JUNE 2023

SAVOIR & COMPRENDRE
AVANCÉES DE LA RECHERCHE

ADVANCES in Duchenne muscular dystrophy and Becker muscular dystrophy

> Duchenne muscular dystrophy (DMD)
> Becker muscular dystrophy (BMD)

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are rare genetic diseases which affect skeletal and cardiac muscle. They primarily occur in males but can also occasionally affect females.
DMD manifests as progressive muscle weakness which begins during childhood, while BMD manifests as less severe muscle weakness which appears during childhood or adolescence, or even later in life.
This document, published to coincide with the AFM-Téléthon General Meeting 2023, presents DMD and BMD research news from the past year (treatments, a selection of ongoing studies and clinical trials, scientific and medical publications, etc.).
It can be downloaded from the AFM-Téléthon website where further information in the scientific, medical, psychological, social and technological fields relating to DMD and BMD can be found:

WEBSITE www.afm-telethon.fr

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Table of contents

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We would like to thank the AFM-Téléthon Groupe d'Intérêt Duchenne-Becker [Duchenne-Becker Special Interest Group] who helped review this document.

Duchenne muscular dystrophy and Becker muscular dystrophy

▪ Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are rare, "X-linked recessive", genetic diseases, meaning they primarily occur in males.
The majority of females who have an X chromosome that carries a *DMD* gene mutation have no symptoms, however, a small number have cardiac and/or muscular manifestations and may even develop actual Duchenne muscular dystrophy. This genetic mutation can be passed down to their children via the X chromosome, with a son being affected by the disease and a daughter a carrier.

*The **prevalence** of a disease is the number of people with that particular disease at a given time.*

▪ **Prevalence.** On average, DMD and BMD affect 4.8 and 1.6 people in every 100,000 respectively according to a meta-analysis based on 25 publications spanning from 1982 to March 2021 involving over 900 million people around the world.
Salari N et al. J Orthop Surg Res, 2022 Feb.

Dystrophin-coding gene mutations

The X chromosome carries the ***DMD* gene** which codes for a protein called **dystrophin.** DMD and BMD are caused by mutations in the dystrophin gene which lead to less or no dystrophin being produced. These diseases are known as dystrophinopathies.
▪ In Duchenne muscular dystrophy (DMD), no dystrophin is produced.
▪ In Becker muscular dystrophy (BMD), dystrophin is produced, but only a small amount, or is an abnormal size and only partially functional.

Little or no dystrophin weakens muscle cells

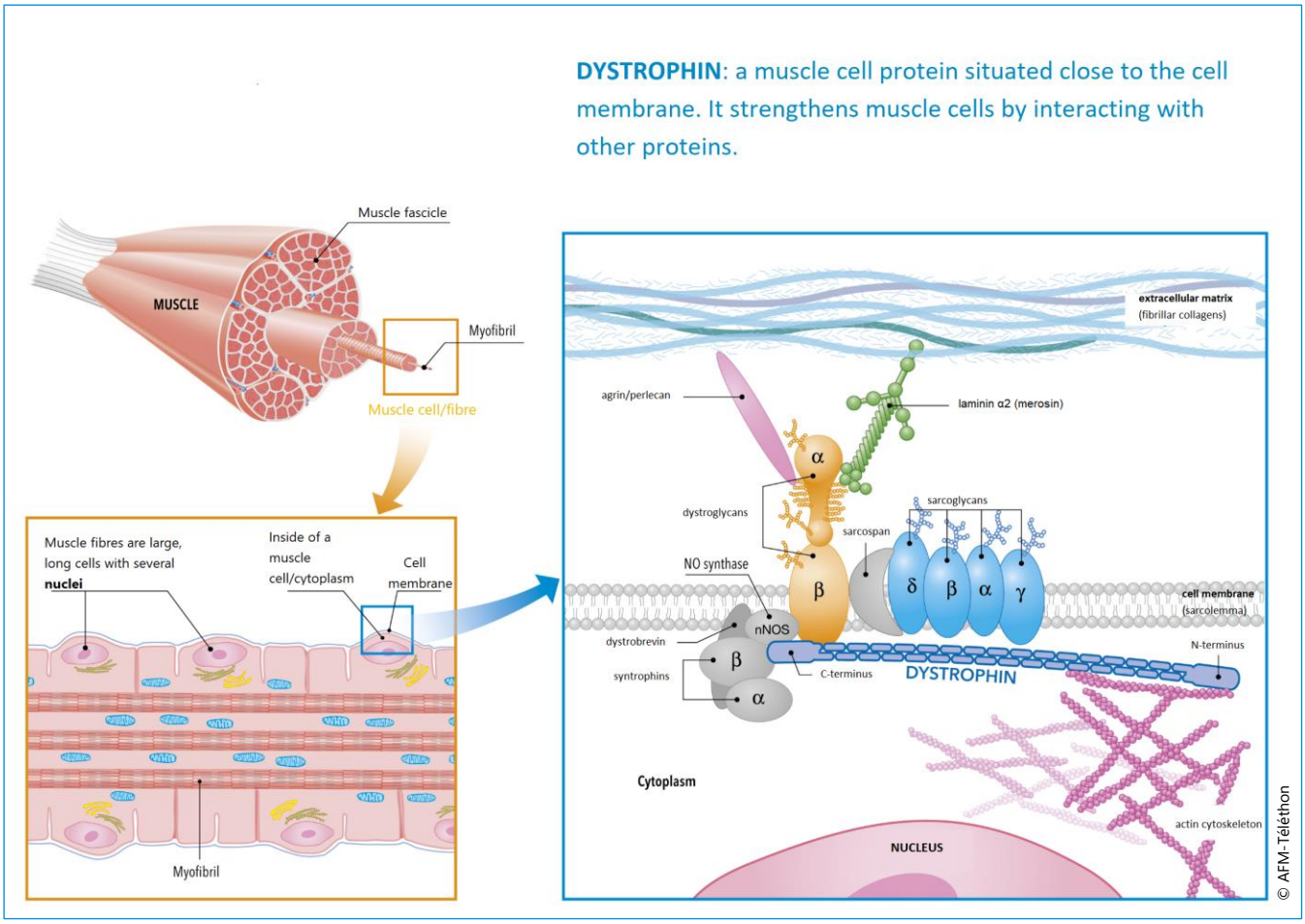
▪ Dystrophin is a structural protein found in muscle cells (also known as muscle fibres) which is localised to the cell membrane (sarcolemma). Several types ("isoforms") of dystrophin of various lengths are made by the same *DMD* gene.
- "Full-length" dystrophin (Dp427) is found in skeletal muscle, smooth muscle, the heart and the brain.
- The smallest isoforms are present in the retina (Dp260), kidneys (Dp140), brain (Dp140, Dp71 and Dp40) and muscles.

*The **extracellular matrix** is a complex network of proteins that bathes cells. It plays an essential role in the composition, maintenance, adherence, movement and regulation of these cells within an organ/tissue. The extracellular matrix of muscle (or myomatrix) is able to absorb the mechanical stress caused by the contraction of muscle fibres.*

▪ Dystrophin binds to the proteins of muscle cells which contributes to their mechanical resistance when the muscle contracts.
- At one end (called the C-terminus), it binds to the dystrophin-associated protein complex which is primarily made up of dystroglycans and sarcoglycans. This protein structure crosses the muscle fibre membrane and attaches to the extracellular matrix.
- At the other end (the N-terminus), it binds to actin, a cell cytoskeletal protein that forms a strong internal mesh.

__Necrosis__ is a cell death mechanism triggered by external factors (lack of oxygen, intoxication, disease, etc.). A cell that is too damaged becomes necrotic - it fills with water until it bursts. Its contents empty into the surrounding environment, causing inflammation and damage to the surrounding tissue.

▪ **The absence, partial deficiency and reduced size of dystrophin** all weaken the membrane of muscle fibres. Repeated muscle contractions eventually lead to muscle fibre necrosis. But muscle fibres are also able to regenerate thanks to stem cells present in muscle called satellite cells. When muscle cells die, satellite cells differentiate into myoblasts which fuse together to reform the muscle. But their supply is not endless and they can lose their ability to self-renew. If lots of muscle cells die, muscle regeneration is less effective.



A little dystrophin helps a lot

A French study supported by AFM-Téléthon divided 90 people with a *DMD* gene mutation listed in the UMD-DMD French database into three groups according to their dystrophin levels:

- **no dystrophin:** 75% of these people developed DMD;
- **dystrophin levels < 5%:** 61% developed BMD;
- **dystrophin levels > 5%:** 57% developed BMD.

An important takeaway here is that **in those with dystrophin levels < 0.5%**, the clinical signs were milder than in the no dystrophin group (loss of ambulation and spinal fusion occurring later in life and a longer lifespan) which shows that even a small amount of dystrophin is enough to improve the manifestations of these dystrophinopathies.

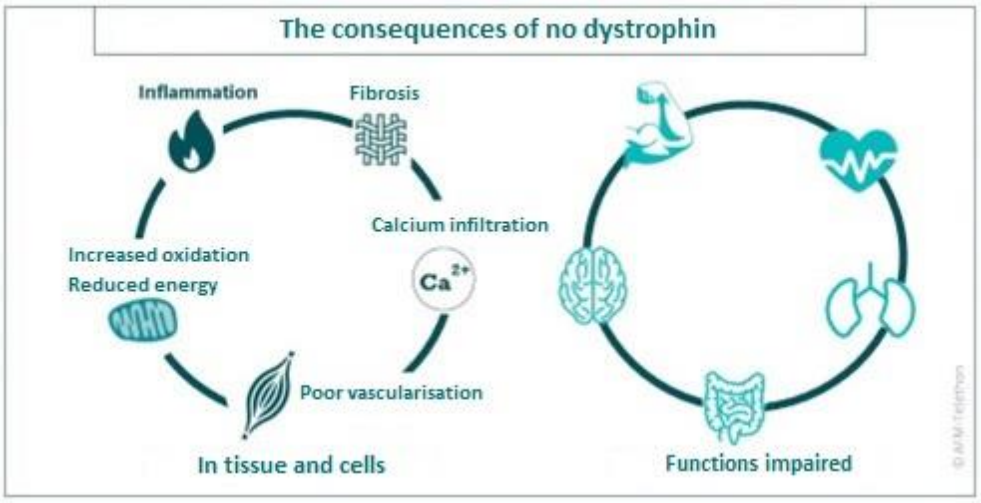
De Feraudy Y et al. Ann Neurol. 2021 Feb.

Harmful effects on tissue and cells

▪ **Calcium, fibrosis, inflammation.** Weakened cell membranes allow large volumes of calcium (Ca^{2+}) to enter their cells which increases the amount of free radicals and oxidative stress. Gradually, muscle is replaced by fibrous tissue (fibrosis) and adipose tissue (fat). Muscle cell necrosis and muscle degeneration take place as well as inflammation. Finally, blood vessels are not able to provide a good supply of blood to the muscles that lack oxygen.

Oxidative stress *contributes to muscle degeneration. It involves the oxidation of various components of the body which is induced by free radicals (reactive oxygen species) produced by the transformation of the oxygen used by cells.*
Free radicals are toxic as they oxidise other cell molecules and contribute to the degradation of cells. They also increase inflammation.

Multiple muscle groups impaired



▪ **Skeletal muscle impairment.** The gradual damage to skeletal muscle causes a decrease in muscle strength and the ability to perform certain movements.

Some muscles become weaker while others compensate for this lack of strength, forcing the body to modify its movements and posture. Muscle and tendon contractures develop, caused by the lack of movement, as well as compensatory postures, muscle strength imbalances, etc. Certain joints and their ligaments become stiff (orthopaedic deformities).

WEBSITE 1 minute pour comprendre : les différents types de muscles [1 minute to explain: the different types of muscles] | AFM-Téléthon (video in French)

Early **orthopaedic treatment** can slow the progression of motor impairment and limit the effects of muscle loss (preserving joint flexibility, preventing contractures, etc.).

▪ **Smooth muscle impairment.** Dystrophin is usually present in the smooth muscle found in the walls of the digestive and urinary tracts. This is why DMD and BMD patients may also suffer from constipation, gastro-oesophageal reflux disease and urinary problems (strong urge to urinate, urinary incontinence, urinary retention, etc.). These conditions are treated using dietary restrictions, abdominal massage and/or medication.

▪ **Cardiac and respiratory impairment.** Cardiac muscle and the muscles involved in breathing (the diaphragm, intercostal and abdominal muscles, etc.) are also impaired.

Gradually, the patient's breathing will lose quality. Mechanical ventilation allows gas exchange (the removal of carbon dioxide produced by the body and the intake of oxygen which can then reenter the blood circulation and the organs) in the lungs to be reestablished.

Damage to cardiac muscle changes how the heart functions. Early **medication** (ACE inhibitors) helps to protect the heart by limiting how hard it has to work.

▪ **Cognitive impairment.** Around 50% of boys with DMD, and a slightly lower number of boys with BMD, have cognitive impairment, which manifests in a variety of different ways in children and adults (problems with memory, executive functions, attention, behaviour and communication). Approximately 17% of children with DMD have autism,

26% have attention and hyperactivity problems, 22% suffer from anxiety and 14% from depression. Around 19% of boys with Becker muscular dystrophy suffer from anxiety and 17% suffer from depression.

Living with these conditions

Adult Duchenne & Becker Days - learning and sharing

On 23 and 24 June 2022 in Créteil (near Paris), AFM-Téléthon held an event dedicated to adult Duchenne and Becker muscular dystrophy for the first time. Adult patients, caregivers and healthcare professionals (196 individuals in total) were able to participate, either at the venue or remotely, in this debut event which gave prominence to stories, expert advice and discussions on very real issues.

This event enabled specific problems which these patients have to cope with on a daily basis to be addressed, especially regarding their health, as well as the fact that there are not enough treatment recommendations for them. These rich discussions can be found in the video recordings of the plenary sessions and most of the workshops which are available online on the AFM-Téléthon YouTube channel.

WEBSITE Journées Duchenne & Becker à l'âge adulte 2022

Not being overweight as an adolescent stems from healthy eating during childhood

A Dutch study involving 48 boys with DMD (with an average age of 10.8), including 22 who could walk ("ambulatory"), showed that 19 were obese. Many of these boys were in the nine to 13 age group and were not able to walk ("non-ambulatory").

The study analysed their diets by age and found that, compared to recommendations, calorie intake was too high in the four to eight year olds (290 kilocalories/day more than required) and too low in the nine to 13 year olds (349 kilocalories/day less than required), while consumption of fibre, nuts (walnuts, hazelnuts, almonds, etc.), meat, fish, eggs and dairy products was insufficient in every age group.

Limiting calorie intake (cakes, sweets, fizzy drinks) and increasing their consumption of fibre (lentils, beans, chickpeas, fruit, vegetables, whole grains, etc.) and protein (meat, fish, eggs, milk, etc.) from an early age may be the best way to prevent them from being overweight later in life.

Dietvorst C.A.W. et al. J. Neuromuscul. Dis. 2022 Nov.

Motor function monitoring and remote rehabilitation

Healthcare professionals are riding the waves of the COVID-19 pandemic and have suggested using telemedicine tools for patient monitoring and rehabilitation.

- **Evaluating motor function.** The movements used by patients to compensate for movements that they are no longer able to do are good indicators of their motor function. Using the **DVA (Duchenne Video Assessment)** mobile app, these movements can be evaluated remotely. Video recordings of patients carrying out preset movements are analysed using a scorecard which makes it possible to link compensatory movements and loss of function.

In order to harmonise practices, a survey of two groups of physiotherapists enabled the relevance of the movements to be performed by the patients during the videos to be evaluated. In the ambulatory patients, climbing stairs, running, getting up from a chair,

jumping and walking all proved to be relevant and were able to show functional changes. In the non-ambulatory patients, turning over in bed, taking off a t-shirt, reaching to pick up their phone, raising a hand over their head, eating 10 mouthfuls, etc. proved to be more appropriate.
Contesse M.G. et al. Physiother Res Int. 2023 Jan.

▪ **Remote rehabilitation is better if it is supervised.** A Turkish study proposed that 19 ambulatory participants with DMD aged six to 15 years old follow a programme consisting of three video-based rehabilitation sessions per week to be performed at home for two months which were either supervised or not supervised remotely by a physiotherapist. The sessions consisted of low- to moderate-intensity movements including flexion and extension of the arms or legs in a seated position.
After two months, the strength of certain muscles in the shoulders, neck, knees and ankles had improved in the participants in the group supervised by a physiotherapist, but not in the unsupervised group. Neither of the two approaches improved motor function.
Kenis-Coskun, O. et al. Acta Neurol Belg. 2022 Oct.

A good quality of life on mechanical ventilation

AFM-Téléthon teams conducted a study of 119 patients on mechanical ventilation who had various neuromuscular diseases, including 59 who had DMD (23 on non-invasive mechanical ventilation and 36 on mechanical ventilation applied via a tracheostomy) and two who had BMD (mechanical ventilation applied via a tracheostomy). The quality of life experienced by these patients was quite good, regardless of age, diagnosis and severity of respiratory impairment (whether they had a tracheostomy or not). It also appears that:
- the longer the time since mechanical ventilation was initiated, the better the quality of life. Perception adjusts over time according to their personal situation, activities, those around them, etc.
- those who have a "home" and are not dependent on their family or partner for their care said that they had a better quality of life as their care needs are taken care of by other caregivers.
Delorme M. et al. Thorax. 2023 Jan.

