Duchenne Parent Project onlus

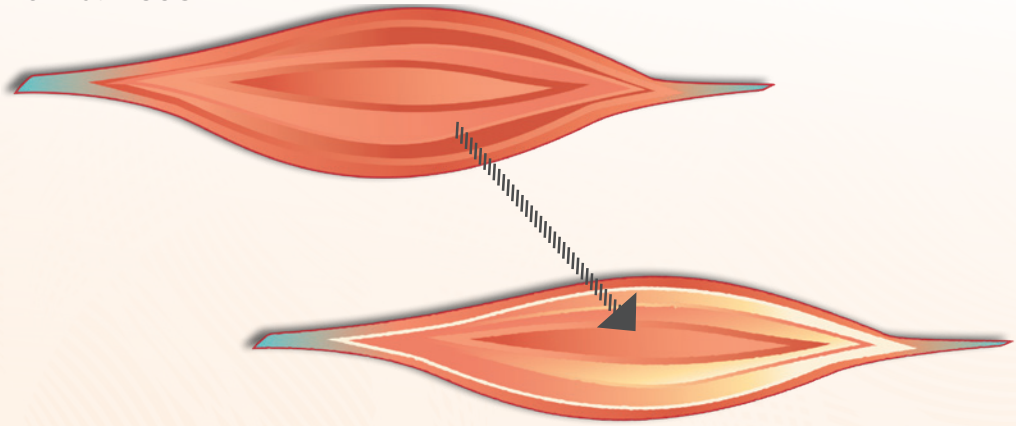
THERAPEUTIC *Strategies*

for Duchenne and Becker
muscular dystrophy

THERAPEUTIC STRATEGIES

Duchenne and Becker muscular dystrophy (DMD and BMD) are rare diseases caused by genetic mutations. A genetic defect underlies a series of complex molecular and cellular events which lead, in a cascading process, to damage and degeneration of the muscle tissue, typical of DMD and BMD.

Normal MUSCLE



DMD MUSCLE

The therapeutic strategies currently in preclinical and clinical development, are designed to act on different targets, specific to these processes.

KEY:
= on trial
= preclinical
= specific mutation
= drug approved
strategies
= gene therapy
= cell therapy
= genome editing
= exon skipping
= NONSENSE
= utrophin induction
= muscle mass
= the mithocondria pathway
= stabilizing the membrane
Ca^{2+} = modulating the transporter channels
= acting on inflammation
= acting on fibrosis

LACK OF DYSTROPHIN

Duchenne and Becker muscular dystrophy are caused by mutations in the dystrophin gene, leading to the total absence, or to reduced levels, of the dystrophin protein. The absence or dysfunction of this protein triggers a complex "cascade" process leading, over time, progressively, to the damage and degeneration of the muscle tissue that characterizing DMD and BMD.

1 **The most ambitious strategy to fight Duchenne is based on innovative approaches aimed at delivering the "healthy" dystrophin gene, in a complete or reduced form, to the muscle tissue.**

GENE THERAPY

The rationale of this strategy is to transmit a healthy copy of the dystrophin gene into muscle tissue, exploiting the natural ability some viruses have to penetrate the muscles. The viruses used are appropriately modified to be harmless and to be able to carry the "therapeutic gene". The huge size of the dystrophin gene hampers its insertion in viral vectors; for this reason different research groups are working to set up gene therapy strategies based on the use of reduced, but still functioning, forms of the gene, the so-called **mini** and **microdystrophins**.

CELL THERAPY

An alternative path to delivery of the healthy copy of the dystrophin gene to muscles, is the infusion of **stem cells from a healthy donor**; a strategy based on the hypothesis that stem cells can colonize the muscle tissue, supplying cells that are able to express the missing dystrophin.

2 **Other innovative strategies are aimed at correcting the specific genetic mutation in order to restore dystrophin production.**

GENOME EDITING

This innovative genetic engineering technique is aimed at modifying the DNA in a specific and permanent way. The strategy is based on an editing system named "**CRISPR**" comprising two simple elements: an enzyme able to cut the DNA (Cas9 for example) and a guide molecule (made of RNA) which specifies the exact position for the cut. This system is able to act on the site of interest with a high level of precision, restoring the production of a **shorter, but still functioning, dystrophin**.

