**Introduction**

This poster is a Research Project Proposal for OTCD (Ornithine Transcarbamylase Deficiency) treatment by TALEN-induced Homologous Recombination.

OTCD, an X-linked recessive disease caused by a mutation in the Ornithine Transcarbamylase gene. It is an Urea Cycle disorder that results in hyperammonemia. Symptoms include vomiting, lethargy, seizures and ataxia, and can lead to coma and death.

**Chronogram**

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<tr>
<th>Year</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
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<tr>
<td>Trimester</td>
<td>1</td>
<td>2</td>
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**Phase 1 - Construction and Validation**

1.1 Plasmid generation

1.2 Hepatocyte isolation and culture

1.3 TALEN cleavage and OT validation

1.4 HR validation

**Phase 2 - Ex Vivo Treatment**

2.1 Partial hepatocyte

2.2 Hepatocyte transduction

2.3 Hepatocyte transplantation

2.4 Follow-up and improvement

**Phase 3 - In Vivo Treatment**

3.1 AAV production

3.2 AAV validation

3.3 AAV injection

3.4 Follow-up and improvement

**Methodology**

Transcription activator-like effector nucleases (TALENs) have emerged as an efficient tool for genome editing by introduction of chromosomal DNA DSBs.

One of their advantages is their total modularity and ease to engineer them to recognize virtually any sequence. A central domain of 33-35 amino acid tandem repeats determines their targeting specificity, through the binding of two variable residues at positions 12 and 13 (RVDs).

The basic idea of this project is the correction and improvement of the already inefficient splicing signal between Exon 4 and Intron 4 by the induction of DSB by TALENs.

**Conclusion**

OTC activity will be restored and controlled by the endogenous promoter and, as a prori, is not expected to be transient as homologous recombination is stable. AAVs and TALENs are both safe and shouldn't be of concern.

In order to ensure enough hepatocytes are corrected, this project proposes a selection method to select them in the ex vivo treatment.

The in vivo treatment might be less successful because it has not been done previously, but could provide proof of concept of in vivo TALEN-based homologous recombination using AAVs.

**Diffusion plan**

OTCD is an illness that has been attempted to be cured several times. This project could provide good results, which can be diffused in:

- Journal publications
- Conference proceedings
- Congresses and meetings

TALENs are a promising technology with a long-term future. Results from this project, and lessons learned in its development, could be used for conveying knowledge in the form of workshops.