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TITLE: The Effect of MEK Inhibitor Treatment on Metabolism in Neurofibromatosis Type 1

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Patients with NF1 are predisposed to the development of plexiform neurofibromas (pNF) and also have other defining clinical features including those related to growth and energy metabolism, such as short stature, low weight, and decreased bone density--findings that are often more prominent in patients with high pNF burden. Interestingly, this phenotype changes in patients who are on Mek-inhibitor treatment. However, the mechanism for this metabolic phenotype in patients with NF1 and its association with pNF tumor burden and change with MEK- inhibitor treatment is not currently understood. In this proposal, we will perform metabolic and metabolomic analyses on NF1-deficient mice that develop plexiform neurofibromas and patients with NF1 who do or do not have plexiform neurofibromas. The effect of Mek-inhibitor treatment on metabolism in NF1 will also be explored. All regulatory approvals have been acquired, and the patient and mouse studies have been initiated.

15. SUBJECT TERMS

Neurofibromatosis Type 1, Metabolism, Plexiform Neurofibroma

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1. Introduction:

Neurofibromatosis Type 1 (NF1) is a neurocutaneous syndrome caused by genetic inactivation of one copy of the tumor suppressor *NF1,* resulting in activation of the Raf/MEK/ERK (MAPK) pathway. Patients with NF1 are predisposed to the development of plexiform neurofibromas (pNF) and also have other defining clinical features including those related to growth and energy metabolism, such as short stature, low weight, and decreased bone density--findings that are often more prominent in patients with high pNF burden. Indirect calorimetry (IC) of adults with NF1 reveals findings consistent with underfeeding (i.e. increased lipid oxidation) at baseline, despite having normal diets. The mechanism for this metabolic phenotype in patients with NF1 and its association with pNF tumor burden is not currently understood. We plan to address these gaps in this study. We hypothesize that germline loss of Nf1 promotes a pro-catabolic state resulting in increased lipid oxidation, and that MEKi treatment reverses this phenotype, promoting anabolic metabolism and weight gain. In addition, we hypothesize that MEKi treatment of pNF alters tumor metabolism, leading to specific tumor and plasma metabolomic signatures containing candidate pharmacodynamics markers of treatment response for future development. In order to test this hypothesis, we will have three Aims. **Aim 1: To establish that MEKi treatment alters clinical metabolic parameters in a pNF mouse model.** Using the *Nf1flox/flox*; *Postn*-cre pNF mouse model, we will examine acute (cohort 1; 4 weeks) and chronic (cohort 2; 8 weeks) effects of MEKi (compared to vehicle) treatment on clinical metabolic parameters in NF1-deficient (cre+) and wildtype (cre -) mice, using weight as a primary outcome and energy expenditure (IC) as an exploratory outcome. **Aim 2: To determine the effects of MEKi treatment on global and pNF metabolomic signatures in a pNF mouse model.** To examine the effect of MEKi on metabolites in NF1, global metabolomic profiling of plasma, nerve (pNF), and liver tissue will be performed on acute and chronic MEKi-treated mice compared to vehicle. Histologic analysis of pNF will be performed for Cohort 2 mice to correlate metabolomics signature with treatment response. We will also explore the effect of MEKi on microbiome-related metabolites by collecting stool from cohort 2 mice. **Aim 3**: **To identify the effects of MEKi treatment on metabolic and metabolomic profiles in patients with NF1.** Using a similar strategy as the mice, we will study the effects of standard of care MEKi treatment on metabolic (weight and IC), metabolomic (plasma) and microbiome (stool) profiles in patients with NF1 and clinically significant pNF (NF1 patients with low/no pNF as controls), and perform a global analysis of all mouse/patient data to detect shared metabolic signatures.