Well-monitored breathing is NIV as soon as it is needed

A study conducted in 24 paediatric centres in the French non-invasive ventilation (NIV)/continuous positive airway pressure (CPAP) network showed that out of 1,447 children treated with this type of ventilation, 77 had DMD with an average age of 15.8 years old. Monitoring enabled respiratory problems to be detected earlier, especially those occurring during sleep (apnoea, drop in oxygen saturation) or unbalanced blood gases during the day, meaning that ventilation could be set up when a patient's breathing was more relaxed. Ventilation was only set up after an acute respiratory episode in just 15% of cases, a positive figure which reflects the value of monitoring for respiratory problems after DMD is diagnosed.
Allaer L. et al. Neuromuscul Disord. 2022 Dec

International guidelines for heart rhythm disturbances

International experts in cardiology have created practical guidelines for heart rhythm disturbances encountered in neuromuscular diseases, in particular in Duchenne and Becker muscular dystrophy, by sharing practices and analysing the literature on the subject.

- In DMD and BMD, ventricular arrhythmias are the most frequently encountered arrhythmias, while bradyarrhythmias (slow heart rate) are less common. Cardiomyopathy and heart failure are also seen in DMD and BMD patients.
- Regular cardiac monitoring is essential, especially when it comes to heart rhythm as heart rhythm disturbances can go undetected.
- The decision to implant a cardiac implantable electronic device to treat a heart rhythm disturbance should be made between the patient, their cardiologist and their family by considering the benefits and risks.

Groh WJ. Et al. Heart Rhythm. 2022 Oct.

*An **arrhythmia** is an abnormal heart rhythm (slows down, speeds up, becomes irregular, etc.). There are different types of arrhythmias which can be treated, depending on their severity, by lifestyle changes, taking medication or surgery.*

***Cardiomyopathy** is a disease of the heart muscle. It can be asymptomatic (causes no visible symptoms) or manifest as significant fatigue, breathing difficulties, heart rhythm disturbances or chest pain (less common).*

Did you know?

Disease-modifying drugs to protect the heart

▪ Angiotensin-converting-enzyme (ACE) inhibitors.

Angiotensin, which is naturally present in the body, increases blood pressure.

Angiotensin-converting-enzyme (ACE) inhibitors limit the synthesis of angiotensin which lowers blood pressure and protects the heart.

- **ACE inhibitors** are prescribed from the age of 10 as a preventative treatment in DMD where they prevent premature death, and as early as possible in BMD depending on the course of the disease.

▪ **Beta blockers** are used to treat established heart failure.

Treatment with beta blockers can be offered in DMD. These drugs protect the heart by slowing the heart rate down and reducing the force at which cardiac muscle contracts.

▪ Beta blockers and ACE inhibitors can be combined to improve efficacy.

Porcher R et al. Eur Heart J. 2021 Mar. - *Stalens C. et al. J Neuromuscul Dis. 2021 Mar.*

WEBSITE *#JADB Atelier : Le cœur, un muscle pas comme les autres [#Journées Duchenne & Becker à l'âge adulte workshop: The heart - a muscle unlike any other] | AFM-Téléthon - YouTube*

Relatively common pains

A Korean study looked into the presence of pain in 148 boys, including 62 who were able to walk and 86 who could no longer do so (35 under the age of 15 and 51 over the age of 15).

- Just under half (45%) had experienced pain in the previous four weeks. This pain was experienced by all age groups.
- Whether they were able to walk or not and whether or not the patients suffered from scoliosis and muscle and tendon contractures made no difference.
- The boys who were over the age of 15 (non-ambulatory) had more pain on a daily basis, especially in the lower back, buttocks, chest and stomach. These pains, which got worse when in a seated position, were eased by changing positions. The impact of these pains on this group's morale was very high.
- In the ambulatory boys, calf pain (triggered or worsened by walking) was the most common type of pain.

Various methods were able to reduce the pain: massage, rest, correction of sitting position, changing positions and pain medication.

Kim A. et al. BMC Musculoskelet Disord. 2022 Jun.

PNDS: good practices that are still relevant

▪ FILNEMUS, the French rare neuromuscular diseases healthcare network, created PNDSs for DMD (2019) and BMD (2020). Free to access on the HAS website, they present the care standards for these conditions, from diagnosis to the treatment of motor, cardiac and respiratory impairment, genetic counselling and the transition of paediatric patients into adult patients and the changes in care that this entails.
Haute Autorité de Santé - Dystrophie musculaire de Duchenne [Duchenne muscular dystrophy] (has-sante.fr)
Haute Autorité de Santé - Dystrophie musculaire de Becker [Becker muscular dystrophy] (has-sante.fr)

The HAS guidelines for tracheostomy (2020) are also available, which were created at the request of AFM-Téléthon.
Haute Autorité de Santé - Place et gestion de la trachéotomie dans la prise en charge de la dépendance ventilatoire des patients atteints de maladies neuromusculaires lentement évolutives [Placement and management of tracheostomies in ventilator-dependent patients with slowly progressive neuromuscular diseases] (has-sante.fr)

*The **PNDS (Protocoles Nationaux de Diagnostic et de Soins [French National Diagnosis and Care Protocols])** are guidelines for healthcare professionals. "The objective of a PNDS is to provide explicit instructions to professionals regarding the current optimal diagnostic and therapeutic management and care pathways for patients with a specific rare disease. Its aim is to optimise and harmonise the care and follow-up of rare diseases throughout the country" (Haute Autorité de Santé [French National Authority for Health]). All PNDSs published are available on the Haute Autorité de Santé (HAS) website.*
WEBSITE Haute Autorité de Santé (has-sante.fr)

A better understanding of Becker muscular dystrophy

An analysis of over 170 studies

Published between 2000 and 2022, these European, Asian and North American studies cover several hundred patients with BMD aged one to 88. In the patients who were over 41, it was revealed that:
- 84% had muscle weakness and 36% needed to use a wheelchair on a daily basis (15% of the 18-40 year olds and 8% of the under 17s required daily wheelchair use);
- 71% had cardiomyopathy (35% for the 18-40 year olds and 27% for the under 17s);
- 56% had respiratory impairment (6% for the 18-40 year olds and 0% for the under 17s);
- 54% had normal cognitive function (12.5% had an impairment).
The average age of symptom onset was 12 years old and 20 years old for muscle weakness.
Mickle AT. et al. MDA Conference March 2023.

Better consideration of women with dystrophinopathies

Dystrophinopathies can occasionally occur in women with a *DMD* gene mutation. Research conducted on these women is increasing, providing a more accurate understanding of these diseases. The aim is to enable them to have access to care which is adapted to the disease and its course. This applies to girls with genuine Duchenne muscular dystrophy as well as those who only have a few symptoms but need to be monitored regularly.

The different profiles of early-onset dystrophinopathy

A Dutch study reviewed the diagnoses and symptoms of 11 women who had signs of dystrophinopathy before the age of 16 and who were monitored for a period of one to 36 years. Clinical checkups performed in 2021 provided information on these patients who were between nine and 56 years old.
- On average, the disease appeared at the age of four.
- Diagnoses were made between the ages of 10 months and 30 years old, six months to 23 years after the first symptoms.

- During the checkups, it was found that eight out of the 11 women could walk, including two who could walk a short distance with assistance and two who had difficulty climbing stairs. The three non-ambulatory women each lost the ability to walk at the age of 20, 36 and 48.
- Some of the women suffered from fatigue, muscle pain in the lower back and/or joints with restrictions in their professional, domestic, sporting and school activities.
- In three women, minor abnormalities were detected by ECGs or echocardiograms.
- The vital capacity, which reflects respiratory function, of just two of the women was below 60%, with one of these women experiencing no symptoms while the other was on non-invasive ventilation.
- All of the participants were being monitored regularly by a neuromuscular disease specialist and had had a cardiology checkup in the last five years. Three participants were being monitored intermittently in rehabilitation and one was receiving physiotherapy.

Houwen-van Opstal SLS. Et al. Dev Med Child Neurol. 2022 Dec.

An ENMC workshop discusses women

In May 2022, the European Neuromuscular Centre (ENMC) brought together around 20 global experts and patient representatives to review the pathophysiology, prevalence and treatment of females with a partial deficiency or complete absence of dystrophin. Key points from the conclusions and recommendations made:
- the need to stop using the term "carrier" of Duchenne muscular dystrophy in favour of a term to be used for females with dystrophinopathy in order to be able to make a distinction between those who have symptoms and those that don't;
- evidence of very limited interest in X-chromosome inactivation studies;
- the importance of regular cardiology follow-up appointments;
- the lack of relevant animal models that would allow researchers to better understand the disease in this context and predict its consequences.

Sarkozy A et al. Neuromuscul Disord. 2023 Mar.

*The **European Neuromuscular Centre (ENMC)** is an international organisation which aims to support research in the field of neuromuscular diseases. It regularly organises meetings on a given topic which bring together scientists and clinicians from around the world.*
WEBSITE https://www.enmc.org/

Monitoring and treating the heart too

British guidelines created by cardiologists for children and adults, doctors and nurses specialising in neuromuscular diseases, as well as patient representatives, detail monitoring procedures and treatments adapted for cardiac involvement in Duchenne muscular dystrophy.
The monitoring procedures used for boys with DMD are the same ones used for female carriers. It is estimated that 7 to 17% of these females will develop cardiac symptoms during their lifetime, regardless of whether or not they have muscle symptoms.
As soon as a dystrophinopathy is diagnosed, a cardiology assessment should be carried out (ECG, echocardiogram, even a cardiac MRI) and **repeated every two years if there are no abnormalities.**
The medical course of action to be taken, before and during the progression of any cardiac symptoms, is adapted to the patient's cardiac health and uses drugs, in particular angiotensin-converting enzyme (ACE) inhibitors and beta blockers, to limit symptoms and protect the heart.

Bourke J. et al. Open Heart. 2022 Oct.

Acting early - when to carry out newborn screening

Newborn screening for a disease consists of routinely looking for it at birth in all newborns.
In France, 13 diseases are part of the country's newborn screening programme.
WEBSITE Programme national de dépistage néonatal [French newborn screening programme] (article in French)

Spinal muscular atrophy (SMA) screening, which is conducted through a pilot project called DEPISMA started by AFM-Téléthon, also paves the way for carrying out newborn screening for other diseases such as DMD. Its aim is to evaluate the feasibility of using newborn genetic screening to screen for SMA when babies are around three days old. This project is already being implemented in two regions in France (Grand-est and Nouvelle-Aquitaine) and relies on finding a genetic mutation (causative *SMN1* gene mutations) rather than a protein (as is the case for other diseases that are subjected to screening).
WEBSITE Haute Autorité de Santé - Dépistage néonatal : s'informer pour décider [Newborn screening: information to help you decide] (has-sante.fr) (article in French)

Early diagnosis helps with decision making

• Over 30 diseases are already part of the Recommended Uniform Screening Panel (RUSP), a national guideline for newborn screening in the United States. The last disease to be added to this programme was spinal muscular atrophy in 2018. Duchenne muscular dystrophy could follow very soon.
• Nearly 70 American families affected by Duchenne or Becker muscular dystrophy were asked about their feelings towards newborn screening. In these families, the average age of diagnosis of a child's disease was four years old while the first symptoms appeared around the age of two.
Only a third of the families would have liked to have known the diagnosis before their child was six months old. This number may seem small, but must be viewed in the context of there being no cure for this disease. Because if there were treatments that worked well, they would be 93% in favour of newborn screening. They also said that learning about their child's diagnosis early would have helped them when making important life decisions.
Crossnohere NL. Et al. Am J Med Genet C Semin Med Genet. 2022 Jun.

DMD and BMD research

Our understanding of Duchenne and Becker muscular dystrophy and research into treatments are advancing. The number of scientific publications and clinical trials or studies is evidence of this.

709 scientific publications
between May 2022 and May 2023

Source: Pubmed

89 DMD/BMD clinical trials underway or in preparation worldwide, including 8 in BMD and 3 in women
as of 15 May 2023

Source: Clinicaltrials.gov

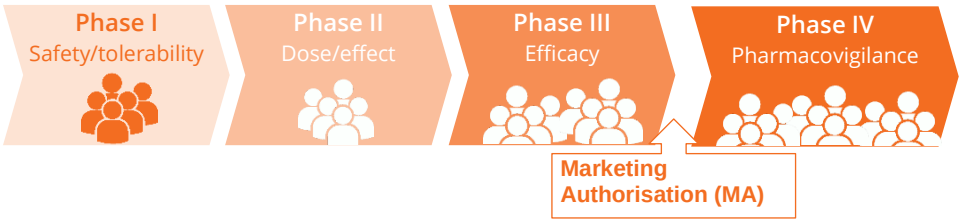
...in France

Treatment approaches and clinical trials

The various phases of clinical trials

Clinical trials consist of evaluating the effects of a potential treatment (drug candidate, medical device, etc.) for a disease in order to ensure that it is well tolerated and effective.

A drug candidate is evaluated in patient cohorts of increasing sizes during successive phases (phases I, II, III and IV). Each phase tries to answer one or a number of questions: is the product well tolerated by patients, what dose seems the best, what effects does it have on the body, is it effective?
In rare diseases, some trials combine several phases (phase I/II, phase I/II/III, etc.), mainly to adapt to the small number of patients represented, while maintaining scientific quality.



Did you know?

Inclusion criteria - essential for the quality of clinical trials

• Set out in the protocol of every clinical trial, inclusion criteria define the population of patients who can participate in a given trial. They are strict and enable researchers to form relatively uniform cohorts of participants so that they are able to interpret the results of the trial. They are decided based on the product, the disease, the disease's course, etc. and on what the trial is trying to demonstrate.
• **In DMD,** the criteria are, for example, genetic mutation, age, sex, indicators of motor function (ambulatory or not, distance able to walk, etc.) and physiological function (lung function, cardiac health, etc.).
This means that if a protocol was designed for ambulatory boys with DMD, it would exclude non-ambulatory patients and those who are too old, as well as symptomatic girls.

*The **sponsor of a clinical trial** is a physical person, company or institution that initiates a clinical trial, assumes its responsibilities and funds its activities.*
They are responsible for the organisation, implementation and monitoring of the clinical trial.

• **Good to know:** if the results of a clinical trial targeting certain criteria are positive, this speeds up the implementation of clinical trials for other patient groups. It can also speed up the release of a drug via an early access system such as the one that exists in France.

Three approaches in DMD and BMD clinical trials

▪ Restoring dystrophin expression

__Gene therapy__ refers to any technique that introduces genetic material into the body in the form of DNA or RNA (gene drugs, antisense oligonucleotides, etc.) for therapeutic purposes.

Gene therapy, exon skipping and stop codon readthrough techniques are designed to provide muscle cells with the genetic or pharmacological tools to make dystrophin. Genome editing (CRISPR) is another approach which is currently in preclinical development for DMD.

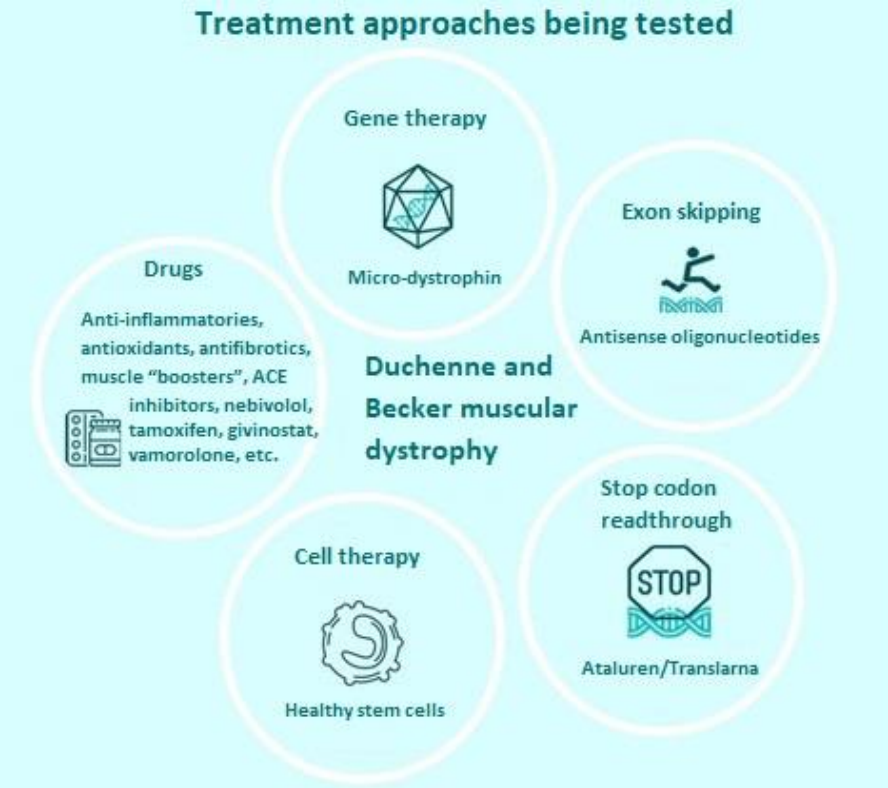
▪ Promoting muscle regeneration

__Cell therapy__ uses living cells. This approach consists of taking cells either from the patient to be treated or a donor, purifying them, modifying them if necessary and then multiplying them. These cells are then put back into the patient to replace deficient or missing cells.

