Guideline on the clinical investigation of medicinal products for the treatment of Duchenne and Becker muscular dystrophy

Draft Agreed by CNS Working Party	January 2013
Adoption by CHMP for release for consultation	21 February 2013
Start of public consultation	1st March 2013
End of consultation (deadline for comments)	31 August 2013
Agreed by CNS Working Party and PDCO	October 2015
Adopted by CHMP	19 December 2015
Date for coming into effect	1 July 2016

Keywords	*Duchenne and Becker muscular dystrophy, paediatric population, genetic neuromuscular disorder, molecular diagnosis, motor function, muscle strength*

---

[1] Typographical errors corrected.

Guideline on medicinal products for the treatment of Duchenne and Becker muscular dystrophy

Table of contents

Executive summary

Recent advances in basic and clinical research have opened new perspectives for future therapeutic options in Duchenne and Becker muscular dystrophy (DBMD). The increasing number of clinical trials that recruit a rather small number of patients and the high degree of variability of disease progression for these disorders have raised a number of issues relating to the study design, including the choice of appropriate clinical efficacy endpoints, the definition of reliable surrogate outcome measures,[1, 2] the need for subgroup analyses within the heterogeneous patient population and the possibility of data extrapolation. These factors may compromise the outcome and conclusiveness of efficacy studies and are challenges that will be considered in the document.

Because of the chronic progressive nature of the disease that is accompanied by several comorbidities, and its poor prognosis with shortened life expectancy, special attention should be paid to the study duration (e.g. long-term treatment goals[3]), the maintenance of effect and long-term safety.

As Duchenne muscular dystrophy (DMD) has an onset in early childhood and substantially shortens life expectancy, while Becker muscular dystrophy (BMD) covers a broader age spectrum, specific difficulties have been identified regarding diagnostic criteria, age and stage of the disease related clinical relevance of efficacy[4] and different safety aspects.

This Guideline is intended to provide guidance for the evaluation of medicinal products in the treatment of DMD and BMD. It is acknowledged that for several aspects the present document cannot give very specific guidance due to the heterogeneity in phenotypes of both diseases and the expected treatment goals that may vary according to disease status and mechanism of action of different products. Published data on natural history of disease, validation of disease-related measurement tools and other aspects are still limited, but under increasing development. Therefore the purpose of the guideline is to provide guidance on some general principles in development of any medicinal product for DMD and BMD, but also to leave room for alternative approaches according to specific products in development. In addition to the general principles as outlined in this guideline, the specific development programme including the patient population, endpoints, and potential room for extrapolation, as well as any specific procedural and regulatory aspects could be discussed with regulators in the form of a scientific advice and a paediatric investigation plan.

The present document should be conceived as general guidance and should be read in conjunction with other relevant EMA and ICH guidelines (see section 3).

1. Background

Duchenne and Becker muscular dystrophies are rare diseases, DMD is life-threatening and shortens patient`s life substantially. DMD and BMD are recessive X-linked forms of muscular dystrophy. With respect to DMD patients, one out of 3500 – 6000 boys is born with this disease[5]. The figures for incidence in girls vary among publications, related to the milder and highly variable clinical presentation. However, presentation of symptoms of DMD in girls is rarer than in boys. Regarding BMD about 1 in 20,000 boys is affected[6].

Duchenne muscular dystrophy is characterised by progressive symmetrical muscular weakness that affects proximal muscles more than distal muscles, often accompanied by calf muscle pseudo-hypertrophy. Symptoms are usually present before five years of age and wheelchair dependency occurs before the age of 13 years. In about one third of the DMD patients there are cognitive and/or behavioural abnormalities. After 18 years almost all patients are affected by cardiomyopathy. Only a few DMD patients survive beyond the third decade; most die because of respiratory complications and heart failure due to cardiomyopathy[7].

Becker muscular dystrophy is characterised by a later onset and a milder clinical course. A substantial variability of clinical expression exists[6]. Patients remain ambulatory for a variable period of their life and the majority are not wheelchair dependant. Most patients develop at some point in time dilated cardiomyopathy that is the most common cause of death. Mean age of death is in the mid-60s and life expectancy of BMD patients is likely to improve further with improved cardiac surveillance.

In DMD patients the dystrophin protein is quantitatively deficient and non-functional, while in BMD patients it has some residual function. The dystrophin gene is mainly expressed in skeletal and heart muscle and in alternative forms in the brain. In the muscle cell dystrophin is part of the dystrophin glycoprotein complex (DGC) that serves to link the muscle fibre cytoskeleton to the cell membrane and further to the extracellular matrix. The loss of dystrophin function causes muscle fragility with muscle fibre loss followed by inefficient regeneration and subsequent progressive replacement of muscular mass with fibrotic and fatty tissue. The progressive damage of the skeletal muscles results in a decrease in muscle strength that starts from the lower extremities and gradually affects all muscles.

The underlying molecular pathogenesis of DMD consists of a variety of mutations in the dystrophin gene. These could be classified into three main categories: gene deletions (mostly in the "hot-spot" central part of the gene; exons 45-53; 60-80%), duplications (7-11%) and small mutations (10-30%) including nonsense mutations, splice-site mutations and small insertions/deletions that disrupt the reading frame[8].

Genetic testing has become more broadly accessible over recent years and is now a common part of the diagnostic process of DMD/BMD in treatment centres in the EU. Other supportive diagnostic methods include serum creatine kinase, muscle biopsy and imaging modalities. With respect to muscle biopsy, there are the typical dystrophic transformations with absence of dystrophin in DMD, while there is a variable decrease of dystrophin in BMD[6]. Due to the highly invasive nature of muscle biopsies, diagnosis of DMD and BMD is increasingly based on genetic testing rather than on qualitative assessment of dystrophin in muscle biopsy.

