1. Abstract

Cystic fibrosis is one of the rare diseases that affect the transmission of chloride ions due to the mutation on CFTR gene(cystic fibrosis transmembrane conductance regulator). In addition to the fact that there are nearly 2000 mutations have been identified on this gene, the most common mutation is F508del. On the other hand, G542X and R553X are Class I mutations which has no modulator treatments appropriate for them. Thus, it is investigated that R553X and G542X mutations can be corrected together with one pegRNA. Therefore, BeeWare focused on these 3 mutations. Utilizing the prime editing technique with the lipid
based delivery for editing 3 common mutations by plasmids that were designed by inserting 2 pegRNA's and Cas enzyme is planned for helping more people with cystic fibrosis. In order to actualize this gene therapy project; ex vivo, animal model and in vivo steps of the project are designed. For the cell line, fibroblast is selected for simplicity and low cost. The animal model of the experiment is determined as ferret because of CF ferret showing higher similarity with human cystic fibrosis symptoms. After these steps are done, the in vivo part, which is delivering the liposome bounded plasmids to human with a nebulizer will be performed. At the last stage of the experimental procedure, next generation sequencing and sanger sequencing are selected for checking the undesired mutations in corrected part of the genome and whole genome. Also, for detecting the presence and expression of CFTR protein in human, immunodetection with flow cytometry will be performed so that the efficiency and usefulness of the project will become more understandable.

2. Problem/Issue

- In vivo gene therapies have higher mutation risk comparing to the ex vivo ones and it might be a problem for our project.
- Cationic lipid may not be efficient as we wish, and the project might not be a lifesaving treatment.
- Ferret is a not usual, traditional model organism as yeast E.coli, mice or Arabidopsis. Thus, making an experiment with this organism maybe more expensive or the permission for doing an experiment on it may be a problem.
- Checking whole genome with Next Generation Sequencing is not cheap as whole people can utilize and pay it.

3. Solution

- We will apply our project first ex vivo with fibroblast cell line and work on ex vivo before the in vivo therapy then we will realize the therapy on animal model and make the therapy for human in vivo.
- If the lipid based technique would not show the required efficiency, we decided

to use the herpes viruses or as second possibility lentiviruses. Since size of herpes simplex virus is bigger than other vectors and also it can be used in epithelial cells; it is useful for in vivo therapy in cystic fibrosis. On the other hand, Lentiviruses have larger capacity comparing to AAV viruses and it also integrate themselves into the genome.

As we have a big plasmid carries 2 pegRNA's and additional sequences we can try to use lentiviruses.

- Instead of ferret, pig's CFTR amino acid sequence is also very similar with human (%92) but not show whole symptoms as good as ferret. However, we can choose pig for model organism, maybe the cost will become cheaper. If the pig would not show the required properties and efficiency, mice can be selected. As mice is not show the lung infection symptom, first we decided to knock out the healthy mice or silence the activity of mice's CFTR, and then apply our project on it.
- For Sanger Sequencing and Next Generation Sequencing more cheaper techniques can be found.