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TITLE: In Vivo Measurement of Drug Efficacy in Breast Cancer

PRINCIPAL INVESTIGATOR: Dr. Randy J. Giedt

CONTRACTING ORGANIZATION: MASSACHUSETTS GENERAL HOSPITAL

BOSTON, MA 02114-2621

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Fort Detrick, Maryland 21702-5012

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October 2015	Annual	30 Sep 2014 - 29 Sep 2015

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In Vivo Measurement of Drug Efficacy in Breast Cancer

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5b. GRANT NUMBER
W81XWH-14-1-0386

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Randy J. Giedt

E-Mail: giedt.randy@mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

MASSACHUSETTS GENERAL HOSPITAL
55 FRUIT ST
BOSTON MA 02114-2621

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The focus of this project is to understand how how nano-encapsulated formulations of common chemotherapies work in vivo by developing and utilizing intravital methods for studying drug and nanoparticle function in mouse breast cancer models. We hypothesize that, firstly, we can develop longitudinal breast cancer specific methods of imaging common chemotherapies and their nanoparticle equivalents. Secondly, we hypothesize that encapsulated drugs will be more effective in terms of specific cell responses as they achieve longer exposure times than unencapsulated drugs. Overall, this work will result in the creation of a breast cancer centered platform for drug development and analysis. At the clinical level, this study will result in pertinent data regarding several agents currently in clinical trials. At the basic science level, we will work to understand the heterogeneity of cell responses to drug treatments. Thus, we believe this project has potential impact in both the near and long term for breast cancer treatment.

15. SUBJECT TERMS

Breast Cancer, Intravital Imaging, Nanoparticles, Pharmacokinetics/ Pharmacodynamics, Chemotherapy, Drug Distribution

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Table of Contents

1. INTRODUCTION

The focus of this project is to understand how how nano-encapsulated formulations of common chemotherapies work in vivo by developing and utilizing intravital methods for studying drug and nanoparticle function in mouse breast cancer models. We hypothesize that, firstly, we can develop longitudinal breast cancer specific methods of imaging common chemotherapies and their nanoparticle equivalents. Secondly, we hypothesize that encapsulated drugs will be more effective in terms of specific cell responses as they achieve longer exposure times than unencapsulated drugs. Overall, this work will result in the creation of a breast cancer centered platform for drug development and analysis. At the clinical level, this study will result in pertinent data regarding several agents currently in clinical trials. At the basic science level, we will work to understand the heterogeneity of cell responses to drug treatments. Thus, we believe this project has potential impact in both the near and long term for breast cancer treatment. During Year 1, this project has focused on goals including setting up and optimizing imaging and image processing methodologies in anticipation of conducting drug testing in year 2 of the project.

2. KEYWORDS

Breast Cancer, Intravital Imaging, Nanoparticles, Pharmacokinetics/ Pharmacodynamics, Chemotherapy, Drug Distribution

3. ACCOMPLISHMENTS

What were the major goals of the project?

The major goals of this project for year 1 have focused on the setup of mouse models, tumor cell genetic reporters, fluorescent drugs, and imaging algorithms. Specific SOW goals for Year 1 and their percentages of accomplishment are shown in Table 1.

Table 1: Specific Tasks for Grant Aim 1

Specific Aim 1: Create and validate a breast cancer centered single cell PK/PD Platform	Months	GSU	% Complete
Subtask 1a-1: Optimize Imaging Models. (5-20 mice).	3-9	Dr. Giedt	100%
Subtask 1a-2: Creation of breast cancer reporter cell lines: MDA-MB-231, MDA-MB-436, HCC1395, HCC1937, HCC38, MCF-7, SKBR3. Source: ATCC.	1-6	Dr. Giedt	100%
Subtask 1b: Development of image processing techniques	3-12	Dr. Giedt	100%
Subtask 1c: Development of mathematical models	9-12	Dr. Giedt	100%
Milestone Achieved: Creation of a breast cancer specific PK/ PD platform	12		

What was accomplished under these goals?