Cell therapy techniques are designed to provide patients with healthy stem cells that colonise and rebuild target tissues, including muscle.

▪ Limiting the consequences of no dystrophin

More traditional drugs tackle inflammation, fibrosis, oxygenation defects, loss of muscle mass, etc. which gradually destroy the affected organs (muscles, heart, gastrointestinal organs, etc.).



If you would like to learn more, an article published in September 2022 reviewed the therapeutic approaches and drugs currently in clinical trials for Duchenne muscular dystrophy.
Markati T. et al. Lancet Neurol. 2022 Sep.

Three important questions regarding DMD and BMD clinical trials

Can these clinical trials be optimised? An ENMC workshop provides an overview

The 269th ENMC workshop (9 to 11 December 2022) brought together 24 manufacturers, clinical neuromuscular disease specialists and patient representatives from five European countries and the United States to work on the challenges, areas of improvement, design and implementation of DMD trials. Here are a few of their "recommendations":

- having **uniform groups of patients who are receiving the study treatment,** determined using strict inclusion and exclusion criteria in order to be able to better understand the disease and its progression and variability.
- **taking prognostic factors into account** (corticosteroids, height, weight, BMI at baseline) when designing trials and analysing their results as they can explain up to 40% of the variability in disease progression.
- **obtaining real-world data** to support clinical trial results.
- **introducing more refined endpoints** than the usual primary and secondary endpoints (six-minute walk test, timed rise from floor, etc.) so that the efficacy of products can be evaluated as accurately as possible.
- taking into account the **significant burden of participating in trials** for patients and their families when hospital visits are frequent, and remedying this by providing clear documentation before the start of the trial on how the trial will run, as well as the tests involved and patient care during the trial.
- reviewing **the issue of long-term placebo groups** (one to two years) and the matter of having to undergo muscle biopsies, particularly in these groups,; implementing strategies so that deteriorating children included in placebo groups can be switched to the group receiving treatment ("rescue strategy"), as is done in other trials of other diseases.

WEBSITE *Clinical trials in DMD: Ten years on, what have we learned? How can we optimize future trial design? 269th ENMC Workshop. 2022 Dec.*

Control groups - a method to be relaxed in DMD?

In order to evaluate the efficacy of a product, a group of patients receiving the study treatment is usually compared to a control group that doesn't receive the study treatment. In DMD trials, these groups also need to have as similar a genotype as possible to the patients being treated, that is, they've been diagnosed with the *DMD* gene mutation. But is this really necessary for forming control groups (especially for exon skipping trials)?

*A **genotype** is the set of genetic characteristics of a living thing. In a way, it's the genetic ID card of an individual.*

An international consortium of researchers and clinicians suggested moving away from this genotype constraint when designing future comparative studies. They emphasized that the genotype of DMD patients only accounts for 2% of variations in function and motor measurements, unlike prognostic factors (age, height, weight, corticosteroid use, motor skills) which explain 30% of variations. This data comes from a one-year follow-up of over 700 DMD patients with distinct genetic mutations (mutations amenable to skipping exon 44, 45, 51 or 53, nonsense mutations or other mutations) included in six natural history studies in various countries.

*Studying the **natural history** of a disease enables researchers to describe different manifestations of a disease and their progression over time without treatment.*

Muntoni et al. Neurology. 2023 Feb.

Which clinical trial should patients chose? A dilemma for patients

Given the unprecedented increase in the number of clinical trials for innovative treatments in DMD (13 exon skipping therapies, including six that are just for exon 51 skipping and five AAV micro-dystrophin gene therapy products currently in clinical trials), an international group of doctors and the Duchenne Parent Project association informed families of the aspects that they should consider before participating in these trials.

These included:

- the investment in the trial: the somewhat long duration (two years, four years, etc.), the constraints of the method of administration (one injection every week, every two weeks, once a month, etc.);
- the type and number of tests, such as biopsies which are sometimes unpleasant;
- the possible benefits and the supposed differences in efficacy between two similar therapies (evaluated in preclinical trials) as well as the benefit-risk ratio;
- the systematic exclusion from participating in other clinical trials in the future, especially if they participate in an AAV micro-dystrophin trial;
- the nature of the trial: double-blind, open-label, both one after the other, and the possibility of receiving the treatment after the trial has finished;
- the pharmaceutical company's willingness to involve patients in the trial design.

Aartsma-Rus, A. et al. Journal of Neuromuscular Disease. 2023 Jan.

*In a **double-blind trial**, neither the patients nor the doctors know which treatment (product being evaluated, placebo, reference product) the patients are taking.*

*An **open-label trial** is a clinical trial in which the doctors and participants are aware of the treatment being given.*

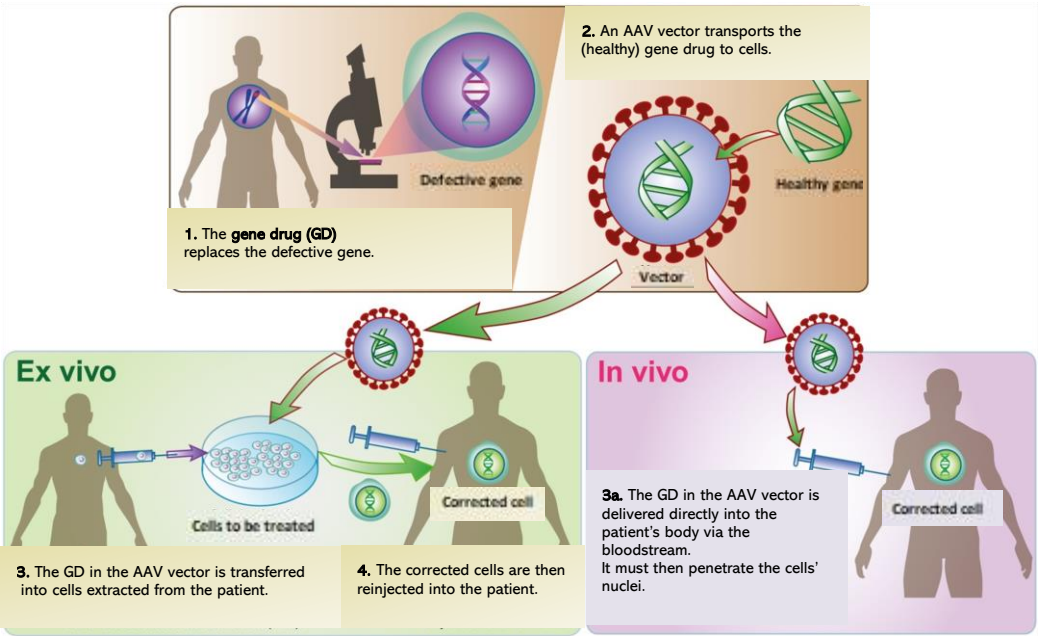
At a glance - a selection of trials, studies and registries

APPROACH	DRUG CANDIDATE	TRIAL	Location
Restoring dystrophin expression			
Gene therapy Micro-dystrophin	• GNT 0004 (Généthon) • SRP-9001 (Sarepta) • PF-06939926 (Pfizer) • SGT-001 (Solid Biosciences) • RGX-202 (Regenxbio) NEW!	Ph. I/II/III Ph. I/II/III Ph. III Ph. I/II Ph. I/II	France Abroad (outside France) France Abroad (outside France) Abroad (outside France)
Exon skipping	• NS-089/NCNP-02 (exon 44 skipping) • AOC 1044 (exon 44 skipping) NEW! • SRP 4045/casimersen (exon 45 skipping) MA (USA) • DS-5141b/renadirsen (exon 45 skipping) • Eteplirsen (exon 51 skipping) MA (USA) • Vesleteplirsen (exon 51 skipping) • PGN-EDO51 (exon 51 skipping) NEW! • Dyne-251 (exon 51 skipping) NEW! • SQY51 (exon 51 skipping) NEW! • SRP-4053/golodirsen (exon 53 skipping) MA (USA) • NS-065/viltolarsen (exon 53 skipping) MA (Japan, USA) • WVE-N531 (exon 53 skipping) • scAAV9.U7.ACCA (exon 2 skipping)	Ph. I/II Ph. I/II Ph. III Ph. II Ph. III Ph. II (Not yet recruiting) Ph. I/II Ph. I/II Ph. III Ph. I, III and IV Ph. I/II Ph. I/II	Abroad (outside France) Abroad (outside France) France Abroad (outside France) France Abroad (outside France) Abroad (outside France) Abroad (outside France) France France Abroad (outside France) Abroad (outside France) Abroad (outside France)
Stop codon readthrough	• Ataluren/Translarna® MA Europe Ambulatory patients over two years old Post-ATU (definition on page 37) in France	Post-marketing surveillance STRIDE Registry	France
Cell therapy	• Transplantation of myoblasts • CAP-1002 – cardiac stem cells • DT-DEC01 – chimeric cells NEW!	Ph. I/II Ph. II Ph. I	Abroad (outside France) Abroad (outside France) Abroad (outside France)
Muscle protection and regeneration			
• Utrophin stimulation • Myostatin inhibition • Combatting muscle loss	• GALGT2 - rAAVrh74.MCK.GALGT2 • Givinostat/DMD • Givinostat/BMD • Sarconeos (BIO101)	Ph. I/II Ph. III (Completed) (Not yet recruiting)	Abroad (outside France) France Abroad (outside France) Abroad (outside France)
Reducing fibrosis	• Tamoxifen • Pamrevlumab • Pamrevlumab	(Completed) Ph. III/ambulatory Ph. III/non-ambulatory	France France France
Reducing inflammation	• Prednisone/prednisolone • Deflazacort MA in the USA, early access in France • Vamorolone (VBP-15) Expanded access (USA, Canada, Israel) MA application in progress (Europe and USA) • Canakinumab (ILARIS) • TAS-205 • ATL1102	Prescribed in B/DMD Ph. IV Ph. III/DMD Ph. II/BMD Ph. I/II Ph. III Ph. IIb	France Abroad (outside France) Abroad (outside France) Abroad (outside France) Abroad (outside France) Abroad (outside France) Abroad (outside France)
• Acting on mitochondria • Limiting oxidation • Protecting myofibrils	• ASP0367 (MA-0211) • Epicatechin BMD • EDG-5506 BMD DMD	(Suspended) (Completed) Ph. I and II / BMD Ph. II DMD	Abroad (outside France) Abroad (outside France) Abroad (outside France) Abroad (outside France)
• Protecting the heart • Limiting how hard it works	• Nebivolol • Ifetroban	(Completed) Ph. II	France Abroad (outside France)
Obtaining clinical and molecular data on DMD and BMD			
• Natural history studies • Registries	• Pre-AAV micro-dystrophin natural history study • BIND study part 1 and 2/cognitive impairment • Registre français des dystrophinopathies [French Dystrophinopathy Registry] • DuchenneConnect Registry	Observational Observational Data collection Data collection	France France France Abroad (outside France)

Clinical trials for dystrophin production

Gene therapy trials

Gene therapy *consists of delivering a therapeutic gene (or gene drug) to cells in which a gene is defective or missing using an adeno-associated virus (AAV) vector so that these cells are able to make a functional protein. The transferred gene is called a transgene and is not incorporated into the genome in DMD and BMD therapies.*
Once inside a cell, the AAV expresses the gene drug (such as micro-dystrophin). Over 250 gene therapy trials involving the use of AAVs are currently taking place in various different diseases.



In order to be able to restore dystrophin expression, gene therapy products contain:
• a micro-dystrophin gene which has the parts of the DMD *gene that are used to produce a protein that is functional and able to bind to muscle cell membrane proteins and the actin cytoskeleton*
• a promoter which orders the production of dystrophin in the target tissue (muscle, heart)
• an adeno-associated virus (AAV) vector such as AAV8, AAVrh74 (a serotype very similar to AAV8) and AAV9, to transport the product into the target tissues (skeletal muscle cells and cardiac cells).
WEBSITE 1 minute pour comprendre : la micro-dystrophine [1 minute to explain: micro-dystrophin] | AFM-Téléthon (video in French)

*A **vector** is a system which enables gene drugs to be transferred into cells in the body.*
By using a vector, gene drugs can access the nucleus of a cell (where the DNA is found), having crossed several biological barriers (vessels and connective tissue) as well as the membranes of the cell and the nucleus.
A vector can be viral or non-viral (plasmids, lipid-based, etc.).

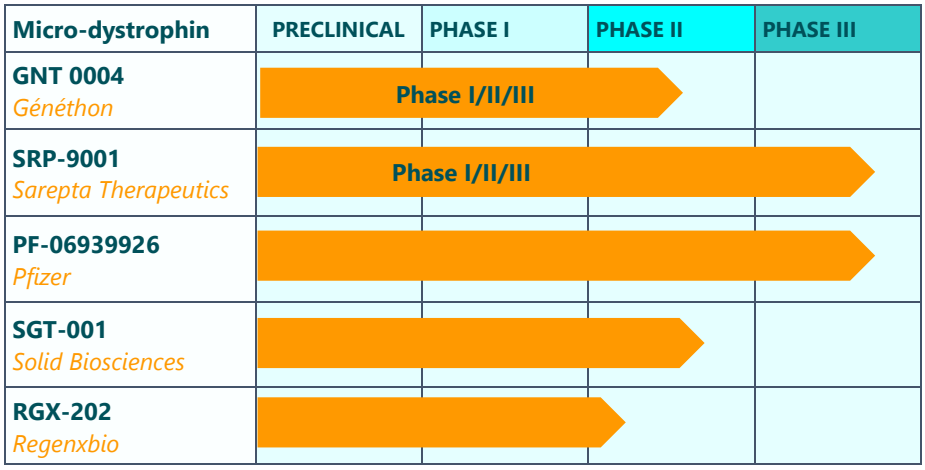
AAVs in gene therapy - the issue of immunity

Adeno-associated viruses (AAVs) are naturally-occurring DNA viruses. They can infect humans without causing disease. Instead, the person infected becomes immune or "seropositive" for this AAV. Administering them a gene therapy treatment that uses this AAV then becomes impossible because they would develop a strong immune reaction to the treatment. It is estimated that between 10 and 40% of people are seropositive for one or another of the AAVs used in micro-dystrophin gene therapies, depending on age and type of AAV.
In order to tackle this immunity issue, researchers and manufactures are exploring different approaches such as using immunosuppressants or products that inhibit antibody activity and desensitise patients who are immune to AAVs, modifying AAV vectors to make them less immunogenic, and even reducing the antibodies circulating in the blood before administering the treatment.
Earley J. et al. Trends in Biotechnology. 2022 Dec. Potter R.A. et al. ASGCT Conference, May 2023. Gross D-A. et al. Front.immunol. 2022 Apr. Lagrue E. et al. Cahiers de Myologie 2021 August.

Five micro-dystrophins developed

Several pharmaceutical companies have developed micro-dystrophin gene therapies to treat DMD, a solution which aims to target the majority of dystrophin gene mutations. Most of these pharmaceutical companies call the gene drug "micro-dystrophin" while Pfizer calls it "mini-dystrophin".
For consistency, we will use the term "micro-dystrophin" henceforth to refer to this gene drug.

*The **mutations** found in genes are usually **deletions** (loss of one or more nucleotides from a segment of DNA), **duplications** (one or more copies of a DNA segment is produced), point mutations (a single nucleotide in a DNA sequence is replaced by another) or **insertions** (addition of one or more nucleotides into a segment of DNA).*



Adjusting inclusion criteria

The first micro-dystrophin trials revealed a link between some *DMD* gene mutations and the serious side effects occurring following gene therapy in certain patients with these mutations. By working together and cross-checking their data, the pharmaceutical companies who sponsor these trials were able to notice the similarity of the side effects and make the connection with these *DMD* gene mutations in the patients receiving the treatment. The collaboration shared this unprecedented finding at the Myology 2022 conference organised by AFM-Téléthon in Nice in September 2022. New inclusion criteria now exclude these mutations from micro-dystrophin trials. The taking of immunosuppressants when the product is being administered in order to prevent immune-related side effects has also been added to protocols.

WEBSITE Avancées et défis des thérapies géniques [Advances and challenges of gene therapies], Braun S. Myology 2022 (video in French)

Wilton-Clark H. et al. Genes 2022 Jan. Philippidis A. Human Gene Therapy. 2022 Mar.

*During clinical trials, the **safety and tolerability of a drug candidate** is evaluated, that is, whether it causes any side effects in the participants (and if it does, which ones).*
Depending on what is observed, product administration conditions may be adjusted, for example, the doses, targeted patients, etc.
In the event of serious side effects, development of the drug may be stopped.

