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TITLE: Novel Postpartum Liver Biology Has Implications for Breast Cancer Liver Metastasis

PRINCIPAL INVESTIGATOR: Pepper Schedin

CONTRACTING ORGANIZATION: Oregon Health & Science University
Portland, OR 97239

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14. ABSTRACT

The poor prognosis of young women diagnosed with BrCa is highest in women diagnosed postpartum, up to 10 years out from a completed pregnancy. Our newer data show that this poor prognosis can be tracked to increased liver metastasis; data that argues strongly for the development of treatments that are effective at blocking metastatic lesions in the liver. Recently, the concept of targeting the metastatic cell niche has gained momentum. However, this approach is seriously hampered by difficulties in finding and characterizing disseminated tumor cells. Here we tackle the problem of defining the liver-BrCa tumor cell niche in models of postpartum breast cancer and explore relevance to women, laying the foundation for rational drug design to treat metastatic BrCa to the liver. Objective/Specific Aims: We identify the lack of understanding of postpartum liver biology as a major obstacle to identifying therapeutic targets aimed at destabilizing the liver metastatic niche in postpartum breast cancer patients. To advance this goal, mechanistic studies and stronger translational rationale are needed. To fill these critical gaps, we propose the following: Aim 1) Use liver metastasis mouse models to decipher the post-intravasation steps of the metastatic cascade that are supported by the involuting liver. Aim 2) Explore the liver metastatic niche in breast cancer patients utilizing tumor and adjacent normal liver tissue obtained from breast cancer patients with liver metastases. Aim 3) Obtain first-of-kind evidence for weaning-induced liver involution in women via a serial MRI imaging study of livers in healthy women across pregnancy and weaning.

15. SUBJECT TERMS-

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

The poor prognosis of young women diagnosed with BrCa is highest in women diagnosed postpartum, up to 10 years out from a completed pregnancy. Our newer data show that this poor prognosis can be tracked to increased liver metastasis; data that argues strongly for the development of treatments that are effective at blocking metastatic lesions in the liver. Recently, the concept of targeting the metastatic cell niche has gained momentum. However, this approach is seriously hampered by difficulties in finding and characterizing disseminated tumor cells. Here we tackle the problem of defining the liver-BrCa tumor cell niche in models of postpartum breast cancer and explore relevance to women, laying the foundation for rational drug design to treat metastatic BrCa to the liver. **Objective/Specific Aims:** We identify the lack of understanding of postpartum liver biology as a major obstacle to identifying therapeutic targets aimed at destabilizing the liver metastatic niche in postpartum breast cancer patients. To advance this goal, mechanistic studies and stronger translational rationale are needed. To fill these critical gaps, we propose the following: **Aim 1)** Use liver metastasis mouse models to decipher the post-intravasation steps of the metastatic cascade that are supported by the involuting liver. **Aim 2)** Explore the liver metastatic niche in breast cancer patients utilizing tumor and adjacent normal liver tissue obtained from breast cancer patients with liver metastases. **Aim 3)** Obtain first-of-kind evidence for weaning-induced liver involution in women via a serial MRI imaging study of livers in healthy women across pregnancy and weaning.

2. **KEYWORDS:** Young women's breast cancer, pregnancy, postpartum breast involution, postpartum breast cancer, metastatic niche, liver growth, tumor microenvironment

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

What were the major goals of the project?

Aim 1) **Use liver metastasis mouse models to decipher the post-intravasation steps of the metastatic cascade that are supported by the involuting liver.**

Liver Metastasis Model 1-Balb C mice/ D2A1-GFP mammary tumor cells,

Year 1, months 1-6: animal studies: *80% complete*
Year 1, months 7-12: tissue sectioning *100% complete*, multiplex IHC: *5% complete*
Year 2, months 1-6: Immunohistochemistry (IHC) data capture and IHC quantitation: 0% complete
Year 2, manuscript preparation: 0% complete

Liver Metastasis Model 2- Balb C mice/ D2OR-GFP mammary tumor cells

Year 1, months 6-12: animal studies: *70% complete*
Year 2, months 1-6: tissue sectioning *100% complete*, multiplex IHC: *25% complete*
Year 2, months 7-12: IHC data capture and quantitation: *30% complete*
Year 3, manuscript preparation: 0% complete

Aim 2) Explore the liver metastatic niche in breast cancer patients utilizing tumor and adjacent normal liver tissue obtained from breast cancer patients with liver metastases.