1. Breast Cancer Reporter Cell Lines (100% Complete):

This goal encompassed two tasks: Firstly, understanding which of the described cell lines in the grant would function as suitable orthotropic models in nude mice, and 2. Creating reporter cell lines with the suitable cells discovered. We therefore began this goal by testing the growth of 6

common breast cancer cell lines in mammary fat pad injections in nude mouse models. Growth results are presented in Table 2:

Table 2: Breast Cancer Cell Line Growth

Cell Line	Growth Characterization
MDA-MB-231	Excellent Growth
MDA-MB-436	Average Growth
HCC-1395	Slow Growth
HCC38	Slow Growth
MCF-7	Slow Growth, estradiol supplement necessary
SKBR3	Average Growth, metastasis noted

Following viability studies of these cell lines, we worked to create fluorescent reporter versions of breast cancer specific cell lines for downstream nanoparticle drug use. As many of the

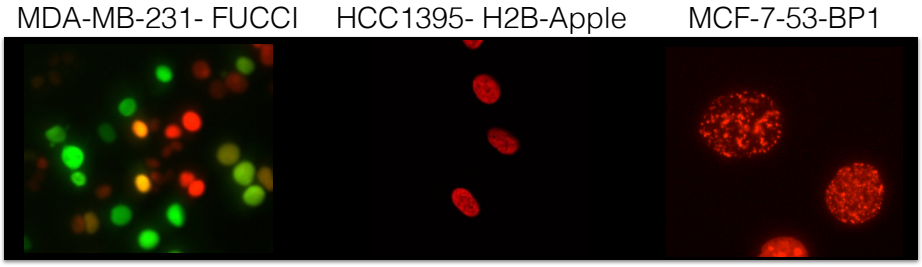


Figure 1: Examples of fluorescent reporters. The Left panel illustrates an example of MDA-MB-231 cells expressing FUCCI, a cell cycle reporter localized to cell nuclei, which changes from Green to Red as the cell cycle advances and cells divide. The center panel shows HCC1395 cells expressing H2B-apple, a general cell location reporter localized to cell nuclei, and the far right panel illustrates MCF-7-53BP1, a reporter for DNA damage. In addition to the displayed cell line reporters, we have tested reporters for cell structural elements such as actin which may be useful depending on results from drug testing.

proposed drugs function as cell cycle or DNA damage inhibitors, we focused on reporters for these specific functions. Figure 1 below shows imaging examples of these fluorescent reporters in newly created cell lines.
To briefly describe the most critical PD reporters:
53-BP-1: To develop an in vivo single-cell pharmacodynamic assay to measure DSBs following olaparib treatment, we chose 53BP1 as a DSB reporter, which has previously been used to measure DNA damage in live cells. Specifically, we fused a truncated portion of 53BP1 (amino acids 1220-1711) to Apple fluorescent protein (53BP1trunc-Apple). Apple fluorescent protein was used for in vivo imaging due to its increased brightness over mCherry, which is critical for successful imaging in live tissue. The truncated version of 53BP1 retains its ability to bind to sites of DSBs, but lacks the known functional domains of 53BP1. Moreover, we show that 53BP1trunc-Apple localizes to sites of double-strand breaks with an antibody targeting the

canonical marker of double-strand breaks, γH2A.X. For additional information please see Yang et. al. 2015.

FUCCI: Fluorescent ubiquitination-based cell cycle indicator (Fucci) is a sophisticated technology which can determine G1 and/or S/G2/M phases of the cell cycle. The technology analyzes living cells in a spatio-temporal manner using a dual color scheme of orange and green. Fucci was successfully established by intelligently utilizing ubiquitin-proteasome protein degradation system. For additional information see Sakaue-Sawano et. al. 2008.

In addition to these PD reporters, we have created a variety of breast cancer cell lines expressing localization reporters such as H2B-Apple, Membrane-Apple, and Actin-Apple, which we anticipate will prove valuable for applications such as drug localization studies.

2. Development of image processing techniques and Mathematical models(100% Complete)

Image processing for this project will utilize modified versions of Matlab code(Giedt et. al. PLoS One, 2012) and recently designed programming designed for automatically measuring

