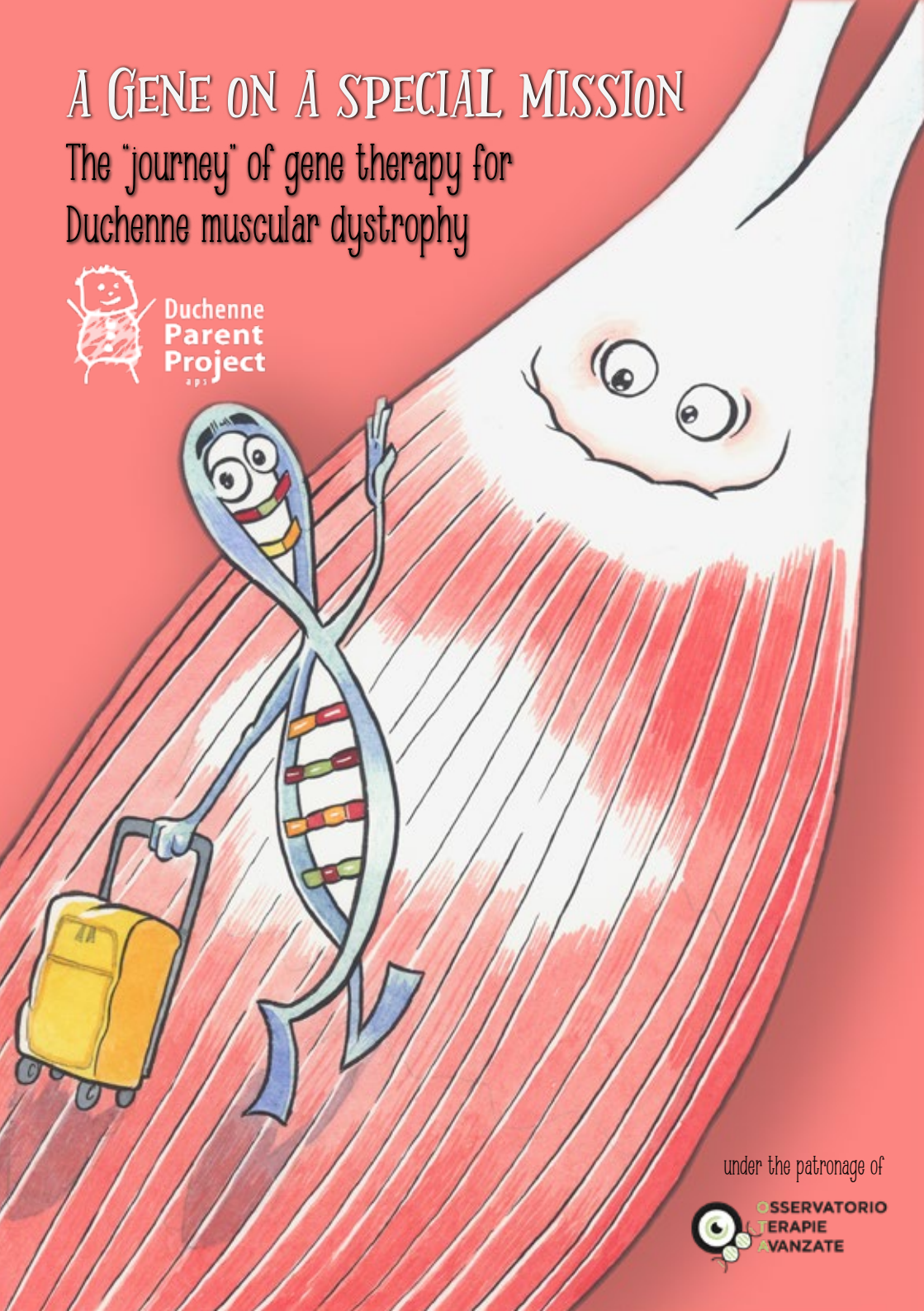
A Gene on a special Mission

The "journey" of gene therapy for Duchenne muscular dystrophy

Duchenne Parent Project



under the patronage of

Osservatorio Terapie Avanzate

Introduction

The concept of gene therapy was born in the 70's with the new technologies of recombinant DNA that allowed researchers to create DNA fragments containing desired gene sequences (a sort of genetic lego). However, it is only in recent years that gene therapy is becoming a reality for some rare genetic diseases.