At present, treatment is almost exclusively symptomatic, including medical and physical therapies to improve cardiac and respiratory function as well as corticosteroids to improve skeletal muscle strength and function. The majority of patients are treated with steroids which have shown to prolong lifespan and delay the onset of cardiac and respiratory complications, as well as delaying the time to loss of ambulation. However corticosteroids are not formally approved for treatment of this disease and their use is often limited due to significant side effects. Moreover, there is a lack of consensus regarding the best treatment scheme, although recent evidences indicates that early and daily treatment is more effective than delayed or alternate day treatment schemes[9,10]. Recently, standard of care guidelines for DMD multi-disciplinary teams have been developed and were published in 2010[5,11]. Additionally therapies exist for orthopaedic corrections. With these interventions, patients are able to remain ambulant for a longer period of time and have a better life expectancy than in previous decades.

Currently no curative treatments for DBMD exist. However, recent advances in basic and clinical research have opened new perspectives for future therapeutic options in DBMD[12] and various potential therapeutic approaches are under development. Gene therapy consists of introducing a transgene coding for full-length or a truncated version of dystrophin complementary DNA (cDNA) in muscles, and pharmaceutical therapies include the use of chemical/biochemical substances to restore dystrophin expression (e.g. the stop codon read-through or exon skipping approach[13, 14, 15]) or through improvement of muscle growth or reduction of muscle damage (e.g. myostatin inhibitors, stem cells therapies) all aiming to alleviate the DMD phenotype[8].

2. Scope

The scope of the guideline is limited to the X-linked recessive dystrophinopathy Duchenne muscular dystrophy (DMD), the most common and severe form of muscular dystrophy, and its milder version, Becker muscular dystrophy (BMD). Other neuromuscular diseases are presently not within the scope of this guideline.

The presented guideline is prepared to provide guidance on general principles in the development of any medicinal product for the treatment in DMD and BMD (symptomatic, disease modifying, among others). General guidance is provided on identification of the target population (e.g. ambulant vs. non-ambulant children and adolescents), study design and choice of efficacy endpoints and safety parameters. Due to the current situation with increasing knowledge based on the ongoing clinical programmes for Duchenne muscular dystrophy specific recommendations in this guideline mostly refer to DMD but some of these might also be applicable for BMD.

3. Legal basis and relevant guidelines

This guideline has to be read in conjunction with the introduction and general principles and part of the Annex I to Directive 2001/83 as amended and relevant CHMP and ICH guidelines (please see "List of relevant guidelines" section at the end of the document), among them in particular:

- Note for Guidance on the Investigation of Drug Interactions - CPMP/EWP/560/95

- Points to consider on application with 1. Meta-analysis; 2. one pivotal study (CPMP/EWP/2330/99)

- Note for Guidance on Clinical Trials in Small Populations (CHMP/EWP/83561/2005)

- Note for Guidance on Clinical Investigation of medicinal products in the paediatric population (CPMP/ICH/2711/99 (ICH E11)

4. Specific considerations when developing products for the treatment of Duchenne and Becker muscular dystrophy

Treatment of DMD and BMD may have different treatment goals:

Improvement of symptoms and improvement of disability in affected patients:

At the present time treatment is mainly symptom-orientated including maintenance of muscle strength and function, prevention of respiratory and cardiac complications, orthopaedic corrections and physiotherapeutic interventions.

To some extent symptomatic treatment may be associated with improvement in disability, although it is not directly related to a delay in disease progression or disease modification. Therefore for regulatory purposes claims on symptomatic treatment and disease modification may require different types of evidence (see section 7.5.).

Modification of the natural course of the disease or prolonging survival:

The concept of disease modification in DMD/BMD is characterised by slowing down or stopping the accumulation and the progression of disability. This includes the delay of symptoms of weakness, or loss of function, in certain muscle groups, the delay of general loss of energy, as well as the delay in time to milestone events (e.g. time to wheelchair, time to loss of upper limb use, time to assisted ventilation). Clinically, a sustained effect on disability progression should be shown.

According to the mechanism of action of a potential medicinal product and the expected treatment goals the clinical development programme may vary with respect to the patient population included, endpoints and trial duration (please refer to section 7).

5. Patients characteristics and selection of patients

5.1. Diagnosis

Definitive diagnosis should be based on the clinical phenotype of DMD/BMD with characteristic clinical signs and symptoms (e.g. proximal muscle weakness, wadding gait and Gowers´ manoeuvre and progressive difficulty in walking), supported by serum CK levels and genetic testing confirming a mutation in the dystrophin gene. The exclusion of other neuromuscular disorders may involve electromyography and emerging imaging modalities (e.g. magnetic resonance spectroscopy); the latter being still in early stage of application.

In the majority of cases the genetic defect can be detected that makes the diagnosis definite. A baseline muscle biopsy as complementary source for the expression of dystrophin in myocytes (e.g. in cases of a possible intronic mutation or with intermediate phenotype), is recommended but not mandated. For patients without a confirmed genetic diagnosis, a combination of clinical symptoms, family history, elevated serum CK concentration, MRI and muscle biopsy is considered sufficient for a clinical diagnosis [6, 7]. However, for inclusion in clinical trials aimed to investigate potential medicinal products targeting certain type of genetic defects, genetic testing is needed.

5.2. Inclusion criteria

Patients to be included in clinical studies should have a confirmed diagnosis through genetic testing according to state of the art methods. This is particularly necessary for inclusion in mutation-specific therapy studies to ensure that only subjects with genetic defects which could be treated with the product are included. Genetic testing will also ensure that subjects with other forms of muscular diseases are not included in the studies which may compromise the homogeneity of the study population (in terms of diagnosis) and may also lead to possibly unnecessary exposure of patients to a drug which is not appropriate for their condition.

The substantial disease heterogeneity between patients with DMD and BMD (e.g. the underlying mutation, the dystrophin level and (residual) functionality, different age of onset, differences in severity) should be reflected in the product development programme. Due to differences in leading symptoms and consequently expected different treatment goals, DMD and BMD patients should be studied separately.

Depending on the objective of the study, different subgroups of patients with respect to the stage of the disease (ambulant and non-ambulant), functional status and developmental stage (e.g. child of pre-school age vs. schoolchild) should be selected a priori. In general, the patient population which is rather limited in numbers should cover a broad range, if possible; normally (but not in all cases) studies should start in older children with a step-down approach. If an effect in specific stages of the disease is expected according to the mechanism of action of the product (e.g. compound acting on fibrotic tissue in muscles), then an alternative approach may be considered. Extrapolation should be discussed in light of potential clinical benefit in other stages of disease, and also in light of potential concerns with regard to safety or dosing for different age groups.