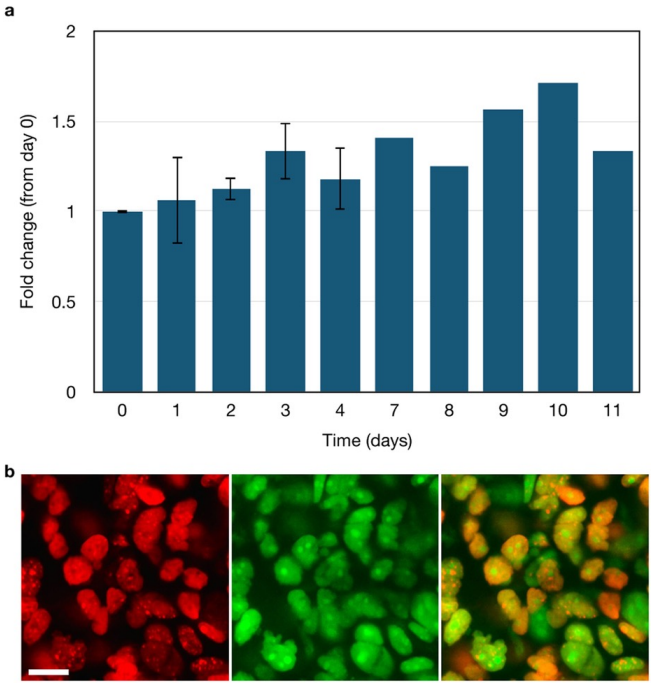


Figure 2: In vivo quantification of the fold change in foci formation in HCC1937 53BP1-Apple cells grown in nude mice. a) The fold change in the number of foci was determined relative to day 0 of treatment with 100 mg/kg olaparib. Error bars represent the standard error of the mean for n = 2 mice. (b) Olaparib-Bodipy FL nuclear uptake in a nu/nu mouse with an HCC1937 tumor. The 53BP1trunc-Apple reporter (red nuclei, left) was imaged 2 hrs after IV administration of olaparib-Bodipy FL (green, center). Images from the 53BP1trunc-Apple (red) channel and the olaparib-Bodipy FL (green) channel were overlaid (merge, right) to show that olaparib-Bodipy FL accumulates in all HCC1937 tumor cell nuclei. Scale bar = 20 µm.

pharmacodynamic output (Yang, Sci. Reports, 2015) that was developed over the course of this year. Our goal is to be able to combine outputs of drug distribution (pharmacokinetics, PK),

which will be monitored via fluorescent tags attached to either drugs, nanoparticles or both, with fluorescent reporters of single cell response (pharmacodynamics, PD) as described. Cell response reporters such as FUCCI for cell cycle inhibitors or 53BP1 for DNA damage (Figure 1) are ideal for the drugs proposed in this grant.

To this end, we have designed optimized codes over this year for analysis of these targets. Figure 2 displays recently published output generated from 53BP1 in breast cancer

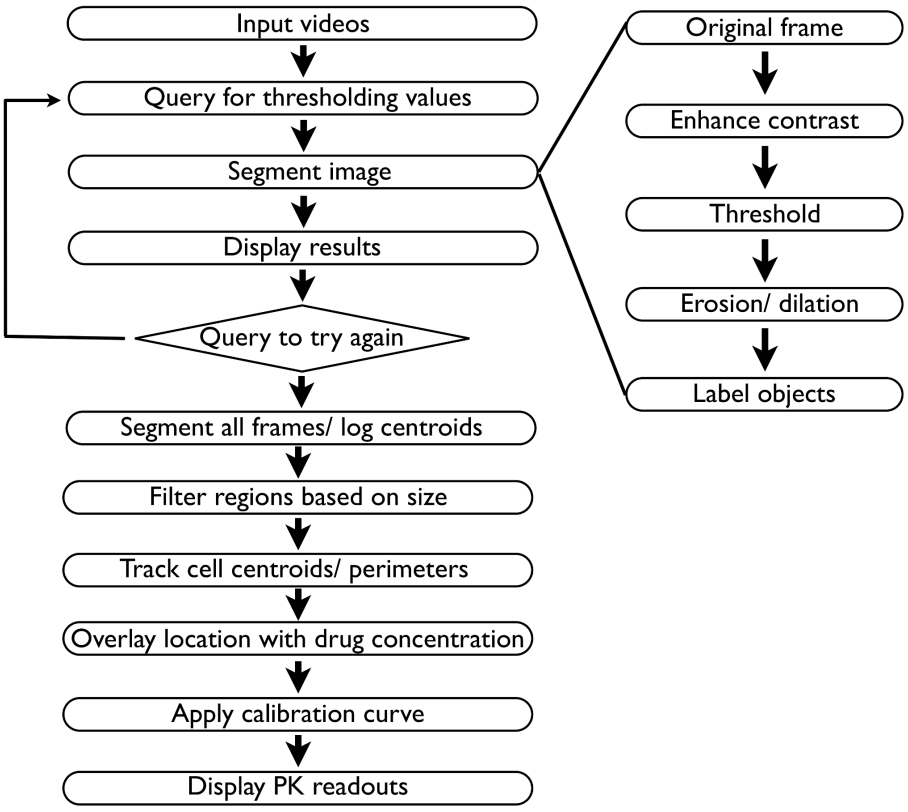


Figure 3. Overview of the image processing method.
The left side of the diagram displays the overall proposed algorithm for analyzing intravital images and determining drug concentration. This algorithm is made up of an iterative section that allows the user to generate the best possible segmentation (top) and a processing module that filters through all videos after satisfactory values have been obtained. On the right, the specific segmentation algorithm used in conjunction with the thresholding method is displayed.

cells imaged in vivo, and analyzed automatically utilizing Matlab code developed in Year 1 of this grant. As shown, treatment with olaparib in vivo resulted in a quantifiable increased in 53BP1 puncta verifying the efficacy of this system for analyzing single cell evaluation of breast cancer tumor cell response to DNA damaging agents such as Olaparib (proposed in Aim 3 of the initial grant). Similarly, FUCCI cells both in vitro and in vivo proved efficacious for follow on studies in a breast cancer model.

