RARE DISEASE CHALLENGE
RaDiChal'21

FİNAL PRE REPORT

TEAM NAME

FMFORCE

TEAM MEMBERS

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TARGET DISEASE

FAMILIAN MEDITERRANEAN FEVER

TEAM LOGO

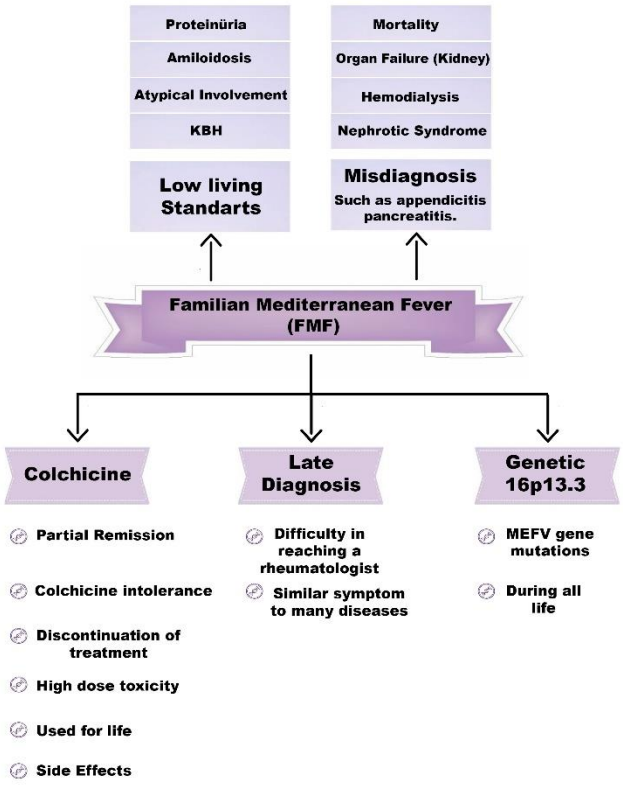
1. Project Summary(Project Description)

SUMMARY

FMF is a autosomal recessive genetic disease seen mostly in Mediterranean populations. FMF, which is the most commonauto inflammatory disease, is mostly seen in Turkey in the world. The main cause of Fmf is the non-functional production of pyrin protein due to single point mutations in the MEFV gene. In this study, we proposed a new treatment idea for FMF disease, which has no definite cure. Our project offers the promise of correcting single point mutations M680L, M680V, M680IGC, M680IGA, G687D, Y688C, Y688F, Y688X,M694V, M694L, M694K, M694I, K695R, K695N, V726A with the Prime Editing 2 system, thus covering 70% of FMF patients. Our study is designed as a study to transfer CD34+ HSCs obtained from the patient to the patient after proving tha tthey are regulated with PE2 system elements in vitroand produce functional pyrin. Transfer of PE2 elements will be done by neon transfection method. In addition, several systems with different pegRNA and electroporation parameters will be tried and the most efficient one will be found. After the in vitro cell studies, animal experiments will be started, and if it is positive, human clinical trials will be started.

Problem/Issue:

Familial Mediterranean Fever (FMF) is a genetic disease characterized by recurrent bouts of fever accompanied by abdominal, chest and joint pain and swelling (1). It is seen in Mediterranean societies such as Jews, Turks, Armenians and Arabs. FMF, which is the most common autoinflammatory disease, is most common in Turkey (1/400-1/1073)(2).



The MEFV gene located at 16p13.3 responsible for FMF disease is a gene consisting of 10 exons that encode the pyrin protein. Mutations in certain regions of the MEFV gene causen on-functional production or in complete expression of the pyrin protein. Various pyrin forms produced in patient swith FMF activity cause in appropriate triggering of neutrophil activation and uncontrolled release of interleukins (IL-1B, IL-18), causing inflammation episodes in the peritoneum, pleura and joints. The variation in clinical appearance and the development of amyloidosis may vary depending on MEFV mutations (3).

The most basic known treatment of FMF is colchicine. This drug is used to reduce the severity and frequency of attacks, in some cases to completely get rid of the attacks and to prevent the development of amyloidosis. Complete remission is seen in 60-65% of patients receiving colchicine treatment, 30-35% partial remission and resistance is seen in 5-10% of patients.

In other words, the effectiveness of colchicine varies from patient to patient, and colchicine cannot provide adequate treatment in some patients (4). In such cases, alternative treatment options such as Canakinumab, Kineret, and Rilanocept are used (5). Unfortunately, since FMF is a genetic disease, patient have toc ontinue the treatment for life. These treatments are temporary treatments that cannot fully cure the patient and aim to increase the quality of life. Symptoms that do not disappear completely due to insufficient treatment, attacks that are not known when, pain and fever prevent the patient from living a quality life.

