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TITLE: New Therapies for Fibrofatty Infiltration

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CONTRACTING ORGANIZATION: UNIVERSITY OF BRITISH COLUMBIA, THE UNIV INDUSTRY LIAISON OFFICE 6190 AGRONOMY RD STE 102 VANCOUVER V6T 1Z3

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this project is to test three classes of compounds in animal models of muscular dystrophy, and evaluate their therapeutic potential in preventing fibrofatty infiltration. Animals and drugs required for the project have been procured. A change has been made to the kinase inhibitor compound to be tested in animal models of disease, as a more efficacious drug was identified with similar substrate specificity.

15. SUBJECT TERMS
Fibrofatty infiltration, drug testing, muscular dystrophy, fibrosis.

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1) INTRODUCTION: The goal of this project is to test three different classes of compounds, stemming from a screen for molecules capable of inhibiting the fibrogenic differentiation of mesenchymal progenitors, in a mouse model of Duchenne's muscular dystrophy and thus assess their therapeutic efficiency.

2) KEYWORDS: Duchenne's muscular dystrophy, fibrosis, bromodomain inhibitors, kinase inhibitors, NFkB inhibitors.

3) ACCOMPLISHMENTS: This report covers the second year of funding. Based on the Statement of work, the following activities were planned for the second year. In general, these year's activities were aimed at initiating the treatments and generating the samples that will be analyzed during the third and final year of funding.

What were the major goals of the project?

For the past year, the milestones to be completed according to the SOW were as follow

a) **Long-term treatment groups fully established. Completed**

b) **Short-term treatment groups fully established. Completed, although with a slight delay (3 months) due to temporary problems with mouse breeding.**

c) **Short-term treatment groups sample processing completed. This has been achieved only partially due to the delays mentioned above. However, promising preliminary results have been obtained for one of our candidate drugs, and the completion of this objective is on track.**

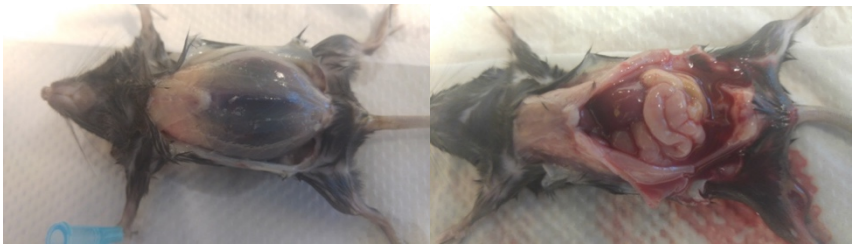
d) **Short term treatment group analysis completed: This milestone is dependent on the milestone describe above in c, and has therefore been affected by the same delays. However, it is on track for completion over the next few months and has already provide preliminary results (see below)**

What was accomplished under these goals?

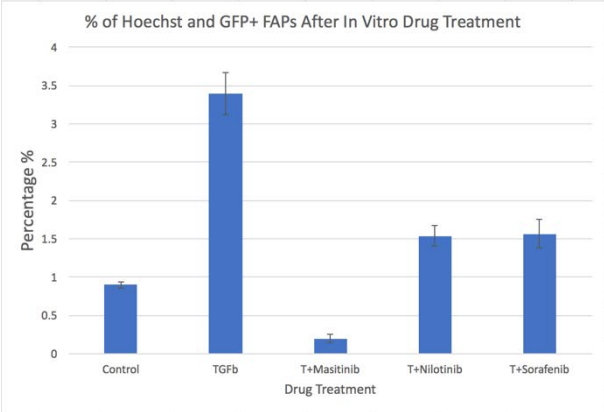
Short term treatment experiments.

The first compound proposed for use in the original application was a synthetic mimetic compound derived from the natural compound withaferin, which showed good activity in dampening the expression of fibrogenic genes in the relevant progenitors in response to TGFβ. Unfortunately, preliminary testing of withaferin in vivo led to severe side effects, and multiple animals injected i.p. with this compound met humane endpoints as defined in our animal protocol and had to be euthanized. Uon necroscopy, we found multiple peritoneal adhesions and, in the majority of cases, evidence of profuse

peritoneal bleeding (see images below). No such event was observed in vehicle treated animals.