Work on drug distribution analysis is based on the published work found in Giedt et. al. PLoS one with minor modifications (Figure 3). Briefly, this code functions by first, acquiring in vivo serial fluorescent images of the distribution of fluorescently labeled drugs injected IV in mice expressing fluorescent marked tumors. Following user inputs for initial values for thresholding, images are run through a brief filtering algorithm to enhance image contrast and

then thresholding. Thresholding for in vivo imaging is conducted by an algorithm based on Ray's method (see Ray N, Saha BN (2009) Edge Sensitive Variational Image Thresholding. ICIP) in which local areas are thresholded without regard to their surrounding environment. Thresholding conducted in this manner has several advantages for in vivo imaging analysis. Firstly, microscope areas with heterogeneous fluorescence are normalized without artifacts from such a methodology. Secondly, cells at varying heights can be either analyzed or not in a binary fashion based on a quick thresholding analysis in a reliable manner with such a method. Finally, this thresholding allows for fully automated image analysis with extremely limited user input, allowing for statistically significant analysis.

Following thresholding, a basic erosion/ dilation method was applied to filter any speckling artifacts found in images, and objects were labeled. Utilizing labeled objects has allowed us to adapt tracking code from a collaborators group (see Jaqaman et. al., Nature Methods 5, pp. 695 - 702 (2008)) to be able to gain information at individual cell level of drug uptake, despite moving cells found in vivo. Analysis of drug uptake in cells is conducted by simple overlay of cell boundaries found from thresholded images onto drug distribution images. While this technique can suffer from possible imaging/ quantification artifacts from Z-plane differences in drug levels, we anticipate verifying drug/ NP concentrations via serial blood draws from mice during the course of experiments during Year 2. In testing this system in vivo with breast cancer models, we were able to successfully segment images and derive drug uptake from fluorescently labeled Olaparib. Work is ongoing for Year 2 to create further fluorescently labeled drug agents.

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

Table 3: SOW Goals for training and professional development.

Major Task 1: Training and educational development in breast cancer research	**Months**	**GSU**	**% Complete**
Subtask 1: Attend bi-weekly scientific talks at MGH focusing on cancer biology.	1-36	Dr. Giedt	100
Subtask 2: Attend monthly scientific talks focusing on apoptosis and cancer (Harvard Medical School - Longwood)	1-36	Dr. Giedt	100
Subtask 3: Attend tri-monthly scientific "T-32 Trainee" scientific talks focusing on cancer biology.	1-36	Dr. Giedt	100
Subtask 4: Attend and present at yearly cancer/ breast cancer meetings (e.g. AACR, ASCO, others).	1-36	Dr. Giedt	100
Subtask 5: Attend breast cancer conferences at the DF Harvard Cancer center (and others if available, e.g. at MSKCC) and present data/posters	1-36	Dr. Giedt	100
Subtask 6: Present ongoing project results for feedback in weekly lab meetings.	1-36	Dr. Giedt	100
Subtask 7: Weekly meetings with mentor and co-mentor	1-36	Dr. Giedt	100

Major Task 1: Training and educational development in breast cancer research	Months	GSU	% Complete
Subtask 8: Attendance at 3 Responsible Conduct of Research Meetings per year. Attend all MGH OCRD (Office of Career Development) meetings on faculty development	1-36	Dr. Giedt	100
Milestone Achieved: Presentation of project data at a national meeting	12,24,36		

This project has provided numerous opportunities for professional development, consistent with the training plan established in the accepted grant proposal. Specific numbers of lecture sessions attended in the MGH/ Harvard community is presented in Table 4 summarizing relevant scientific discussions. Briefly, these lectures focused on Breast Cancer to the extent possible, cancer in general, and new technologies and animal manipulation methods which are directly applicable to project goals.

Table 4: Number of Lectures attended for required educational opportunities.