If the main treatment target is improvement in motor function, patients in whom the evaluated motor function is preserved up to a pre-defined degree could be included into the same clinical studies. Since this may mean including patients of different stage of disease, e.g. ambulant and non-ambulant patients, stratification according to the stage of disease should be considered.

With due consideration of the progressive nature of the disease, different cut-off scores for an appropriate scale should be used to assure sensitivity to change. Thresholds for clinical severity of motor function impairment, respiratory and cardiac symptoms, associated cognitive deficits, as well as further relevant co-morbid symptoms should be defined a priori.

5.3. Exclusion criteria

- initiation of systemic corticosteroid therapy within 6 months or changes in dosing within 3 months prior screening (for efficacy assessment)

- any change in relevant concomitant therapies within 3 months prior to start of study treatment (for efficacy assessment)

- other neurological diseases or relevant somatic disorders that are not related to DMD/BMD, especially pre-existing pulmonary and cardiac disorders not attributed to DMD/BMD (consideration should be given to the use of a minimum standard of respiratory function as an inclusion criteria (e.g. FVC) to reduce the drop-out rate throughout the trial)

- lack of confirmed mutation in the dystrophin gene; subjects with another neuromuscular disease

- intake of other concurrent investigational medications

6. Methods to assess efficacy

6.1. Efficacy variables

The objectives of each study should be well defined according to the expected stage and functional status-related improvement in certain symptom domains, e.g. walking, daily functioning, maintaining ambulant stage, use of upper limb in non-ambulant subjects, time to assisted ventilation or survival.

Functional improvement (or at least delay of progression and deterioration) is considered as the most relevant outcome measure for patients affected by DMD and BMD. Improvement or stopping/delaying the deterioration in motor function could be achieved by different mechanisms of action such as correcting or counter-acting the underlying genetic defect to restore the expression of dystrophin, or by increasing muscle growth and regeneration, or by modulating inflammatory responses. Therapeutic approaches targeting increase of the dystrophin protein that are currently under development include gene or dystrophin protein replacement, dystrophin-splice-modulation therapy, specific drug treatment (e.g. the stop-codon read-through approach) and stem cell therapy.

The primary pathophysiological effect of DBMD is a decline in muscle strength and motor function and these are therefore important parameters to measure. Muscle strength and motor function are closely related but quite distinct motor system parameters. Many additional factors other than muscle strength may influence the ability to function (e.g. the patient`s ability to walk is affected by other factors than muscle strength alone)[16]. Although it is well known and recognised that there is no linear correlation between muscle strength and function in boys with DMD, for total evidence of clinical efficacy an effect on motor function should be supported by an effect on muscle strength. In addition, treatment effects on functionality should be backed up by effects in the activities of daily living (ADL).Similarly, a compound aiming at increasing or maintaining muscle mass should show in addition to efficacy on

muscle strength also an effect (or at least no detrimental effect) on function to evaluate the clinical relevance.

Two endpoints should be selected from the domains muscle strength (depending on the functional status and the compound tested) and motor function. According to the motor system parameter estimated to be particularly affected, one should be selected as primary endpoint and the other as secondary endpoint. Effects on the single selected primary endpoint should be supported by results from the most relevant secondary endpoints for consistency. Considering the relatively small number of patients in such studies in this context reference is also made to the Guideline for Small Populations. Depending on the treatment goals, e.g. in DMD-associated dilated cardiomyopathy effects on cardiac or respiratory function measured with relevant tools, could also be selected as relevant primary endpoints.

In reference to the estimated primary treatment goal, secondary outcome measures should include change from baseline in activities of daily living (ADL), respiratory and cardiac function, health-related quality of life and caregivers survey. Although physical dependence, especially in DMD, is ultimately to be expected, maintenance of ADL (e.g. using the computer and/or a smart phone, communication, eating, dressing, going to the toilet) is considered as important treatment goal[17]. The reduction of corticosteroid use is another potentially relevant outcome parameter but should be considered carefully, as the expected efficacy on the primary efficacy outcome could be compromised if corticosteroid-reduction adversely affects the effectiveness of the test treatment. However, due to the variability in clinical practices and the heterogeneity of the patient population in this respect, this may be assessed as an exploratory endpoint. The same applies to the measurement of effects on cognition.

The results should be discussed not only in terms of statistical significance but also in terms of clinical relevance. Presentation of the proportion of patients with a pre-specified degree of improvement /maintenance on a symptom score is valuable and a responder analyses should be presented for the primary and the most relevant secondary endpoints. A clinically relevant cut-off for these proposed endpoints has to be defined for each clinical study and there may be a number of ways to define a "responder"/"non-responder". The definitions of responders should be pre-specified in the protocol and should be clinically convincing and logical.

6.2. Efficacy measurements

There are several measurement tools that are used in assessing motor functioning and disability. These are reflected in muscle function testing that encompass e.g. measurement for upper and lower limb activity or walking speed (rather representing motor function on a lower level of muscular performances), as well as effects on ADL that more clearly represents the status of a certain muscle dysfunction, thus disability. However, further data are needed in order to establish how parameters such as quantitative muscle testing (QMT), forced vital capacity (FVC) or timed activities correlate with quality of life, time to death and other life-changing events (e.g. time to wheelchair). The perspectives of patients and carers should be taken into account in the development of new measurement tools.

From a regulatory point of view, no specific recommendation for the choice of measurement tools can be made. Information should be obtained both from a reliable informant (e.g. parent or carer) and from the affected subject. Although self-reporting in children may not always be reliable, the development of measurement tools in this respect is strongly encouraged. Measurement tools should establish different limits according to subject age and/ or stage–related phenotype of the disease. Co-morbid symptoms should be rated with proper scales.