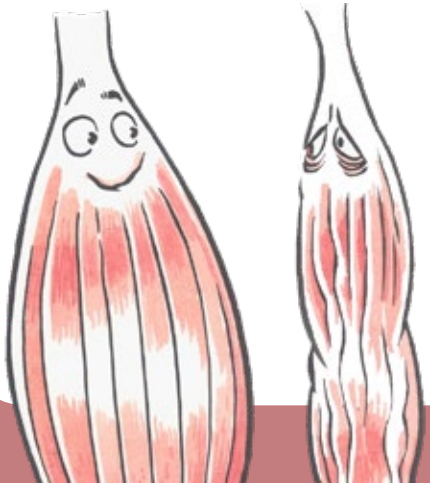
Among the different advanced and precision therapies, gene therapy is one of the first to be developed. The aim of gene therapy is to treat a disease by directly targeting its genetic basis. The basic concept of this therapeutic strategy is **to provide the body with a correct copy of the defective gene**, as a "spare part", or to provide a different gene designed to compensate for the defect in the diseased cells. Gene delivery to the target organs and tissues is **mediated by viral vectors**, i.e. modified viruses, acting in this scenario as vehicles.

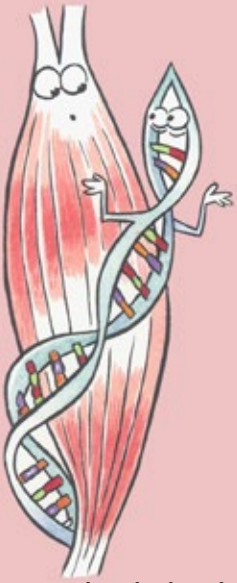
Although the concept may seem simple, the actual application of gene therapy in patients poses challenging obstacles, some of which - as in the case of Duchenne muscular dystrophy - are imposed by specific peculiarities of the disease.

Duchenne muscular dystrophy (DMD) is a rare genetic disease affecting the muscle tissue. Patients with DMD show weakness in all their muscles, progressing over time and leading to a gradual loss of muscle function.
Over time, in addition to the loss of function of lower and upper limbs, heart and diaphragm are also affected, leading to a deficit of cardiac and respiratory functions.

The genetic causes behind DMD were clarified in 1986, with the identification of the dystrophin gene. Genetic defects, technically called mutations, localized in this gene are responsible for the disease manifestation.
The gene provides the necessary information to produce the dystrophin protein, which is crucial for the integrity and function of muscle cells. The absence of this protein leads to the muscle degeneration observed in patients with Duchenne.

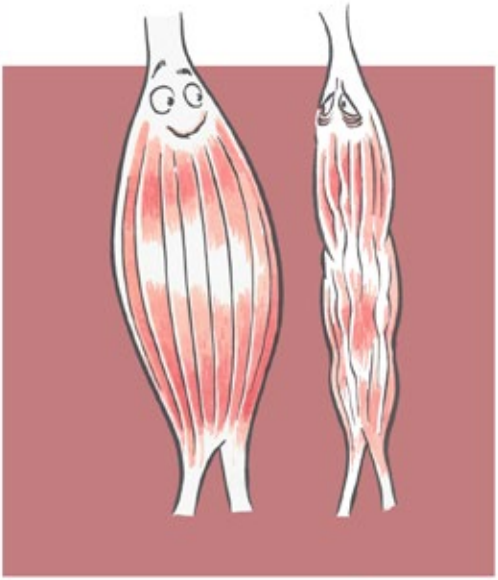
The aim of a gene therapy for Duchenne is therefore to transfer a functional version of the dystrophin gene that is able to restore the protein production in all muscles. This becomes a real challenge when we consider there are hundreds of muscles in our body.



