AD_____

AWARD NUMBER: W81XWH-17-1-0015

TITLE: Understanding the Role of Matrix Microstructure in Metastasis

PRINCIPAL INVESTIGATOR: Catherine Kuo

RECIPIENT: University of Rochester

REPORT DATE: Feb 2020

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Feb 2020	Annual	02/01/19 – 1/31/20

4. TITLE AND SUBTITLE
Understanding the Role of Matrix Microstructure in Metastasis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0015

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Edward Brown and Catherine Kuo

E-Mail: Catherine.K.Kuo@rochester.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Department of Biomedical Engineering
University of Rochester
201 Robert B. Goergen Hall
P.O. Box 270168
Rochester, NY 14627

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this project we will address the underlying mechanisms by which certain light-scattering properties of the tumor ECM predict metastasis. Understanding the mechanisms of this novel phenomenon may yield novel insights into metastatic processes, leading to new treatments for metastatic breast cancer. It may also uncover additional prognostic indicators, improving our predictive ability and further reducing overtreatment. The light scattering phenomenon in question is the direction that second harmonic generation light scatters from collagen fibers, the "F/B ratio". F/B is sensitive to the diameter of fibrils that are bundled into collagen fibers, as well as the spacing and disorder of their packing within the fiber, altogether known as a fiber's "microstructure". To begin to address why collagen microstructure, reported by F/B, predicts patients' metastatic outcome, we must first determine how that microstructure is defined by cells within the tumor, and the cues that influence those cells to do so.

15. SUBJECT TERMS
Microscopy, metastasis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1.INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Overtreatment of breast cancer is a pressing clinical problem as patients are subjected to the toxic side effects of chemotherapy even though they were not going to experience any post-surgical metastases. We recently discovered an innovative method to predict metastatic outcome in invasive ductal carcinoma (IDC) using light scattering from tumor collagen as an independent prognostic indicator. In this project we will address the underlying mechanisms by which these light-scattering properties predict metastasis. Understanding the mechanisms of this novel phenomenon may yield novel insights into metastatic processes, leading to new treatments for metastatic breast cancer. The light scattering phenomenon in question is the direction that second harmonic generation light scatters from collagen fibers, the "F/B" ratio. F/B is sensitive to the diameter of fibrils that are bundled into collagen fibers, as well as the spacing and disorder of their packing within the fiber, altogether known as a fiber's "microstructure". To begin to address why collagen microstructure, reported by F/B, predicts patients' metastatic outcome, we must determine how that microstructure is defined by cells within the tumor, and the cues that influence those cells to do so, as well as how that microstructure affects tumor cells' metastatic behavior. We believe that tumor cells and fibroblasts respond to presented matrix cues, including collagen subtypes, crosslink density, and the presented microstructure itself, to modify collagen microstructure (and hence F/B) by digestion of existing fibrillar collagen with MMPs, synthesis of fibrillar collagen, and LOX establishment of new crosslinks. We also believe that the microstructure of the tumor collagen in turn affects tumor cell metastatic ability at least in part by influencing tumor cell motility. This project will significantly impact the fight against breast cancer because understanding the mechanisms by which F/B predicts metastasis may uncover additional metastasis predictors, which will improve our ability to identify who should receive adjuvant chemotherapy. Improved predictive formulas can be incorporated into our ongoing development process and rapidly moved towards the clinic, thereby accelerating and enhancing the impact. Mechanistic understanding may also yield novel insights into metastatic processes. We are exploiting a novel way to study metastatic processes (by exploring the role of collagen microstructure) and thus newly discovered mechanisms affecting metastasis will likely themselves be novel. We will then be poised to explore druggable targets for antimetastatic therapies based upon these insights.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Metastasis, collagen, microenvironment, microscopy

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project? *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or*

phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The Specific Aims of this three-year grant are as follows, and are unchanged from the original submission:

Aim 1: Determine how F/B is related to collagen properties in IDC ER+ LNN primary tumors.
- 1a. Evaluate F/B vs collagen I:III and I:V ratios in IDC tumor samples.
- 1b. Evaluate F/B vs collagen crosslink density in IDC tumor samples.

