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PRINCIPAL INVESTIGATOR: Markus Grompe

CONTRACTING ORGANIZATION: Oregon Health & Science University

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6. AUTHOR(S)
Markus Grompe

E-Mail: grompem@ohsu.edu

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Oregon Health & Science
University
3181 SW Sam Jackson Park Rd
Mailcode: L106OPAM
Portland, OR 97239-3098

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This award pertains to the treatment of the inherited bone marrow failure syndrome Fanconi's Anemia. Specifically, the commonly used diabetes drug metformin will be tested by itself and in combination with the current standard of care, anabolic steroids.

15. SUBJECT TERMS

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1. Introduction

This award pertains to the treatment of the inherited bone marrow failure syndrome Fanconi's Anemia. Specifically, the commonly used diabetes drug metformin will be tested by itself and in combination with the current standard of care, anabolic steroids.

2. Keywords

Fanconi Anemia, bone marrow failure, treatment, drug, small molecule, DNA damage, metformin

3. Accomplishments

Major Goals:

Specific Aim 1: To evaluate metformin for the treatment of FA
Metformin is a biguanide drug widely used for the treatment of type 2 diabetes. In humans, it is known to have cancer chemoprevention properties. We found that MET enhances hematopoiesis specifically in *Fancd2*-/- mice, but how it does so is unclear. Here, we will determine whether MET acts via AMPK activation or as an aldehyde scavenger. We will ascertain whether it can prevent inflammation-induced anemia and whether it is beneficial when BMF has already started.

Specific Aim 2: To evaluate the combination of anabolic androgens with metformin for the treatment of FA.
Many FA patients respond to androgens by significant improvement of their blood counts. We now understand how androgens work and predict that there is no overlap with the mechanism of action of MET. Androgen therapy does not cure FA and we will therefore determine whether the combination of oxymetholone with metformin can synergistically improve hematopoiesis in FA.

Goals achieved:
Substantial progress was made on both Aims, although the work has not yet been completed. However, we have NIH funding through 2021 for this project and this will enable us to fully complete the work in 2019.

Aim 1:

Anemia reversal: To address whether metformin can reverse already existing anemia, we generated a cohort of Fancd2 mutant mice to be aged until they display bone marrow failure. We plan to determine whether metformin can reverse already established anemia. Anemia developments takes 12-18 months from birth and hence the metformin treatment will start at that time, which will be this year (2019).
Importantly, we helped design a clinical trial in human patients with Fanconi anemia and early bone marrow failure. The clinical trial has opened at Boston Childrens hospital. Six patients have been enrolled and are being treated with metformin. Neutrophil counts have improved in 3/6 patients, but it is too early to determine whether this result is statistically significant. The study was based on preclinical data from my laboratory and we are receiving credit for our contribution in the form of co-authorship. DoD funding will be acknowledged when this work is published.

Aldehyde scavenger hypothesis: We found that Fancd2/Aldh2 double mutant mice did not develop bone marrow failure and hence were not suitable for intervention studies. We therefore generated Fanca/Adh5 double knockout mice on the 129Sv background. These animals accumulate formaldehyde in their tissues and become anemic spontaneously by only 4 weeks of age and will be treated with metformin to determine whether the compound can block formaldehyde induced DNA damage. In addition, we generated human Fanconi cell lines deficient in Adh5 to perform in vitro experiments in parallel. Now that the required cell and animal reagents are finally in hand, we expect to complete the experiments this year.

Aim 2:

Metformin + androgen combination: The proposed work is nearly complete and we will have definitive results this year. It took us 18 months to generate the required mice. In total, 8 cohorts of 30 mice each were generated. Half the animals (120) are Fancd-/- mutant and half (120) are wild-type littermate controls. Each genotype is receiving four different kinds of treatment: 1) Oxymetholone + metformin; 2) Metformin alone; 3) Oxymetholone alone and 4) controls, receiving placebo (no drug). All cohorts recently reached the 12 month treatment timepoint. Peripheral blood counts show no adverse effects of the combination of metformin and oxymetholone. Half the animals (16 from each group) have been sacrificed and subjected to the final analyses proposed in our application: CFU-S assay, FACS analysis of bone marrow stem cell number and cell cycle status. In addition, the extent of DNA damage will measured using histological stains on relevant tissues. The experiments have been done, but the detailed statistical analysis of this large data set is not yet complete. We expect the data analysis to be complete in one month. This will give us the first look at the effects of combining the current standard of care (androgens) and metformin. If no adverse effects of the combination is seen in mice our collaborators in Boston plan to open the metformin trial to patients that are currently on androgen therapy. Currently, androgen treatment is an exclusion criterium.

