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Successful Correction of a Pathogenic Mutation Causing Lipoprotein Lipase Deficiency, Using a CRISPR/Cas9 Base Editing Therapeutic Approach

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Overview

- CRISPR/Cas9 base editors provide a novel approach to treat > 6000 known genetic diseases.[1]
- Safety and efficacy need rigorous testing and optimization
- We designed a proof-of-principle disease model to evaluate and optimize the *in vitro* and *in vivo* therapeutic potential of CRISPR/Cas9 base editing
- We successfully demonstrated efficient repair of a pathogenic LPLD mutation in HEK293 cells via base editing

Background

Lipoprotein Lipase Deficiency (LPLD)

Autosomal recessive genetic disease caused by mutation in *LPL* gene[3]

- LPL hydrolyses triglycerides in blood serum
- Patients with LPLD present extreme hypertriglyceridemia, chronic abdominal pain, hepatosplenomegaly and pancreatitis
- Childbearing strongly discouraged for female patients due to LPLD related complications

Fig. 2: Clinical Features of LPLD

Limited therapeutic options[4]

- Currently an extreme no-fat diet is the best therapeutic option, however in spite of strict adherence, hospitalizations due to LPLD related complications do continue
- Enzyme replacement therapies are not effective due to the short half-life of LPL in blood serum

Rationale for Gene Therapy

Dr. Ross and others developed an innovative gene therapy to treat LPLD, using AAV delivery of the function LPL gene → alipogene tiparvovec (Glybera®)[5-7]

- First gene-augmentation therapy to receive regulatory approval
- Limitations (including the high cost of the AAV production driving the pricing to over $1 million USD per treatment) led to its withdrawal from the market[8]

Hypothesis

We hypothesize that CRISPR/Cas9 base editing delivered using lipid nanoparticles can successfully repair the mutant *LPL* gene and demonstrate superior therapeutic benefit over the previous approach, by both circumventing the issues of dysregulated gene expressed and by allowing for repeat administration.[9,10,11,12]

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Background photo from: Haley Bennett, https://eic.rsc.org/feature/crispr-the-cutting-edge/2500411.article

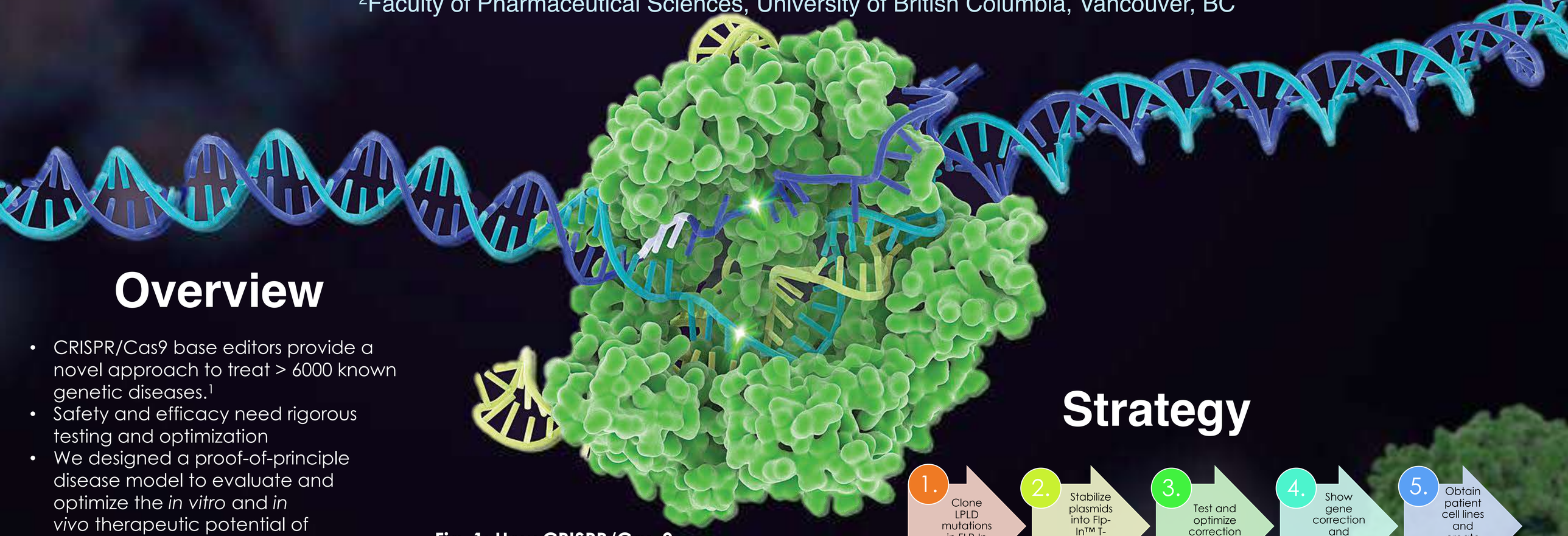
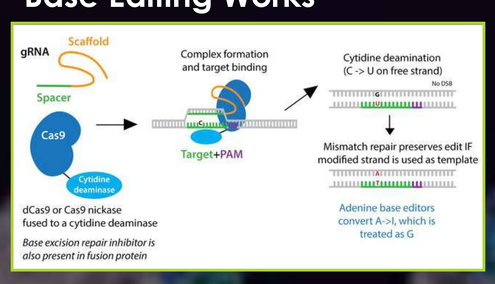