Aim 2: Determine what parameters influence F/B modification activity by the cells in a primary tumor.
- 2a. Alter collagen ratios (I;III and I:V) in collagen gels and quantify resultant F/B modification by tumor cells and by fibroblasts.
- 2b. Alter crosslink density in collagen gels and quantify resultant F/B modification by tumor cells and by fibroblasts.
- 2c. Test mechanisms by repeating 2a,b after inhibiting formation of new crosslinks, collagen synthesis, and MMP activity.
- 2d. Repeat 2a-c in decellularized tumor tissue.

Aim 3: Expand our ability to predict metastatic outcome in patient biopsy samples.
- 3a. Add quantification of collagen I:III and I:V ratio, and crosslink density, to our predictive formula, and assess its predictive strength.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

This project began with the observation that a light-scattering phenomenon, specifically the directionality of second harmonic generation quantified as "F/B", is prognostic of metastasis free survival time in untreated patients with invasive ductal carcinoma (IDC) that are estrogen receptor positive (ER+) and do not yet have significant visible signs of tumor cells in their lymph nodes (N0). The ultimate goal of the work is two-fold: 1) to propel that observation into a clinically useful tool for patients that will facilitate patient-tailored therapy by identifying who should, and should not, receive chemotherapy, and 2) to study the mechanisms underlying the relationship between F/B and metastasis in order to learn more about the metastatic process and hence develop anti-metastatic therapies. Aim 3 of the grant addresses goal #1, while Aims 1 + 2 of the grant address goal #2. To accomplish goal #1 broadly speaking we wish to first confirm our results (that F/B predicts metastasis) in a US-based population with the modern distribution of neoadjuvant and adjuvant chemotherapy (the original discovery was made with a Dutch-based population who received neither adjuvant nor neoadjuvant chemotherapy), optimize our methodology of determining F/B, evaluate additional related predictors such as Col I/III immunolabeling, then ask the more direct question if F/B plus related predictors can predict who will, and will not, respond to

chemotherapy, then generate and validate the appropriate predictive model, then move to a multicenter trial to test that predictive model. While accruing patients with curated samples and data sets for Aim 3, we were approached by a clinical collaborator with an exciting and highly relevant *pre-existing* data set: a set of formalin-fixed paraffin-embedded needle biopsy sections with the associated patient data denoting subsequent response to neoadjuvant chemotherapy recorded as the Residual Cancer Burden (RCB) score. This sample set allows us to rapidly gain insight into the question as to whether F/B, in addition to predicting metastasis, can also predict response to chemotherapy, albeit strictly in the neoadjuvant setting. This allows us to "skip a step" and see if F/B predicts chemoresponse as well as metastatic outcome, again with the caveat that it asks that question in the neoadjuvant setting. Once we have accomplished our accrual of our full data set of archived samples from primary tumor excision and associated data on neoadjuvant and adjuvant chemotherapy, we can expand our study to the adjuvant setting and also expand our study numbers. I will describe our additional progress with this new neoadjuvant data set as my first numbered point below, then continue with numbered updates of other progress on this project:

1) For Aim 3 (Performed in Brown Lab): Breast cancer patients with IDC who receive neoadjuvant chemotherapy primarily fall into two groups: HER2+ and triple negative (TNBC). Some luminal A patients also receive neoadjuvant chemotherapy but their numbers continue to remain small because few of them respond positively. *If a subset of luminal A patients can be identified who are likely to respond to neoadjuvant treatment it would likely be more widely prescribed.* With funding from this grant we recently published our study of F/B and response to neoadjuvant chemotherapy in HER2+ and TNBC patients (that work was reported last year). This year we identified a cohort of luminal A core needle biopsies with accompanying response data. We performed F/B assessment on these specimens in our usual manner (Burke et al. 2013, 2015). In brief, SHG images in the forward (F) and backward (B) direction are generated for each field of view. A blinded observer selects an intensity-based threshold for all "F" and all "B" images in the data set (i.e. one threshold for each of the two sets of images) which sets pixels to "1" if they are within collagen fibers and sets them to "0" if they are within background regions. These two masks are multiplied together to create a final intensity-based mask to select collagen pixels for study. This mask is then multiplied by all F/B ratio images and the average value of all nonzero pixels is calculated to determine the average F/B value of the imaged region. Using this method, F/B is calculated from 3 fields of view in the tumor/host interface, as well as 3 fields of view in the tumor bulk, of needle biopsy sections from luminal A patients and compared the results to the subsequent RCB score. RCB scores of 0, 1, 2, 3, and 4 correspond to progressively poorer response, and patients whose RCB scores are 0 or 1 (complete or significant response, respectively) have the same clinical outcomes. Therefore, we pooled RCB scores of 0 and 1, as well as RCB scores of 2 and 3 for this study. In luminal A patients, *we find that there is a statistically significant correlation between F/B and RCB score category when F/B is assessed in the tumor/host interface* (Figure 1a) but not in the tumor bulk (Figure 1b).