EXON SKIPPING

This strategy aims to correct a series of mutations, thereby restoring the **reading frame of the dystrophin gene**. Unlike genome editing, exon skipping does not act directly on DNA but on messenger RNA, using short RNA molecules called **antisense oligonucleotides** (AONs) to skip an exon. It is not a definitive and irreversible correction. The dystrophin produced will be a shorter but still functioning protein.

Strategy for NONSENSE MUTATIONS

A therapeutic approach that attempts to act on the mechanisms involved in the decodification of genes and their translation into proteins, rather than acting directly on DNA. More specifically, this strategy seeks to avoid the premature stop of the dystrophin gene when a **"nonsense mutation"**, also called stop mutation, is present.

MUSCLE FRAGILITY AND WEAKNESS

The dystrophin protein is anchored to the internal membrane of muscle fibers and plays a crucial role for the mechanical stabilization of the membrane during muscle contraction. Its absence, or dysfunctioning, causes **muscle fragility and weakness.**

1 **Some experimental strategies to fight Duchenne muscular dystrophy do not act on the genetic defect but are aimed at compensating the dystrophin loss acting on the downstream molecular mechanisms that affect *muscle strength and functionality.***

UTROPHIN INDUCTION

Utrophin is a protein that is very similar to dystrophin, it is normally produced during fetal development. During this phase, utrophin is part of the protein complex in charge of maintaining the stability and functionality of muscle fibers. Over time, utrophin gradually decreases, up to almost completely disappearing after birth, when it is replaced by dystrophin. Using specific molecules able to reactivate utrophin production, it is theorized that dystrophin loss may be compensated.

ENHANCING MUSCLE MASS

Among the different approaches to fight muscle weakness, a specific one is aimed at enhancing muscle mass in order to compensate for fragility and improve muscle function. This strategy targets mainly two proteins: **myostatin**, that is produced by muscle cells and acts as a negative regulator of muscle growth, and **follistatin** which, on the other hand, promotes muscle mass enhancement. The molecules that have been developed for this strategy are therefore of two types: the molecules able to positively regulate follistatin and those able to inhibit myostatin.

THE MITHOCONDRIA PATHWAY

An alternative strategy to counteract muscle weakness is to act on the functional deficit of mithocondria, that is typical of dystrophic muscle cells. Mithocondria are cellular organelles working as the **cell's power station** and a deficit in their activity contributes to muscle failure. Some investigations are based on the development of therapies aimed at increasing the number of mithocondria and/or at enhancing their functionality.

Calcium and inflammation

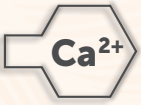
A lack of dystrophin causes membrane instability, leading to the formation of microscopic tears. At cellular level, this causes an abnormal flow, with fundamental substances for muscle functionality exiting the cell, and deleterious substances, like **calcium**, entering the cell. This situation rapidly leads to muscle cell death, triggering **an inflammatory response, which over time counteracts the normal self-repair mechanisms.**

1 Some experimental strategies are designed to *limit the entrance of calcium* in muscle cells.

Stabilizing the membrane

Some molecules act directly on the microscopic tears created in the cellular membrane as a result of dystrophin lack or dysfunction. These molecules act as a "glue" which restores the membrane impermeability to calcium ions.

Modulating the transporter channels



Calcium is one of the several substances that cross the cell membrane through transporter channels, which are made up of proteins. Different molecules have been developed to modulate the activity of the channel's proteins in order to regulate the calcium flow in muscle cells.

2 One of the most studied strategies in the Duchenne and Becker field aims at fighting muscular inflammation using innovative molecules.

