TALEN-based Gene Correction for Ornithine Transcarbamylase Deficiency in Spf^{ash} Mice

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Introduction

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

OTCD, is 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 comma and death.

Hypothesis

1. In the Spf^{ash} mouse model, a **mutation in the splicing signal** between exon 4-intron 4 is responsible of the disease.
2. Treatment should be **administered at a perinatal stage**, as this is the period when it is more life-threatening.
3. Correction should be **persistent in time** and at least have **a 3-5% of WT OTC activity**.
4. Treatment should not trigger **immune response**.

Objectives

1. To correct and improve the splicing sequence by **TALEN-induced homologous recombination**.
2. To develop an ***ex vivo*** **treatment for adult** mice using autologous transplantation of corrected hepatocytes, in order to avoid immune reaction.
3. To develop an ***in vivo*** **therapy in neonatal** Spf^{ash} using AAVs, as partial hepatectomy is not feasible at neonatal stages.

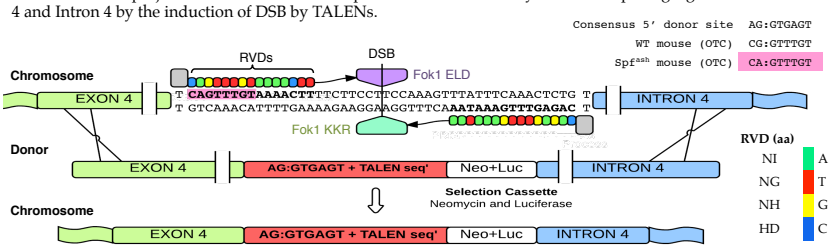
Chronogram

Year	2015				2016				2017			
Trimester	1	2	3	4	1	2	3	4	1	2	3	4
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 hepatectomy												
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).

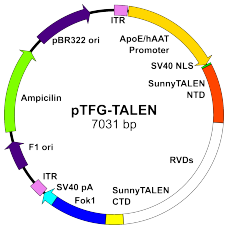
The basis of this project is the correction and improvement of the already inefficient splicing signal between Exon 4 and Intron 4 by the induction of DSB by TALENs.

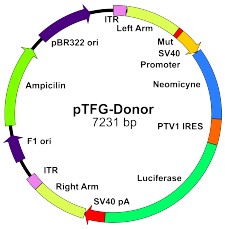


Phase 1 — Construction and Validation

1.1 Plasmid generation
RVDs will be assembled using the GoldenGate cloning system and then cloned into an ITR-flanked SunnyTALEN scaffold plasmid with heterodimeric FokI architecture. The ApoE/hAAT promoter is an strong, liver-specific promoter.

Donor vector Homology Arms will be cloned from WT mouse by genomic PCR, then ligated to a floxed selection cassette and cloned into a plasmid with ITRs.





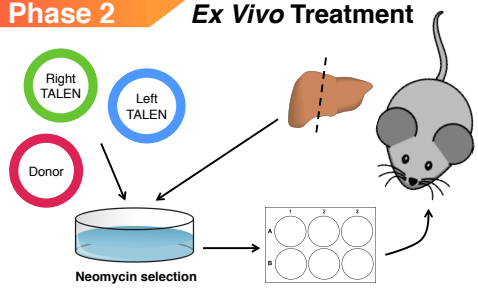
1.2 Hepatocyte isolation and culture
Hepatocytes will be isolated from adult WT and Spf^{ash} mice by hepatectomy and lobule digestion with collagenase.

1.3 TALEN cleavege and OT validation
TALENs plasmids will be transfected in pairs to cultured hepatocytes. DSBs will be corrected by NHEJ.
The surveyor nuclease assay will be carried on at 48 hours post-transfection to test the percentage of DSB of each pair.

Off-Target effects (OT) will be assessed with an Integration Defective Lentiviral Vector coding for GFP, which can be trapped in DSB by NHEJ. 48 hours post-transfection, GFP-positive cells will be selected by FACS and then analyzed by both classical and nonrestrictive LAM-PCR (nrLAM-PCR).

1.4) Homologous Recombination validation
TALENs and donor plasmids will be transfected to Spf^{ash} mice hepatocytes, cultured for 48 h and selected in bulk using neomycin. Then, they will be seeded at low density, cultured for 48 hours and screened for correct HR by PCR, using a primer that can only anneal in the chromosome and another one that can only anneal to the mutated TALEN's target sequence: only correctly edited cells will amplify.
Phenotype correction, ammonia metabolism, will be assessed using a commercial kit.

Phase 2 — *Ex Vivo* Treatment

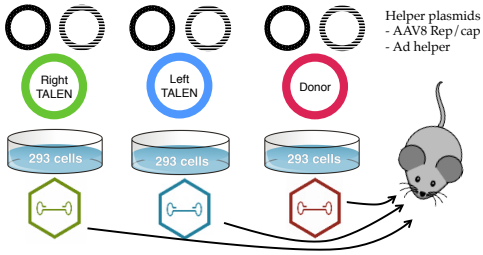


2.1) Partial hepatectomy
A 70% partial hepatectomy will be performed in adult (12 weeks) Spf^{ash} mice.

2.2) Hepatocyte transduction
Hepatocytes will be transfected in vitro with the TALENs and the donor plasmids, cultured for 48 hours and selected with neomycin.

2.3) Hepatocyte transplantation
Hepatocytes will be transfused no later than two weeks after partial hepatectomy, as total liver regeneration occurs within this time. Cells will be screened prior transfusion.

Phase 3 — *In Vivo* Treatment



3.1) AAV production
AAVs will be produced by triple transfection (calcium phosphate) of 293 cells. 3 days after transfection, cells will be lysate and AAVs purified with a cesium chloride gradient

3.2) AAV injection
All three AAVs (right and left TALEN and Donor) will be added to cultured hepatocytes. After 48 hours, correctly edited hepatocytes will be selected with neomycin. This stage could be useful for optimum MOI determination and relative proportion of each AAV.

3.3) AAV injection
Newborn Spf^{ash} mice (1.5 days) will be infected with all three AAVs by IP injection.

Follow-up and improvement

Orotic aciduria and plasma ammonia concentration will be analyzed once per week during the first two months, and then every two weeks. Mice will be challenged with the ammonia challenge 21 days post-injection and at 3 months. Behavior will also be scored.
Liver samples will be harvested at 1, 2, 3, 5, 7, 14 days, and once every three months. Samples will be used to test OTC activity and to analyze pattern of expression using immunohistochemistry.

Provisional results might be used for retrospective improvement of techniques and methodology (number of cells transfused, number of transfusions, viral genomes injected, among others), as well as assessment of new parameters (such as zonation expression OTC activity).

Future challenges

If these approaches are successful, then a humanized mouse model for OTC could be produced or treatment could be tested in human OTCD hepatocytes.

TALENs are a really new technology and there is scope for improvement. This project could help understand more about them and be the basis for future projects.

Conclusion

OTC activity will be restored and controlled by the endogenous promoter and, 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.

Cunningham, S. C. et al. AAV2/8-mediated correction of OTC deficiency is robust in adult but not neonatal Spf(ash) mice. Mol. Ther. **17**, 1340–6 (2009).
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