Equivalence of danazole and oxymetholone: Many human FA patients are receiving danazole instead of oxymetholone. We therefore thought it important to directly compare the hematopoietic effects of oxymetholone and danazole. FA mice were treated with either oxymetholone or danazole for 3 months. The mice were sacrificed and the cell cycle status of hematopoietic stem cells was ascertained by FACS analysis. Oxymetholone led to increased HSC cycling as previously reported and importantly, danazole had the same effect. We conclude that danazole and oxymetholone are interchangeable in terms of their effects on hematopoietic stem cells.

Summary: Due to the difficulty of generating the required numbers of FA mice, it has taken significantly longer than anticipated to fully populate our proposed in vivo studies. However, we have the resources to complete the work and the results will be very important for human clinical trials. Already our experiments have informed one human clinical trial, currently ongoing in Boston. We anticipate that the completion of Aim 2 will lead to a second clinical trial, combining androgens and

Training opportunities:
Nothing to report.

Results dissemination:

We have published several abstracts, but full-sized publications.

4. Impact

Principal discipline:
Metformin has never been considered for the treatment of bone marrow failure before. Our preclinical data in mice are sufficiently compelling that a clinical trial of children with Fanconi Anemia with metformin is being implemented at Boston Childrens Hospital. The study opened in the summer of 2018.

Other disciplines:
Although hundreds of papers have been written about metformin and although many cancer prevention clinical trials are ongoing, metformin has never before been reported to block DNA damage and enhance genome integrity. The aldehyde scavenging effects of metformin have also not been previously reported. We believe that our findings provide a potential mechanism for the cancer prevention effects of metformin. This is a novel paradigm in the field.

Technology transfer:
Nothing to report.

Society:
Nothing to report.

5. Changes

Nothing to report.
We are happy to report that our experimental plans are coming off without a hitch and are on time.

6. Products

Presentations:

Spanish Fanconi Anemia Society Meeting, Madrid, Spain, June 2017
“Small Molecule Therapy of Fanconi Anemia”.
Author: M. Grompe

Fanconi Anemia Research Foundation Annual meeting, 2018
“Hyperactive TGF-β pathway signaling is required for viable gestation during the development of Fanconi anemia mice”.
Authors: Alfredo Rodríguez, Chunyu Yang, Michael Epperly, Larissa Sambel, **Markus Grompe**, Kalindi Parmar, Joel Greenberger and Alan D’Andrea

Fanconi Anemia Research Foundation Annual meeting, 2018
“TGF-β pathway inhibition rescues clonogenic growth of primary bone marrow from Fanconi anemia patients”
Authors: Alfredo Rodríguez, Benilde García de Teresa, Elissa Furutani, Melissa Ruiz-Gutierrez, Patricia Flores, Silvia Sánchez, Chunyu Yang, Larissa Sambel, Markus Grompe, Akiko Shimamura, Sara Frías, Kalindi Parmar and Alan D’Andrea

North American Pediatric Aplastic Anemia Consortium (NAPAAC), 2018 Spring Meeting
"Pilot Study of Metformin in Patients with Fanconi Anemia"
Authors: Elissa Furutani, MD1; Erica Esrick, Edie Weller, Maggie Malsch, Ashley Kuniholm, **Qingshuo Zhang**, **Markus Grompe**, Alan D'Andrea, David Williams, Shimamura, Akiko

Web-sites:
Nothing to report

Technologies:
Nothing to report

Inventions/patents:
Nothing to report

7. Participants and other collaborating organizations.

Individuals on the project during entire award period

Name	*Markus Grompe, M.D.*
Project Role	*Principal investigator*
Researcher ID	*0000-0002-6616-4345*
Person month worked	*2*
Contribution	*Overall experimental design. Oversight of project personnel; communication with funding agencies; manuscript writing.*
Funding support	
Name	*Qingshuo Zhang, Ph.D.*
Project Role	*Senior Research Associate*
Researcher ID	
Person month worked	*17*
Contribution	*Dr. Zhang is the project leader in the lab; He designs most experiments (in collaboration with Dr. Grompe), performs experimentation himself, collects data and oversees the research assistants working on the project.*
Funding support	
Name	*Leslie Wakefield*
Project Role	*Research Assistant*
Researcher ID	
Person month worked	*6*
Contribution	*Ms. Wakefield is in charge of our mouse animal colony (breeding, genotyping etc.) and assists Dr. Zhang with hands on experimentation.*
Funding support	

Name	*Carolyn Loughran*
Project Role	*Student worker*
Researcher ID	
Person month worked	*1*
Contribution	*Prepared chemical solutions, DNA isolation, plasmid preps*
Funding support	
Name	*Sean C. Nygaard*
Project Role	*Research Associate*
Researcher ID	
Person month worked	*2*
Contribution	*Laboratory manager*
Funding support	

Changes:
Nothing to report

Other organizations:
Nothing to report

8. Special Reporting Requirements

Nothing to report.

9. Appendices

Nothing to report.