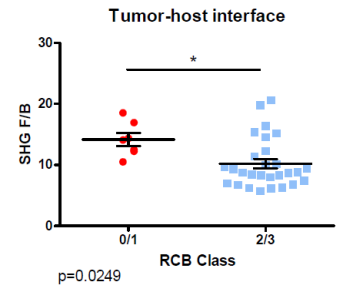

Figure 1a) F/B versus Residual Cancer Burden (RCB) score in luminal A needle biopsy sections. Measured at the tumor/host interface. p=0.0249, N=35.

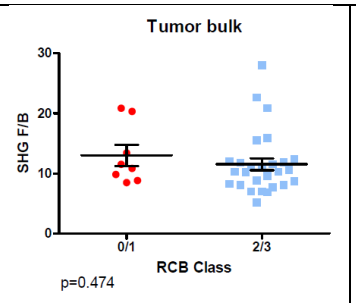

Figure 1b) F/B versus RCB score in luminal A needle biopsy sections. Measured in the tumor bulk. p=0.474, N = 35.

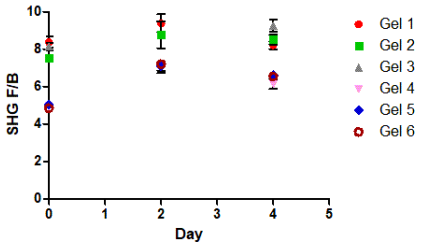

Figure 2) F/B versus time in pure collagen gels. SHG imaging was performed pre-, and 2- and 4-days post-seeding with 10^4 4T1 cells. F/B was calculated in 3 regions where cells had accumulated and manipulated collagen fibers.

2) <u>For Aim 2 (performed in Brown lab):</u> After our last report, it became clear that changes in clinical practice here at the University of Rochester have made it extremely difficult to get excess human breast tumor samples for decellularization, and that this is a permanent situation. Our partners in the Rochester Cancer Library, the ultimate source of our samples, report that this is because our surgeons are becoming more aggressive at excising smaller tumors earlier, hence there is little to no tissue to spare. While we are still working on accumulating those samples, we have expanded our efforts in related experiments that address the same fundamental questions: In figure 2 we see a study of the modifications of collagen F/B made by 4T1 murine mammary adenocarcinoma cells to pure collagen gels. At the 4 day time point it appears that 4T1 cells do not significantly modify pure collagen gels with this F/B value.

3) <u>For Aim 2</u> (performed in Brown Lab and Kuo Lab): To further understand the impacts that putative microstructure modification by tumor cells may have on the metastatic process, we have been tracking the motility of tumor cells after they have been applied to collagen gels, monitoring their motility over short time scales, before they themselves have had a chance to modify the collagen F/B. To distinguish microstructure effects from effects of ECM elastic modulus (i.e. stiffness) we have optimized a method to create mixed collagen/polyacrylamide gels where microstructure is tuned by modifying collagen polymerization conditions while elastic modulus is held stable with admixture of polyacrylamide (PA) (Figure 3). We applied two different cell lines to these gels for motility analysis, the 4T1 murine mammary adenocarcinoma (a model of TNBC) and its sister cell line (from the same original tumor) the 67NR murine mammary adenocarcinoma (a model of luminal disease). We found that the 4T1 exhibited a motility that increased with F/B at two different values of gel elastic modulus, while 67NR exhibited a motility that decreased with F/B at two different values of gel elastic modulus (Figure 4). As expected for these values of modulus,

TNBC cell (4T1) motility varies with stiffness while luminal cell (67NR) motility does not (i.e., blue and green lines overlap).

Previously, we have shown that the average F/B of primary IDC tumor biopsies is prognostic of metastatic outcome. Specifically, in ER+ IDC tumors (i.e. including the luminal subtypes), patients whose tumors had higher F/B experienced fewer distant metastases in 10-year follow-up data (Burke et al., 2015). Conversely, in ER- IDC tumors (i.e. including the basal triple negative and HER2-enriched subtypes) the relationship was inverted and patients whose tumors had higher F/B experienced more distant metastases. Interestingly, these results are mirrored in our in vitro data (Figure 4) wherein the motility of 4T1 cells (a model of TNBC) increased with F/B while that of 67NR cells (a model of luminal disease) decreased.