Motor function:

Ambulation is a relevant milestone in DMD patients. In several DMD trials the 6-minute walk test (6MWT), originally developed as an assessment of cardiac and respiratory insufficiency, has been used in a modified version as an outcome measure [23, 24]. It has been validated in paediatric subjects above the age of 5 years; normative data are available. By measuring endurance and the ability to walk, the test measures walking parameters that are relevant only in the ambulant stage of DMD [21]. There are however some issues identified with using the 6MWT as an outcome measure, including a learning effect, inter- and intra-personal variability, the impact of age at baseline and the interference of a growth effect, as well as the lack of long-term data for assessment in case of loss of ambulation. These issues and the cut-off estimated to be clinically relevant according to the population studied should be addressed in the study protocol. If the 6MWT is selected as primary endpoint, results on efficacy should be supported by consistent favourable results on complementary meaningful clinical outcomes such as timed-function tests, pure motor function tests, muscle strength, disability, activities of daily living or cardio-pulmonary function testing.

For both ambulant and non-ambulant patients, the Motor Function Measure Scale (MFM) is a 32-item validated global scale for children from 6 years of age with neuromuscular disorders including DMD [18]. It offers a continuous assessment regardless of disease severity and ambulatory status [19], thus allowing the inclusion of children with a wide range of disease stages and facilitating long-term assessment. Three dimensions are assessed, D1: standing position and transfers, D2: axial and proximal motor function, D3: distal motor function. A short form, the MFM-20 (20 items only excluding those not applying in young children) is available and should be considered in children as young as two years of age [20].

Alternatively, for ambulant boys with DMD the disease-specific North Star Ambulatory Assessment (NSAA) that also includes timed items and the Hammersmith Motor Ability Scale (HMAS) [21, 22] can be used.

Other functional assessment grades are the Vignos´ lower limb score, the Brooke upper limb score and the GSGC (gait, stairs, Gowers, chair) assessment, which, among others, may be considered if adequately validated.

Timed-function tests to assess timed activities exist for climbing a short flight of steps, walking a short, predefined distance (usually 10 meters), rising from the floor, and sit-to-standing from a chair. Timed-function tests continue to be used clinically and in study programmes because they provide relevant information. There can be problems with assessors' error in timing (especially for very brief tests as sit-to-stand from a chair), as the observed value of any measures is equal to the true value plus the degree of random error or bias [16]. Due to typically huge baseline variability and small changes from baseline, the clinical relevance of results is often difficult to evaluate. However, by incorporating such activities into standardised functional rating scales (e.g. NSAA includes timed rise from floor and timed 10 meter run) and by using appropriate calibrated timing devices, it may be possible to increase confidence in the accuracy of these tests, if well substantiated.

Recently, also specific measures for non-ambulant DMD patients have been developed with the aim to provide data on clinically meaningful changes, for example the Performance of Upper Limb scale (PUL) [25]. Functional ability over the long-term can be measured with the validated composite Egen Klassifikation (EK) scale [26].

Most of these tools have their shortcomings related to the use of sum scores [27] in the context of inter- and intra-personal variability, the definition of a clinically relevant cut-off, and the difficulties in generating long-term data. Taking into account the heterogeneity in DMD and BMD, disease-specific scales and tools that cover a broader range of disease severity should be applied in parallel. It is also

recommended to use different assessment tools, e.g. a functional scale and a timed-function test, to sufficiently assess relevant changes in motor function (e.g. endurance) and to transfer results into clinical relevance.

Muscle strength:

Muscle strength should be evaluated using a validated tool. Options include quantitative muscle testing (QMT) scores such as hand-held-dynamometry (HHD) and manual muscle testing (MMT).

Both tools have their shortcomings. HHD is often a preferred measure as it provides quantitative parametric data, whereas MMT is a subjective measurement method that depends on the perception of the assessor. The clinical significance of HHD data may, however, be less obvious than that of MMT as the correlation of a value in Newtons or kilograms with a change in grade of muscle strength, or a change in functional ability is not clear[16].

New tools have been developed to assess upper limb strength in non-ambulant patients (e.g. pinch (MyoPinch), grip (MyoGrip) and wrist flexion and extension (MyoWrist) strength) [28], which may be considered if adequately validated.

Activities of daily living (ADL):

In the past, deficits of ADL were studied with the Functional Independence Measure (FIM)[29]. For wheelchair-dependent patients, the Barthel Index aims at quantifying the degree of functional assault for activities of daily living. Generally, the chosen tool should assess the age- and stage- related activities that are of most importance for the included patient population (e.g. climbing stairs, computer use, eating, bathing, clothing). Specifically designed actimeters (Actimyo) are currently developed for neuromuscular disease (NMD) patients and are currently used in several trials or natural history studies.

Survival and time to treatment failure:

Time to death or loss of a milestone (e.g. loss of ambulation) is relevant for drugs expected to delay disease progression. Alternatively, time to tracheostomy or permanent continuous ventilation is considered a relevant endpoint in advanced stages of disease. As studies with this endpoint would necessarily be of very long duration, unless only patients in advanced stages of disease are included, such assessments might be performed post approval. Decision criteria for tracheostomy and continuous ventilation should be pre-specified and unified for participating study sites, since these can vary among countries and regions.

Respiratory function:

All trials should include measurement of respiratory function as appropriate, especially in non-ambulant patients. As the main impact of the illness is the loss of lung volume, suitable parameters should be volume- related parameters. Measurement of forced vital capacity (FVC), vital capacity (VC), as restriction- related parameters may be suitable efficacy parameters to show an effect on pulmonary function and should be performed in accordance to current standards. As properties might change with patient`s age and disease stage, for younger patients expiratory flow (PEFR (peak expiratory flow rate)/FEV1 (forced expiratory volume in 1 second)) would be better while for older patients it might be lung volume.

It is acknowledged that pulmonary function tests are difficult to perform in non-ambulant patients and have poor reproducibility. Therefore only a small number of the most sensitive tests should be selected to provide relevant information without extreme burden for the patients participating in the study.

Cardiac monitoring:

Cardiac pathologies, e.g. secondary dilated cardiomyopathies, as well as change in cardiac function during the trial can be assessed using e.g. echocardiography and cardiac magnetic resonance imaging (CMRI). Assessment of left ventricular volumes, ejection fraction (LVEF) and dimensions should be complemented by ECG and measures of heart rate and blood pressure.