The final goal is to find new therapies to replace corticosteroids, or at least reduce them as soon as possible, as they currently represent the only anti-inflammatory gold standard for DMD. However, several side effects are associated with corticosteroids. The different molecules under investigation in this field act on one or more levels of the complex molecular mechanism responsible for the inflammatory process. In any case, the aim is to obtain a more efficient treatment combined with a reduction of corticosteroid side effects.

Fibrosis

The damage to the muscle tissue is linked to an immune response that recognizes the cellular "debris" of muscle cells as a foreign object and triggers an "attack" leading to an inflammatory process, further contributing to the damage. During the fibrotic process, the muscle tissue is replaced by connective and adipose tissues, creating a scar that permanently prevents its functionality. This is a self-perpetuating process that becomes chronic and ultimately results in the death of all muscle cells.

Acting directly on fibrosis

Many of the experimental molecules developed to counteract inflammation have, in addition, an important effect on the fibrotic process. However, a series of approaches focuses specifically on fibrosis inhibition. More specifically, they are designed to inhibit fibrotic tissue deposition and to enhance the natural capacity of the damaged muscle cell to repair itself.

Respiration and heart

Duchenne and Becker muscular dystrophy involves all muscles, including respiratory muscles (diaphragm and intercostal muscles) and heart. As the condition progresses, these muscles gradually weaken and respiratory failure and cardiomyopathy - which are the main causes of a reduction in life expectancy - occur.

Most of the therapeutic strategies in development focus mainly on enhancing the functionality of skeletal muscle tissue, used for ambulation and mobility. These therapies are potentially useful also for respiratory and cardiac functionality.

On the other hand, there are approaches targeting heart and respiration directly.

THERAPEUTIC *Strategies*

LACK OF DYSTROPHIN

Duchenne and Becker muscular dystrophy are caused by mutations in the dystrophin gene, leading to the **total absence, or to reduced levels, of the dystrophin protein.** The absence or dysfunction of this protein triggers a complex "cascade" process leading, over time progressively, to damage and degeneration of the muscle tissue that characterizes DMD and BMD.

MUSCLE FRAGILITY AND WEAKNESS

The dystrophin protein is anchored to the internal membrane of muscle fibers and plays a crucial role for the mechanical stabilization of the membrane during muscle contraction. Its absence, or dysfunctioning, causes **muscle fragility and weakness.**

