**Therapeutic approach design for Duchenne muscular dystrophy**

**Autologous ex vivo cell therapy and in vivo gene therapy**

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**INTRODUCTION**

Duchenne muscular dystrophy (DMD) is the most severe and common myopathy, affecting one in every 3,500 newborn males. DMD is a recessive lethal X-linked disorder caused by the absence of dystrophin in muscle fibers. The main function of dystrophin is to stabilize myofibers during muscle contractions. DMD is characterized by progressive muscle degeneration, which is replaced with adipose and fibrotic connective tissue (Fig. 1). Central nervous system (CNS) and bones are also affected. The clinical progression of DMD is reflected in Figure 2.

This disorder is caused by an alteration in the frameshift of the dystrophin gene whereby result in a premature stop codon, which could be generated by these three main categories (Fig. 3).

The size of the dystrophin gene is 2.4 Mb, the biggest human gene. It contains 79 exons that code for 448k mRNA and has 7 isoforms (Fig. 4).

For these reasons, the treatment of DMD with gene therapy is difficult and still a challenge nowadays.

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**STATE OF THE ART**

There are currently no curative treatments for this disease, but different ameliorating therapeutic approaches are being studied. Table 1 shows current treatments and approaches for DMD.

<table>
<thead>
<tr>
<th><strong>OTHER APPROACHES</strong></th>
<th><strong>PHARMACOLOGY</strong></th>
<th><strong>DYSTROPHIN RESTORATION APPROACHES</strong></th>
<th><strong>GENE THERAPY</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiotrophic (stem)</td>
<td>Urophil up regulators</td>
<td>Muscle strength with hypertrophy and hyperplasia</td>
<td>Antisense oligonucleotide</td>
</tr>
<tr>
<td>Function</td>
<td>Strangulated</td>
<td>Introduction of antisense oligonucleotide to continue the mRNA translation</td>
<td>Nucleases (ZFN, TALEN, CRISPR)</td>
</tr>
<tr>
<td>Problems</td>
<td>Strangulated</td>
<td>Easy skipping due to the interaction with splicing signal in pre-mRNA</td>
<td>Restoration of the dystrophin reading frame by micro-injection or micro-viruses.</td>
</tr>
<tr>
<td>Side effects such as</td>
<td>Strangulated</td>
<td>- Only used for preclinical murine’s patients (10-30%)</td>
<td>Difficulty to access to all target locations using an ex vivo approach.</td>
</tr>
<tr>
<td>weight gain and fat</td>
<td>Strangulated</td>
<td>- Continuous administration</td>
<td>- There are lots of strategies to tackle DMD, either using viral vectors or non-viral ones.</td>
</tr>
<tr>
<td>degeneration.</td>
<td>Strangulated</td>
<td>- Continuous administration</td>
<td>- It is under investigation.</td>
</tr>
</tbody>
</table>

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**THERAPEUTIC APPROACH DESIGN**

**Autologous ex vivo cell therapy**

- Patient cells → donor cells → immune response
- DMD patients have 
  - myocytes or satellite cells
- Somatic cells → iPSCs

**In vivo gene therapy**

- DMD patients’ cause of death: cardiopulmonary failure
- Target tissues: myocardiocytes (skeletal muscles) (differentiated cells)
- Minimal levels of functional dystrophin expression to ameliorate symptoms of DMD: 40-50%

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**IMMUNORELOGICAL REMARKS**

- If the patient has anti-AAV antibodies before treatment, he cannot be accepted for the therapy because the treatment is not going to be effective.
- Immunomodulatory have to be administered until patient eliminates viral capsid.

Depending on the genetic alteration, the immune systems of patients could react against new dystrophin (ex vivo cell therapy) or synthetic abbreviate dystrophin (in vivo gene therapy). For these reasons, some patients have to take immunosuppressants in the lifelong.

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**REFERENCES**

1. Pelinowski et al. Mol Ther 2011;19:830-40
5. Skates et al. Mol Ther 2010;18:523-32

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**MATERIALS AND METHODS**

In this project, the most recent publications related to DMD treatment and its therapeutic approaches, either researches or clinical trials were studied. Data has been obtained using the searching engine PubMed. The search was focused by means such as Duchenne, gene therapy and DMD. These articles were read and summarized.

Althowards, different strategies and methods were compared based on risks and benefits and the best ones were selected and explained.

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**CONCLUSIONS**

- Nowadays, it does not exist a clinical strategy which completely restores healthy phenotype in Duchenne patients.
- Nevertheless, an optimal therapeutic approach has been determined. This one ameliorate locomotor function and prolong life expectancy, like clinical characteristics of Becker muscular dystrophy patients. More studies in mice and canine (canine) models are needed to further develop this therapy.