Quality of Life:

A disease specific module of the PedsQL (Pediatric quality of life inventory), the PedsQL 3.0 Neuromuscular Module (NMM) is available that could be administered together with the PedsQL 4.0 Generic Core Scales [4, 30]. Also selected domains of the Pediatric Outcomes Data Collection Instrument (PODCI) [31] are considered useful outcome measures in ambulatory DMD patients.

Cognitive impairment:

Cognitive deficits or behavioural problems are present in many DMD patients. Therefore, improvement in the cognitive or behavioural domains might be a relevant clinical outcome. Neuropsychological tests should be used to assess cognitive function and/or behavioural changes. However, as experience with neuropsychological tests in clinical trials for DBMD is limited their use should currently be considered exploratory.

Muscle composition and muscle damage:

Serum CK levels, muscle dystrophin expression[32] and reduction in inflammatory infiltrates still have limitations as surrogate efficacy measures. The currently existing methodologies to quantify dystrophin from muscle biopsies are debatable regarding the robustness and reproducibility of quantification of extremely low levels of dystrophin. Therefore, quantification of dystrophin protein from repeated muscle biopsies currently could only be considered as an exploratory endpoint for clinical efficacy. However, ethical aspects are likely to preclude repeated muscle biopsies for exploratory assessment. In cases where the mechanism of action of the therapy is related to the restoration of dystrophin expression, detection of dystrophin in muscle tissue could provide valuable supportive information as a pharmacodynamic marker for proof of concept.

At this stage, there are no suitable biomarkers that could serve as a primary or key secondary endpoint in phase III studies, but development of such biomarkers is strongly encouraged.

MRI measures of muscle T2 and lipid fraction as well as Nuclear Magnetic Resonance Imaging (NMRI) and Nuclear Magnetic Resonance Spectroscopy (NMRS) are under development as measurement tools for muscle dystrophy[33, 34, 35]. Their use as exploratory or secondary endpoints in clinical trials is encouraged for generating additional data. Whether and which MRI examinations could be used as surrogate endpoints for efficacy can be decided as part of a procedure for Qualification of Novel methodologies by the CHMP.

CK is not considered a useful parameter to follow disease progression given its inconsistency in the course of disease.

7. Strategy and design of clinical studies

7.1. Pharmacokinetics

The usual PK programme may be replaced by an adapted one according to the mode of action of the new compound e.g. applicability in healthy volunteers. If feasible, pharmacokinetic studies may start with adults for safety reasons, e.g. first experience. Based on extrapolation approach and PK/PD

modelling and simulation, these first exposure data would in principle allow a reduction in the number of children needed.

A Population-PK approach with sparse sampling using optimally sensitive methods is recommended in younger children. Based on adequate support by pre-clinical data and PK modelling and simulation, extrapolation of PK data across different age groups might be sufficient. However, if pharmacokinetic differences in children, adolescents as well as young adults are expected, investigation of the pharmacokinetic profile for each age cohort is needed.

7.2. Pharmacodynamics

The proposed mechanism of action of a new product should be described and discussed in relation to possible testing in available animal models. The widely used mdx mouse and the golden retriever muscular dystrophy dog are of value for pathology studies and for pre-clinical tests of therapeutics. Adoption of standard operating procedures and of controlled experimental settings is highly recommended to minimise the known limitations of both models and enhance predictability of data.

In addition, changes in biological parameters seen in patients or healthy volunteers (if appropriate) should be addressed. It also should be explored, whether the pharmacodynamic effect is similar in different stages of the disease (e.g. restoration of dystrophin in early and advanced disease).

The dystrophin protein (with truncated but functional variants) is accepted as surrogate marker for proof of concept studies in products aiming at inducing dystrophin synthesis. Biopsies should be minimised, but performed when necessary. The obtaining, storing, transport of muscle biopsies and the assessment of protein expression should be standardized and performed according to international standards.

7.3. Interactions

The note for guidance on drug interactions should be followed to investigate possible pharmacokinetic and pharmacodynamic interactions. Data on pharmacodynamic interactions with other treatments of the disease are important (in particular corticosteroids, cardiac and pulmonary medications).

If applicable, the Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007) and the Guideline on safety and efficacy follow-up-risk management of advanced therapy medicinal products (EMEA/149995/2008) should be followed as well.

7.4. Exploratory studies

Proof of concept and dose-finding for a new product should be established in a preferably homogeneous patient group without relevant co-morbidities.

7.5. Therapeutic confirmatory studies

Patient population

In confirmatory trials, the efficacy and safety of the product should be studied in the broad range of patients (e.g. with respect to comorbidities such as pulmonary diseases, or various manifestations of the disease) that the investigational product is intended to treat.

Characteristics of patients to be included in the studies may vary according to the mechanism of action of the product and its expected effect. This can differ according to the underlying mutation,

characteristics of abnormal dystrophin (if present e.g. in BMD), stage of disease and hence different treatment goals and measurement tools.

Separate studies are preferred according to the disease stage and/or the outcome parameters, or at least those groups should be studied in a single trial with pre-specified stratification of subgroups including sufficient number of patients to allow for comparison in the different disease stage groups. However, consistency over the subgroups would add to supportive evidence.

In studies for symptom or disability improvement, the patient population to be included should be characterised by clear symptoms that might improve. In contrast, the patient population for disease modifying therapies could also include phenotypic unobtrusive patients (with no or only few symptoms) which may be prone to deterioration.

7.5.1. **Short-term studies**

Study design

Confirmatory trials to show symptom or disability improvement should be randomised, double-blind, parallel-group controlled trials.

A time to event design where the event is defined as worsening on a functional or symptom scale or time to milestone event might be an option to show a disease modifying effect or survival increasing.

Choice of control group

In general at present, for a product with a new mechanism of action, the test product should be compared to placebo in a two way design as add-on to stable standard of care. If the study population includes both, patients treated and not treated with corticosteroids, stratification for corticosteroid treatment will be required.

The decision to include a placebo control will be influenced by the number of affected patients and the availability of some data from other compounds with the same mechanism of action (please refer also to section 7.7).