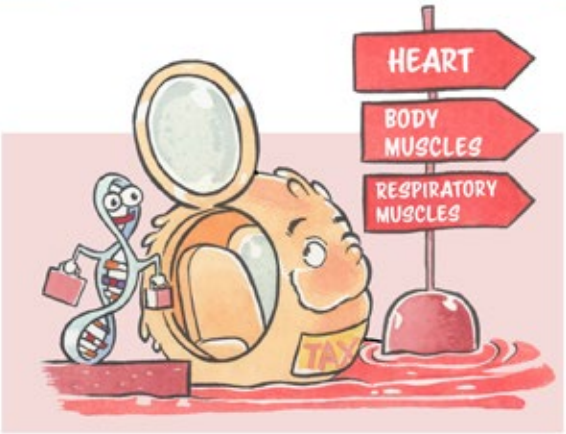


Duchenne muscular dystrophy is a rare genetic disease affecting the muscle tissue.

Over time, muscles progressively lose their function.



The cause is a defect in the dystrophin gene.



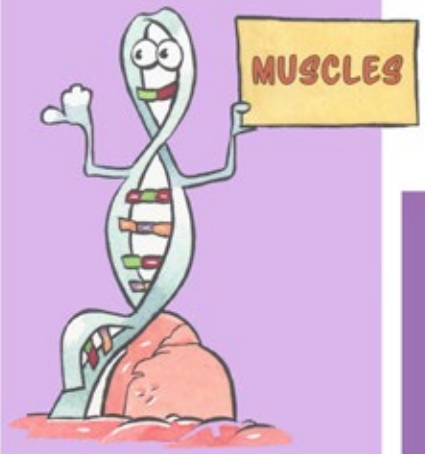
The aim of a gene therapy for Duchenne is to transfer a functional version of the dystrophin gene to all the muscles.

THE FINAL DESTINATION OF OUR DYSTROPHIN GENE IS THUS THE MUSCLE. BUT HOW WILL IT BE ABLE TO GET THERE?

The gene needs a vehicle that can take it "on board" and give it a ride inside the body. Here's where viruses come into play: with adequate modifications, they become suitable vectors for gene transport.

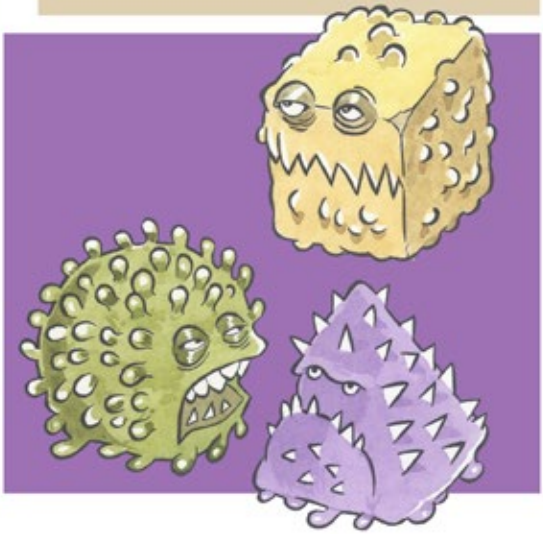
There are many types of viruses and each of them has preferences for a specific cell type. It is thus important to choose the right viral vector to vehiculate our gene to its final destination. Some subtypes of a family of viruses called adeno-associated viruses (AAV), for instance, have a remarkable preference for muscle cells and, in addition, do not have pathogenic effects.

However, before the gene gets on board, the AAV virus needs to be modified. Viruses are very small and to enable them to host any gene, it is necessary to make room by removing their viral genes first.
At the same time, this transforms the virus into a harmless vehicle, unable to replicate and without undesired activities, making it useful for the goal of gene therapy.