Year 1, months 1-6: IRB submission and approval: *100% complete*
Year 1, month 7-12: Begin patient accrual: 0% complete
Year 2, months 1-6: First tissue batch of three/block sectioning, multiplex IHC: 0% complete
Year 2, months 7-12: batch #1 IHC data capture and quantitation: 0% complete
Year 2, months 7-12: Second tissue batch of three/block sectioning, multiplex IHC: 0% complete
Year 3, months 1-6: batch #2 IHC data capture and quantitation: 0% complete
Year 3, months 1-6: Third tissue batch of three/block sectioning, multiplex IHC: 0% complete

Year 3, months 7-12, batch #3, data capture and quantitation: 0% complete
Year 3, manuscript preparation: 0% complete

Aim 3) **Obtain first-of-kind evidence for weaning-induced liver involution in women via a serial MRI imaging study of livers in healthy women across pregnancy and weaning**

Year 1**:** Continue to enroll new participants to the Moms LIVEr study by contacting participants enrolled in the parent Baby Bump study: *100% complete*
Years 2-3**:** Continue monthly contact with enrolled participants to determine their anticipated time of weaning: *90% complete*
Years 2-3: At time of weaning, schedule their postpartum liver MRI scan, Bodpod for body composition analysis, and dietary, physical activity and lactation questionnaire visits: *85% complete*
Years 1-2: Interim data analyses-including compilation of liver volumetric data, body composition, dietary and physical activity surveys, lactation survey data collection and calculation of breast/liver work load during lactation: *40% complete*
Years 2-3: manuscripts preparation: *20% complete*

What was accomplished under these goals?

Aim 1: Major Activities and Specific Objectives

Liver Metastasis Model 1-Balb C mice/ D2A1-GFP mammary tumor cells

- Animal studies with D2A1-GFP tumor cells to investigate post-intravasation steps of the metastatic cascade in nulliparous and involution animals are 80% complete with endpoints of 90 minutes, 1 day, 3 days, and 5 days post tumor cell injection
- Tissue sectioning for Model 1 (described in Y1Q1 report)
- IHC protocol development is ongoing to identify D2A1-GFP+ tumor cells. We encountered a technical problem in that GFP in D2A1 cells is either downregulated or has a different protein conformation that is not recognized by our standard anti-GFP antibodies. For this reason, we advanced our studies in Model 2 as described below.

Liver Metastasis Model 2- Balb C mice/ D2OR-GFP mammary tumor cells

- Animal studies with D2OR-GFP tumor cells to investigate post-intravasation steps of the metastatic cascade in nulliparous and involution animals are 70% complete with endpoints of 90 minutes, 1 day, and 3 days post tumor cell injection
- Tissue sectioning for Model 2 is complete for the finished animal studies
- Single IHC staining and quantitation to identify GFP+ tumor cells is complete for the finished animal studies
- Multiplex IHC staining and quantitation to identify proliferating and dying GFP+ tumor cells is complete for 1 and 3 days post-injection cases

Aim 1: Significant Results and Key Conclusions

The overt metastatic advantage found in the involution host liver is NOT observable at early time points after tumor cell injection, as we had predicted. Opposite of anticipated results, we find increased burden of tumor cells in the nulliparous host 1 and 3 days after portal vein injection (Figure 1). While these results confirm our hypothesis that the nulliparous and involuting livers provide different metastatic niches, the direction of the data were opposite of expectations. Multiplex IHC for tumor cell proliferation and tumor cell death at 1 and 3 days after tumor cell

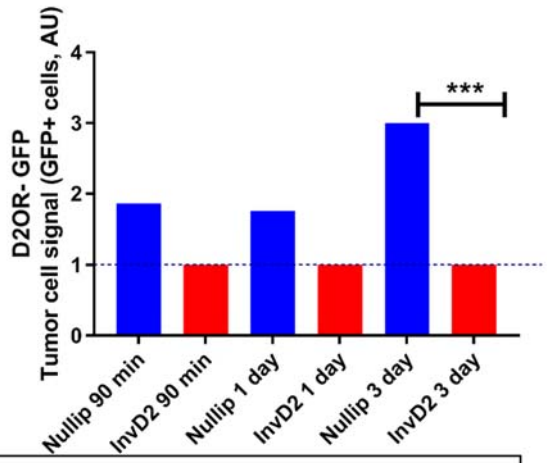

Figure 1.Quantification of tumor cell signal based on percent area positive for GFP; one-way ANOVA with Tukey's Multiple Comparisons Test, $p<0.001$

injection show no differences in the rate of cell growth in nulliparous and involution group mice.