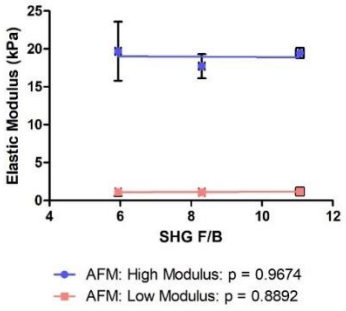

Fig 3. Elastic modulus as a function of mixed gel F/B for both high and low modulus collagen/PA mixed gels. Linear regression p>0.05 (n=3). These AFM results were confirmed with rheology (data not shown).

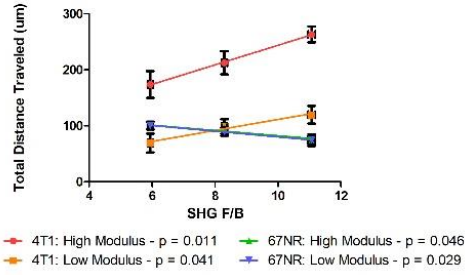

Fig. 4. Cell distance traveled versus mixed gel F/B when elastic modulus is held constant. P values <0.05 signify each slope is nonzero, revealing that collagen FIS influences motility independently of stiffness.

4) For Aim 3 (performed in Brown Lab): In order to use F/B as a prognostic tool we must develop the best method to assess it from patient tissue sections. In pursuit of this we have secured 96 pathology slides in the typical format (i.e. not a tissue microarray disk), with followup data, and for each patient imaged in three locations in the tumor bulk and three in the tumor/host interface (these regions are suggested by our recently published work on neoadjuvant chemotherapy Desa et al. 2019 after consultation with our clinical pathologist). Then we evaluated three different methods of determining F/B from those images. In each method a mask is produced which identifies pixels for inclusion in F/B calculation (presumably those pixels which are in collagen fibers). First a blinded user identified an intensity-based threshold for each image. Second, in collaboration with Dr. Wencheng Wu from the Rochester Data Sciences Institute, the Otsu method was applied to determine a threshold from the overall pixel count histogram of each image. While that method reduces user input (and possible bias) it neglects heterogeneity within images. Therefore, Third, an "adaptive thresholding method" wherein each pixel was evaluated in comparison to the average pixel value of a small box of pixels surrounding it, and included or excluded from the mask depending upon its value compared to the average in that box. Optimum box size was chosen based upon an analysis of the typical size of dark regions in all images. The results are presented in Figure 5a-c. Comparison of the p values of log-rank tests for trend in each Kaplan-Meier plot reveals that the best method is the adaptive thresholding method and hence this will be our preferred method moving forward.

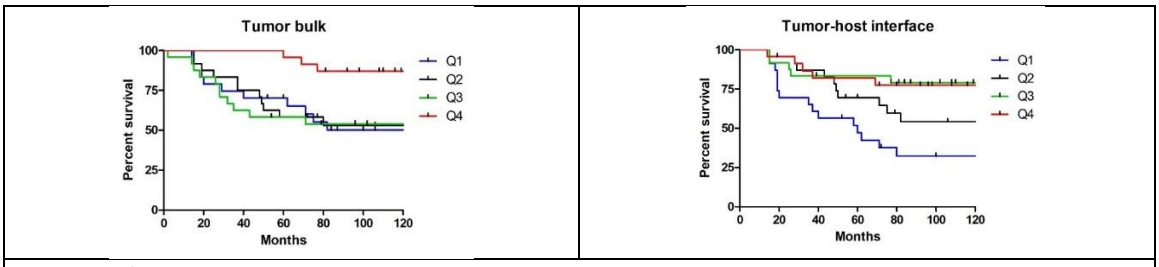

Fig. 5a. F/B measured in the tumor bulk and tumor-host interface of primary breast tumor sections is prognostic of MFS. SHG F/B values were produced by a user-defined threshold for each individual image, using images from the a) tumor bulk and b) tumor-host interface are shown. Patients were split into four equal quartiles (Q1=lowest F/B) based on the natural log of average F/B, and the percentage of each quartile surviving without metastasis then plotted versus time. Tick marks represent censoring events caused when a patient dies of a cause other than cancer or is lost to followup. Logrank test for trend p=0.023 (tumor bulk) and p=0.0007 tumor-host interface).