2. Keywords

Neurofibromatosis Type 1, Metabolism, Mek-inhibitor

3. Accomplishments

a. Major tasks of the project – completed

Major Task 1: Collect weight data from pNF mice

Subtask 1: Submit documents for IACUC approval (CNH and JHU- completed)

Subtask 2: Submit IACUC approval and documentation to ACURO for approval (CNH-completed)

Milestone 1: IACUC and ACURO approval received (goal month 6)

- CNH IACUC approved 8/9/2021
- CNH ACURO approval 12/2/2021
- JHU (subcontract) IACUC approval 7/19/2019

- JHU ACURO approval 2/4/2022

Major Task 5: Obtain Metabolomic parameters and samples for metabolomic and microbiome studies

Subtask 1: Submit documents for local IRB review (Months 1-4) – Completed

Subtask 2: Submit IRB approval and necessary documents for HRPO review

Subtask 3: Establish data transfer agreement between CNH and GWU and CNH and JHU

- No human data will be exchanged between CNH and JHU, thus DTA was determined not to be necessary
- De-identified data transfer to GWU was approved by the IRB 6/27/2021

Milestone 7: HRPO approval received

- IRB approval 6/27/2021
- HRPO approval 11/3/2021

b. Major tasks of the project - ongoing

Major Task 1: Collect weight data from pNF mice

Subtask 3 (Cohort 1- JHU): Collect weight data (Month 8-18)

- Progress: Breeding cages have been set up to generate the cohort of mice to be used in these experiments

Subtask 4 (Cohort 2- CNH): Collect weight data (Month 8-18)

- Progress: Mice were obtained from JHU through an MTA to ensure all experiments are done on mice with the same genetic background, breeding cages have been set up to generate the cohort of mice to be used in these experiments

Major Task 2: Collect Energy Expenditure Data from pNF mice (Month 12-18)

Subtask 1 (Cohort 1- JHU) (Month 12-18)

- Progress: Experiment scheduled for either December 2022 or January 2023

Major Task 3: Collect plasma and tissue samples from pNF mice to send for metabolomic analysis

Subtask 1 (Cohort 1- JHU): Collect plasma and tissue samples following acute tx (Month 14-18)

- Progress: Pending

Subtask 2 (Cohort 2- CHMC): Collect plasma and tissue samples from untreated baseline and following chronic treatment (Month 14-18)

- Progress: Pending

Subtask 3: Submit mouse plasma and tissue samples for metabolomics analysis (Month 20)

- Progress: Pending

Milestone 2: Obtain metabolomic data from pNF tumor mice (Month 22)

- Progress: Pending

Subtask 4: Perform analysis of metabolomic data from pNF tumor mice (Month 22-24)

- Progress: Pending

Major Task 4: Collect stool samples from pNF mice to send for microbioma analysis (Month 12-20)

Subtask 1 (Cohort 2- CNH): Collect samples for microbiome studies using the treated (MEKi or Veh) mice (Month 12-20)

- Progress: Pending

Subtask 2: Submit mouse stool samples for microbiome analysis (Month 12-20)

- Progress: Pending

Milestone 3: Obtain microbiome data from pNF tumor mice (Month 14-22)

- Progress: Pending

Subtask 3: Perform Global analysis of initial metabolomic and microbiome pNF mouse studies (Month 22-26)

- Progress: Pending

Milestone 4: Submit abstract to national meeting with preliminary mouse metabolomic/microbiome data (Month 22-26)

- Progress: Pending

Milestone 5: Co-author manuscript on paired omics functional integration analysis of microbiome and metabolomic data in mice (Month 24-26)

- Progress: Pending

Milestone 6: Submit collaborative grant with co-Is for additional funding to support analysis of additional tissue sources (i.e. muscle, heart, brain) (Month 22-28)

- Progress: Pending

Major Task 5: Obtain Metabolomic parameters and samples for metabolomic and microbiome studies

Subtask 4: Recruit, consent and enroll 69 patients/human subjects to the pre-clinical study (Month 9-33)

- Progress: Enrollment was delayed as we were advised not to enroll patients on "non-therapeutic trials" between December and February due to new Covid outbreak in the US; enrollment started in April 2022 and 5 patients have been enrolled

Subtask 5: Submit microbiome specimens for analysis (Month 9-33)

- Progress: Pending

Milestone 8: Obtain data for patient microbiome studies (Month 9-33)

