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Elena Ravano

UFFI supporters

Summary of project presentation March 21st 2023

Dear Elena Ravano,

Dear UFFI supporters,

On March 21st 2023 we had a joint video conference to discuss the current state of the project supported by UFFI:

Development of an enzyme replacement therapy of transglutaminase-1 deficient ARCI

Recently, you asked us to provide the essential slides of this video conference and a short summary so that this material can be posted on the UFFI homepage or other UFFI related social media. Therefore, we now give a short summary explaining the slides.

The first slides introduce the members of the two teams that are now involved in the project in the university of Münster, Germany and previous collaborators. At the department of dermatology these are Dr. Henning Wiegmann, a molecular biologist, Dr. Kira Süßmuth, a dermatologist who just moved on May 1st this year to the department of Dermatology of Helios Klinikum Berlin Buch / Medical School Berlin, and Professor Heiko Traupe, a dermatologist who is now affiliated as a retired guest scientist with the university hospital in Münster. At the Institute of Pharmaceutical Technology and Biopharmacy (IPTB) Professor Klaus Langer and Mr. Kris Paul are involved. Professor Langer is a pharmacist and head of the IPTB and Mr. Kris Paul holds a master´s degree in Pharmaceutical Sciences. After he finished his master thesis in the topic of developing a formulation with the transglutaminase 1 (TG1), he further works on his PhD thesis for the project **(slide 3)**. The project is mostly supported by financial means from UFFI and also receives some additional financial support from the German patient organization “Selbsthilfe Ichthyosis” and from the Dutch patient organization for ichthyosis.

In previous work we had also a longstanding cooperation with Dr. Margitta Dathe in Berlin from the Leibniz Institute of Molecular Pharmacology (FMP) who helped with cutaneous delivery of TG1 using liposomes until 2021. She has now retired and can no longer assist us in this project. We are very grateful for her tremendous help in the past. We also have a cooperation with Dr. Fernando Larcher, who is a molecular biologist and works at the "Centro Investigaciones Energéticas, Medioambientales y Tecnológicas" (CIEMAT) in Madrid. He runs a lab that is specialized in doing experiments with immunodeficient mice that received transplants from human cell cultures having TG1 deficient ARCI. If the transplants are successful these mice then recapitulate the human disease and the human grafts can be treated **(slide 3)**.

The next slide shows a schematic picture of the healthy epidermis and how it produces a stratum corneum (horny layer). This development process is called "terminal differentiation" and for TG1 deficient ARCI we want to achieve a normal epidermis **(slide 4)**.

The next two slides refer to previous work done in cooperation with the lab of Dr. Dathe and Dr. Larcher **(slides 5 and 6)**. In Madrid the mouse model with transplants from ichthyosis cell cultures (skin equivalents) were produced. Two grafted mice were treated with empty liposomes lacking TG1, two animals were treated with liposomes containing 200 ng TG1 and two animals were treated with a higher dosage of 800 ng TG1. Only one of these two latter animals showed a partial effect. This is far less than we had expected **(slide 5)**. After obtaining these unexpected results our first reaction was to repeat the experiment with a higher dosage and a longer treatment period for example four weeks instead of two weeks. Unfortunately, this was not possible as the mouse model in Madrid turned out to be unstable. Therefore, in the immediate future we want to use human 3D skin models with keratinocytes from patients with TG1 deficient ARCI as an alternative **(slide 6)**.

Due to the challenging results we intensified our cooperation with the lab of Professor Langer **(slide 7)**. In his master thesis Mr. Paul in the Langer lab had already identified protein instability of TG1 as a major problem that could explain the unexpected results of the mouse experiments. The next slide discusses what TG1 instability actually means and that we are faced with two aspects. One is aggregation of the protein which is a well known challenge in the pharmaceutical industry. It prevents enzyme activity and complicates to define the true dosage applied. The second aspect is that TG1 disintegrates into subunits which is a physiological process that even increases enzyme activity, but when occurring we expect, it makes it impossible to direct the enzyme to its site of action that is binding with its anchoring domain to the inner side of the cell membrane of the keratinocytes **(slide 8)**.

In **slide 9** we describe the current project plan. Currently Mr. Paul is focusing on the topics of enzyme production and improving protein stability. **Slide 10** summarizes what has already been achieved in the last year. In particular we succeeded in developing a bacterial expression system that is cheap and allows the production of the entire protein and its subunits. **Slide 11** summarizes the challenges relating to protein stability. We are working on identifying the perfect environment for the protein for its cutaneous delivery in a stable form. **Slide 12** summarizes the time table (chronogram) of the project. The financial means of the current project will support it until spring 2025. We hope to overcome protein instability and to develop a hydrogel and possibly even reach the point where we ideally can show a positive effect in human cell cultures at the end of the current project. Then we will provide a report. If this report and the results obtained by that time are favorable, it makes sense to continue the project and

to put the focus once more on mouse model studies. For the pharmaceutical industry convincing mouse model studies will be a key issue for their decision to adopt the project and to take it over. In the discussion of the videoconference Professor Gianluca Tadini from Milano pointed out that our actual problems with the ichthyosis grafts in the current mouse model may relate to the fact that the human cell cultures used for the transplantation were rather old (on average more than ten years) and that fresh skin biopsies and fresh cell cultures may be needed if one wants to use this model system.

Summary

Actually we had a certain drawback in the experiments with the mouse model that right now no longer is stable and therefore did not allow to test the whole range of dosage and treatment periods with the TG1 formulation. The project now has the focus on the inexpensive production of TG1 in a bacterial system and on improving protein stability. A further aim is to develop a hydrogel and ideally to test this in cell cultures/ three dimensional skin equivalents. If this is successful, it will make sense to start a follow-up project dealing with a renewed mouse model in order to gain convincing data for the pharmaceutical industry to take over further drug development.

We thank all UFFI supporters for their cooperation and support!

Heiko Traupe

Klaus Langer

Kira Süßmuth

Kris Paul

Henning Wiegmann