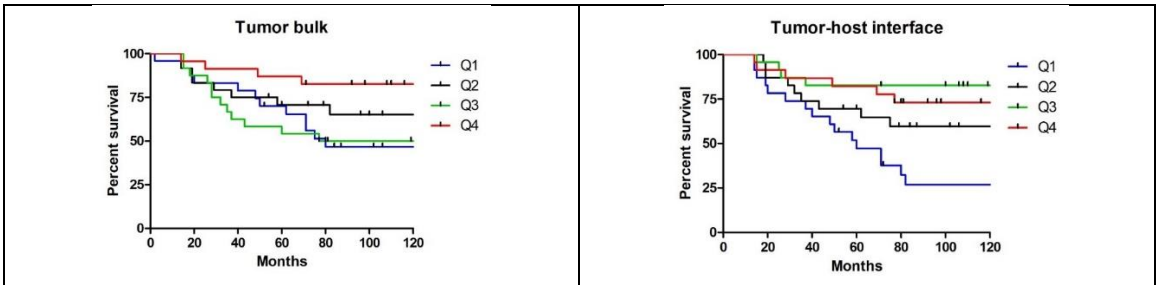

Fig. 5b. F/B using histogram-based thresholding (the "Otsu method) predicts MFS in the tumor-host interface of primary breast tumor excisions. MFS was plotted as a function of F/B from the a) tumor bulk and b) tumor-host interface are shown. Logrank test for trend p=0.082 (tumor bulk) and p=0.001 (tumor-host interface).

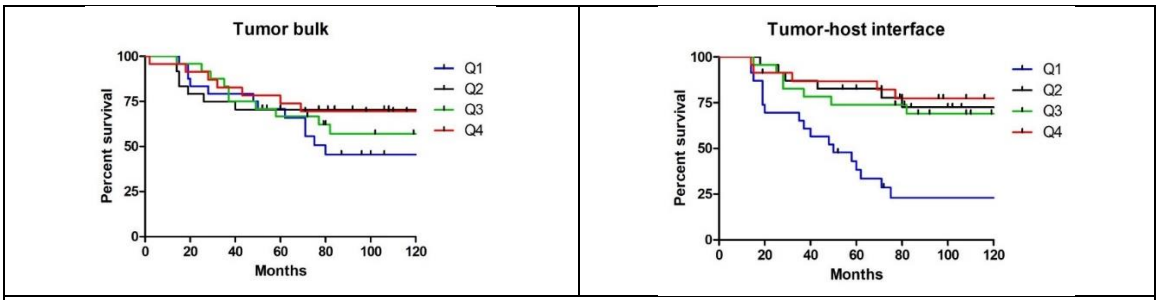

Fig. 5c. F/B generated using automated adaptive thresholding (i.e. each pixel compared to the average of a box of pixels surrounding it) predicts MFS in the tumor-host interface of primary breast tumor excisions. MFS was plotted as a function of F/B from the a) tumor bulk and b) tumor-host interface are shown. Logrank test for trend p=0.29 (tumor bulk) and p=0.0005 tumor-host interface).

In clinical practice, F/B may be used in conjunction with current genomic indicators such as OncotypeDX to predict which patients will metastasize and which patients are better candidates for chemotherapy. Based upon the recent TailorX study, patients who have an OncotypeDX score less than 26 are not recommended for chemotherapy while patients who have a score of 26 or

greater are recommended for chemotherapy. Using available genomic data and a publicly available surrogate OncotypeDX calculator, we first divided up patients depending upon if their surrogate Oncotype score (SODX) is less than or greater than 26 and then generated Kaplan-Meier plots of those cohorts based upon F/B (Fig 6). Interestingly, we find that F/B generated from the tumor-host interface can identify a subset in the SODX<26, i.e. allegedly "low risk" who has an extremely poor outcome.