Generally, the use of historical controls is not considered an ideal approach due to a huge variability in patient populations, standard of care and co-medication during various times and treatment centres. The use of recent natural history cohorts[37] could be an option for small patient populations with a very rare genetic defect in which controlled studies are not feasible and if the use of such controls can provide an adequate comparison. However, it should be motivated what would generate the most evidence: a small placebo controlled trial with a type I error increased to reflect the rareness of the disease or a single arm trial with a justification that comparison with historical controls can be adequate.

Study duration

The duration of the studies should correspond to the mechanism of action of the investigational product and the intended treatment goal. Trials investigating symptomatic treatment should usually last 3 months. Trials to show an improvement of disability should be of at least 6 months duration; experience to date indicates that a longer duration of at least 1 year may be necessary to ensure separation from placebo and demonstration of efficacy on measures of disability.

Confirmatory studies with products intended to modify the course of the disease or to increase survival should be of sufficient duration to show a clear effect on disability progression, with all patients to be studied as much as possible, with a follow-up of at least one or two years recommended.

Methodological considerations

The population to be studied will consist of a substantially heterogeneous study population with respect to the stage of the disease, co-morbid symptoms, concomitant supportive care and corticosteroid treatment. Therefore, stratified randomisation for the most influential factors and stratified analyses for the other factors should be considered. In particular, the effect of the investigated product has to be clearly separated from effects received from concomitant medication (e.g. steroids, pulmonary or cardio-protective agents). To this end, baseline care should be unified as much as possible to prevent results from being confounded by variable supportive care such as clinical care, physiotherapy, orthopaedic, respiratory, psychosocial management of DBMD and cardiovascular medications. If appropriate, stratification could be considered according to background therapy. However, in all cases stratification according to corticosteroid use is requested.

The number of required patients that can reasonably be included in clinical studies will depend on the number of affected patients, but should primarily be based on the treatment effect that is considered and justified as clinically relevant.

Care should be taken to ensure that follow-up of patients is as complete as possible for as many patients as possible, even after discontinuation of treatment.

Some specific recommendations

To illustrate the above mentioned considerations a few examples are given below although the list is not exhaustive:

Clinical studies to demonstrate efficacy for a symptom improving agent could include patients with different stages of disease and should last 3 months. Primary endpoints should be selected from domains corresponding to the symptoms of relevance.

In ambulant boys clinical studies to demonstrate efficacy of a disease modifying agent (e.g. enhancing some level of functional dystrophin or delaying muscle dystrophy) the study duration is dependent on the sensitivity for the event of the population included. Primary endpoints should be focused on mobility; activities of daily living should be selected as important secondary endpoint.

Clinical studies to demonstrate efficacy of a disease modifying agent in non-ambulant patients (advanced stage of disease) should be of sufficient duration to detect a difference in the selected endpoints. Primary endpoints would be measurements of upper limb function or muscle strength. Again, ADL should be selected as important secondary endpoint. In more advanced disease stages the primary endpoint should derive from the domain of cardiac and/or pulmonary capacity and survival.

7.5.2. <u>Long-term studies</u>

Because of the chronic and progressive course of DMD/BMD, long-term effects on safety and efficacy (e.g. neutralisation of effect) need to be investigated. This may vary depending upon the investigated agents' profile. If considered necessary (e.g. for medical products intended for symptom improvement), long-term data collection may be warranted in the post-approval setting.

7.6. *Studies in special populations*

For DMD the paediatric population is considered to be the central target population as the disease has an onset during early childhood. BMD is characterised by a later onset. In this context adults (and rarely elderly) are considered a special population. The age of inclusion is in principle unlimited in adults, although elderly subjects are not expected to be available for clinical investigation.

Special ethical considerations and safety concerns in children have to be followed. Alternative strategies for dose-finding may be necessary in the youngest age group.

If certain subgroups are not studied (e.g. extremes of clinical severity) extrapolation should be justified in the dossier.

7.7. Extrapolation

In general, extrapolation is considered a challenge and needs adequate justification. The extent of extrapolation that might be accepted will depend on the demonstrated mechanism of action of the product and the efficacy data available.

The question of extrapolation concerns two different aspects: The first is the extrapolation of efficacy to various degrees of disease severity in a population with the same (group) gene defect (e.g. that can be corrected by the same exon skipping strategy). This also includes the extrapolation from older to younger patients, e.g. due to the lack of available clinical endpoints especially in the youngest children, or from ambulant to non-ambulant patients. Currently there is lack of information whether the efficacy and safety of a certain mechanistic approach is comparable within different stages of the disease or within different age groups. As therapies (e.g. exon skipping) that make use of dystrophin transcripts rely on muscle quality, these therapeutics might be expected to have more impact on muscle strength and function in younger children than in older children with DMD (i.e. due to earlier than more advanced stages of muscle dystrophy). Collection of specific data gathered in other ages are thus desirable. A further difficulty is that inclusion for example of children below 7 years of age in clinical studies is likely to be difficult after MAA approval based on efficacy data in older patients. Therefore, if supported by the mechanism of action, extrapolation from older to younger (or from younger to older) patients might be discussed in the context of additional real life data needed to be collected post-authorisation.

The second question of extrapolation is the extrapolation of efficacy results for products with the same mechanism of action but different molecules (e.g. exon skipping with antisense oligonucleotides targeting different regions of the gene and hence patient populations with different groups of mutations). These aspects are increasingly discussed but for any further consideration a CHMP scientific advice should be sought to discuss the most appropriate strategy for development.

With respect to differences in the underlying gene defect, differences in disease onset, the progressive course of the disease and different phenotype in DMD and BMD, it is impossible to extrapolate results of efficacy and safety from exploratory trials or benefit-risk evaluation from BMD (mainly adolescents/young adults) to DMD (mainly paediatric patients) or vice versa. Hence separate clinical programmes for both patient populations are considered mandatory unless a clear justification on a joint approach could be provided.

8. Clinical safety evaluation

8.1. General recommendations

In general the content of ICH E1 should be taken into consideration.