"For" accelerated approval

American researchers and clinicians and the scientific director of AFM-Téléthon advocated for using the amount of micro-dystrophin produced in the muscles of patients treated with AAV micro-dystrophin gene therapy as a way of evaluating therapeutic benefit instead of whether patients improve clinically (using assessments of their motor and respiratory function, for example) for the Food and Drug Administration's (FDA) accelerated approval pathway.

- This pathway, which is applicable in the United States, is available to drug candidates for serious and potentially fatal diseases which have an effect on a parameter (biological, molecular, etc.) that is likely to predict clinical benefit. According to the authors, micro-dystrophin fits this description.

Here is their rationale:
- the "small dystrophins" produced by gene therapy in muscle are believed to be effective as they contain the parts that are essential for them to function. And you don't need a large amount. As evidenced in real life, levels of a shorter dystrophin at 20% of normal is enough to moderate the severity of Becker muscular dystrophy and 5 to 10% lead to less severe DMD.
- micro-dystrophin is also produced very quickly after treatment - its levels are able to be measured as early as two months after treatment. This period of time would allow its safety and tolerability to be ensured.
Once this accelerated approval has been obtained, the evaluation of clinical efficacy continues in the form of confirmatory trials - an essential condition. For example, the four antisense oligonucleotides (eteplirsen, casimersen, golodirsen and viltolarsen) authorised for DMD in the United States (exon skipping) are still in clinical trials.

Chamberlain J.S. et al. Human Gene Therapy 2023 Mar.
Boehler J.F. et al. Neuromuscular disorders 2022 Dec.

GNT 0004 (Généthon)

Designed by **Généthon** researchers and the AFM-Téléthon laboratory in collaboration with Prof. George Dickson's team at the University of London, this gene therapy combines an AAV8 vector and a micro-dystrophin (**AAV8.Spc5.12.micro-dystrophin**). This product was codeveloped by Généthon and Sarepta Therapeutics and approved in preclinical trials as a result of its long-term effects in dogs.

Milestones achieved for GNT 0004

- A clinical trial programme which includes:
  - a **natural history study**: preinclusion and clinical monitoring stage for boys with DMD ("baseline" study) in order to collect baseline data and to help interpret the results of the trial that will follow it. This trial is currently still recruiting using the new inclusion criteria from the 2022 protocol.
  - a phase I/II/III **clinical trial:** designed for children who were preincluded in the natural history study who met the trial's inclusion criteria.
- The trial, which was suspended in April 2021, received approval to start again on 1 March 2022 with an adjusted protocol and inclusion criteria (deletions in exons 1 to 17 of the *DMD* gene prevent patients from participating in the trial and immunosuppressants are administered at the start of the trial).
- Two patients have been included since the trial resumed in 2022 and there have been no serious adverse drug reactions to date.

A preinclusion natural history study

*Studying the **natural history** of a disease enables researchers to describe different manifestations of a disease and their progression over time without treatment.*

Natural history study of Duchenne muscular dystrophy

In France, the United Kingdom, Israel and the United States

100 ambulatory participants (5 to 9 years old)

Recruiting

3 months to 3 years of follow-up

June 2019 – June 2023

NCT03882827

A double-blind trial where all participants receive the study treatment

Phase I/II/III trial of GNT 0004

In France, the United Kingdom, Israel and the United States

50 preincluded participants (6 to 10 years old) who meet the clinical trial criteria

Recruiting

5 years of follow-up

January 2021/March 2022 – December 2027

2020-002093-27

Phase I
Safety/tolerability

Phase II
Dose/effect

Phase III
Efficacy

A three-part trial: this trial consists of a first part which enables the optimal dose of the product to the determined, a second double-blind, placebo-controlled part which evaluates the safety and efficacy of this dose, and a third part during which all the patients receive a dose of the treatment and are monitored for five years. The treatment is administered via IV infusion.

The first part is currently taking place and includes three patients in France who have received one injection of the gene therapy product.

*A **placebo** is a product whose appearance is identical to a particular medicine but does not contain any active substances. In a clinical trial, a placebo is used to measure the true action of a medicine by comparing the effects of the medicine (which contains the active substance) to those of the placebo.*

SRP-9001 (Sarepta)

SRP-9001 (delandistrogene moxeparvovec/ **rAAVrh74.MHCK7.micro-dystrophin**), developed by the pharmaceutical company Sarepta Therapeutics, is made up of an AAVrh74 vector (a serotype very similar to AAV8) which has an affinity for skeletal and cardiac muscle cells, and the muscle-specific promoter MHCK7.

Four SRP-9001 trials including over 200 patients

Treatment administered as a single infusion in all four trials.

1. **A phase I/II open-label trial** (completed): four ambulatory patients (four to seven years old) treated then monitored for five years in the United States (NCT03375164).
2. **A phase II double-blind placebo-controlled trial** (ongoing): 41 ambulatory patients (four to seven years old) treated then monitored for four years in the United States (expected to be completed in April 2026) (NCT03769116).
3. **A phase Ib open-label trial (ENDEAVOR)**: 38 ambulatory or non-ambulatory patients (children over three years old, adults) treated then monitored for five years (expected to be completed in January 2028) (NCT04626674)
4. **A phase III trial (EMBARK)**

Phase III placebo-controlled DMD trial (EMBARK)

In Germany, Belgium, the United States, Spain, Hong Kong, Italy, Japan, the United Kingdom and Taiwan

126 participants (4 to < 8 years old)

Not recruiting

1 year of follow-up after treatment

October 2021 – November 2024

NCT05096221

Phase III
Efficacy

*In the United States, it falls to the Food and Drug Administration (FDA) to authorise, or not authorise, the sale of new drugs. The "**fast track** drug development program" was set up in 1997 with the aim of facilitating development and accelerating the regulatory review of marketing applications for new drugs for serious diseases which do not yet have a treatment.*

Milestones achieved for SRP-9001

▪ **An application for accelerated approval filed with the FDA** in the United States is currently being reviewed (decision expected by 22 June 2023).
- Aimed at ambulatory DMD patients aged four to five years old.
- If the approval is granted, confirmatory results of clinical efficacy from the phase III EMBARK trial (late 2023 at the earliest) will be needed to support it.
- Depending on these results, the indication could be extended to other age groups.

▪ **Over 200 patients treated with SRP-9001** in four clinical trials:
- Ambulatory patients between the ages of three and seven.
- Ambulatory and non-ambulatory patients of "all ages".

▪ **Results one year after administration of the treatment**

Integrated data from trials 1, 2 and 3 conducted on 52 patients confirmed:
- a significant functional improvement in the four to seven year olds compared to external controls (NSAA scale, timed rise from floor and 10-metre walk test);
- safety and good tolerability of the product, despite some adverse drug reactions at the start of treatment which can be controlled.

▪ **Long-term results (four years after treatment)**

Data from trial 1 conducted on four patients aged four to seven years old showed:
- significant, long-term improvement in functional assessments and better preservation of motor function compared to external controls.
- a long-term, durable expression of SRP-9001.

Zaidman C. et al. 27th Annual Congress of the WMS. Oct. 2022
Proud C. et al. MDA Conference March 2023
Lowes L.P. et al. 26th Annual Meeting of the ASGCT. May 2023
Sarepta press releases: 24 May 2023 – 28 November 2022 – 6 July 2022

WEBSITE Dossier submitted to the FDA by Sarepta

PF-06939926 (Pfizer)

The pharmaceutical company Pfizer developed an AAV9.CK.mini-dystrophin gene therapy product made up of an AAV9 vector and a micro-dystrophin gene which is under the control of a muscle-specific promoter called PF-06939926 (fordadistrogene movaparvovec). It is the subject of four clinical trials covering all ages of ambulatory and non-ambulatory patients with DMD.

One of the PF-06939926 trials is taking place in France.

Phase III
Efficacy

Phase III double-blind trial of PF-06939926 (CIFFREO)

In France (Nantes, Paris) and abroad

99 participants (4 to 7 years old)

Not recruiting

5 years of follow-up after gene therapy

November 2020 – April 2029

NCT04281485

The other three trials are being conducted in the United States and in other countries.

Phase I
Safety/tolerability

▪ **A phase Ib open-label trial in the United States**

This trial includes 23 ambulatory or non-ambulatory participants over the age of four (recruitment completed) with five years of follow-up after PF-

06939926 administration. It is due to be completed in March 2026 (NCT03362502).

Phase II
Dose/effect

- **A phase II open-label trial in children** in the United States and Australia. This trial includes 10 patients (two to three years old) who are monitored for five years following treatment. It is due to be completed in July 2028 (NCT05429372).

Phase III
Efficacy

- **A phase III international trial with long-term follow-up** of all patients previously included in another PF-06939926 trial. This trial includes 250 patients of all ages who will be monitored for five to 10 years following treatment. It is due to be completed in May 2039 (NCT05688164).

Milestones achieved for PF-06939926

- **Over 200 patients treated** across four ongoing clinical trials.
- **All ages are represented** in these trials, as well as both ambulatory and non-ambulatory patients.
- Mutations between exons 9 and 13, 56 and 71 and 29 and 30 of the *DMD* gene are excluded from these trials to prevent serious side effects. These mutations are present in 15% of patients.
- **One year after treatment**, data from 19 boys aged six to 13 (with an average age of 8.8) showed:
  - promising results in terms of NSAA scores which increased by one point compared to control patients whose NSAA scores decreased by four points;
  - micro-dystrophin production in muscle cells;
  - safety and good tolerability of the product.

Butterfield R. et al. MDA Conference March 2023

The North Star Ambulatory Assessment (NSAA) *scale measures a patient's ability to perform various tasks (walking, running, jumping, climbing stairs, getting up from the floor, etc.). This scale provides a fairly accurate interpretation of the experiences of patients in daily life.*

SGT-001 (Solid Biosciences)

SGT-001 (AAV9-CK8-microdystrophin), developed by the pharmaceutical company Solid Biosciences, is made up of an AAV9 vector, a truncated dystrophin gene (micro-dystrophin) and a muscle-specific promoter (CK8). The micro-dystrophin produced contains the protein domain that binds to the nNOS complex in muscle cells.

Milestones achieved for SGT-001

- A phase I/II trial of SGT-001 (IGNITE DMD) is still taking place.
- **Nine patients treated:**
  - Three at a high dose, monitored for at least one year,
  - Six at a lower dose, monitored for more than three years.
- **A three-year follow-up of the six patients treated at the lower dose showed:**
  - stabilisation of functional and respiratory abilities as well as movements (upper limbs, transfers, etc.) evaluated at home compared to the natural progression of the disease (control patients).
  - safety and good tolerability of the product.
- **Continuation of SGT-003 development** with a vector that is more effective at targeting muscle cells than SGT-001 and a clinical trial planned for 2023.

Phase I/II open-label trial of SGT-001 (IGNITE DMD)

Phase I
Safety/tolerability

Phase II
Dose/effect

In the United States

16 participants (4 to 17 years old)

Not recruiting

2 years of follow-up

December 2017 – December

NCT03368742

Walking faster one year later

Results presented at the World Muscle Society (WMS) conference in November 2022 showed that nine patients over the age of five were able to walk quicker one year after treatment with SGT-001 (two doses evaluated) compared to three patients who were not treated.
SV95C, an indicator of walking speed, increased by nearly 10% compared to measurements taken before treatment, and by more than 26% compared to DMD patients included in the trial or from a natural history study who were not treated.
Both doses of SGT-001 produced an improvement in motor function, as indicated by the increase in SV95C, while motor function decreased in the patients who were not treated and the reference patients being monitored in the CINRG natural history study, results which were confirmed at the Muscular Dystrophy Association (MDA) conference in March 2023.

Dreghici R.D. et al. MDA Conference March 2023
Gonzalez P. et al. Poster 83 MDA Conference March 2023
Solid Biosciences press release Oct 2022

Did you know?

SV95C - measuring function using an electronic device

SV95C (stride velocity 95th centile) is a variable which measures top walking speed using a Syde wearable electronic device (a modernised version of the ActiMyo device) which is made up of two connected bracelets. Since 2019, SV95C has been recognised by the European Medicines Agency (EMA) as a secondary endpoint in DMD clinical trials for patients over the age of five. Its benefit is that it reflects a patient's ability to move and how quickly in daily life, and measures rapid progress. It could also be reclassified as a primary endpoint.
Haberkamp M. et al. Neuromuscul Disord. 2019 Jul.

Lasting effects three years after treatment

Results after three years from six patients treated with the lower dose in the IGNITE DMD trial showed maintained motor function (NSAA scale, six-minute walk test), pulmonary function, as well as movements in general which were evaluated at home using the PODCI questionnaire, compared to the natural history of the disease.
Dreghici R.D. et al. Poster 81. MDA Conference March 2023

***The North Star Ambulatory Assessment (NSAA)** scale measures a patient's ability to perform various tasks (walking, running, jumping, climbing stairs, getting up from the floor, etc.). This scale provides a fairly accurate interpretation of the experiences of patients in daily life.*

*The American **PODCI (Pediatric Outcomes Data Collection Instrument) questionnaire** evaluates the functional health of children with musculoskeletal disorders.*

SGT-003 - a next-generation gene therapy with improved muscle targeting

SGT-003, developed by Solid Biosciences, is a micro-dystrophin gene therapy product which is very similar to SGT-001 but has a modified vector (AAV-SLB101) which enables it to better reach muscle cells.
Preclinical studies in mdx mice and muscle cells from patients with DMD confirmed this with an increased production of micro-dystrophin after treatment with SGT-003.
The ability of SGT-003 to better target muscle tissue without too much being delivered to other tissues such as the liver enables the dose of the vector injected to be reduced all while achieving higher levels of efficacy. A clinical trial should be starting soon (the authorisation applications are currently being assessed by the FDA).
Solid Biosciences press release 17 October 2022

RGX-202 (Regenxbio) NEW!

The American pharmaceutical company Regenxbio has developed a gene therapy product which combines its proprietary AAV8 vector, a micro-dystrophin containing the extended C-Terminal domain found in naturally occurring dystrophin and Spc5-12, a skeletal and cardiac muscle-specific promoter. RGX-202 administered to mdx mice (the mdx mouse is a mouse model of DMD) led to dystrophin production in muscle and improved strength. With this proof of concept and the required approvals, Regenxbio started a phase I/II trial in the United States.

Phase I/II open-label trial of RGX-202 (AFFINITY DUCHENNE)

In the United States

18 participants (4 to 11 years old)

Recruiting

1 year of follow-up

January 2023 – December 2025

NCT05693142

Phase I
Safety/tolerability

Phase II
Dose/effect

▪ **An observational study (AFFINITY BEYOND)** is running until December 2024 alongside the AFFINITY DUCHENNE trial and evaluating the presence of anti-AAV8 antibodies in 200 DMD patients under the age of 11 in order to identify potential participants for the gene therapy trial (NCT05683379).

Milestones achieved for RGX-202

▪ **A phase I/II dose escalation trial** which initially involves enrolling six patients into two cohorts, each evaluating a different dose of the product, followed by more patients being enrolled into each cohort to reach a total of 18 participants.
▪ **A single administration of the product** via IV infusion.
▪ **Criteria evaluated:**
- The safety and tolerability of RGX-202 over one year.
- The production of dystrophin three months after treatment.
- Functional assessments after one year (NSAA, walk test, etc.).

Dastgir J. et al. Neurom. Dis. 2022 Oct.
Regenxbio press release 23 January 2023

Exon skipping trials

Exon skipping in the *DMD* gene

Exon skipping is a technique which uses **antisense oligonucleotides (ASOs)** to restore the expression of a gene whose message has become unreadable.

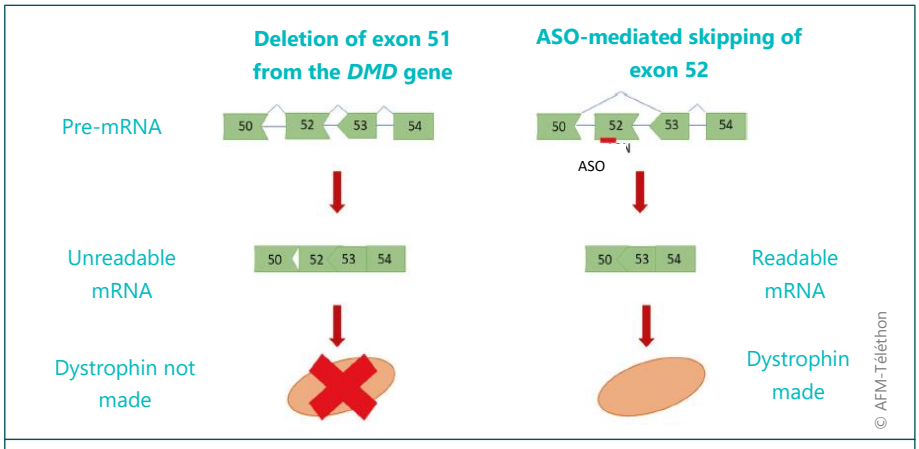
Reformulating the message of a gene to enable it to be expressed

Antisense oligonucleotides act during mRNA production, a stage in the process during which proteins are made from a gene. Pre-mRNA, a direct copy of the gene, is produced first. During its maturation into messenger RNA, only the parts that are used to make the protein (the exons) are kept and strung together.