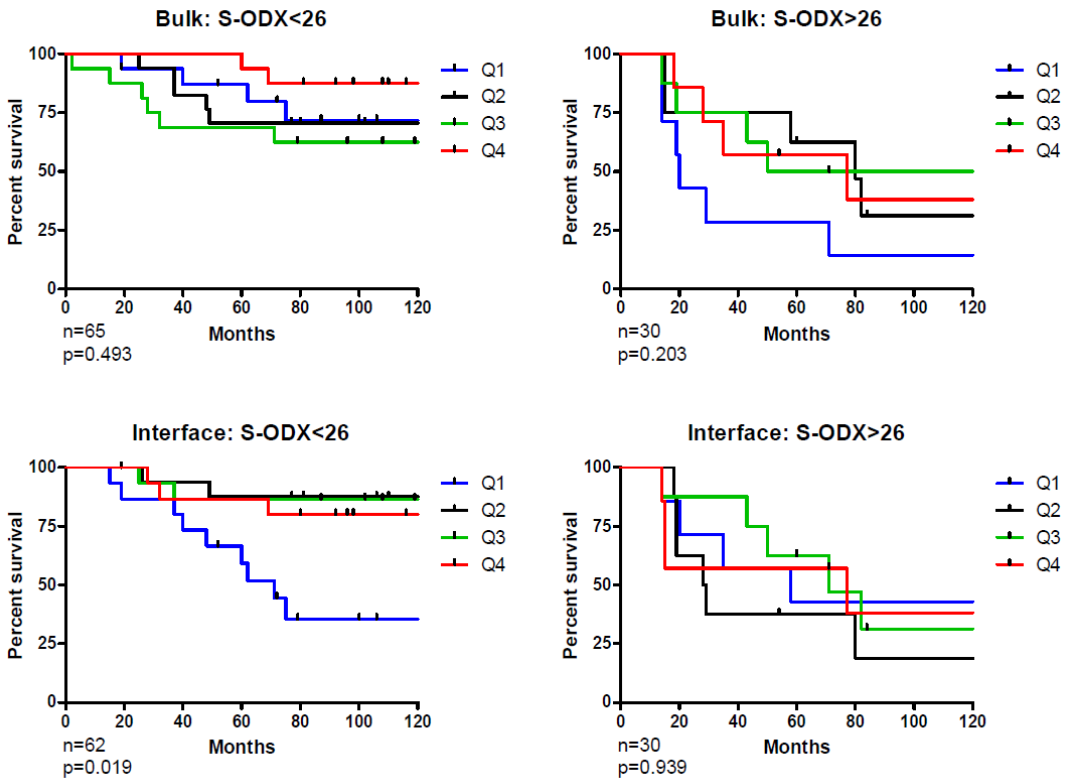

Figure 6. F/B measured in the tumor-host interface of primary breast tumor sections is prognostic of MFS in SODX<26 patients and can identify a cohort with poor outcome. SODX score was calculated from genomic data and patients split into a low risk group (SODX<26, left column) and a high risk group (right column). Then SHG F/B values were produced by a user-defined threshold for each individual image, using images from the tumor bulk (top row) and tumor-host interface (bottom row) are shown. Patients were split into four equal quartiles (Q1=lowest F/B) based on the natural log of average F/B, and the percentage of each quartile surviving without metastasis then plotted versus time. Logrank test for trend p values are shown, revealing that F/B in the tumor-host interface is prognostic in the SODX<26 "low risk" group and identifies a cohort with poor prognosis.

In summary, we are making progress towards our goal of understanding the role of collagen microstructure (as reported by F/B) in tumor metastasis, and its use as a predictor of metastasis. We have received a one-year no cost extension and believe that in the remaining seven months we will make significant strides in achieving our remaining goals.

project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We have been awarded a one year no-cost extension to help us accomplish our objectives. We expect to finish Aim 1 and determine how F/B is related to collagen properties in IDC tumors. We expect to finish Aim 2 and gain insight into tumor cell modulation of microstructure in mixed collagen/PA gels as well as in decellularized tissue. We also expect to finish sample acquisition for Aim 3 and analyze the predictive power of F/B in those samples alone and with immunolabeling for Collagen 1, 3, and 5.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

What seemed to be a brief slowdown in our supply of fresh excess human breast cancer tissue has now become a long drought, and we have heard from our clinical sources that this will be a permanent change due to changes in clinical practice. While frustrating, we do not think this need cause a major change in our goals as we can switch to using collagen gels, and also expand our investigation from just the causes of microstructure manipulation to include the consequences of that microstructure (i.e. on tumor cell motility).

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

See above.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Desa D, Bhanote M, Hill R, Majewski J, Buscaglia B, D'Aguiar M, Strawderman R, Hicks D, Turner B, Brown E. (2019) Second harmonic generation directionality is associated with neoadjuvant chemotherapy response in breast cancer core needle biopsies. Journal of Biomedical Optics. 24(8):1-9. Published, federal support acknowledged.