To get to the muscles the gene needs a lift...

... here's where viruses come into play.



The adeno-associated viruses (AAV) have the right features for use as a vector.

The viral genes are removed transforming the virus in a harmless vehicle, i.e. a vector, able to transfer the gene to target muscles..

The viral vector is ready and all seems done, but we need to solve a 'little' problem... **How can we combine the huge size of the dystrophin gene with the limited space available in the virus?**

The dystrophin gene is actually the largest gene in our genome and the capacity of a virus such as AAV would need to be much larger to accommodate it all. Since these viruses have this limited capacity, the only possible way to solve this problem is to reduce the gene size without compromising its function.

An impossible mission? Not really, because there are unusually small but functional forms of the dystrophin gene in nature, identified in some Becker muscular dystrophy (BMD) patients with unexpected mild symptoms. This inspired scientists to create shorter versions of the gene that were the smallest possible versions capable of still maintaining their function. This is how, the idea of mini- and micro-dystrophins was born.

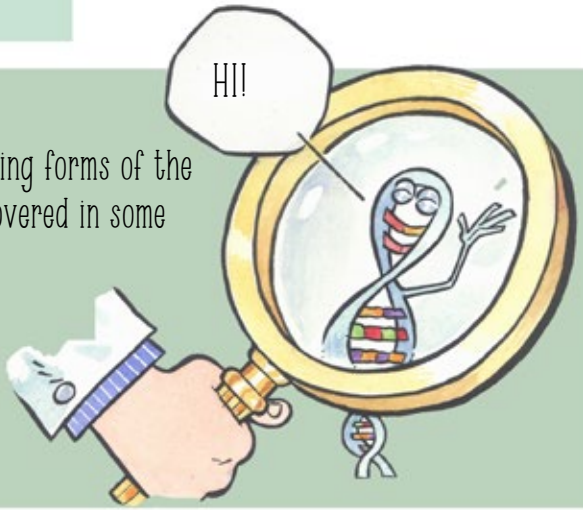
These versions of the gene are therefore obtained by removing regions less relevant for protein function and maintaining the essential regions. But watch out: although our "mini/microgene" was optimized, we cannot yet know the extent to which it will be able to perform its function... only time and clinical trials currently in progress will provide a full answer to this question.

The viral vector is ready...

... but the gene is TOO BIG!!!

Gene size must be reduced.

Unusually small but functional naturally occurring forms of the dystrophin gene were discovered in some BMD patients.
This is how the idea of mini- and micro-dystrophins was born.



The dystrophin gene is finally on board and ready to begin its journey:

WHERE DOES IT START FROM?

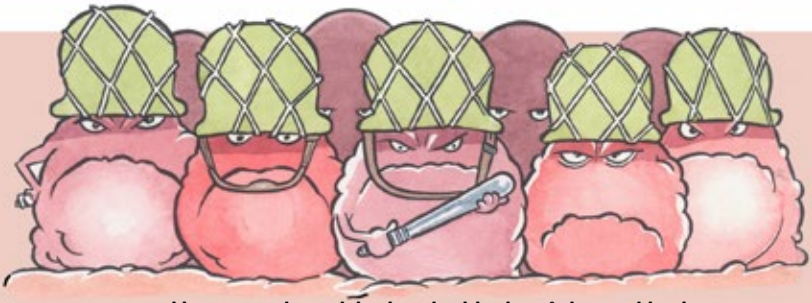
The best route to reach every muscle is through the bloodstream: the journey starts with an intravenous injection that moves vector and gene into the bloodstream. The vector entrance doesn't go unnoticed, though: it is, by all accounts, still an intruder, immediately recognized as a stranger by the immune system of the recipient. Some cells promptly activate to try to eliminate it, while others get organized to file its identity, creating specific antibodies ready to block any possible subsequent "attack".