Lecture Series	Location	Number of Lectures Attended
Center for Systems Biology Scientific Talks	Mass General, Simches, 3rd Floor	9
Mass General Hospital Cancer Center Grand Rounds	Mass General, Simches, 3rd Floor	~ 20
NIH T32 Post-doctoral Fellow Lectures	Mass General, Haber Auditorium, Blake 1	4
Harvard Department of Systems Biology Apoptosis Meeting	Harvard Medical School, 563 Warren Alpert	11
Mass General Hospital Responsible Conduct of Research Lectures	Mass General, Variable	4
Mass General Hospital Office of Career Development Lectures	Mass General, Variable	3
MGH Systems Biology Lab Meetings	Mass General, Simches, 5th Floor	~ 40
JAX Onsite Animal Training	Mass General, Simches 3rd Floor	2

In addition to regular lectures, this grant also enabled travel to one American Association for Cancer Research Meeting (held jointly with the Society of Nuclear Medicine and Molecular Imaging), titled “State of the Art Molecular Imaging in Cancer Biology and Therapy”. The focus of this meeting was highly relevant to the grant aims presented here, as sessions included a

focus on molecular imaging along with emerging treatment and therapeutic methods across a wide spectrum of cancer research.

How were the results disseminated to communities of interest?
Thus far preliminary results from this project have been discussed in weekly lab group lab meetings. Primary results from this project will be published with the completion of imaging experiments. Dissemination of peripheral work for this project (imaging methods, math models) took place at a joint conference between AACR-SNMMI with a poster presentation titled, "Automated analysis of drug distribution in intravital imaging" as well as publications noted below.

What do you plan to do during the next reporting period to accomplish the goals?
During the next reporting period, we plan to focus on drug and Nanoparticle development for several firstling drugs (taxol, cisplatin, PARP inhibitors, potentially vineblastine and other chemotherapy agents as described in the initial grant) and testing results in mouse breast cancer models. Work is currently ongoing focused on generation of fluorescently labeled versions of common chemotherapy agents which will be combined with fluorescent nanoparticles described in the initial grant application.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
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?
We anticipate the approach will remain the same as described in the original award.

Actual or anticipated problems or delays and actions or plans to resolve them?
No delays or problems were encountered during this reporting period.

Changes that had a significant impact on expenditures?
There were no changes to expenditures.

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

There are no changes to report in the use of animals, biohazards or select agents.

6. PRODUCTS

Journal publications
Due to this training award, PI was published on the following papers during this funding period:
Published Papers:

- Turetsky A, Lee K, Song J, **Giedt RJ**, Kim E, Kovach AE, Hochberg EP, Castro CM, Lee H, Weissleder R. On chip analysis of CNS lymphoma in cerebrospinal fluid. Theranostics. 2015 Apr 17;5(8):796-804. doi: 10.7150/thno.11220.
- **Giedt RJ**, Sprachman MM, Yang KS, Weissleder R. Imaging cellular distribution of Bcl inhibitors using small molecule drug conjugates. Bioconjug Chem. 2014 Nov 19;25(11): 2081-5. doi: 10.1021/bc500433k.
- Yang KS, Kohler Rh, Landon M, **Giedt RJ**, Weissleder R. Single cell Pharmacodynamic imaging of Parp inhibitor efficacy. Sci Rep. 2015; 5:10129.

Submitted Papers:

- Dubach JM, Kim E, Yang K, Cuccarese M, **Giedt RJ**, Vinegoni C, Weissleder R. Quantitating drug-target interaction in single cells in vivo.
- C. Leon Swisher, C. Vinegoni, P. Fumene Feruglio, **RJ Giedt**, DL Rousso, R Weissleder. Real-time high dynamic range laser scanning microscopy.

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

Other publications, conference papers, and presentations
Conference Presentation:
“State of the Art Molecular Imaging in Cancer Biology and Therapy” - AACR Meeting
“Automated Analysis of Drug Distribution in Intravital Imaging”. Randy J Giedt, Ralph Weissleder. - February 15, 2015.

Websites or other Internet site(s)
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 & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Randy J Giedt
Project Role	PI/ Research Fellow
Researcher Identifier	ORCID 0000-0001-8327-6069
Nearest person month worked	12
Contribution	Dr. Giedt is the PI of this post-doctoral Fellowship and as such has conducted all research on this grant as well as attending required trainings as described in the original application.
Funding Support	DOD BCRP Post-doctoral Fellowship

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

No changes have occurred in the PIs funding for this project.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Quad Chart:

