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14. ABSTRACT

Localized skeletal disorders, such as fracture pseudarthroses, in children with Neurofibromatosis Type 1 (NF1) are associated with somatic loss-of-heterozygosity (LOH) at the NF1 gene locus. Such LOH events are associated with other non-skeletal manifestations of NF1, including cafe-au-lait pigment macules and plexiform neurofibromas, for example. LOH events in fracture pseudarthroses and NF1-associated cancers are associated with dramatic increases in the activation of numerous genetic signaling pathways. These same signaling pathways are targeted by drug compounds that have been tested in clinical trials, including in children with NF1-associated cancers. Our study is testing whether these compounds can rescue the molecular dysregulation inherent in patient fracture pseudarthrosis-derived primary cells, with the goal to repurpose these compounds to treat NF1-associated fracture pseudarthrosis.
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1. Introduction

Localized skeletal disorders, such as fracture pseudarthroses, in children with Neurofibromatosis Type 1 (NF1) are associated with somatic loss-of-heterozygosity (LOH) at the *NF1* gene locus. Such LOH events are associated with other non-skeletal manifestations of NF1, including cafe-au-lait pigment macules and plexiform neurofibromas, for example. LOH events in fracture pseudarthroses and NF1-associated cancers are associated with dramatic increases in the activation of numerous genetic signaling pathways. These same signaling pathways are targeted by drug compounds that have been tested in clinical trials, including in children with NF1-associated cancers. Our study is testing whether these compounds can rescue the molecular dysregulation inherent in patient fracture pseudarthrosis-derived primary cells, with the goal to repurpose these compounds to treat NF1-associated fracture pseudarthrosis.

2. Keywords

Neurofibrormatosis Type 1, pseudarthrosis, fracture, targeted therapy

3. Accomplishments

What are the major goals of the project?

The major goal of this project is to test the response of patient fracture-derived primary cells to pharmacologic compounds targeting genetic pathways that our preliminary data suggested were activated in the disease. Major Task #1 tested patient-matched fracture- and control bone-derived primary cells, while Major Task #2 tested an independent cohort of patient fracture-derived primary cells without patient-matched control samples. Both utilize transcriptome profiling (RNA-seq) to evaluate molecular changes in response to each compound. If successful, results from this study may implicate one or multiple compounds as novel non-surgical treatments for this disease.

What was accomplished under these goals?

Thus far during the project period, we have completed all cell culture experiments testing two MEK inhibitors (MEKi; selumetinib and trametinib), including samples pertaining to both Major Task #1 and

#2. RNAseq has been completed for Major Task #1 samples, and we will soon send MEKi samples for Major Task #2 for sequencing.

In addition to the MEKi compounds, we are completing preliminary testing of all other drugs proposed in the study. Ongoing experiments will determine the optimal concentration of each compound, then each will be used to treat patient-derived primary cells as part of Major Task #1. Following, we will complete these experiments as part of Major Task #2.

	Progress
Specific Aim 1: Compare the pharmacologic response of fracture-derived bone stromal cells (BSCs) to patient-matched control BSCs	
Major Task 1 –Perform ribonucleic acid (RNA) sequencing (RNA-seq) in 7 untreated human iliac crest-derived BSCs and in 7 treated and untreated human patient-matched pseudarthrosis-derived BSCs.	
Subtask 1 – IRB/HRPO approval	Completed
Subtask 2 – Culture, expand, and extract RNA from 7 human iliac crest-derived BSCs treated with vehicle.	2/6 compounds completed
Subtask 3 – Culture, expand, and extract RNA from 7 human pseudarthrosis-derived BSCs treated with vehicle and each pharmacologic agent.	2/6 compounds completed
Subtask 3 – Perform RNA-seq and analysis of all samples in Major Task 1.	2/6 compounds completed
Milestone(s) Achieved:	
Specific Aim 2: Replicate pharmacologic responses in an independent cohort of patient fracture-derived BSCs	
Major Task 2– Perform RNA-seq in 8 treated and untreated human patient pseudarthrosis-derived BSCs.	
Subtask 1 – Culture, expand, and extract RNA from 8 human pseudarthrosis-derived BSCs treated with vehicle and each pharmacologic agent.	2/6 compounds completed
Subtask 2 – Perform RNA-sequencing and analysis of all samples in Major Task 2, including integrating results between Major Tasks 1 and 2.	0/6 compounds completed

What opportunities for training and professional development has the project provided?

The use of these pharmacologic targeted therapies for *in vitro* study is new to our laboratory. We have designed the experiments such that we can evaluate both the response of patient fracture-derived primary cells to each compound and also the response of control bone-derived primary cells. Moreover, we are

comparing vehicle-treated cell data to our historical results without drug or vehicle. In this way, we are able to dissect the molecular response of these primary cells to vehicle and drug treatments.

How were the results disseminated to communities of interest?

We have not yet published or presented results from this study. We continue to analyze the first of the RNA-seq data and have planned supportive *in vivo* experiments to further interrogate the impact that MEKi may have to treat fracture pseudarthrosis. The *in vivo* studies are not part of this proposal, are being performed independently, and are not expected to delay completion of this study.

We have discussed preliminary results from this study with colleagues of the Neurofibromatosis Clincial Trials Consortium (NFCTC) and the Response Evaluate in Neurofibromatosis and Schwannomatosis (REiNS) groups.

What do you plan to do during the next reporting period to accomplish the goals?

We anticipate completing all remaining Tasks within the next reporting period. We were recently awarded a no-cost extension to complete this study. Initial testing of all remaining compounds is ongoing. Once optimal concentrations are identified, all experiments will be performed in parallel.

4. Impact

What was the impact on the development of the principal discipline of the project?

Our efforts toward completing this study are currently ongoing, and we anticipate the study being completed within the extension period. At the completion of this study, our results may identify specific targeted therapies that may be repurposed to improve treatment of fracture pseudarthroses in children with NF1.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. Changes/Problems

Changes in approach and reasons for change.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them.

Nothing to report. We have not encountered any problems. We selected the two MEKi included in this study to investigate first. This work is nearing completion and we are now focusing on the other remaining compounds included in our study.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

6. Products

Publications, conference papers, and presentation

Nothing to report.

Website or other Internet site

Nothing to report

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other products

Nothing to report.

7. Participants and other collaborating organizations

Name	Jonathan Rios
Project Role	Principal Investigator
Researcher Identifier	ORCID: 0000-0002-0969-2184
Nearest person month worked	2

Contribution to project	Dr. Rios meets regularly with the study team and performs RNA-seq data analysis.
Funding support:	

Name	Nandina Paria
Project Role	Research Scientist
Researcher Identifier	
Nearest person month worked	4
Contribution to project	Dr. Paria performs all experimental aspects of this study and meets regularly with Dr. Rios.
Funding support:	

Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period.

Nothing to report.

What other organizations were involved as partners.

RNA-seq is performed at a Genome Technology Access Center core facility at the McDonnell Genome Institute of Washington University in St. Louis. This is an academic Institute that provides core genomic services.

Name: McDonnell Genome Institute

Location of Organization: St. Louis, MO

Partner's contribution to the project:

Collaboration: RNA-seq is performed at this core facility as a fee-for-service

8. Special reporting requirements

Nothing to report

9. Appendices

Nothing to report