This self-defense mechanism, so useful for our survival, can represent a big obstacle and risk for viral vector-mediated gene therapy. If a person has already developed antibodies for a specific virus, a gene therapy treatment with a vector derived from that virus will trigger a strong immune response that will not only elicit its immediate removal but may also pose some risks for the patient's health.

For this reason, not only can a specific gene therapy treatment be administered only once, but all those who previously came into contact with the same type of AAV virus cannot receive the treatment. This scenario may apply to approximately 20 to 50% of the population and can vary depending upon the specific vector used.

The journey begins: vector and gene are injected into the bloodstream, but their entrance doesn't go unnoticed...

Some cells of the immune system try to eliminate it...



... others create antibodies to block a future attack.
For this reason a specific gene therapy treatment can be administered only once...

... and all those who previously came into contact with the same type of AAV virus cannot receive the treatment.

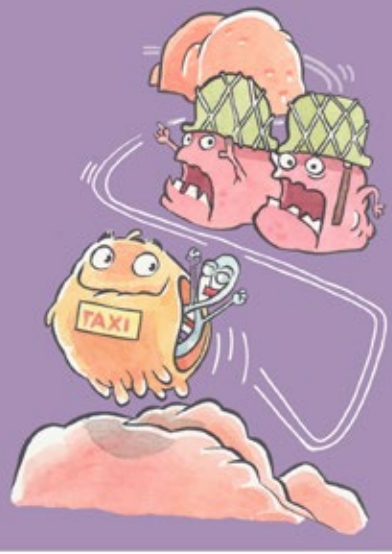
Overcoming various adventures in their journey, vector and gene reach the muscle cells where they eventually part ways. While our viral vector is demolished, the gene remains intact and free to place itself next to the cell DNA where it can finally start to work on its mission: producing the mini/micro dystrophin.

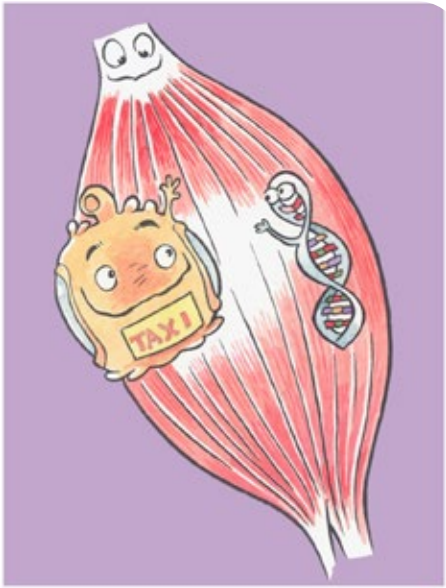
Beware though: How many vectors will actually overcome the challenges encountered in the journey and How many muscle cells will be reached?

To optimize the chances of success, as many muscle cells as possible must be reached. For this reason, the treatment involves the administration of very high levels of viral vectors, high enough to be as effective as possible while still being safe.

The issue of the necessary amount of vector introduces yet another challenge in the path of gene therapy for DMD.
Indeed, the technologies available today, make it possible to satisfy the production of the amount of viral vector required to carry out clinical studies in a few patients. However, in order to produce amounts suitable for a potential extended use, additional investment, resources and time are needed and are currently in the process of being addressed.

Overcoming various adventures in their journey...





... vector and gene reach the muscle cells and part ways.