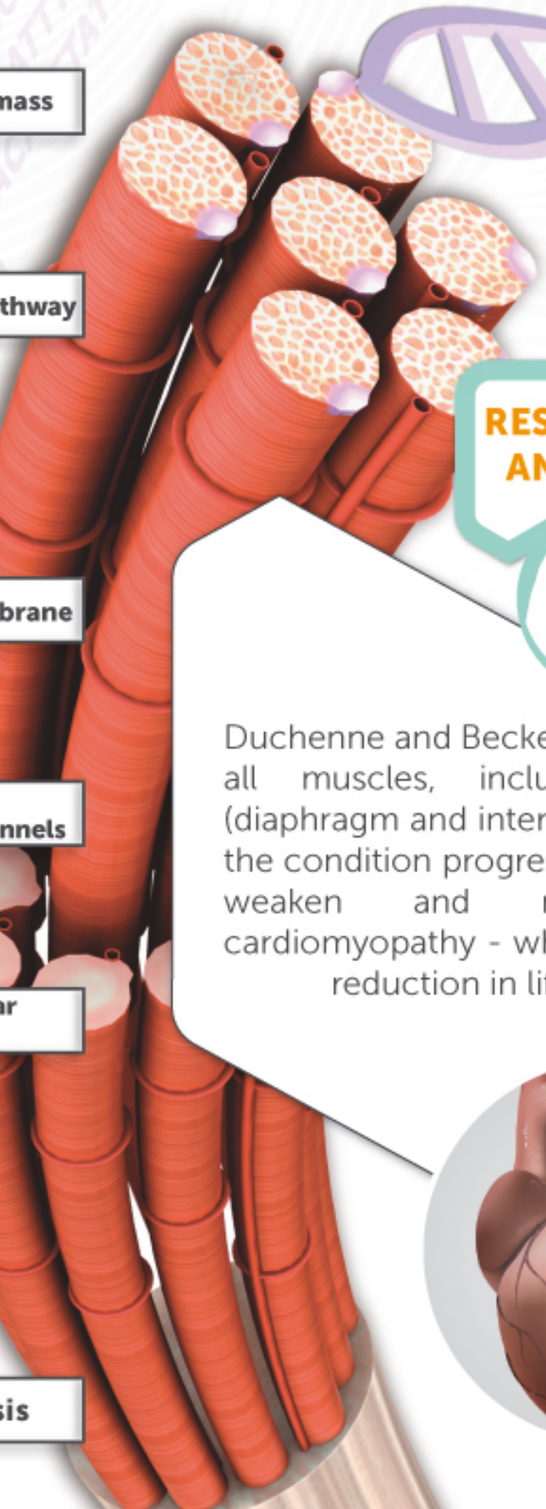
CALCIUM AND INFLAMMATION

A lack of dystrophin causes membrane instability, leading to the formation of microscopic tears. At cellular level, this causes an abnormal flow, with fundamental substances for muscle functionality exiting the cell, and deleterious substances, like **calcium**, entering the cell. This situation rapidly leads to muscle cell death, triggering **an inflammatory response, which over time counteracts the normal mechanisms of self-repair.**

FIBROSIS

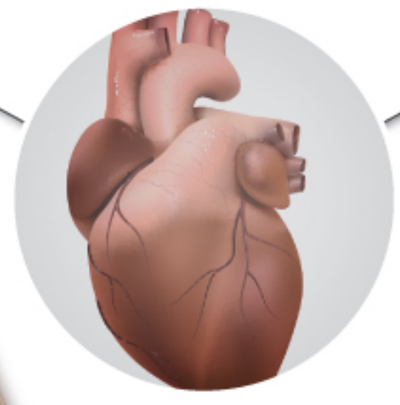
The damage to the muscle tissue is linked to an immune response that recognizes the cellular "debris" of muscle cells as a foreign object and triggers an "attack" leading to an inflammatory process, further contributing to the damage. During the fibrotic process, the muscle tissue is replaced by connective and adipose tissues, creating a scar that permanently prevents its functionality. This is a self-perpetuating process that becomes chronic and ultimately results in the death of all muscle cells.

- gene therapy
- cell therapy
- genome editing
- NONSENSE
- exon skipping
- utrophin induction
- enhancing muscle mass
- the mithocondria pathway
- stabilizing the membrane
- Ca^{2+} modulating the transporter channels
- fighting muscular inflammation
- fighting fibrosis



RESPIRATION AND HEART

Duchenne and Becker muscular dystrophy involve all muscles, including respiratory muscles (diaphragm and intercostal muscles) and heart. As the condition progresses, these muscles gradually weaken and respiratory failure and cardiomyopathy - which are the main causes of a reduction in life expectancy - occur.



KEY:

- *strategies*
- = on trial
- = preclinical
- = specific mutation
- = drug approved

Over the past ten years there has been an exponential growth of therapeutic strategies designed to fight Duchenne and Becker muscular dystrophy. Some of these strategies target specific mutations and are therefore directed to a restricted population of DMD/BMD patients. Others are potentially "universal", meaning that they are indicated for all patients regardless of their mutation. To date, more than 30 human trials, aiming to evaluate the safety and efficacy of these strategies, are ongoing globally. The present scenario strongly suggests that certain and sufficient improvement may come from a synergic action of different molecules and innovative strategies, combined with the recommended multidisciplinary preventive and rehabilitative management.

Text: *Scientific Office - Parent Project onlus*
Artwork: *Communication Office - Parent Project onlus*
Editing support: *Deborah Robins*

Parent Project onlus
Via P. De Francisci, 36 - 00165 - Roma
tel. 06 66182811 - fax 06 66188428
Centro Ascolto Duchenne 800 943 333
www.parentproject.it

Follow us on

Produced with the unconditional contribution of