In Vivo measurement of drug efficacy in breast cancer

BCRP-132081

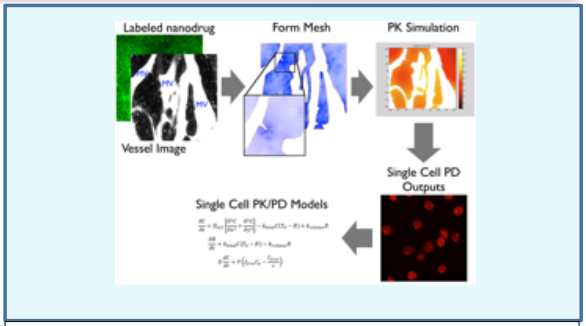
PI: Giedt, Randy J **Org:** Massachusetts General Hospital/ HMS **Award Amount:** $516K

Study/Product Aim(s)

- Training and educational development in breast cancer research
- Create and validate breast cancer PK/PD platform
- Investigate targeted nanoformulations for drug delivery
- Measure PK/PD of a model PARP inhibitor in breast cancer

Approach

The focus of this project is to understand how how nano-encapsulated formulations of common chemotherapies work in vivo by developing and utilizing intravital methods for studying drug and nanoparticle function in mouse breast cancer models. We hypothesize that, firstly, we can develop longitudinal breast cancer specific methods of imaging common chemotherapies and their nanoparticle equivalents. Secondly, we hypothesize that encapsulated drugs will be more effective in terms of specific cell responses as they achieve longer exposure times than unencapsulated drugs. Overall, this work will result in the creation of a breast cancer centered platform for drug development and analysis. At the clinical level, this study will result in pertinent data regarding several agents currently in clinical trials. At the basic science level, we will work to understand the heterogeneity of cell responses to drug treatments. Thus, we believe this project has potential impact in both the near and long term for breast cancer treatment.



Accomplishment: In the first year of this project we have successfully demonstrated the creation of a platform for analysis of nanoparticle and drug distribution in vivo in mouse breast cancer models.

Timeline and Cost

Activities / CY	15	16	17
Training in Breast Cancer			
Create/Validate PK/PD platform			
Investigate targeted Nanoform.			
Measure PK/PD of Parp			
Estimated Budget ($K)	**$172k**	**$172K**	**$172K**

Updated: (10-26-2015)

Goals/Milestones

CY15 Goal – Create Validate PK/PD Platform

☑ Demonstrate new methods and analysis software in mouse model

CY16 Goals – Investigate targeted Nanoformulations

☐Test common chemotherapy agents in NP formulations

CY17 Goal – Measure PK/PD of Parp inhibitors

☐ PK/PD Drug Response Measurements

☐ **Comments/Challenges/Issues/Concerns**

- N/A

9. APPENDICES

1. PI Updated CV

Randy James Giedt

EDUCATION

The Ohio State University
Columbus, OH
Doctor of Philosophy, Biomedical Engineering
June 2012

The Ohio State University
Columbus, OH
Master of Science, Biomedical Engineering
June 2009

South Dakota State University
Brookings, SD
Bachelor of Science, Mechanical Engineering, *Cum Laude*
May 2007

- *Minor degree*, Biology

RESEARCH EXPERIENCE

Massachusetts General Hospital, Harvard Medical School
Boston, MA
Post-doctoral Fellow/ Research Fellow
August 2012 – Current

The Ohio State University, Davis Heart and Lung Research Institute
Columbus, OH
Graduate Research Associate
2007 - 2012

Hub City Manufacturing, Engineering Department
Aberdeen, SD
Engineering Intern
Summers 2005, 2006

TEACHING EXPERIENCE

The Ohio State University, Department of Biomedical Engineering
Columbus, OH

Graduate Teaching Assistant
2009-2010

- Assisted with courses including Numerical Simulations in BME, Biomaterials, Biotransport and Introduction to Matlab.

The Ohio State University, Department of Mechanical Engineering
Columbus, OH
Graduate Teaching Assistant
Fall 2007

- Instructed engineering Measurements Lab (Signal processing, pressure measurement, and fluid flow measurement).

Fellowship and Grant Support

1. 2014 – 2017 Congressionally Directed Medical Research Programs (CDMRP) Department of Defense Breast Cancer Research Program Post-Doctoral Fellowship **Role: PI**

2. 2011-2012 American Heart Association Pre-doctoral Fellowship . **Role: PI**

AWARDS AND HONORS

1. 2011 Best Overall Presentation, The Ohio State University Biomedical Engineering Department Research Conference.

2. 2010 1st Place: The Ohio State University Edward F. Hayes Graduate Research Forum, Science and Technology Poster Division.

3. 2009 Travel Award from the Biomedical Engineering Society (BMES).

4. 2009 Best Poster Presentation under the "Molecular, Cellular, & Tissue Engineering" track, The Ohio State University Biomedical Engineering Department Research Conference.