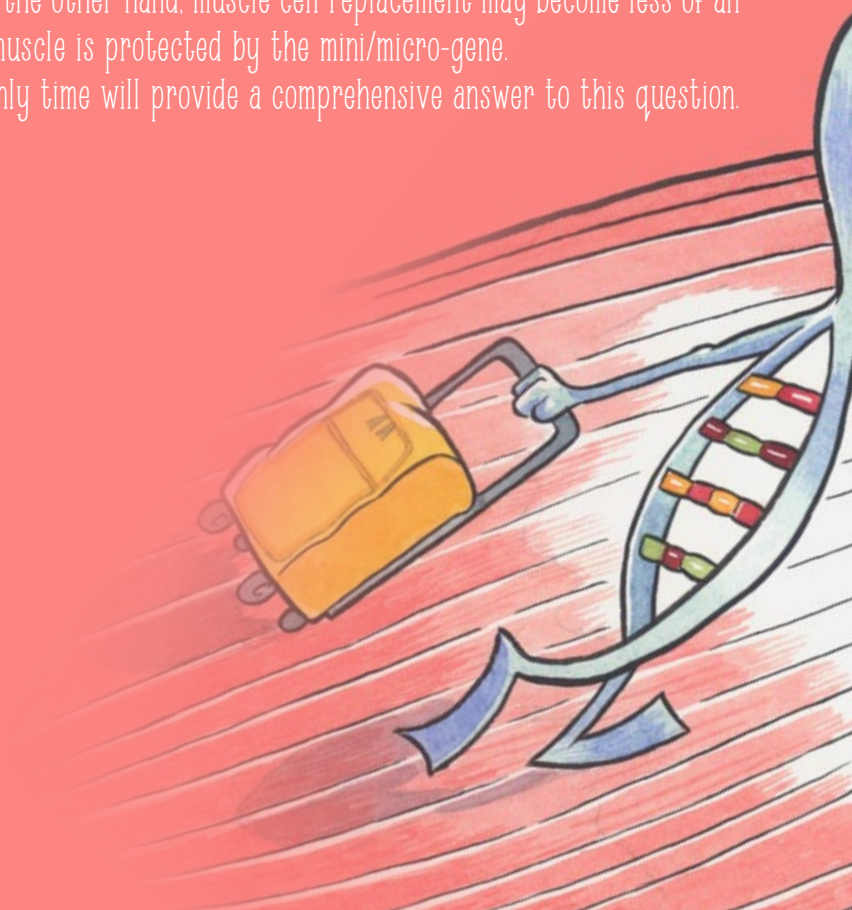
The gene places itself next to the cell DNA. where it can finally start to work on its mission.

There are still many unanswered questions

There is one further question and, again, only time will provide an answer:

How long will the therapeutic effect last?