When a gene loses certain exons ("deletion"), this can shift the reading frame of the mRNA's message. Exon skipping using antisense oligonucleotides reformulates this message and makes it readable so that the corresponding protein can be made.

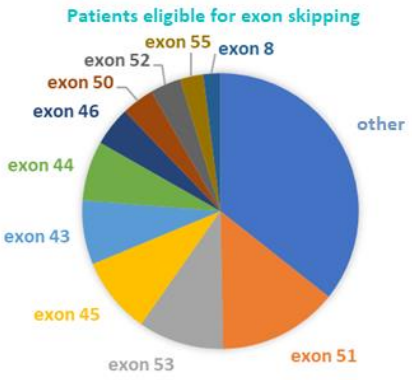
__Messenger RNA (mRNA)__ is a copy of a gene's DNA from which the protein is made. The mRNA's nucleotide sequence dictates the protein's amino acid sequence, composition and structure.

Every gene is organised into an alternating arrangement of coding sequences (__exons__) and non-coding sequences (__introns__). The term "coding" is used to refer to the portions of genes that are used by cellular machinery as a blueprint for making proteins, therefore only exons are translated into proteins.



Skipping exon 52 of the dystrophin gene using an antisense oligonucleotide joins up exons 50 and 53 and makes the mRNA readable.

The dystrophin (*DMD*) gene contains 79 exons. Deletions account for nearly 70% of all mutations in this gene and 11% affect more than one exon. Over 60% of deletions occur between exons 45 and 55, the "hotspot" region of the *DMD* gene. The majority of antisense oligonucleotides developed target this region. Skipping either exon 44, 45 or 51 is applicable to nearly 30% of all Duchenne muscular dystrophy patients.



Skipped exon	*DMD* gene deletions potentially treated
7	2-6, 8-11, 8-17, 8-43, 8-45
8	4-7, 5-7, 6-7, 3-7
17	12-16, 18, 18-20, 18-22, 18-25, 18-27, 18-29, 18-33, 18-36, 18-38, 18-41, 18-44
44	10-43, 19-43, 30-43, 35-43, 36-43, 40-43, 42-43, 45, 45-54
45	12-44, 18-44, 44, 46-47, 46-48, 46-49, 46-51, 46-53, 46-55
50	51, 51-53, 51-55
51	45-50, 47-50, 48-50, 49-50, 50, 52
52	53, 53-55, 53-57, 53-59, 53-60
53	10-52, 45-52, 46-52, 47-52, 48-52, 49-52, 50-52, 52
55	47-54, 48-54, 49-54, 50-54, 52-54, 54, 56, 56-62

WEBSITE Duchenne.com: Exon Skipping

Thirteen ASOs being developed with four receiving authorisation

Thirteen antisense oligonucleotides developed by various pharmaceutical companies are currently in clinical trials. Four of them have obtained conditional marketing authorisation (MA) in the United States and Japan, but not in Europe for the moment; confirmatory trials are underway for these four antisense oligonucleotides.

Exon to be skipped	Oligonucleotide/drug candidate	Status
Exon 44	• NS-089/NCNP-02 • AOC 1044	• Clinical trial (Japan) • Clinical trial (United States)
Exon 45	• Casimersen (Amondys 45®/SRP-4045) • Renadirsen (DS-5141b)	• MA in the United States Clinical trial (France) • No trials currently underway
Exon 51	• Eteplirsen (Exondys 51®/AVI-4658) • SRP-5051 • SQY51 • DYNE-251 • PGN-EDO51	• MA in the United States Clinical trial (France) • Clinical trial (United States) • Clinical trial (France) • Clinical trial (United States) • Clinical trial (Canada)
Exon 53	• Golodirsen (Vyondys 53®/SRP-4053) • Viltolarsen (Viltepso®/ NS-065/NCNP-01) • WVE-N531	• MA in the United States Clinical trial (France) • MA in Japan and the United States International trial • Clinical trial (Canada, United Kingdom)
Exon 2	• scAAV9.U7.ACCA	• Clinical trial (United States)

Optimising antisense oligonucleotides through constant research

The chemical structure of antisense oligonucleotides (ASOs) affects their cell targeting abilities (e.g. their ability to target muscle cells) and their efficacy in inducing exon skipping.

After finding that the first ASOs developed (2'O-methyl-phosphorothioate or **2OMePS** antisense oligonucleotides) were not very well tolerated, researchers optimised their structure to help them perform better and make them less toxic, which led to new generations of ASOs being developed.

• **PMOs** (phosphorodiamidate morpholino oligomer) or "**morpholinos**" are less toxic and more effective (eteplirsen, casimersen, golodirsen, viltolarsen, renadirsen, etc.) but their tissue targeting abilities are insufficient.

• **PMOs** can also be conjugated to other compounds in order to improve their ability to reach muscles and penetrate into cells so that doses can be lowered without losing efficacy. The conjugated compound could be:

- a peptide (to make a peptide-conjugated phosphorodiamidate morpholino oligomer or "PPMO") (e.g. SRP-5051, PGN-EDO51)
- a fragment antibody (Fab) that binds to TfR1 (transferrin receptor 1) (e.g. DYNE-251, AOC 1044)

• **Tricyclo-DNA antisense oligonucleotides** have a higher mRNA affinity and better resistance to nuclear enzymes (e.g. SQY51).

• **Stereopures** have a chemical structure which limits their toxicity and increases their efficacy (e.g. WVE-N531).

• Another approach consists of using an AAV vector to deliver a **small nuclear RNA (snRNA) called U7** combined with a promoter to muscle.

enabling it to be produced there. It's the U7 snRNA which induces exon skipping (e.g.: scAAV9.U7.ACCA (AT-702)).
Filanova G, Aartsma-Rus A. Expert Opin Biol Ther. 2023 Feb.
Egli M.,Manoharan. M. Nucleic Acids Research 2023 Mar.

NS-089/NCNP-02 - exon 44 skipping

In addition to developing viltolarsen which targets exon 53 skipping, the Japanese pharmaceutical company NS Pharma, a subsidiary of the pharmaceutical company Shinyaku Co. Ltd, is also developing another antisense oligonucleotide (a PMO called NS-089/NCNP-02) which this time targets exon 44 skipping.

Well tolerated and effective

A phase I/II clinical trial (NCT04129294) evaluated four doses of NS-089/NCNP-02 which were administered via IV injection once a week for six months to six DMD patients between the ages of four and 17. An open-label extension of this completed trial is currently underway.
The initial results from the trial were announced at the Muscular Dystrophy Association (MDA) conference in March 2022. They showed an increase in dystrophin production of 10 to 15% of normal rates after 24 weeks of treatment with the two highest doses (40 and 80 mg/kg). The product was well tolerated.
National Center of Neurology and Psychiatry press release March 2022.
Ishizuka T. et al. medRxiv 2023 Feb.

Phase I
Safety/tolerability

Phase II
Dose/effect

Open-label extension of the phase I/II trial of NS-089/NCNP-02

In Japan

6 participants (4 to 17 years

Not recruiting

5 years of follow-up

June 2021 – July 2026

NCT05135663

AOC 1044: morpholino + antibody - exon 44 skipping NEW!

Avidity Biosciences developed AOC 1044, a PMO conjugated to an antibody that binds to TfR1 (transferrin receptor 1) on the surface of muscle cells and helps penetrate into them.

- AOC 1044 is currently being evaluated in a phase I/II clinical trial called EXPLORE44 (NCT05670730) which is being conducted in the United States in healthy volunteers first, then DMD patients aged seven to 55 years old (ambulatory or non-ambulatory) in a phase which will determine the most suitable dose of the product. The results from the first part of the trial are expected to be released by the end of 2023.
Data from animal models was presented at the MDA conference in March 2023.
Usue Etxaniz U. et al. MDA Conference March 2023

Casimersen (Amondys 45®/SRP-4045) - exon 45 skipping

This PMO was developed by Sarepta Therapeutics. Clinical trials are currently taking place.

Milestones achieved for casimersen (Amondys 45®/SRP-4045)

- **Treatment authorised (conditional MA) in the United States (2021).**

Casimersen targets mutations that respond to exon 45 skipping. It is administered intravenously once a week.

- **No MA in Europe** due to insufficient efficacy results according to the European Medicines Agency (EMA).
- **Two trials currently taking place (ESSENCE and its extension)** with sites in France. Discussions are ongoing regarding prolonging access to the drug for the participants in these trials.
- Results after one year of treatment in 27 patients aged six to 13 years old:
  - The number of muscle fibres containing dystrophin was 3.5 times higher than in control patients, showing effective exon skipping.
  - The treatment is well tolerated, including in the non-ambulatory patients.
  - The clinical benefit remains to be seen.

Susan Iannaccone et al. 27th Annual Congress of the WMS Oct 2022

Two clinical trials still taking place

Phase III trial of SRP-4045 and SRP-4053 (ESSENCE) (and its extension)

Phase III
Efficacy

In France and abroad

229 participants (6 to 13 years old)

Not recruiting

3 years of follow-up

September 2016 – October 2025

NCT02500381

Extension of the phase III open-label trial of SRP-4045 and SRP-4053

In France and abroad

260 participants (7 to 23 years old)

Enrolling by invitation

7 years of follow-up

August 2018 – August 2026

NCT03532542

Renadirsen (DS-5141b) - exon skipping 45

Developed by the Japanese company Daiichi Sankyo, DS-5141 is an oligonucleotide that is highly resistant to nucleases (enzymes that can destroy them) and has a higher affinity for targeted mRNA molecules.

- Preclinical results have shown its ability to induce exon 45 skipping in muscle and the diaphragm in an mdx mouse model.
- The first phase I/II trial of DS-5141b (NCT02667483) involved seven DMD patients aged five to 10 years old. It was followed by a phase II open-label extension (NCT02667483) involving the same patients. These trials are complete. The initial results showed dystrophin production in

all of the patients who received the treatment and good product tolerability. No other trials have started since.
Daiichi Sankyo press release Jan 2021 *Ito K. et al. Curr. issues Mol. Biol. 2021 Sept.*

Eteplirsen (Exondys 51®) - exon 51 skipping

The PMO eteplirsen (Exondys 51®) was developed by the pharmaceutical company Sarepta Therapeutics.

Milestones achieved for eteplirsen (Exondys 51®/AVI-4658)

▪ Authorised in the United States (conditional MA) in 2016, eteplirsen targets mutations that respond to exon 51 skipping; it is administered intravenously once a week.
▪ No MA in Europe at present.
▪ **Eleven clinical trials** including a total of over 300 patients between the ages of six months and 21 years old have been analysed. A phase III trial (MIS51ON) is still taking place.
▪ **Long-term eteplirsen treatment (over seven years)** in patients over the age of seven, compared to controls, showed:
- a clear effect on their ability to walk (time to loss of ambulation reduced by two years on average at least until the age of 15) irrespective of corticosteroid use,
- respiratory benefits (vital capacity declined half as fast),
- good tolerability of the treatment in general, particularly in boys aged six months to four years old, an argument in favour of starting treatment earlier in order to increase efficacy, with treatment starting as soon as the diagnosis is made being preferable.

▪ **Lifespan increased by 5.4 years on average**: this finding comes from data collected **on real-world use in 575 patients** compared to control patients (natural history). Being treated at a younger age and for a longer time (over two years at least) increases survival.
The effect of eteplirsen on survival is better when treatment is started before the age of 28.
Exondys, EMA, 2018
Iff J. et al. 27th Annual Congress of the WMS. Oct. 2022
Mitelman O. et al. J Neuromuscular Dis. 2022 Jan.
McDonald CM. et al. J Neuromuscul Dis. 2021 Jun.

The MIS51ON trial - evaluating higher doses of eteplirsen

A phase III trial involving dose escalation from 100 to 200 mg/kg/week (part 1) and comparison of these doses with the 30 mg/kg/week dose used so far in all eteplirsen trials (part 2).

Phase III
Efficacy

Phase III open-label (part 1) and double-blind (part 2) trial of high-dose eteplirsen (MIS51ON)

In France and abroad

154 participants (4 to 13 years old)
Recruiting
3 years of follow-up
July 2020 – November 2024

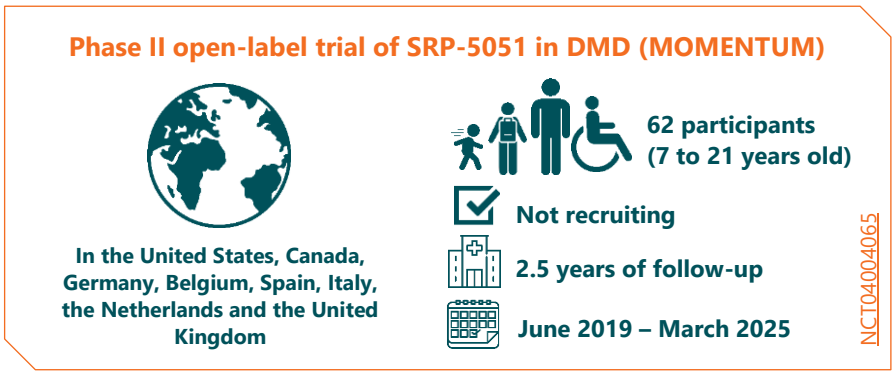
NCT03992430

Vesleteplirsen (SRP-5051) - exon 51 skipping

To make it more effective, the pharmaceutical company Sarepta Therapeutics added a peptide to eteplirsen which helps it penetrate into muscle cells. The resulting PPMO (SRP-5051 (vesleteplirsen)) is only injected once a month, limiting the cumulative dose administered.

Sheikh O. & Yokata T. Archives of Toxicology. 2021 Nov.

- Two doses of SRP-5051 are evaluated in the MOMENTUM trial.



WEBSITE Momentum | Clinical Trials (sarepta.com)

The initial results of the MOMENTUM trial presented during the MDA conference in March 2022 showed that the treatment penetrated well into muscle tissue and that dystrophin production was 3 to 6% of normal rates after three months for the 20 and 30 mg/kg doses.

Campell C. et al. MDA Conference March 2022.

SQY51: a tricyclo-DNA antisense oligonucleotide - exon 51 skipping NEW!

Developed by the French pharmaceutical company SQY Therapeutics, SQY51 is a tricyclo-DNA antisense oligonucleotide that was designed to have a higher affinity for targeted RNA molecules and better resistance to nuclear enzymes. It is also able to reach targeted organs more easily as demonstrated in preclinical animal studies; following administration in mice and monkeys, SQY51 was well distributed in skeletal muscle and the heart, and obtained satisfactory levels of exon skipping.

The first single-site trial of SQY51 is underway

The first ever phase I/IIa clinical trial (AVANCE1) evaluating SQY51 is being conducted at a single site in France (Hôpital Raymond-Poincaré [Raymond-Poincaré Hospital], Garches). It will evaluate the safety and tolerability of the product, determine its initial effects on functional assessments and measure the amount of dystrophin produced in muscle in 12 ambulatory or non-ambulatory patients. The first "dose escalation" part lasts 13 weeks and will be followed by a second part which will run for 32 weeks. The product is administered via IV infusion.

SQY Therapeutics press release 6 February 2023

WEBSITE https://www.youtube.com/watch?v=6E83e4DTYMQ

Phase I/IIa open-label trial of SQY51 in DMD (AVANCE1)

In France
One site (Garches)

12 participants (over 6 years old)

Recruiting

1 year of follow-up

March 2023 – February 2025

NCT05753462

Tricyclo-DNA antisense oligonucleotides have an effect on memory and anxiety in mice

▪ In an initial study, the administration of a tricyclo-DNA antisense oligonucleotide targeting the mRNA of exon 23 of the *DMD* gene in mdx mice produced dystrophin levels 10 to 30% of normal levels in certain regions of the brain and the long-term memory of the mice who were treated improved compared to that of the control mice.

▪ In a second study in mdx52 mice (a model that is more representative of the human disease), administration of a tricyclo-DNA antisense oligonucleotide targeting exon 51 of the *DMD* gene restored 5 to 15% of dystrophin expression in the hippocampus, cerebellum and cortex of the mice who were treated; fear, anxiety and emotional memory also improved.

Peak dystrophin expression was between six and 10 weeks.

Zarrouki F. et al. Ann. Neurol. 2022 Aug.
Saoudi A. et al. Mol Ther Nucleic Acids. 2023 Mar.

Better distribution of dystrophin in muscle fibres

The amount of dystrophin in muscle is not the only factor that affects function. Its distribution in muscle cells is important too!

Researchers from the Université de Versailles Saint-Quentin-en-Yvelines [University of Versailles Saint-Quentin-en-Yvelines], with help from Généthon and the Institut de Myologie [Institute of Myology], carried out experiments on transgenic mice which showed:

- compartmentalisation of naturally-occurring dystrophin in muscle fibres, more so at the muscle–tendon junctions; the amount of dystrophin in these locations may be regulated by the presence of the more numerous nuclei while mechanical protection needs to be increased as the physical requirements are greater at these sites.
- spatial distribution of dystrophin in mdx mice improved after they were treated with **tricyclo-DNA antisense oligonucleotides**.