PEER REVIEWED PUBLICATIONS

1. Yang KS, Kohler RH, Landon M, **Giedt RJ**, Weissleder R. Single-cell Pharmacodynamic imaging of Parp inhibitor efficacy. *Sci Rep.* 2015; 5:10129.

2. Turestsky A, Kyungheon L, Song J, Castro C, **Giedt RJ**, Kovach A, Hochber E, Lee H, Weissleder R. On chip analysis of CNS Lymphoma in Cerebrospinal Fluid. *Theranostics.* 2015; 5(8): 796-804.

3. **Giedt RJ***, Sprachman MM*, Yang KS, Weissleder R. Imaging Cellular Distribution of Bcl Inhibitors using Small Molecule Drug Conjugates. *Bioconjugate Chem*. 2014 Nov 19; 25(11): 2081-5.

4. Alieva M, L Ritsma, **Giedt RJ**, Weissleder R, van Rheenen J. Imaging windows for long-term intravital imaging: General overview and technical insights. *Intravital.* 11 Aug. 2014.

5. Meimetis LG, Carlson JC, **Giedt RJ**, Kohler RH, Weissleder R. Ultrafluororenic coumarin-tetrazine probes for real-time biological imaging. *Angew Chem Int Ed Engl*. 2014 Jul 14;53(29): 7531-4.

6. Kim E, Yang KS, **Giedt RJ**, Weissleder R. Red Si-rhodamine drug conjugates enable imaging in GFP cells. *ChemComm*. 2014 May 4:50(34):4504-7.

7. Coffey SE*, **Giedt RJ***, Weissleder R. Automated analysis of clonal cancer cells by intravital imaging. *Intravital*. 2013 Jul;2(3).

8. **Giedt RJ**, Koch PD, Weissleder R. Single Cell Analysis of Drug Distribution by Intravital Imaging. *PLoS One*. 2013 Apr 10;8(4).

9. **Giedt RJ**, Pfeiffer DR, Matzavinos A, Kao CY, Alevriadou BR. Mitochondrial dynamics and motility inside living vascular endothelial cells: role of bioenergetics. *Ann Biomed Eng*. 2012 Sep;40(9):1903-16.

10. **Giedt RJ**, Yang C, Zweier JL, Matzavinos A, Alevriadou BR. Increased mitochondrial fission in endothelial cells following simulated ischemia/reperfusion: Role of nitric oxide and mitochondrial reactive oxygen species. *Free Radical Medicine and Biology.* 2012 Jan 15; 52(2): 348-56.

11. Han Z, Varadharaj S, **Giedt RJ**, Zweier JL, Szeto HH, Alevriadou BR. Mitochondria-derived reactive oxygen species mediate heme oxygenase-1 expression in sheared endothelial cells. *J Pharmacol Exp Ther*. 2009; 329(1):94-101.

* Denotes Equal Project Effort.

BOOK CHAPTERS

1. **Giedt RJ,** Yang KS, Weissleder R. *Imaging Drug Distribution and Effects at the Single Cell Level In Vivo*. Advances in Intravital Microscopy: From Basic to Clinical Research. Weigert R. Springer Publishing Company, New York City New York, 2014, pp. 263 – 280.

2. B.R. Alevriadou, C.I. Jones 3rd, **R.J. Giedt**. *Nitric oxide and endothelial mitochondrial function: Implications for ischemia/reperfusion*. Hemodynamics & Mechanobiology of Endothelium, Hsiai TK, Blackman B, and Jo H, World Scientific Publishing Co., Singapore & USA, September 2010, pp. 153-177.

MANUSCRIPT REVIEWS

Ad hoc peer reviewer for manuscripts submitted to:

1. *Molecular Pharmaceutics*

SELECTED PRESENTATIONS

- **Giedt RJ**, Feruglio PF, Pathania D, Mitchison TJ, Weissleder R. Single Cell Analysis of Mitochondrial Morphology in Cancer. Harvard Systems Biology Departmental Retreat, May 2015 (Seabasco Bay, ME).

- **Giedt RJ**, Weissleder R. Mitochondrial Morphology: Predictor for Cancer Therapy. Harvard Systems Biology Apoptosis Meeting, March 2014 (Boston, MA).

- **Giedt RJ,** Koch PD, Weissleder R. Single Cell Analysis of Drug Distribution by Intravital Imaging. Scientific Advisory Committee Meeting, Massachusetts General Hospital, May 2013 (Boston, MA).

- **Giedt RJ,** Weissleder R. Intravital Imaging Effects of Bcl2 Inhibitors. Harvard Systems Biology Apoptosis Meeting, November 2012.