Schilling K, El Khatib M, Plunkett S, Xue J, Xia Y, Vinogradov S, Brown E*, Zhang X*. (2019) Electrospun Fiber Mesh for High-Resolution Measurements of Oxygen Tension in Cranial Bone Defect Repair. ACS Applied Materials & Interfaces. 11(37):33548-33558. Published, federal support acknowledged.

Dawes R, Burke K, Byun D, Xu Z, Stastka P, Chan L, Brown E*, Madden K*. (2020) Chronic stress exposure suppresses mammary tumor growth and reduces circulating exosome TGF-β content via β-adrenergic receptor signaling in MMTV-PyMT mice. Accepted for Publication: Breast Cancer: Basic and Clinical Research.

Shares B, Sheu T, Schilling K, Sautchuk R, Paine A, Beutner G, Shum L, Smith C, Gira E, Brown E, Awad H, Eliseev R. (2020) Inhibition of the mitochondrial permeability transition exerts sex-specific stimulatory effect on fracture repair. Accepted for Publication: Bone.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Exploration of the Mechanisms Underlying the Relationship Between SHG Scattering and Metastasis. Tresa Elias, Danielle Desa, Song Yuanhui, Danielle Benoit, Catherine Kuo, Bradley Turner, Edward Brown. Annual Meeting of the Biomedical Engineering Society. October 2019. Philadelphia PA

Second-harmonic generation scattering from breast cancer core needle biopsies is associated with neoadjuvant chemotherapy response. DE Desa, M Bhanote, RL Hill, JB Majeski, B Buscaglia, M D'Aguiar, R Strawderman, DG Hicks, BM Turner, EB Brown. Photonics West Annual Meeting. February 2020, San Jose CA

Nguyen PK, Deng F, Assi S, Paco P, Fink S, Stockwell C, Kuo CK, “Phenotype Stability and Expansion Potential of Embryonic Tendon Progenitor Cells In Vitro,” Orthopaedic Research Society Annual Meeting, Phoenix, AZ, February 7-11, 2020. Podium Presentation.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Edward Brown
Project Role:	co-PI
eraCommons:	EBBROWN
Nearest person month worked:	1
Contribution to project:	Dr. Brown has provided general oversight in all aspects of data generation and analysis with particular focus on imaging as well as planning, writing, revising, and submitting IRB protocols.

Name:	Catherine Kuo
Project Role:	co-PI
eraCommons:	ckuo01
Nearest person month worked:	1
Contribution to project:	Dr. Kuo has provided general oversight in all aspects of data generation and analysis with particular focus on mixed gel generation/analysis and crosslink density analysis.

Name:	Bradley Turner
Project Role:	co-Investigator
eraCommons	BMTMDMPH
Nearest person month worked:	1
Contribution to project:	Dr. Turner has assisted with sample assessment, archived sample acquisition, and overall clinical relevance of the project.

Name:	Rachel Farkas
Project Role:	co-Investigator
eraCommons	N/A
Nearest person month worked:	1
Contribution to project:	Dr. Farkas assists with acquisition of human tumor samples and overall clinical relevance of the project.

Name:	Robert Strawderman
Project Role:	co-Investigator
eraCommons	STRAWDERMAN
Nearest person month worked:	1
Contribution to project:	Dr. Strawderman assists with statistical analysis of data and project planning.

Continued on Next Page

Name: Danielle Desa
Project Role: Graduate Student
eraCommons N/A
Nearest person month worked: 12
Contribution to project: Ms. Desa has assisted with decellularization procedures, collagen gel creation, immunolabeling, imaging, and creation of a curated human samples dataset.
Funding Support: Wilmot Cancer Institute, University of Rochester

Name: Tresa Elias
Project Role: Undergraduate Student
eraCommons N/A
Nearest person month worked: 2
Contribution to project: Ms. Elias has assisted with collagen gel creation and AFM analyses.
Funding Support: Worked for class credit.

Name: Phong Nguyen
Project Role: Graduate Student
eraCommons N/A
Nearest person month worked 9
Contribution to project: Mr. Nguyen optimized polyacrylamide gel formulations for specific stiffnesses, trained the Brown Lab on making polyacrylamide gels, and performed atomic force microscopy to evaluate gel stiffnesses. He also worked on IHC staining for collagens, but this was interrupted by the pandemic.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.