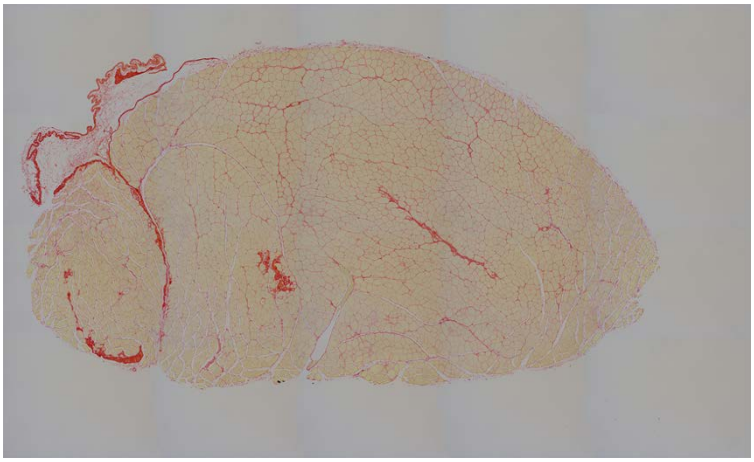


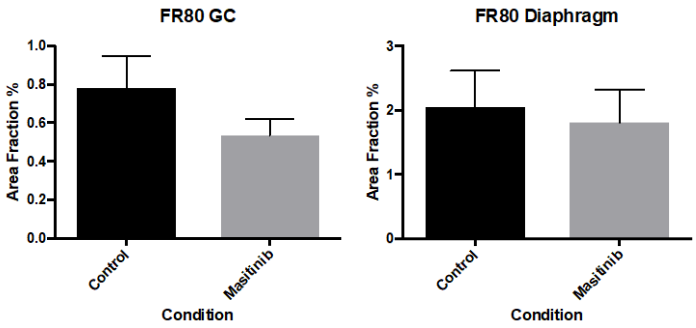
Work on this compound has now been stopped. This compound has been replaced with a new candidate, the kinase inhibitor masitinib, that showed excellent ability to inhibit collagen expression in fibrogenic progenitors in vivo. These are represented in the figure below, showing the percentage of cells positive for green fluorescent protein driven by a collagen 1 enhancer. Notice that Masitinib appear more powerful than Nilotinib or Sorafenib in this setting.



Short term treatment groups for Masitinib have been established and are been harvested, and their analysis has started. Preliminary results indicate that treatment may have been successful in reducing the area of collagen deposition in treated animal vs vehicle-treated controls, as shown in the figure below. However, the effect of treatment appears more pronounced in certain muscles such as the quadricep (left panel) than in others such as the diaphragm (right panel). More samples need to be analyzed in order to verify that these differences are statistically relevant, which will

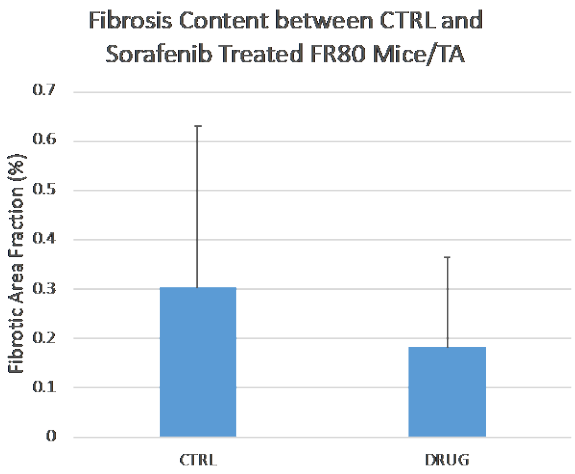
take place in the next couple of months.

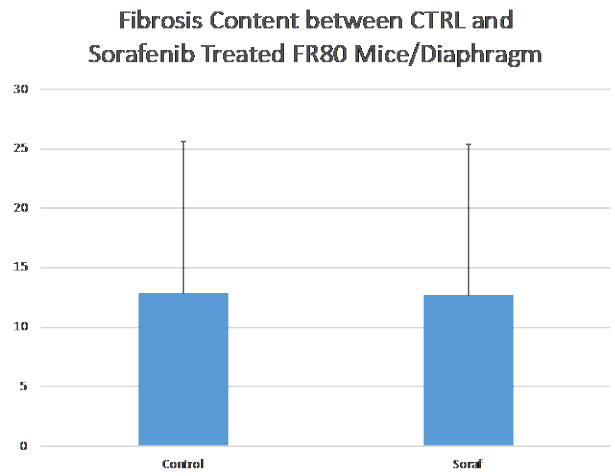




Experiments using the second candidate compound, the bromodomain inhibitor JQ1, have suffered from delays in procuring the drug (it was backordered and took much longer than expected to produce). However, the experiment is now proceeding as planned. Osmotic pumps were implanted in vehicle and drug treatment groups and the harvesting of these animals is ongoing. No preliminary results is yet available for this compound.