- **Giedt RJ**, Yang C, Zweier JL, Matzavinos A, Alevriadou BR. Mitochondrial Changes in Endothelial Cells Due to Mechanochemical Stimuli. Presented at the Biomedical Engineering Society (BMES) Annual Meeting, October 2011 (Hartford, CT).

- **Giedt RJ**, Pfeiffer DR, Matzavinos A, Kao CY, Alevriadou BR. Image analysis of dynamic changes in mitochondrial motion and shape inside living vascular endothelial cells: Role of bioenergetics. Presented at the Davis Heart and Lung Research Day, October 2011 (Columbus, OH).

- **Giedt RJ**, Pfeiffer DR, Matzavinos A, Kao CY, Alevriadou BR. Image analysis of dynamic changes in mitochondrial motion and shape inside living vascular endothelial cells: Role of bioenergetics. Presented at the Ohio State University Biomedical Engineering Conference, September 2011 (Columbus, OH).

- **Giedt RJ**, Praetorius-Ibba M, Matzavinos A, Alevriadou BR. Mitochondrial network morphology in post-ischemic vascular endothelial cells. Presented at the Biomedical Engineering Society (BMES) Annual Meeting, October 2010 (Austin, TX).

- **Giedt RJ**, Yang C, Matzavinos A, Praetorius-Ibba M, Zweier JL, Alevriadou BR. Mitochondrial network morphology changes, mechanisms and consequences in postischemic vascular endothelial cells. Presented at the Davis Heart and Lung Institute Research Day, October 2010 (Columbus, OH).

- **Giedt RJ**, Yang C, Matzavinos A, Praetorius-Ibba M, Zweier JL, Alevriadou BR. Mitochondrial network morphology changes, mechanisms and consequences in postischemic vascular endothelial cells. Presented at the Ohio State University Biomedical Engineering Conference, September 2010 (Columbus, OH).

- **Giedt RJ**, Matzavinos A, Alevriadou BR. Analysis of mitochondrial morphology in cells experiencing a heart attack. Presented at the Edward F. Hayes Graduate Research Forum, May 2010 (Columbus, OH).

- **Giedt RJ**, Matzavinos A, Alevriadou BR. Analysis of mitochondrial morphology in postischemic vascular endothelial cells. Presented at the American Heart Association 2010 Young Researchers Reception, April 2010 (Columbus, OH).

- **Giedt RJ**, Jones CI, Alevriadou BR. Mitochondrial superoxide radical generation in endothelial cells exposed to hemodynamic forces. Presented at the Biomedical Engineering Society (BMES) Annual Meeting, October 2009 (Pittsburgh, PA).

- **Giedt RJ**, Jones CI, Galbraith VK, and B.R. Alevriadou. Real-time detection of mitochondrial superoxide radicals in endothelial cells exposed to ischemia/reperfusion injury. Presented at the Ohio State University Biomedical Engineering Conference, May 2009 (Columbus, OH).

- **Giedt RJ,** Jones CI, Galbraith VK, Alevriadou BR. Mitochondrial superoxide levels in endothelial cells exposed to changes in flow and oxygen tension. Presented at The Ohio State University Conference "Engineering and Medicine: The Prescription for an Aging Population", November 2008 (Columbus, OH).

- **Giedt RJ**, Jones CI, Galbraith VK, Alevriadou BR. Mitochondrial superoxide levels in endothelial cells exposed to changes in flow and oxygen tension. Presented at the Davis Heart and Lung Institute Research Day, November 2008 (Columbus OH).

- **Giedt RJ**, Jones CI, Galbraith VK, Alevriadou BR. Mitochondrial superoxide levels in endothelial cells exposed to changes in flow and oxygen tension. Presented at the BMES Annual Meeting, October 2008 (St. Louis, MO).

SERVICE

- 2011 Assistant at University Community Health Care Day – University Hospital East (assisted persons without health care coverage in getting free screenings).

- 2010 Ray Travel Award Judge (Examined graduate student research applications for merit to determine graduate school allocations of travel funds).

- 2007 Brookings County Youth Mentorship Program (Mentored at risk community youth).

PROFESSIONAL MEMBERSHIPS

- 2008 Biomedical Engineering Society.

- 2007 Engineering in Training (E.I.T.) Certification.

- 2005 Tau Beta Pi (Engineering Honor Society, awarded to top 1/8 of Junior Class).

- 2004 Pi Tau Sigma (Mechanical Engineering Honor Society, awarded to top 1/4 of Junior Class).

- 2002 American Society of Mechanical Engineers (ASME).