A modest restoration of dystrophin but a more appropriate distribution of it would have a great effecter on function.

Morin A et al. Proc Natl Acad Sci 2023 Jan.

***Muscle fibres** are "syncytial" cells, i.e., they are formed by fusion and then the maturation of several cells (myoblasts) and contain several nuclei which are distributed along the length of their plasma membranes. But they are not distributed evenly.*

Small molecules that improve the efficacy of tricyclo-DNA antisense oligonucleotides

▪ A team of researchers from the Université Paris-Saclay [Paris-Saclay University], the pharmaceutical company SQY Therapeutics and Hôpital Raymond-Poincaré in Garches showed that combining a small molecule called an oligonucleotides enhancing compound (UNC7938) with a tricyclo-DNA antisense oligonucleotide increases exon skipping in mdx mice. UNC7938 releases oligonucleotides that have been captured by

endosomes. Compared to treatment with tricyclo-DNA antisense oligonucleotides alone, this co-treatment multiplies exon skipping levels by four as early as three days after being administered in mice; two weeks after receiving the injection, three times more dystrophin was found in the hearts of mdx mice who received the combined treatment compared to those who received antisense oligonucleotides alone. After three months, their cardiac function was comparable to that of healthy mice.
Bizot F. et al. Cells. 2023 Feb.

Histone deacetylase inhibitors (HDACi) too

The same team of researchers also showed that administrating histone deacetylase inhibitors (HDACi), which regulate gene expression, can significantly improve the efficacy of exon skipping in DMD treatments by increasing the amount of dystrophin transcripts. In mdx mice, adding valproic acid and givinostat (two HDACi) led to dystrophin restoration levels of up to 74% compared to mice treated with antisense oligonucleotides alone.
Bizot F. et al. Mol Ther Nucleic Acids. 2022 Nov.

DYNE-251: morpholino + antibody - exon 51 skipping NEW!

Developed by the pharmaceutical company Dyne Therapeutics, DYNE-251 is a PMO conjugated to an antibody that binds to TfR1 (transferrin receptor 1) on the surface of muscle cells and helps penetrate into them. Its efficacy has been demonstrated in mouse models of DMD.

The first clinical trial conducted in children and adolescents

Phase I/II double-blind DMD trial (DELIVER)

In the United States, Canada, Belgium, Spain and the United Kingdom

46 participants (4 to 16 years old)
Recruiting
5 years of follow-up
August 2022 – November 2026

NCT05524880

WEBSITE Dyne Therapeutics press release 6 September 2022

PGN-EDO51: morpholino + peptide - exon 51 skipping NEW!

Developed by the pharmaceutical company PepGen, PGN-EDO51 is a PMO conjugated to a next-generation Pip6 peptide. This antisense oligonucleotide has a certain affinity for skeletal, cardiac and smooth muscle as well as the central nervous system.

Encouraging results from a trial in healthy volunteers in Canada

- Four different doses of PGN-EDO51 administered via a single IV infusion were evaluated in 32 healthy volunteers compared to a placebo. In muscle biopsies performed 10 and 28 days later, the amount of PGN-EDO51 produced and increases in exon skipping (1.5 to 2% for the highest dose) were proportional to the dose injected.

The product was well tolerated and the side effects were mild and reversible.

Those pesky endosomes! *Once they reach a cell, antisense oligonucleotides are captured by endosomes, structures that transport them to the compartment where they are supposed to act. But they can also partly sequester them, making them unavailable to act. Small molecules called* ***oligonucleotides enhancing compounds (OECs)****, which can be coadministered with antisense oligonucleotides, can help them break free from endosomes so that they can reach their targets in the nucleus.*

▪ A new phase II trial in DMD patients was announced by the pharmaceutical company PepGen for the second half of 2023.
Larkindale J. et al. Abstract 1038, 26th Annual Meeting of the ASGCT May 2023
PepGen press release 18 May 2023

Golodirsen (Vyondys 53®/SRP-4053) - exon 53 skipping

SRP-4053 is a PMO developed by Sarepta Therapeutics.

Milestones achieved for golodirsen (Vyondys 53®/SRP-4053)

▪ **Authorised in the United States (conditional MA)** in 2019, golodirsen targets mutations that respond to exon 53 skipping; it is administered intravenously once a week.
- **No MA in Europe** (insufficient efficacy results according to the EMA).
• An international clinical trial (ESSENCE, NCT02500381) and its open-label extension (NCT03532542). Discussions are ongoing regarding prolonging access to the drug for the patients included in these trials.
• **Efficacy results after nearly four years** from 25 patients aged six to 15 years old from 2022 (no recent update) showed:
- significant dystrophin production in muscle,
- slowing of the decline in breathing capacity and walking ability.
Servais L. et al. Nucleic Acid Ther. 2022 Jan.

Viltolarsen (Viltepso®/NS-065/NCNP-01) - exon 53 skipping

NS-065/NCNP-01 is a PMO which targets exon 53 skipping. Developed by the Japanese pharmaceutical company Shinyaku Co. Ltd, NS Pharma and the National Center of Neurology and Psychiatry, its chemical makeup has been optimised so that it is able to target pre-mRNA more effectively.

Milestones achieved for viltolarsen (Viltepso®/NS-065/NCNP-01)

• Conditional MA in Japan and the United States (2020) for DMD.
• A post-MA access programme (USA) and four confirmatory trials.
• **Encouraging results after four years of treatment**
- Obtained from completed phase II trials (NCT02740972 and NCT03167255);
- Two doses (40 and 80 mg/kg) administered as weekly infusions evaluated in 16 participants aged four to 10 years old at enrolment compared to control patients in the CINRG natural history cohort;
- **Stabilisation of motor function after two years of treatment** and significant slowing of disease progression after the following two years of treatment.

(Motor function was assessed using time to stand from supine, time to climb stairs, six-minute walk test, and the NSAA scale).
- **Good tolerability and safety profile for viltolarsen**
- Significant dystrophin production in muscle cells localised to the membrane following treatment.

Clemens PR. et al. J Neuromuscul Dis. 2023 May.
Clemens PR. et al. J Neuromuscul Dis. 2022 Jul.

WEBSITE https://www.viltepso.com/patient/about-viltepso

Viltolarsen continues to be evaluated in DMD.

▪ **A post-MA access programme in the United States**

Patients between the ages of three and 12 years old who are eligible for exon 53 skipping can be treated with viltolarsen/Viltepso (weekly 80 mg/kg infusion) and monitored as part of this programme (NCT04337112).

▪ **A phase IV real-world trial in the United States and Canada**

The **VILT-502** trial includes nine participants who already received treatment in the completed phase II trial, who will continue their treatment with viltolarsen and be monitored for 10 years; the trial is expected to be completed in October 2032 (NCT04687020).

Phase IV
Pharmacovigilance

▪ **Three international trials in ambulatory or non-ambulatory patients**

- The phase II **Galactic53** trial of viltolarsen includes 20 ambulatory or non-ambulatory participants over the age of eight years old with one year of follow-up; the trial is expected to be completed in September 2023 (NCT04956289).

Phase II
Dose/effect

- The phase III double-blind trial **RACER 53** and **RACER53-X,** its open-label extension (recruitment for both trials completed), include 74 ambulatory participants aged four to seven years old with a total follow-up period of three years; due to be completed in December 2024 (NCT04060199) and June 2026 (NCT04768062).

Phase III
Efficacy

WVE-N531 - exon 53 skipping

Developed by Wave Life Sciences, WVE-N531 is an exon 53 skipping therapy. This new generation of antisense oligonucleotide has a chemical structure which has been modified to limit toxicity. They are called "stereopures".

Phase I/II open-label trial of WVE-N531 in DMD

In Canada and the United Kingdom

15 participants (5 to 18 years old)

Recruiting

8 weeks of follow-up

September 2021 – December 2022

NCT04906460

*An **open-label trial** is a clinical trial in which the doctors and participants are aware of the treatment being given.*

Modest initial results

The first part of the trial involved three ambulatory boys who received escalating doses of WVE-N531 over six weeks. Analysis of their muscles six weeks after the first dose showed that the product reached the nuclei of cells; the average exon skipping rate was 53% and dystrophin production was 0.27% of normal.

Wave Life Sciences press release 19 December 2022.

scAAV9.U7.ACCA (AT-702) - exon 2 duplication

scAAV9.U7.ACCA is an antisense and gene therapy product designed to produce an exon-skipping molecule in muscle cells. It targets exon 2 duplications - one of the most common duplications found in DMD patients.

A "long-lasting" solution
scAAV9.U7.ACCA combines an AAV9 vector and a U7 snRNA equipped with a promoter which enables the antisense oligonucleotide to be produced in cells. A single administration of scAAV9.U7.ACCA is enough to sustain its production.

▪ Nationwide Children's Hospital developed scAAV9.U7.ACCA and demonstrated the feasibility of this approach in mouse models of Duchenne muscular dystrophy. A phase I/II trial was set up together with the pharmaceutical company Astellas Gene Therapies, which has now withdrawn from the project.

Phase I
Safety/tolerability

Phase II
Dose/effect

Phase I/II trial of scAAV9.U7.ACCA in DMD

In the United States

3 participants (6 months to 13 years old)

Not recruiting

2 years of follow-up

January 2020 – November 2025

NCT04240314

Data from the youngest of the three participants, who was treated at seven months old, showed that dystrophin expression in his muscles remained high 12 months after treatment (88% of normal), an encouraging reason for continuing trials of this approach.
Waldrop M.A. et al. Abstract 802, 26th Annual Meeting of the ASGCT May 2023

Stop codon readthrough

Ignoring stop codons

*A **stop codon** is a codon, that is, a piece of DNA made up of three bases (three "letters"), which designates the end of a genetic message and therefore determines the end of the synthesis of a protein.*

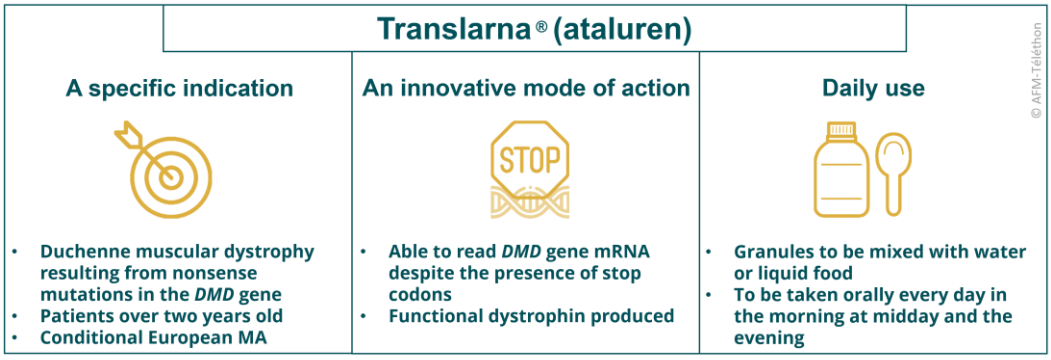
__Messenger RNA (mRNA)__ is a copy of a gene's DNA from which the protein is made. The mRNA's nucleotide sequence dictates the protein's amino acid sequence, composition and structure.

Just under 15% of Duchenne and Becker muscular dystrophy patients have *DMD* gene "nonsense" mutations that introduce a premature "stop codon" into messenger RNA (mRNA) which stops normal dystrophin from being made.

▪ **Stop codon readthrough.** Some drugs are able to forcer these stop codons to be ignored so that normal dystrophin can be produced. This treatment strategy allows the mRNA to be read and enables the cellular machinery to assemble the protein's amino acids despite the stop signal. It only targets nonsense mutations in the *DMD* gene that introduce a premature stop codon.

Ataluren (Translarna®, PTC124)

Ataluren is a direct application of this approach. Developed by PTC Therapeutics, this drug is used in Duchenne and Becker muscular dystrophy to restore **functional dystrophin** production.



Milestones achieved for ataluren (Translarna®)

• **Conditional European MA renewed** until 5 August 2023 which is contingent on the interpretation of the latest results from the confirmatory trial.

• **In France, prescriptions can be given** from the age of five in a hospital setting (post-ATU) and on a case by case basis from the age of two, as well as to non-ambulatory patients and women with dystrophinopathies.

• **The results of a phase III confirmatory trial** (NCT03179631) announced in June 2022 showed, besides that the safety profile is still good, that:
- disease progression slowed down;
- performance on the six-minute walk test did not improve in children aged between seven and 16 years old whose ability to walk was preserved but not stabilised;
- patients with a six-minute walk distance of 300-400 metres before treatment saw more benefits.

• **Findings from the STRIDE registry (real-world study)** published in April 2023 showed that the age at which loss of ambulation took place was delayed by four years and the age at which respiratory decline occurred was delayed by two to two and a half years (patients over the age of five, 70% of whom could walk).

PTC Therapeutics press release 20 June 2022
Mercuri E. et al. J Neurol. 2023 Apr.

WEBSITE	https://www.ema.europa.eu/en/medicines/human/EPAR/translarna
WEBSITE	https://www.has-sante.fr/jcms/p_3118134/fr/translarna-ataluren
WEBSITE	Translarna: Summary of Product Characteristics

Autorisation temporaire d'utilisation (ATU), *a type of approval in France which enables certain categories of patients to use drugs that have not yet received marketing authorisation, can be granted by the Agence nationale de sécurité du médicament et des produits de santé (the French medicines agency) to a new drug while it waits to receive marketing authorisation. This exceptional scheme is used exclusively for drugs that aim to prevent or treat serious or rare diseases for which no other appropriate treatments exist. Drugs with ATU can only be dispensed by a hospital pharmacy.*

The European STRIDE registry is still running

European STRIDE registry - routine clinical follow-up

14 European countries including France

316 participants (girls and boys over 2 years old)

Not recruiting

5 years of follow-up

April 2015 – May 2025

NCT02369731

Data from the European STRIDE registry

As of 31 January 2022, 307 patients from 14 countries were being monitored by the registry (17 French neuromuscular consultations involved).

- The first symptoms of the disease appeared around the age of three in these patients and the diagnosis was made on average when they were about four and a half years old.
- Data from 268 of these patients (over the age of five years old, 70% of which could walk) was used to evaluate ataluren.
- The treatment was administered for an average of four and a half years.
- It significantly delayed the age at which loss of ambulation took place by four years and the age at which respiratory decline (forced vital capacity) occurred by nearly two and a half years.
- Ataluren was well tolerated with only mild adverse events.

Mercuri E. et al. J Neurol. 2023 Apr.

Three clinical trials still taking place

Phase III
Efficacy

- A phase III trial in the United States: 160 ambulatory or non-ambulatory participants of all ages monitored for eight years while receiving the treatment (NCT01247207).
- An international phase III confirmatory trial: 360 participants over the age of five years old monitored for just under four years (NCT03179631).

Phase II
Dose/effect

- A phase II study conducted in children in the United States: 10 participants aged six months to two years old (NCT04336826).

Cell therapy trials

Stem cells as treatment

Cell therapy consists of replacing diseased cells with healthy stem cells. Transplanting stem cells in Duchenne muscular dystrophy patients should promote muscle regeneration and improve motor function. Several trials are currently underway.

CAP-1002: cardiac stem cells still being tested

__Stem cells__ possess both the ability to multiply to produce identical new stem cells (auto-renewal) and the ability to give rise, under specific conditions, to differentiated cells (blood cells, liver cells, muscle cells, etc.).

A cell therapy developed by the American pharmaceutical company Capricor Therapeutics aims to promote the cellular regeneration of cardiac muscle tissue by using donor cardiac stem cells. CAP-1002 is administered into the arteries that supply blood to the heart muscle, and its effects on the heart and upper limbs are studied. Two trials, **HOPE-2 and 3** (which stands for **H**alt cardiomy**OP**athy progr**E**ssion in Duchenne), are still taking place in over 80 ambulatory or non-ambulatory patients in total.

The results of the HOPE-2 trial of CAP-1002 published in 2022 showed a significant improvement in finger strength, grip, and a more general improvement in overall motor function in the upper limbs (shoulders, arms, hands) as well as an improvement in cardiac morphology.

McDonald C. et al. The Lancet. 2022 Mar.

Phase II open-label trial (HOPE-2-OLE) - extension of HOPE-2

In the United States

13 participants (over 10 years old)

Enrolling by invitation

1 year of follow-up

July 2020 – March 2024

NCT04428476

Phase III double-blind trial (HOPE-3)

In the United States

68 participants (over 10 years old)

Recruiting

1 year of follow-up

May 2022 – December 2025

NCT05126758

Phase III
Efficacy

DT-DEC01 - dystrophin-expressing chimeric cells

The American pharmaceutical company Dystrogen Therapeutics has developed a cell therapy approach called DT-DEC01 which uses dystrophin-expressing chimeric cells created by fusing two myoblasts (precursor stem cells of muscle cells), one from a healthy donor and the other from DMD patients. These patients are then treated with these "personalised" chimeric cells.