The third compound we proposed to test is the kinase inhibitor sorafenib. Short term treatment groups for this drug were established as proposed and harvested. Similar to what observed with masitinib, a reduction in fibrosis was observed for leg muscles but not for diaphragm (See figure below). In this case however, and despite the fact that the analysis is not yet complete and more samples need to be quantified, the differences reached statistical significant ($p<0.05$).





Long term treatment experiments.

Despite a delay due to difficulties in procuring one of the candidate drugs to be tested, all long term groups have been established and their analysis will proceed during the last year of the grant.

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

A very talented summer student has played a key role in gathering the data form some of the experiments presented here. As a result of his positive experience in the laboratory, he has applied for and been admitted to a PhD program at our institution.

How were the results disseminated to communities of interest?

The PI has given talks covering the subject of this award at multiple international meetings, see below.

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

- Complete the analysis of the short-term treatment groups
- Analyze the long-term treatment groups
- Write up the results and publish them

4) IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The study is ongoing and despite promising results, it has not yet yielded results that would impact the field. Nothing to report.

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.

The main change has been the elimination of a synthetic withaferin analogue as a candidate compound and its replacement with the kinase inhibitor masitinib. As explained in the progress report above, the Withaferin analogue triggered significant side effects in the recipient group that strongly limited its potential as a candidate therapeutic.

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

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report

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

The local IACUC approval is currently undergoing renewal for the coming year, with an expected expiration in Sept 2019.

Significant changes in use or care of human subjects.

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6) PRODUCTS:

Publications, conference papers, and presentations

Journal publications. No publication resulted from this work yet. Nothing to report

- **Books or other non-periodical, one-time publications.** Nothing to report

Other publications, conference papers, and presentations.

The PI presented our progress as an invited speaker at the following international conferences:

Gordon conference tissue repair and regeneration., New London, New Hampshire, United States

Advanced Mechanisms of Growth and Repair. Gordon Conference Myogenesis, Italy

2nd Int'l Conference on Tissue Repair, Regeneration, and Fibrosis, Greece

Website(s) or other Internet site(s)

N/A

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Nothing to report

Other Products

Nothing to report

7) PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Fabio Rossi*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	orcid.org/0000-0002-0368-2620
Nearest person month worked:	*2.4*
Contribution to Project:	*Dr Rossi is the PI on the project*
Funding Support:	N/A

Name:	*Marcela Low*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Dr Low has designed the currently ongoing experiments. During the past year, she has left the laboratory and the country*
Funding Support:	

Name:	*Elena Groppa*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*9*
Contribution to Project:	*Dr Groppa has permanently replaced Dr Low in coordinating the project and analyzing the samples*

	collected so far as Dr. Low has left the lab and the country
Funding Support:	

Name:	*ChihKai Chang*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Mr Chang has been working on procuring the animals required for testing, breeding enough of them, performing osmotic pump implantation surgeries*
Funding Support:	

▪

Name:	*Andrew Wu*
Project Role:	*Graduate Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Mr Wu has been working through the summer to analyze results from short term treatment groups.*
Funding Support:	Other PI funds

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

Dr. Rossi has received a grant from Genome Canada to develop therapeutic monoclonal antibodies as anti-fibrotic therapeutics in collaboration with a local startup. No budgetary overlap with the current project.

What other organizations were involved as partners?

AB Sciences has provided us with (masitinib).

Organization Name: AB Sciences
Location of Organization: France
Partner's contribution to the project: providing a proprietary kinase inhibitor (masitinib) for testing in animal models of muscular dystrophy.
Financial support; N/A
In-kind support: Providing drug for testing.
Facilities N/A;
Collaboration N/A;
Personnel exchanges N/A
Other. N/A

8) SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS: N/A
QUAD CHARTS: N/A

9) APPENDICES: Nothing to Report