Identified adverse events (AE) should be characterised in relation to age, dose, duration of treatment and other relevant variables. Assessment of adverse events, especially those that could represent adverse reactions predicted by the pharmacodynamic properties of the investigational product, should

be performed using a systematic methodology. Appropriate laboratory tests and ECG recordings should be conducted.

8.2. Specific potential adverse reactions

Specific adverse effects related to off target effects of (gene) therapy should be monitored according to signals from the preclinical and early studies.

A major category of products developed or tested in DBMD are considered to target the primary pathophysiological defect by restoring expression of dystrophin. For antisense oligonucleotides, which alter the synthesis of a particular protein, special attention to accumulation of that protein should be given, including renal and hepatic effects. With respect to gene replacement therapy, special attention should be given to the occurrence of immunological side effects (e.g. serious infections and autoimmune disease).

Clinical exacerbation or deterioration could be expected if treatment is stopped. Due to the relatively long half-life of the dystrophin protein acute effects would not be expected. This should be anticipated and followed in studies accordingly.

Central Nervous System (CNS) effects:

Behavioural changes should be assessed if effects on CNS are expected.

Cardiovascular effects:

Special attention should be paid to cardiotoxicity, e.g. arrhythmias and conduction disorders. The need for ECG tracing before starting the investigational product should be addressed. Depending on the class of the investigated medicinal product it might be necessary to closely monitor cardiac safety in all patients. In patients with dilated cardiomyopathy, deterioration in cardiac function could be due to lack of efficacy on cardiac function (of the test treatment), to natural course of disease, or to an adverse effect. The distinction of these might be problematic.

Endocrinological effects

Special attention should be paid to weight gain and growth (retardation) in children. Distinction should be made between the effect of corticosteroid therapy and the test therapy.

Depending on the pharmacological properties of the new therapeutic agent, the investigation of neuroendocrinological parameters (e.g. delayed puberty) may be necessary over an adequate period of time.

Hepatic adverse reactions:

Special attention should be paid to hepatic safety; transaminases are known to be abnormal in patients with dystrophin mutations and muscular dystrophies in general, so liver toxicity due to treatment should be distinguished from this background elevation of liver enzymes.

8.3. Long-term safety

Since DMD is a chronic progressive disease with onset in early childhood, and lifelong treatment is anticipated, studies addressing the long-term safety of the therapeutic interventions should be carefully designed. Special attention should be drawn towards the effects on the developing brain and body (in particular the endocrine system and CNS). Careful consideration should also be given to AEs related to long-term exposure and accumulation of the test drug (in particular relevant for oligonucleotides) in parenchymal organs.

Long-term safety data can be generated in open extensions of short-term studies and/or by specific long-term trials. Studies should last for at least 12 months, and prospective follow-up for a longer period of time should be part of the Risk Management Plan (RMP) post-licensing. A registry is advised as part of the Risk Management Plan.

For substances that are already approved in another indication, extrapolation of parts of the safety data to the DBMD population could be considered.

Definitions

Exons: The portions of a gene which contain coding DNA sequences.

Introns: The parts of a gene containing non-coding DNA sequences. Adjacent exons are separated by introns, which are later removed from the RNA transcript via the splicing mechanism.

Splice-modulation: This procedure aims at correcting genetic defects by molecular manipulation of the pre-messenger RNA. This is mostly mediated by antisense oligonucleotides (AO) or other short complementary sequences. The aim is to modulate the pre-m RNA splicing which results in a different mRNA (with exclusion of one or more exons).

Exon skipping: A mechanism based on masking part of the pre-mRNA in such a way that the splicing machinery skips over one or more exons. As a result, mRNA lacking some exons is produced which codes for a shorter protein.

References

1 Wagner K.R.: Approaching a New Age in Duchenne Muscular Dystrophy Treatment. Neurotherapeutics 5: 583-591 (2008)

2 Mayhew J.E. et al.: Reliable surrogate outcome measures in multicenter clinical trials of Duchenne muscular dystrophy. Muscle & Nerve 35:36-42 (2007)

3 Aartsma-Rus A. et al.: Effects of long-term treatment and combination therapeutics for neuromuscular disorders. Neuromuscular Disorders 21, 151-156 (2011)

4 Mercuri E., Mazzone E.: Choosing the right clinical outcome measure: From the patient to the statistician and back. Neuromuscular Disorders 21, 16-19 (2011)

5 Bushby K. et al.: The diagnosis and management of Duchenne Muscular Dystrophy – Part 1 Lancet Neurology 9(1): 77-93: 2010

6 Burgunder J.-M. et al.: EFNS guidelines for the molecular diagnosis of neurogenetic disorders: motoneuron, peripheral nerve and muscle disorders. European Journal of Neurology 18:207-217 (2011)

7 Darras B.T. et al.: Dystrophinopathies. GeneReviews (Internet). Seattle (WA): University of Washington, Seattle; 1993-2000 Sep 05 (updated 2008 Mar 21).

8 Pichavant C. et al.: Current status of pharmaceutical and genetic therapetic approaches to treat DMD. Mol Ther. 2011 May; 19(5):830-40

9 Ricotti V., et al: Long-term benefits and adverse effects of intermittent versus daily glucocorticoids in boys with Duchenne muscular dystrophy. J Neurol Neurosurg Psychiatry 2013; 84:698-705

10 Ricotti V., et al: The NorthStar Ambulatory Assessment in Duchenne muscular dystrophy: considerations for the design of clinical trials. J Neurol Neurosurg Psychiatry 2015; 0:1-7

11 Bushby K. et al.: The diagnosis and management of Duchenne Muscular Dystrophy – Part 2 Lancet Neurology 9(2): 177-189: 2010

12 Ferrier A. et al.: New Directions in Biology and Disease of Skeletal Muscle, Meeting Report, 5-8 May 2010, Ottawa, Canada Neuromuscular Disorders 21, 157-159 (2011)

13 Mendell J.R.et al.: Eteplirsen for the treatment of Duchenne Muscular Dystrophy. Ann Neurol 2013 74: 637-647

14 Cirak S. et al.: Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. 2011, Lancet 378, 595-605

15 Järver,P. et al.: A Chemical View of Oligonucleotides for Exon Skipping and Related Drug Applications. Nucleic Acid Ther. 2014; 24, 37-47