Our data gathered during year 1 are contrary to our initial hypothesis, i.e., that the metastatic advantage in the involution host would be due to an advantage early after tumor cells enter the liver and rather, support an alternative hypothesis of differential immune cell clearance of tumor cells between nulliparous and involution hosts. Differential tumor cell clearance would be expected to be manifest between 7-10 days post tumor cell injection. To investigate the role of the adaptive immune system, we have undertaken the following experiments – 1) repeat the overt metastases experiments in the background of immune compromised mice, and 2) - extend the tumor time-course to 14 days after tumor cell injection, as this is time would be just after when the adaptive immune system would be activated by tumor cells. These studies are ongoing.

Aim 2: Major Activities and Specific Objectives

- A new IRB protocol and supporting documents were written and approved by OHSU's Institutional Review Board (IRB) in order to collect liver tissues from women with breast cancer liver metastasis
- An Independent Research Monitor was identified and contracted to oversee the research study in accordance with DoD policy
- All of the required human protecting documents are submitted to DOD and await final approval.

Aim 2: Significant Results and Key Conclusions

Nothing to report at this time as patient accrual has not yet begun.

Aim 3: Major Activities and Specific Objectives

- Have collected first and third trimester liver scans on N=47 pregnancy women, all of whom were contacted to participate in the postpartum study
- We wrote and gained approval for a letter informing previously enrolled participants of new funding source (e.g DoD BC170206)
- To increase participation, a re-contact letter was written and approved in order to reach out to eligible participants from the parent "BabyBump" study who had not responded to request for enrollment in the post-weaning study
    - 8 Babybump study participants were contacted to determine interest in participating in the weaning cohort. Of these 8 women, 3 responded and were enrolled for the post-weaning time point.
- Completed follow up and post-weaning liver imaging visits for 16 study participants
- Measured liver volume by segmentation on MR scans and volume calculation for 16 post-weaning liver imaging visits
- Actively following final 3 enrolled participants until time of weaning or scheduled study visit or weaning

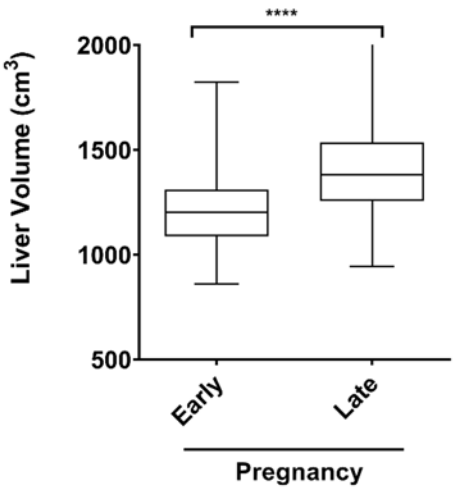

Figure 2. Liver volume (cm^3) calculated from MR scans at early and late pregnancy; N=47 pairs, paired t-test p<0.0001

Aim 3: Significant Results and Key Conclusions

Previously we found that liver volume increases by an average of 15% between early and late pregnancy (in the "BabyBump" portion of this study, **Figure 2**). However, not all women have increased liver volume during pregnancy; some have not change and for a small group liver volume is actually lost between first and third trimesters (**Figure 3**). Further, the participants who gained liver volume during pregnancy (**Figure 4 left panel**)

were more likely to have a reduction in liver volume post-weaning compared to participants who lost or did not change liver volume during pregnancy (**Figure 4 right panel**).

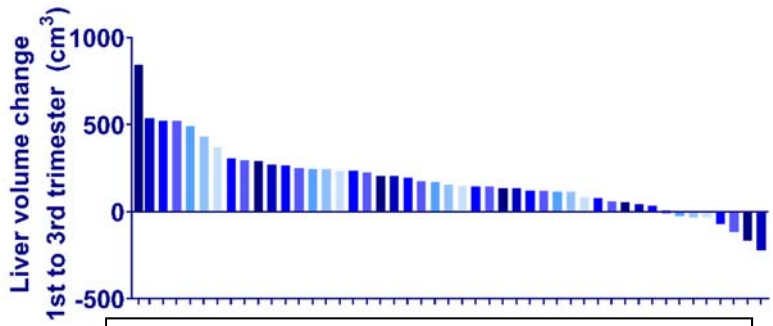

Figure 3. Change in liver volume (cm^3) from early to late pregnancy; each bar represents 1 woman