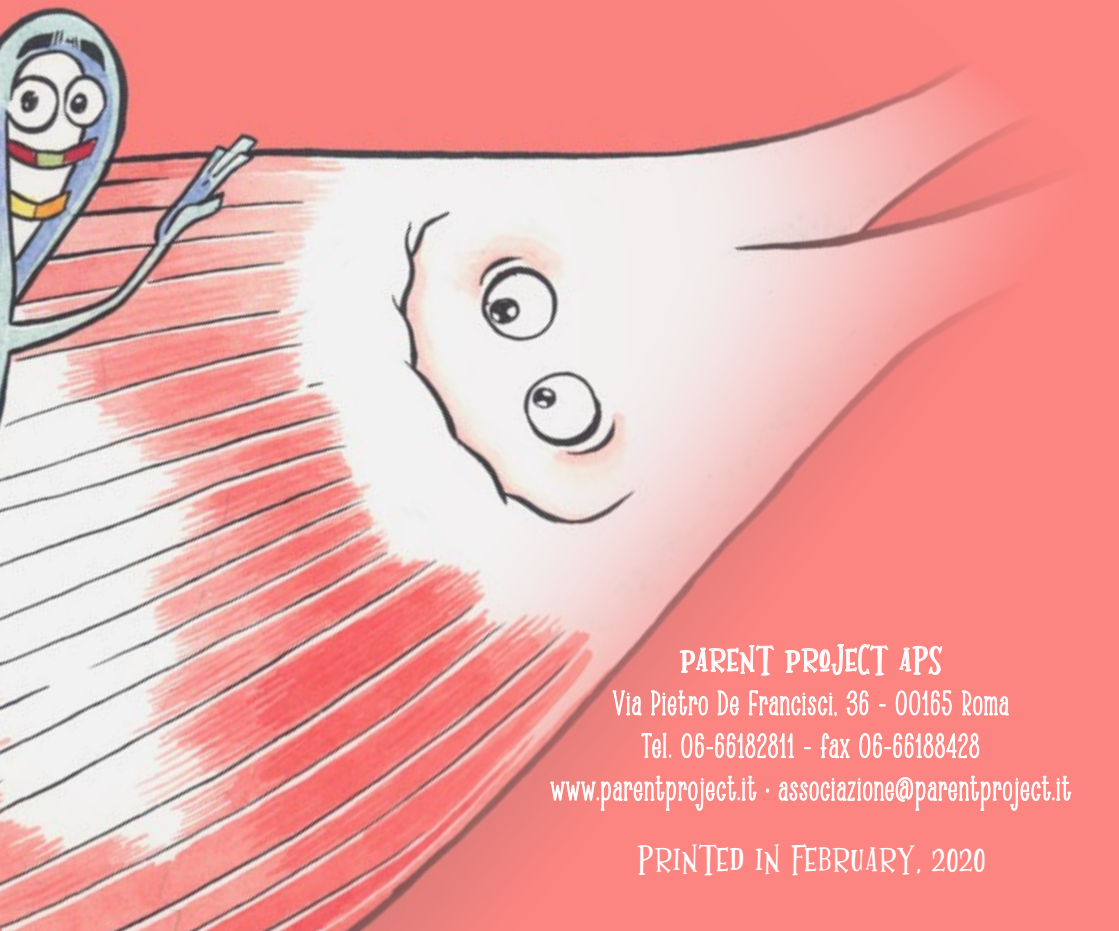
Since the transferred mini/microgene does not insert into cellular DNA, when the muscle grows or when it needs to be regenerated following a trauma, there is no possibility to also increase the quantity of mini/microdystrophin originally transferred. Over time, the muscle as a whole will thus contain proportionally smaller amounts of it. This would mean that the administration of gene therapy to younger patients, although convenient because it is aimed at protecting the muscles at a time when the damage is still not too severe, could exhaust its therapeutic potential as the patient grows up. On the other hand, muscle cell replacement may become less of an issue if the muscle is protected by the mini/micro-gene.
Eventually, only time will provide a comprehensive answer to this question.



Looking ahead

Expectations for gene therapy are very high and patient communities follow the progress of these programs with strong interest. However, it is important to bear in mind that we are still in the clinical trial phase and we don't have answers to all the questions.

Synergic collaboration between the scientific community and patient associations keeps moving forward so that, as recently happened for other rare diseases, gene therapy for Duchenne can also become a reality.



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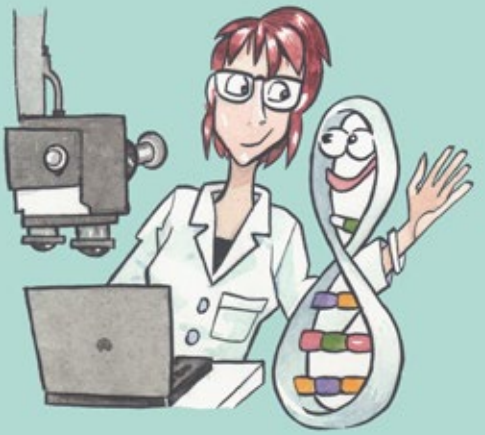

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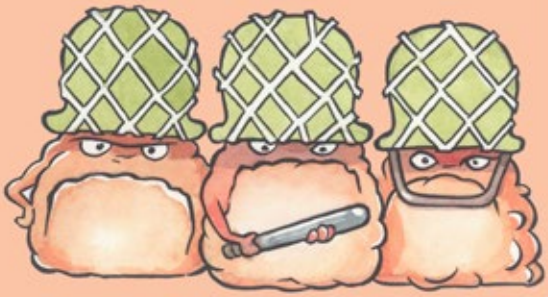

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