***Myoblasts** are muscle precursor cells.*

- Preclinical results obtained from an mdx/scid mouse model of DMD 180 days after administration of DT-DEC01 showed a restoration of dystrophin expression and an improvement in cardiac, respiratory and muscle function.

Siemionow, M. et al. Stem Cell Reviews and Reports 2022 May.

- An ongoing study is currently evaluating DT-DEC01 in three boys aged between six and 15 years old (only one of them has already lost the ability to walk). They received a single intraosseous injection of DT-DEC01.

Six months later, performance in the six-minute walk test (6MWT) of the two that could still walk had improved, as had their functional abilities (NSAA). The three participants achieved better scores on "PUL" tests, which evaluate mobility, muscle strength and resistance to fatigue in the upper limbs, after treatment compared to their initial scores.

The treatment was well tolerated with no adverse drug reactions in the 14 months after treatment.

Heydemann A. et al. Stem Cell Rev Rep. 2023 Mar.

Transplantation of myoblasts - another approach being tested

A trial taking place in Canada until 2024 is evaluating the safety and efficacy of transplanting myoblasts (grown from the muscle biopsy of a donor and kept frozen) into an arm muscle of recipients with DMD, while a saline solution is injected into the same muscle on the opposite arm (control). The transplantation is maintained using immunosuppressants. Muscle strength is measured at three and six months after the transplantation.

Phase I
Safety/tolerability

Phase II
Dose/effect

Phase I/II trial of myoblasts in DMD

In Canada

10 participants (over 16 years old)

Recruiting

6 months of follow-up

May 2014 – January 2024

NCT02196467

Clinical trials of muscle-targeting therapies

Other therapeutic approaches in Duchenne and Becker muscular dystrophy consist of targeting the consequences of *DMD* gene mutations on muscle health.

- **Stimulating the production of molecules or proteins** which will functionally replace dystrophin, such as utrophin, directly or indirectly using gene therapy.

- **Correcting or reducing disease manifestations**, e.g., increasing muscle mass, reducing fibrosis, improving muscle strength, preventing cardiac involvement, reducing inflammation and reducing oxidative stress.

Utrophin stimulation

Utrophin, a protein that is very similar to dystrophin (nearly 80%), is naturally produced in the human body during the formation of muscles and is coded for by chromosome 6. When muscle matures, **utrophin hands over to dystrophin** in muscle cells and its production is suppressed. A small amount of utrophin is still produced in muscles affected by dystrophy and the idea of stimulating this production continues to make its way into research projects. Studies also show that the absence of utrophin increases the severity of the disease.
Guiraud S. & Davies K. Med. 2023 Apr.

GALGT2 gene transfer

The *GALGT2* gene codes for a neuromuscular junction enzyme involved in the transfer of sugar (glycosylation) to molecules such as dystroglycan which activates them.
In mouse and great ape models, increasing the expression of *GALGT2* also stimulates utrophin expression and lessens the severity of the disease.

The results of an ongoing phase I/II gene transfer trial which is evaluating this approach (developed by Prof. Kevin Flanigan's team at Nationwide Children's Hospital in United States) in two patients were published at the end of 2022. The product was injected into vessels in both thighs (at a low dose for the nine-year-old patient and at a high dose for the seven year old).
Two years after the injection, the treatment is well tolerated.
Muscle biopsies analysed three or four months after treatment showed increased **GALGT2 protein levels** compared to initial levels.
With regard to efficacy, motor function improved in the younger participant, who was treated with a high dose, but not in the older patient who received a low dose. The small number of patients means that conclusions cannot be drawn regarding a potential therapeutic effect, but these results will guide future trials.
The phase I/II gene transfer trial of the *GALGT2* gene evaluating this approach in DMD is still taking place in these two patients in the United States. It is due to be completed in October 2023 (NCT03333590).
Flanigan KM. Et al. Mol Ther Methods Clin Dev. 2022 Sep.

Tubastatin A inhibits HDAC6 and also increases utrophin levels

Laurent Schaeffer's team in Lyon, in collaboration with a Canadian team in Ottawa, showed that administering tubastatin A, a histone deacetylase 6 (HDAC6) inhibitor, to mdx mice increases their strength, reduces muscle atrophy and fibrosis, increases utrophin and β-dystroglycan levels and improves microtubule and neuromuscular junction organisation. These effects are achieved through a more targeted action on muscle via the TGF-β signalling pathway and an action on Smad3 which increases muscle growth.
Osseni, A. et al. Nat Commun 2022 Nov.

Increasing muscle mass by blocking myostatin

Myostatin is a muscle growth inhibitor which is naturally produced in the human body. Myostatin inhibition is being explored as a possible treatment avenue to promote muscle growth and limit degeneration.
In an mdx mouse model of DMD, inhibiting myostatin reduces muscle dystrophy. In humans, increasing muscle mass is not enough to improve function.

Givinostat trials in DMD

Givinostat acts on muscle through a cascade of actions, the first involving the inhibition of histone deacetylases (HDACs), enzymes which activate or repress certain genes. It therefore increases the production of follistatin, a muscle protein which counteracts myostatin and increases muscle mass.

Delayed loss of ambulation and maintained respiratory function

- The latest results from the phase III EPIDYS trial (NCT02851797) which compared givinostat to a placebo were announced at the end of June 2022. This international trial, which had sites in France, enrolled 179 boys who had an average age of nine years old. The results are from 120 of these boys who were treated for 18 months. They showed a less significant decline in motor function in those who were treated compared to the placebo group (timed four-stair climb test). Their

functional test scores (NSAA and timed rise from floor) went in the same direction.
Previous results showed that the participants treated with givinostat and corticosteroids as background therapy lost their ability to walk two and a half years later than those on corticosteroids only who were being monitored in the CINRG natural history study. Givinostat reduces fatty infiltration in muscle. It was also well tolerated.
Italfarmaco press release 25 June 2022
Italfarmaco press release 22 February 2021

Phase II
Dose/effect

Phase III
Efficacy

Extension of the phase II/III trial of givinostat in DMD

In France and abroad

206 participants (over 7 years old)
Enrolling by invitation
1 year of follow-up
October 2017 – December 2023

NCT03373968

Givinostat and Becker muscular dystrophy - mixed results

In BMD, givinostat does not reduce fibrosis in muscle tissue, the main criterion that needs to be achieved to measure it efficacy. Functional results are similar. This is what was revealed by the completed phase III trial (NCT03238235) which was conducted in 51 Italian and Dutch participants with an average age of 37.5 who were treated for one year and compared to a placebo group. Givinostat also caused mild and moderate adverse drug reactions which led 11 participants to withdraw from the trial vs only one in the placebo group.
On the plus side, MRI images showed stabilisation of muscle fat fraction in the thigh, particularly in the quadriceps, in the givinostat group while it increased in the placebo group.
Comi GP. et al. Front Neurol. 2023 Jan.

Bacterium expressing human myostatin in mice
BLS-M22 consists of a bacterium (*Lactobacillus casei)* which contains a vector that expresses modified human myostatin. This orally-administered product increased the amount of anti-myostatin antibodies in mdx mice. The mice's weight increased and their motor function improved. The safety and therapeutic efficacy of this product still needs to be evaluated in humans. A BLS-M22 study has been conducted in healthy volunteers.
Sung DK. et al. Int J Mol Sci. 2022 Aug.

Combatting muscle loss

Sarconeos (BIO101) - a new protocol submitted in 2023

Developed by the pharmaceutical company Biophytis, Sarconeos (BIO101) is a molecule which combats loss of muscle mass due to age (sarcopenia) and in neuromuscular diseases. It promotes muscle regeneration and maintains muscle strength by stimulating protein synthesis and energy production.
- The product's clinical development plan (called MYODA) had been authorised in the United States by the FDA, then in Belgium in March 2020 with a protocol of continuous trials from phase I to phase III.

Phase I
Safety/tolerabil

Phase II
Dose/effect

Phase III
Efficacy

The MYODA study was then postponed. A clinical trial application for a phase I/II trial for non-ambulatory DMD patients with respiratory involvement will be submitted at the end of 2023.

▪ Preclinical studies of mouse models of DMD treated with Sarconeos (BIO101), supported by AFM-Téléthon, have produced positive results in terms of muscle, mobility and respiratory function.

Duchenne muscular dystrophy: MYODA clinical programme – Biophytis

WEBSITE 1 minute pour comprendre : la sarcopénie [1 minute to explain: sarcopenia] | AFM-Téléthon (video in French)

AP-13 - a hormone that stimulates muscle regeneration

AP-13 is a 13-amino acid peptide and an isoform of a hormone called apelin which has several functions in the body, such as improving the use of sugars in skeletal muscle and promoting blood vessel formation. Canadian and Swiss researchers showed that AP-13 was able to mobilise muscle stem cell activity in mouse models of muscular dystrophies in order to promote muscle regeneration. According to the authors, this makes it a good therapeutic candidate for stimulating muscle repair in muscular dystrophies.

Le Moal E. et al. bioRxiv 2022 Sept.

Reducing fibrosis

The replacement of muscle tissue by inelastic, fibrous scar tissue occurs during muscle degeneration. This mechanism is part of the dystrophic process of muscle necrosis/regeneration. One treatment option is to limit fibrosis in order to preserve the muscle and retain its strength.

Tamoxifen - TAMDMD trial inconclusive

Tamoxifen is an anti-oestrogen drug which has been used for a number of years to treat some types of cancer, in particular breast cancer. This drug has been "repositioned" for DMD (drug repositioning means using a drug for an indication different from the initial indication). Tamoxifen has been evaluated in DMD.

A phase III trial (TAMDMD) was conducted at two sites in France to evaluate tamoxifen in DMD (NCT03354039).

Milestones achieved for tamoxifen in DMD

▪ **The phase III TAMDMD trial has been completed:**

- 93 ambulatory DMD patients (6.5 to 12 years old) receiving corticosteroid therapy, and 16 to 20 non-ambulatory patients (10 to 16 years old) not being treated with corticosteroids.
- Treatment consisted of tamoxifen 20 mg/day for 11 months.

▪ **It did not provide any proof of tamoxifen's efficacy.**

- Tamoxifen was well tolerated after more than two years of treatment.
- The course of the disease in patients treated was no different to that of patients who were not treated.

▪ Duchenne UK, who helped implement the TAMDMD trial, announced these results on 22 July 2022 and stated that it would not continue developing this approach.

A statement is currently being prepared.

▪ Marketing authorisation has not been granted for tamoxifen to be used in Duchenne or Becker muscular dystrophy.

▪ Use outside the terms of its marketing authorisation requires medical supervision.

Duchenne UK press release Sept 2021.
Duchenne UK press release July 2022.

***Orphan drug designation** is a status given by health authorities to a drug candidate that has been developed to treat a rare disease. This procedure encourages the development of treatments for rare diseases. Clinical trials of the drug candidate need to provide the proof of efficacy, tolerability and quality required for it to be granted marketing authorisation (MA) in the future. The pharmaceutical company whose product is designated as an orphan drug benefits from facilitatory conditions at the various stages of its development.*

A review published in November 2022 provided an update on projects focusing on tamoxifen in DMD.
Botti V. et al. Front Pharmacol. 2022 Nov.

Pamrevlumab (FG-3019) - clinical trials still taking place

Pamrevlumab was developed by the pharmaceutical company FibroGen. It is a monoclonal antibody which has been shown to inhibit fibrosis and improve muscle function in mdx mouse models of Duchenne muscular dystrophy. This drug candidate was granted orphan drug designation in the United States in 2019 (FDA).
FibroGen press release 15 April 2019

Two phase III trials of pamrevlumab are still taking place
These double-blind, placebo-controlled trials (which have sites in France) are being conducted in DMD patients who are being treated simultaneously with corticosteroids. The product is administered intravenously.

Phase III
Efficacy

Phase III trial of pamrevlumab (FG-3019) in ambulatory DMD patients

In France
(Strasbourg, Garches, I-Motion in Paris),
Europe and the United States

70 participants (6 to 11 years old)
Not recruiting
1 year of follow-up
December 2020 – March 2024

NCT04632940

Phase III trial of pamrevlumab (FG-3019) in non-ambulatory DMD patients

In France
(Strasbourg, Garches, I-Motion in Paris),
Europe and the United States

92 participants (over 12 years old)
Not recruiting
1 year of follow-up
July 2020 – May 2024

NCT04371666

Another pamrevlumab trial is taking place in the United States.
This phase II trial involves ambulatory or non-ambulatory patients over the age of 12 and has a two-year follow-up period (NCT02606136).

Reducing inflammation

In Duchenne muscular dystrophy, chronic inflammation of the muscles contributes to muscle fibre necrosis. Several anti-inflammatories are being studied to help limit this as well as muscle necrosis and to protect muscles.

Corticosteroids - a safe bet despite the side effects

Prednisone/prednisolone and deflazacort are used in DMD as long-term oral corticosteroid therapy from the age of four or five years old. This early treatment is part of the medical recommendations for this disease, in addition to other types of care.

- Prescribed from childhood, it delays loss of ambulation by three years on average and protects respiratory and cardiac function.
- Daily (0.75 mg/kg/day) or intermittent regimens (10 days of treatment at the same dose followed by 10 days without treatment and so on) can be offered for prednisone.
- Adverse drug reactions caused by corticosteroid therapy are common but are often able to be controlled with appropriate treatment and dose adjustments.

***Corticosteroids** are drugs that have chemical structures similar to that of hormones in the human body, which have essential functions. Corticosteroid therapy is the name given to any treatment involving corticosteroids.*

Corticosteroids prescribed for DMD

Prednisone/prednisolone and deflazacort are prescribed according to country-specific guidelines.

- **French guidelines currently recommend prednisone or prednisolone which are available from pharmacies and reimbursed by medical insurance.**
- **However, doctors can now also offer deflazacort by applying for compassionate access.**

ATU/RTU - Dezacor - ANSM (sante.fr)*

*Recommandation temporaire d'utilisation (RTU) is a type of approval in France which enables certain drugs to be used outside the terms of their marketing authorisation.

- **Doctors can prescribe the most appropriate drug and adjust doses depending on the patient's needs and in response to any side effects experienced (weight gain, behavioural problems, etc.).**

Deflazacort or prednisone - the debate continues

According to the authors of one study which analysed dozens of articles on the subject, the real difficulty in deciding between these two drugs is down to the variability of Duchenne muscular dystrophy from one boy to another. This makes it more complicated to accurately assess changes in movement, walking or strength produced by corticosteroids.

- While deflazacort generally seems to be a bit more effective at preserving the ability to walk, in particular time to stand from supine, studies which have analysed the efficacy of both drugs do not seem to entirely agree regarding motor function. For some, deflazacort and prednisone have the same efficacy on motor function, while others think that deflazacort is more effective.
- To decide, it might be necessary to look at them over the long term, where the two drugs may also have similar effects.
- Bone fragility, cataracts and slow growth are all worse with deflazacort but not with prednisone, which in turn causes weight gain and worsens behavioural issues.
- Regarding dosing frequency, corticosteroid therapy taken daily is the most effective, with no difference between the two drugs.

The choice is obvious - early corticosteroid treatment preserves the ability to walk on average until the age of 13 years old vs 10 years old without it.

Biggar WD. et al. J Neuromuscul Dis. 2022 Jul.

Continuing to take corticosteroids is still helpful after loss of ambulation

There is no consensus on this yet, however, the results look promising. A study compared the progression of DMD in 86 boys with or without corticosteroid therapy (prednisone or deflazacort) who had a follow-up every six months for three years. The participants were between three and 18 years old and had lost their ability to walk before the study or during it.

- Corticosteroids slowed declines in cardiac and respiratory function.
- They preserved upper limb function for a longer time - the ability to bring their hand to their mouth was better preserved at the age of 15, and the boys treated with deflazacort were more likely to eat independently without arm support. Finally, turning in bed unaided was easier and this ability was retained for a longer time.

McDonald CM. et al. J Neuromuscul Dis. 2023 Jan.

Vamorolone in DMD - well tolerated and effective too

Vamorolone (VBP15) is a steroid analogue codeveloped by the pharmaceutical companies ReveraGen BioPharma and Santhera Pharmaceuticals. This anti-inflammatory acts like a glucocorticoid but with fewer side effects. It has been granted orphan drug designation in the United States and Europe.

Milestones achieved for vamorolone

• **Long-term trials show that vamorolone:**
- is well tolerated;
- has an effect on muscle and motor function in four to seven year olds (similar to that of corticosteroids) after two and a half years of treatment;
- maintains bone health better than corticosteroids;
- also increases weight gain, especially at high doses, just like corticosteroids do;
- does not stunt growth, unlike corticosteroids.

• **Two marketing authorisation applications have been submitted**, one in Europe (EMA) and one in the United States (FDA). Response expected by the end of 2023.

Santhera-ReveraGen press release 25 April 2023
Santhera-ReveraGen press release 27 October 2022

Expanded access for vamorolone in certain countries

In the United States, Canada and Israel, children, adolescents and adults with DMD are able to be given vamorolone as part of an expanded access programme and special protocol.