Fig. 1: How CRISPR/Cas-9 Base Editing Works[2]

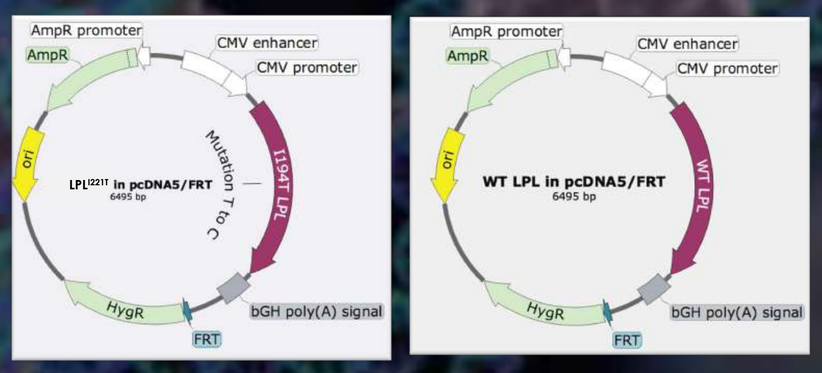


Strategy

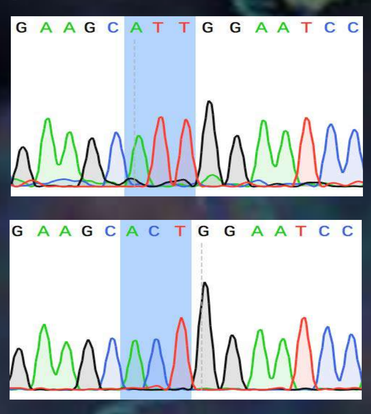
1. Clone LPLD mutations in FLP-In plasmids
2. Stabilize plasmids into Flp-In™ T-Rex™ 293 cells
3. Test and optimize correction efficiency
4. Show gene correction and functional restoration
5. Obtain patient cell lines and create mouse model

Results

1. **Figure 3. Schematic of Cloning of LPL expressing plasmids (mutant and wildtype)**



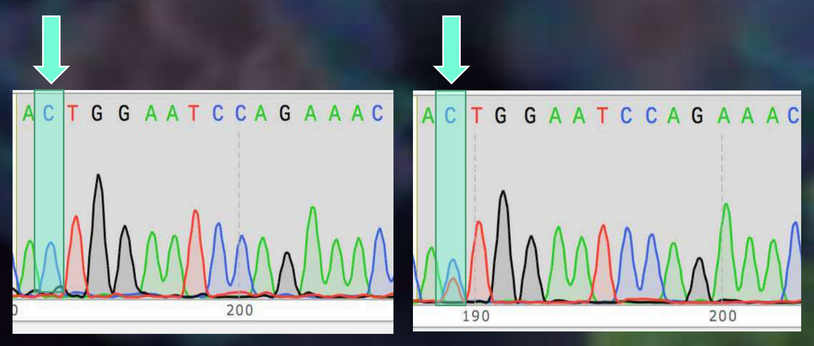
2. **Figure 4. Results of Stabilized LPL[I194T] expression in Flp-In™ T-Rex™ 293 cells**



3. Optimization: A variety of base editors were tested alongside commercially available gRNA formulations. Our results indicated that only Sakkh-BE3 plasmid (Addgene #85170) and sgRNA cloned onto a plasmid (Addgene #70709) worked efficiently for our target site. In addition, we identified cell toxicity induced by increasing amounts of Lipofectamine® 3000 as a key limitation.

4. **Figure 5. Rescue of wildtype variant using CRISPR/Cas9 Base Editing**

Transfection of base editors into Flp-In™ T-Rex™ LPL (mutant or wildtype) expressing HEK293 cells using Lipofectamine® 3000. Results via Sanger Sequencing.



I221T mutation: ACTGGAATCCAGAAACCAG
Wildtype sequence: ATTGGAATCCAGAAACCAG

Discussion

We initially tested mutation correction efficiency using a transient transfection approach. We saw very limited correction (<10%). We then moved into stabilized cell lines in order to ensure equal expression in both wildtype control and mutation cell lines. When transfected with base editor plasmids and the corresponding guides, our mutant cell lines showed superior correction than previously demonstrated. Using Sanger sequencing we have observed ~35% correction of the LPL[I221T] mutation. Due to the substantial change observed between the two experiments, we concluded that the stabilized cell lines were a better model to move forward with due to the ability to limit wildtype expression to a single stabilized copy.

5. ## Future Directions

These cell lines are the basis for a series of optimization experiments using lipid nanoparticle (LNP) delivery of Base Editing constructs. We are currently quantifying protein restoration using an LPL specific assay in conjunction with ELISA (data not shown). We are also obtaining both a patient cell line to demonstrate proof-of-principle in diseased cells as well as generating a mouse model using the I221T mutation as the basis. This model will enable us to test our base editing approach *in vivo* and optimize the best delivery approaches using LNPs.

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