Complication ssuch as amyloidosis, proteinuria, nephrotic syndrome, hemodialysis, atypical involvements and chronic kidney diseases may develop in patients with late diagnosis or in whom treatment does not work. Complications can even lead to organ failure and death.

In order to prevent all these complications and to treat FMF disease completely, a genetic treatment method is needed.

Solution

Our study consists of 3 main steps; Including in vitro HSC studies, animal trials and human clinical trials. The following steps will be followed for the study of editing single point mutations causing FMF disease.

1-) The patient who has been found to have the mutations we intend tocorrect; CD34+ hematopoietic stem cells from peripheral blood will be isolated by stem cell apheresis.

2-) It will be reproduced with the isolated cells.

3-) Elements specific to the target mutations (pegRNAand Cas9) of the Prime Editing 2 system will be prepared.There are multiple mutations targets at this stage. For this reason, pegRNA designs with different lengths and different mutation targets will be tried and the most efficient one will be preferred.

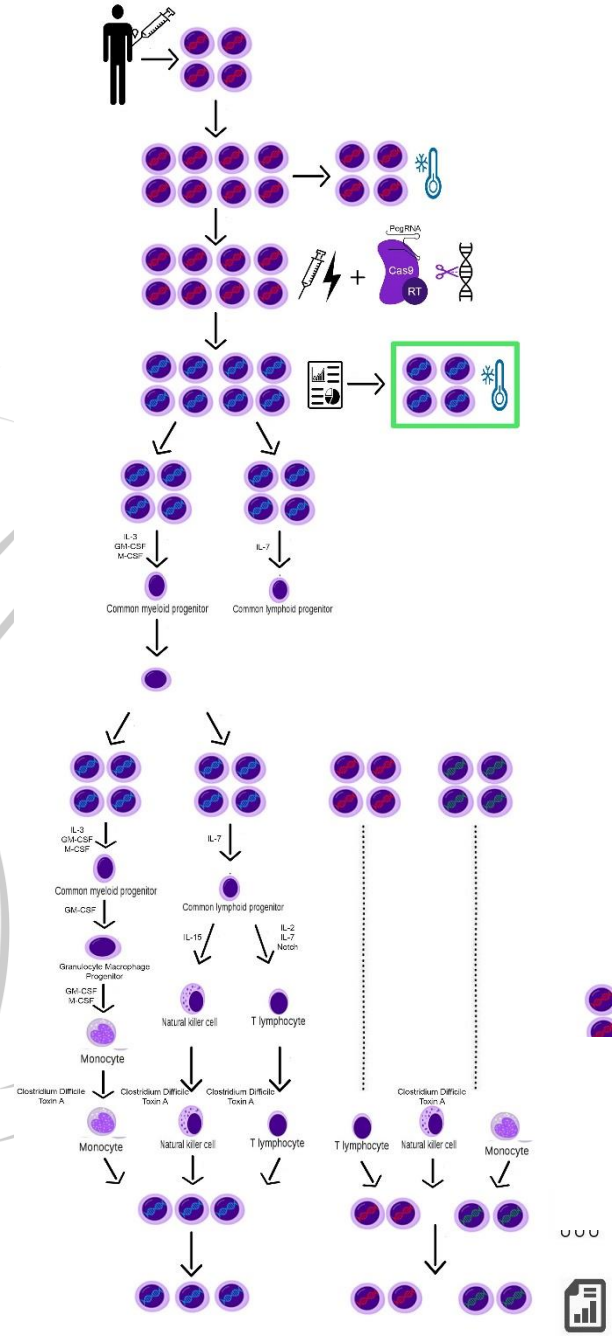
4-) The prepared PE2 elements will be added to there suspended CD34+ hematopoietic stem cells and they will be allowed to enter the cells through the Neon Transfection System. At this stage, the efficiency of the system with 9 different electroporation parameters will be evaluated in order to increase the transfer efficiency and reduce cell death.

5-) The sequences of the cells that have been rearranged will be extracted by the Sanger sequencing method, and the results will be analyzed.
We will continue with the cells that have proven to be successful in editing, and some will be reserved for later use.

6-) CD34+ hematopoietic stem cells will be differentiated into monocyte, T lymphocyte and natural killer cells by giving the necessary cytokines. Differentiating cells will be stimulated with toxin A.

7-) After stimulation, pyrin will be isolated from pyrin producing cells, its functionality and amount will be analyzedby ELISA and Werstern Blot methods.

8-) Cells that have been proven to be successful in editing will be transferred to the patient again.



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