Vamorolone is effective and well tolerated

The results of two trials evaluating short- and long-term vamorolone use were published in 2022:

• one involved 23 patients aged four to seven years old who were treated for two and a half years with vamorolone 2 or 6 mg/kg/day compared to boys from the CINRG natural history study who were not treated with vamorolone but received corticosteroids outside the trial.

• the other involved 121 boys aged four to seven years old who received vamorolone (2 or 6 mg/kg/day), prednisone (0.75 mg/kg/day) or a placebo for six months.

Vamorolone seems to be as effective as prednisone in maintaining muscle and motor function after six months of treatment, as well as after

two and a half years. It is well tolerated in the long term (blood tests and kidney and liver function tests show minimal impact). The adverse events which have occurred are not serious and are as frequent as with prednisone.

Vamorolone causes weight gain like prednisone (the direct comparison in the second trial shows this). It does not stunt growth, whereas patients from the natural history study who did not receive vamorolone and patients treated with prednisone did have stunted growth. It also maintains bone health.

Guglieri M. et al. JAMA Neurol. 2022 Oct. *Mah JK. Et al. JAMA Netw Open. 2022 Jan*

Clinical trial underway in young patients in Canada

A Canadian team are evaluating the tolerability and efficacy of several doses of vamorolone over one year in 44 boys between the ages of two and four and seven and 18 years old. The trial is expected to be completed in December 2023 (NCT05185622).

Vamorolone in BMD - one trial underway

A new vamorolone trial is currently taking place in ambulatory adults with BMD in the United States. No results have been shared to date.

Phase II placebo-controlled trial of vamorolone in BMD

Phase II
Dose/effect

In the United States

39 participants (18 to 64 years old)

Recruiting

28 weeks of follow-up

June 2022 – December 2024

NCT05166109

Canakinumab (Ilaris®) - an interleukin inhibitor

Canakinumab is an anti-interleukin 1 beta (IL1β) antibody used in children with inflammatory diseases such as juvenile idiopathic arthritis and familial Mediterranean fever. Its use is currently being evaluated in DMD.

An ongoing phase I/II trial (ILARIS, NCT03936894) is analysing changes in inflammatory biomarkers in blood taken from boys with DMD treated with two successive doses of canakinumab (2 mg/kg then 4 mg/kg if the first dose is well tolerated).

TAS-205 - a prostaglandin D2 synthase inhibitor

TAS-205 is being evaluated in Duchenne muscular dystrophy due to its effect on inflammation and muscle necrosis. It inhibits haematopoietic prostaglandin synthase, an enzyme which enables the production of prostaglandin D2. Prostaglandin D2 is found in the necrotic muscle of people with DMD.

TAS-205 is well tolerated. It showed positive effects on walking ability in boys over the age of five who received TAS-205 over 24 weeks in a phase II trial. A phase III trial is currently taking place.

Komaki H et al. Ann Clin Transl Neurol. 2020 Feb.

Phase III trial of TAS-205 (REACH-DMD)

In Japan

80 participants (over 5 years old)

Recruiting

1 year of follow-up

November 2020 – May 2027

NCT04587908

ATL1102 - an antisense oligonucleotide that reduces inflammation

ATL1102, developed by the Australian pharmaceutical company Antisense Therapeutics, is an antisense oligonucleotide which does not target *DMD* gene mRNA but instead targets CD49d (a subunit of VLA-4 which codes for a key molecule in the inflammation process) mRNA in order to reduce its levels.ATL1102, by destroying these mRNA molecules, helps reduce muscle inflammation.

- ATL1102 was well tolerated in a phase II study in which it was administered once a week to DMD patients.
- A clinical development plan for ATL1102 as a DMD treatment was presented by Antisense Therapeutics in September 2022. An international, phase IIb, placebo-controlled trial in 45 non-ambulatory boys is being planned. A clinical trial application has been submitted in Europe (United Kingdom, Bulgaria, Turkey).

WEBSITE ATL1102 for DMD – Antisense Therapeutics

WEBSITE ATL1102 for DMD: Revised Clinical Study Plans, Antisense Therapeutics, September 2022

WEBSITE Antisense Therapeutics press release 19 December 2022

- In addition to these trials, Antisense Therapeutics is also developing an approach which combines the administration of ATL1102 and exon skipping drugs which have already been authorised in DMD in the United States. Mdx mice have been treated with this combination.

WEBSITE Antisense Therapeutics press release 12 September 2022

Acting on mitochondria and tissue oxidation

Mitochondria *are the powerhouses of the cell. Their respiratory chain provides energy for the cell to use. The number of mitochondria in a cell is variable and depends on the cell's energy needs, with numbers ranging from a few hundred to nearly a million. Muscle fibres, which have a high energy demand, contain several thousand mitochondria.*

In DMD, mitochondrial dysfunction is one of the first cellular changes observed in muscle fibres, which occurs before the onset of the muscle disease, and is evidenced by reduced mitochondrial function, abnormal morphology and impaired mitophagy, a mechanism which breaks down damaged mitochondria. Dysfunctional mitochondria release high levels of reactive oxygen species (ROS) which can activate inflammatory pathways and worsen the disease.

Reid AL. et al. Life 2021 July.

ASP0367 (MA-0211) increases number of mitochondria

Developed by the Japanese pharmaceutical company Astellas Pharma Inc., ASP0367 (bocidelpar sulphate) increases fatty acid oxidation and the amount of mitochondria in muscle cells (mitochondrial biogenesis).

- Initial results from a phase I trial of ASP0367 in healthy volunteers showed that it is well tolerated.
- A phase II trial in the United States in eight DMD patients between the ages of eight and 16 years old was terminated early (NCT04184882).

Mototsugu Ito et al. Muscle Nerve 2021 Oct.

Olesoxime and early mitochondrial stress in D2.mdx mice
Treatment with olesoxime maintains respiratory and locomotor muscle creatine sensitivity in D2.mdx mice. These are the conclusions drawn by researchers from INSERM (Institut National de la Santé et de la Recherche Médicale [French National Institute of Health and Medical Research]) and two American universities, which suggest the involvement of early mitochondrial stress in the myopathy observed in these D2.mdx mice.
Bellissimo CA. et al. Am J Physiol Cell Physiol. 2023 May

Antioxidants - awaiting updates

Antioxidants are molecules that are able to protect the body against the toxic effects of free radicals. They are not made by the body and are instead found mainly in food.
Several antioxidant drugs have been evaluated over the years in DMD and BMD and some, such as idebenone, have been abandoned.

▪ **Epicatechin is an antioxidant** from the flavonoid family that is present in certain foods such as cocoa, apples, cherries and even green tea. An American team showed that receiving epicatechin for eight weeks improved mitochondrial biogenesis and skeletal muscle regeneration in seven ambulatory participants with Becker muscular dystrophy (18 to 60 years old). It also regulated muscle growth and increased the expression of protective muscle fibre membrane proteins, effects that are similar to those observed after a short aerobic exercise programme. *McDonald CM. Et al. Muscle Nerve. 2021 Feb.*

▪ **Flavonoids** combined with omega 3 (FLAVOmega β) have also been studied in mouse models of DMD through in vitro and in vivo assays. They reduced inflammation and fibrosis and modified the metabolism of muscle cells. Mitochondrial activity, vascularisation and fatigue resistance increased in the mice.
Tripodi L. et al. Cells 2021 Oct.

Protecting muscle fibres via myofibrils

EDG-5506 - protecting the muscles of Becker muscular dystrophy patients

EDG-5506 is a small molecule developed by Edgewise Therapeutics which inhibits an enzyme called ATPase. ATPase targets the myosin in fast-twitch muscle fibres. This limits the recruitment of fast-twitch muscle fibres, which are particularly affected in dystrophies. EDG-5506 prevents their degradation.

▪ EDG-5506, taken orally in tablet form every day, is well tolerated as shown by a phase Ib trial in healthy volunteers and BMD patients which saw some adverse drug reactions, the most common being mild and temporary dizziness.
It significantly decreased key biomarkers of muscle damage, depending on treatment time (creatine kinase (CK) and fast skeletal muscle troponin (TNNI2) decreased by 71% and 83% respectively). EDG-5506 therefore seems to protect muscle
which has encouraged other large-scale trials involving ambulatory participants to be set up.
Edgewise Therapeutics press release 5 January 2022

__Myofibrils__ are contractile structures found in muscle fibres. They are comprised of myosin and actin, filaments that slide over each other during muscle contraction. There are __slow-__ and __fast-twitch muscle fibres__. Fast-twitch muscle fibres, which are recruited for powerful and fast movements, __contain fast myosin.__ Slow-twitch muscle fibres are recruited for endurance activities.

A __biological marker__, also referred to as a __biomarker__, is a measurable characteristic that indicates a normal or pathological biological process. The identification of new biological markers for a disease is very important in monitoring the progression of the disease and the efficacy of new treatments. These markers are physiological (change in blood pressure, heart rate, etc.) or molecular (change in the expression of a protein, etc.).

▪ A **phase I open-label trial (ARCH)** is evaluating EDG-5506 over two years in 12 BMD participants aged between the ages of 18 and 55 years old in the United States. It is due to be completed in April 2024 (NCT05160415).

▪ A **phase II placebo-controlled trial (CANYON)** is evaluating five different doses of EDG-5506 over one year in 66 ambulatory participants with BMD aged between 12 to 50 years old in the United States, the United Kingdom and the Netherlands. It is due to be completed in December 2023 (NCT05291091).

EDG-5506 is being studied in Duchenne muscular dystrophy too

▪ Since the end of 2022, the **phase II placebo-controlled trial (LYNX)** has been evaluating four different doses of EDG-5506 over one year in 27 ambulatory participants with DMD aged between four and nine years old in the United States. It is due to be completed in June 2024 (NCT05540860).

Cardiac muscle - a crucial muscle to protect

In Duchenne and Becker muscular dystrophy, heart muscle damage (cardiomyopathy) can occur at various ages. Drugs that are already used to treat classic heart failure (ACE inhibitors, beta blockers) are being evaluated in these diseases.

Ifetroban - limiting how hard the heart has to work

Phase II
Dose/effect

Ifetroban inhibits the thromboxane receptor, a molecule that occurs naturally in the body which has vasoconstrictive effects. It inhibits these effects, lowering blood pressure and limiting how hard the heart has to work. A phase II trial is currently taking place in the United States in children over seven years old, adolescents and adults (NCT03340675).

Nebivolol - awaiting results

A French trial evaluated the efficacy of this beta blocker (alone or combined with ACE inhibitors) on cardiac involvement in DMD over at least five years. The results are still being analysed.

Other treatment avenues: Where are we with CRISPR therapy?

In DMD, CRISPR therapy in humans is still a long way off. But this approach is teeming with studies targeting the dystrophin gene. They have managed to confirm its feasibility and efficacy in animals and various preclinical models by successfully correcting the *DMD* gene.

Did you know?

The CRISPR/Cas9 system

Like a pair of molecular scissors, this is an approach that is able to remove, repair or modify a DNA sequence or gene by cutting at specific locations in the genome in any cell.

- This molecular tool targets the region to be modified using a small guide RNA.
- Treatment strategies using this approach make it possible for a piece of DNA to be removed, a mutation to be corrected, the reading frame of a gene to be modified, a splicing site to be changed in order to induce exon skipping, and even a piece of DNA to be added to a gene.

A gene is like a sentence in which all the words contain three letters (codons). A gene starts with a "capital letter" (a start codon) and ends with a "full stop" (a stop codon). This determines the ***reading frame*** *of the gene codon by codon (three "letters" by three "letters"). This reading of the genetic message "word for word" leads to the formation of a functional protein.*

Splicing *is a stage in the protein production process. During the first stage (transcription), the gene's message is "transcribed" into messenger RNA (a bit like a photocopy of the DNA region that bears the gene). During the second stage (splicing), the messenger RNA is "spliced", i.e., some parts (the introns) are cut out and the remaining pieces (the exons) are joined up into one single strand of mature messenger RNA which only contains the information necessary to direct the synthesis of the protein.*

Various approaches

Two reviews dating from May 2023 offer an overview of the innovations and results of genome editing technology applied to muscular dystrophies, including DMD. The authors emphasized the challenges that still need to be overcome in order to be able to progress to clinical application in humans with DMD, but believe that these efforts will be worth it. These include targeting satellite cells (muscle "stem cells" that enable muscle to regenerate), making genome editing more effective in skeletal and cardiac muscle tissue, better delivery of treatments to tissues to be repaired using vectors that enable them to penetrate tissues more easily, and controlling immune responses to treatments.

Chemello F. et al Hum Gene Ther. 2023 May
Fatehi S. et al. Hum Gene Ther. 2023 May

Registries, databases and other studies

The French registry on dystrophinopathies

The French registry on dystrophinopathies is a database for Duchenne and Becker muscular dystrophy. Set up in 2019, coordinated by Prof. Isabelle Desguerre (Hôpital Necker-Enfants Malades [Necker Children's Hospital], Paris) and funded by AFM-Téléthon, this registry is used in over 50 adult and child neuromuscular consultation sites. Over 1,000 DMD and BMD patients have already joined it.

- The aim of the registry is to enable us to better understand the natural history of the disease, fuel research projects and make it easier for patients to access clinical trials.

It records their clinical and genetic data, as well as information on their treatments and participation in clinical trials.
The patients are able to access to their data.

WEBSITE Le registre Dystrophinopathies | AFM Téléthon (afm-telethon.fr)

Newsletters are shared on the registry's portal:

WEBSITE https://portailafm.voozanoo.net/portailafm#! > newsletter

The TREAT-NMD Global Registry

*The **TREAT-NMD Alliance** is an international network for neuromuscular diseases which brings together scientists, clinicians and patient groups. Originally supported by the European Commission as an EU Network of Excellence, the TREAT-NMD Alliance facilitates conditions which ensure that the most promising research reaches patients. It also seeks international recognition of the best current care practices for people with neuromuscular diseases.*
WEBSITE TREAT-NMD

Created in 2009 by the TREAT-NMD Alliance, the TREAT-NMD Global Registry collects data on patients with neuromuscular diseases, including DMD and BMD, to make it easier for clinical trials to be set up around the world and patients to be recruited to them and to adapt treatment standards.
WEBSITE https://treat-nmd.org/patient-registries/what-are-the-treat-nmd-global-registries/

Treat-NMD also runs DMD and BMD registries in Europe:
WEBSITE Duchenne/Becker muscular dystrophy - TREAT-NMD

The PPMD DuchenneConnect Registry

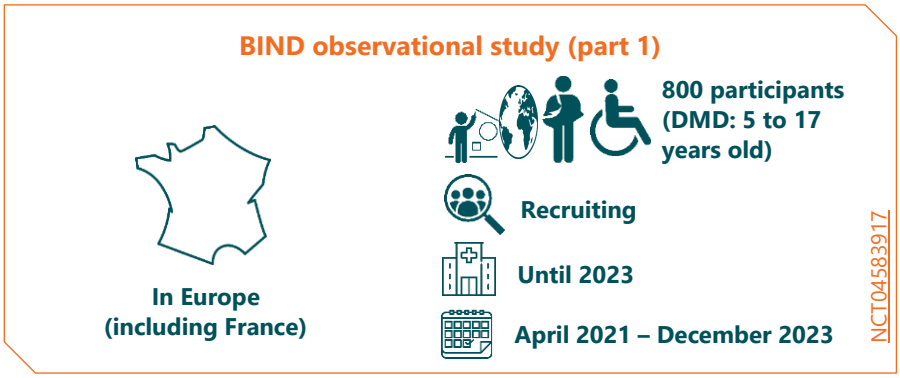
This online American registry set up in 2007 by Parent Project Muscular Dystrophy (PPMD) (NCT02069756) involves a 40-year follow-up period and includes over 10,000 patients with Duchenne or Becker muscular dystrophy and women with dystrophinopathies. It collects genetic, therapeutic and clinical data on these patients in order to improve treatment, care and access to clinical trials.
WEBSITE https://www.duchenneregistry.org/

The collaborative data analysis project called c-TAP (collaborative Trajectory Analysis Project), led by CureDuchenne, aggregates follow-up data from DMD and BMD patients as well as data from specific studies in order to optimise research into future treatments and facilitate the implementation of clinical trials. Source: http://www.ctap-duchenne.org/

The BIND study part 1 and 2

__Cognitive functions__ are functions coordinated by the brain. These include language, know-how, visual recognition and executive functions, i.e. functions that organise and control voluntary actions. During every action or activity (intellectual or manual), different cognitive functions, and therefore different parts of the brain, are called upon.

The European Brain INvolvement in Dystrophinopathies (BIND) study, coordinated in France by Prof. Isabelle Desguerre (Hôpital Necker-Enfants Malades, Paris), started in 2021. It records the neurocognitive, neurodevelopmental and behavioural issues seen in people **with DMD and BMD** in order to correlate them with the various mutations that can occur in the *DMD* gene.



- **Part 2 of the BIND study** is also currently recruiting in France (NCT04668716). It needs to recruit 270 participants of the same age as those participating in part 1 of the BIND study and is due to be completed in May 2024.

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Keep up to date on neuromuscular disease research news throughout the year on the AFM-Téléthon website: **WEBSITE** www.afm-telethon.fr