- Progress: Pending

Subtask 6: Submit plasma samples from patients (stored in -80C) for metabolomic analysis (Month 33)

- Progress: Pending

Milestone 9: Obtain data for patient metabolomic studies (Month 35)

- Progress: Pending

Subtask 7: Begin global analysis of patient and mouse microbiome and metabolomic data (Month 33-36)

- Progress: Pending

Milestone 10: Submit abstract to national meeting with mouse/patient data incorporating metabolic, microbiome and metabolomic data (Month 32-36)

- Progress: Pending

Milestone 11: Begin manuscript on global analysis of metabolism in mouse/patient incorporating metabolic, microbiome and metabolomic data (Month 32-36)

	Year 1				**Year 2**				**Year 3**			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Site 1 (CNH)				6	10	10	10	10	10	8	5	
Target Enrollment (cumulative)				**6**	**16**	**26**	**36**	**46**	**56**	**64**	**69**	

c. Accomplishments from goals

At this time, data is still being collected so there are no major accomplishments or findings to report

d. Opportunities for training and professional development
Nothing to report

e. Dissemination to communities of interest
Nothing to report

f. Plan for the next reporting period
During the next reporting period the short and long term MEKi treatment studies, murine metabolic studies, and murine microbiome studies will be performed at the two sites (CNH and JHU). This will allow for completion of Major task 1, 2 and part of 3. Patient enrollment will continue with a goal to enroll 10 patients during each quarter for the upcoming year. Although I am on target so far with enrollment goals, in order to help generate interest in the project, an informational flyer with study information will be generated and approved by the IRB so this can be disseminated to patients both at CNH and JHU (partner site through Verena Staedtke). Microbiome samples will also be collected (stool samples), and submitted for analysis in batches.

4. Impact
a. Impact on development of the principal disciplines of the project
Nothing to report

b. Impact on other disciplines
Nothing to report

c. Impact on technology transfer
Nothing to report

d. Impact on society beyond science and technology
Nothing to report

5. Changes and Problems
a. Changes in approach and reasons for change
Nothing to report

b. Changes that have had a significant impact on expenditures
The mouse costs during the first year were much less than expected at CNH because the mice did not arrive from JHU until June 2022. In order to avoid delay in the generation of data/experiments during year 2, more breeding cages will be set up so experiments can be done in parallel, and therefore the anticipated mouse costs will likely be greater during the 2nd year than anticipated.

The IC testing supplies that were budgeted for in year 1 were provided in kind by Dr. Kimberly Chapman (Genetics). These will be required during year 2 of the award, however, so the funding not used during year 1 will be used in year 2.

The PI of the grant had additional funding to cover travel during year 1 of the award, and therefore the funding budgeted for this was not used. This will be used in year 2 or year 3 of the award as results are disseminated to the scholarly community.

c. Significant changes in the use or care of human subjects
Nothing to report

d. Significant changes in the use or care of vertebrate animals
Nothing to report

e. Significant changes in the use of biohazards and/or select agents
Nothing to report

6. Products
a. Publications, conference papers, and presentations
Nothing to report

b. Website or other Internet sites
Nothing to report

c. Technologies or techniques
Nothing to report

d. Inventions, patent applications, and/or licenses
Nothing to report

e. Other products
Nothing to report

7. Participants and Other Collaborating Organizations

a. Individuals working on the project

Name	Miriam Bornhorst
Project Role	Principal Investigator
Researcher Identifier	0000-0002-7595-7726
Nearest person month worked	4
Contribution to Project	Mouse studies – metabolic/metabolomic including setting up cages, generating mice with genotype for studies, NF1 patient metabolic/metabolomic studies

Name	Kathryn Lemberg
Project Role	Co-investigator
Researcher Identifier	0000-0003-1511-9322
Nearest person month worked	0.5
Contribution to Project	Mouse studies – metabolic/metabolomic including setting up cages, generating mice with genotype for studies

b. Change in active other support of the PD/PI or senior/key personnel since last reporting period
Nothing to report

c. Organizations involved as partners
Nothing to report

8. Special Reporting Requirements: None

9. Appendices: None