-individual variability also begged the question: what are the determinants of variable disease course and manifestation? It was described by recent publications [6,15,21] that different DMD mutations follow a variable course, showing that genotype bears some role in phenotypic expression. Even within cohorts amenable to exon skipping, those deletions skippable by exon 44 follow a milder course than those skippable by exons 51 or 53. Although these differences may only become evident after 2 years of observation, clinical trial design needs to account for such variability. Novel techniques of dystrophin quantification [22–25] have recently allowed quantifying accurately the resulting protein product also across laboratories [26]. This has facilitated the use of dystrophin quantification as a treatment-response-related biomarker in the setting of clinical trials [27–32]. Furthermore, they allowed understanding the role that even low residual protein expression may have in contributing to a less severe phenotype [33–35]. Recently described gene disease modifiers also play a role in prolonging ambulation [36,37] and influence treatment response to steroids [38]. However, these results may need to be reproduced and further explored in larger scale populations; the variability in genotype studied for these disease modifiers is so wide that their application in clinical trials with smaller cohorts to date is rather limited, as their specific role especially in different ethnicities still needs to be fully evaluated. Finally, in spite of the efforts made to standardise treatment approaches such as steroid therapy, different regimens are currently being used and therapy is introduced at different ages, impacting on the effect that steroids can have on the disease course during the ambulant phase [2] but possibly also after the age of loss of ambulation [39,40].

4. Impact of clinical trial design and outcome measures

With all these considerations in mind the design of clinical trials for DMD needs to take the dynamic variables of this condition into account. In such a panorama, biomarkers play a very important role. Biomarkers can be used to demonstrate proof of concept, as for example dystrophin quantification in dystrophin replacement therapies. Biomarkers can also be used to stratify patients, for example genetic disease modifiers (e.g. LTBP4, SPP1) [41] could guide stratification of less/more severely affected subjects, if cohorts are sufficiently large. In addition, imaging biomarkers, such as the assessment of fatty transformation of skeletal muscle by MRI and MRS can be implemented to explore the variability of the phenotype and in future to create more homogenous cohorts for clinical trials. Finally, these novel biomarkers can potentially be used to monitor treatment response. For-example, fragments of myomesin-3, a myofibrillar structural protein, which are measured at abnormal levels in the sera of DMD subjects, have been found to be sensitive to monitor response to therapy in *mdx* mice treated with morpholino AON [42]. Similarly, longitudinal data on serum matrix metalloproteinase-9 showed increased levels with age and disease progression in DMD patients [43]. In relation to the brain, monitoring of the bioelectrical response of the retina by ERG may be a convenient and non-invasive biomarker for monitoring the effects of CNS dystrophin restoration in retinal neurons [44]. This is also pertinent for AAV-based gene correction therapy because AAV9 has previously been shown to effectively transduce the retina after systemic injection [45].

A recent publication [46] reported improvement in skeletal muscle MR indices in Golden Retriever Muscular Dystrophy dogs injected with AVV-mediated U7snRNA-coupled antisense sequences promoting exon skipping. The injected limb after three months showed improvement in T2w intensity; ^{31}P NMS demonstrated a decrease in phosphodiester (PDE) signal and decrease in phosphocreatine (PCr) signal. The PDE/PCr ratio has been described to characterise muscle membrane metabolism and could potentially represent an effective biomarker of sarcolemma integrity [47]. Moreover, it was recently reported that the determination of the skeletal muscle fat fraction by MRI/MRS was able to detect the beneficial effects of steroid therapy in DMD subjects within 3 months of treatment [48].

MRI and MRS are currently included as exploratory endpoints in clinical trial protocols for Duchenne muscular dystrophy (www.skip-nmd.eu) and are able to monitor the relentlessly progressive fatty transformation, now documented for lower [49,50] and upper extremities [51,52], which can be used as a baseline ruler of the disease progression. It is foreseeable that in the future MRI/MRS may become a primary endpoint for clinical trials in DMD and other myopathies.

5. The use of poly-therapies and a place for registries

In neuromuscular disorders, specifically DMD, we are now most certainly entering an era when speeding-up of therapies is required. Clinical trial design needs to be adapted to the new disease course, and novel outcome measures need to be