16 Scott E., Mawson S.J.: Measurement in Duchenne muscular dystrophy: considerations in the development of a neuromuscular assessment tool. Neurology 2006, 48: 540-544

17 Fujiwara T. et al.: Activities of Daily Living (ADL) Structure of Patients with Duchenne Muscular Dystrophy, Including Adults. Keio L Med 58 (4) 223-226, December 2009

18 Bérard C. et al.: A motor function measure scale for neuromuscular diseases. Construction and validation study. Neuromuscul Disord. Jul;15(7):463-70 (2005)

19 Vuillerot C. et al.: Monitoring changes and predicting loss of ambulation in Duchenne muscular dystrophy with the Motor Function Measure. Dev Med Child Neurol 2009; 52:60-5

20 de Lattre C., et al: Motor function measure: Validation of a Short Form for Young Children With Neuromuscular Diseases. Archives of Physical Medicine and Rehabilitation; Volume 94, Issue 11, November 2013:   2218–2226

21 Mazzone E. et al.: North Star Ambulatory Assessment, 6-minute walk test and timed items in ambulant boys with Duchenne muscular dystrophy. Neuromuscular Disorders 20 (2010) 712-716

22 De,Sanctis R. et al.: Suitability of North Star Ambulatory Assessment in young boys with Duchenne muscular dystrophy. 2015, Neuromuscul. Disord. 25, 14-18

23 McDonald C.M. et al.: The 6-minute walk test as a new outcome measure in Duchenne muscular dystrophy. Muscle Nerve 41: 500-510; 2010

24 Voit,T. et al.:. Safety and efficacy of drisapersen for the treatment of Duchenne muscular dystrophy (DEMAND II): an exploratory, randomised, placebo-controlled phase 2 study. 2014, Lancet Neurol. 13, 987-996

25 Mayhew A. et al: Development of the Performance of the Upper Limb module for Duchenne muscular dystrophy. Dev Med Child Neurol. 2013 Nov: 55(11):1038-45

26 Steffensen B et al: Validity of the EK scale: a functional assessment of non-ambulatory individuals with Duchenne muscular dystrophy or spinal muscular atrophy. Physiother Res Int 2001; 6(3): 119-34

27 Merkies I.S.J.: Outcome measures in Duchenne muscular dystrophy: are we ready for the new therapeutic era? Neuromuscular Disorders 19 (2009) 447

28 Servais L. et al: Innovative methods to assess upper limb strength and function in non-ambulant Duchenne patients. Neuromuscular Disorders 2013 Feb; 23 (2):139-148

29 Uchikawa K. et al.: Functional status and muscle strength in people with duchenne muscular dystrophy living in the community. J Rehabil Med 2004; 36: 124-129

30 Davis S.E. et al.: The PedsQL in pediatric patients with Duchenne muscular dystrophy: feasibility, reliability, and validity of the Pediatric Quality of Life Inventory Neuromuscular Module and Generic Core Scales. J Clin Neuromuscul Dis. 2010 Mar; 11(3):97-109

31 McDonald C. et al.: Relationship Between Clinical Outcome Measures and Parent Proxy Reports of Health-related Quality of Life in Ambulatory Children with Duchenne Muscular Dystrophy. J Child Neurol. 2010 September; 25(9): 1130-1144

32 Aartsma-Rus A.: Dystrophin Analysis in Clinical Trials. 2014, Journal of Neuromuscular Diseases 1, 41-53

33 Forbes S.C. et al: Magnetic Resonance Imaging and Spectroscopy Assessment of Lower Extremity Skeletal Muscles in Boys with Duchenne Muscular Dystrophy: A Multicenter Cross Sectional Study. PLoS One. 2014 Sep 9,9(9)

34 Willcocks R.J. et al: Longitudinal measurements of MRI-T_2 in boys with Duchenne muscular dystrophy: effects of age and disease progression. Neuromuscul. Disord. 2014, 24, 393-401

35 Arpan,I., Willcoc et al.: Examination of effects of corticosteroids on skeletal muscles of boys with DMD using MRI and MRS. Neurology 2014, 83, 974-980

36 Muntoni F. et al.: Dystrophin and mutations: one gene, several proteins, multiple phenotypes. 2003, Lancet Neurol. 2, 731-740

37 Pane,M. et al.: Long Term Natural History Data in Ambulant Boys with Duchenne Muscular Dystrophy: 36-month changes. 2014, PLoS. One. 9, e108205

List of relevant guidelines

- Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95 (ICH E6))

- Note for Guidance on General Considerations for Clinical Trials (CPMP/ICH/291/95 (ICH E8))

- Dose-Response Information to Support Drug Registration (CPMP/ICH/378/95 (ICH E4))

- Pharmacokinetic studies in man – EudraLex vol. 3C C3A

- Statistical Principles for Clinical Trials (CPMP/ICH/363/96 (ICH E9))

- Choice of Control Group in Clinical Trials (CPMP/ICH/364/96 (ICH E10)

- Points to consider on adjustment for baseline covariates (CPMP/EWP/2863/99)

- Guideline on Missing Data in Confirmatory Clinical Trials (CPMP/EWP/1776/99)

- Points to consider on multiplicity issues in clinical trials (CPMP/EWP/908/99)

- Note for Guidance on Population Exposure: The Extent of Population Exposure to Assess Clinical Safety (CPMP/ICH/375/95 (ICH E1A)

- Ethical considerations for clinical trials on medicinal products conducted with the paediatric population, Recommendations of the ad hoc group for the development of implementing guidelines for Directive 2001/20/EC relating to good clinical practice in the conduct of clinical trials on medicinal products for human use (Final 2008)

- Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications (EMEA/CHMP/SWP/169215/2005)

- Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007)

- Guideline on safety and efficacy follow-up – risk management of advanced therapy medicinal products (EMEA/149995/2008)

List of Abbrevations

ADL: Activities of daily living

DMD: Duchenne muscular dystrophy

BMD: Becker muscular dystrophy

DBMD: Duchenne/Becker muscular dystrophy

CK: creatinine kinase

AEs: adverse events

FVC: Forced vital capacity