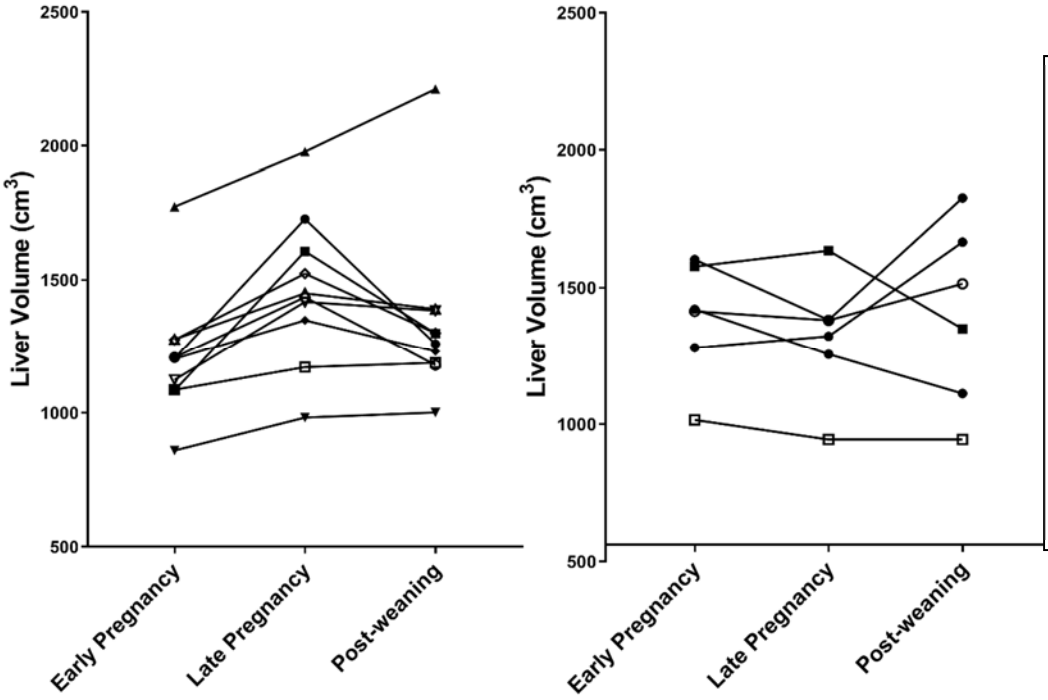

Figure 4. Liver volume at early pregnancy, late pregnancy, and post-weaning for participants who completed all three time points (n=16); data is divided by participants who gained liver volume during pregnancy (LEFT) and those who did not gain volume during pregnancy (RIGHT).

We also find that the liver volume in the first trimester is highly correlated to a women's body size overall (**Figure 5**). Since a woman's' body weight at early pregnancy is similar to her pre-pregnant body, these data fit the dominant hypothesis that liver size is regulated by body size. However, surprisingly, we did not see that third trimester body size correlated well with liver size, indicating that the mechanism by which liver size changes during pregnancy is unlinked to overall body size. We have yet to find a clinical correlate that predicts liver volume change during pregnancy.

Preliminary data from rodent studies show that bile acid synthesis is elevated during pregnancy and lactation, and is reduced post-weaning. Based on literature showing that bile acid levels can modulate liver size, we hypothesize that liver size change during pregnancy, lactation, and post-weaning may be driven by increase and subsequent decrease in bile acids. We recently received additional OHSU internal pilot funding to develop a

robust assay to measure bile acids in human serum using LC-MS/MS methods. Collection and analysis of these data are ongoing.

Finally, we find that the liver returns to its "pre-pregnancy" size after weaning (that is, using first trimester liver size as a surrogate for pre-pregnancy size), regardless of liver size increase or decrease with pregnancy **(Figure 6**). These data are consistent with a liver size homeostatic mechanism that is dysregulated during pregnancy/lactation, but reestablished after weaning.

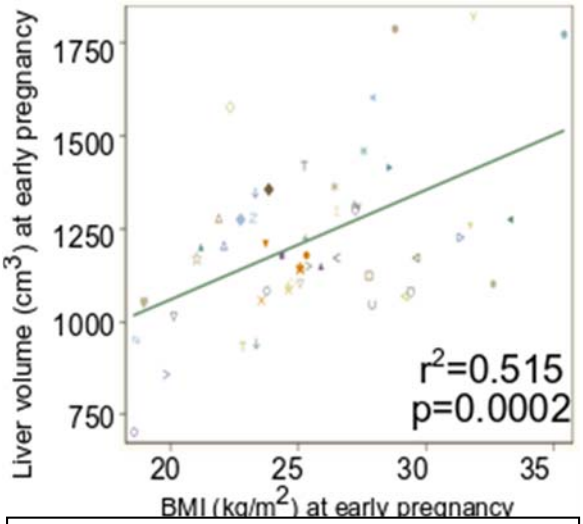

Figure 5. Correlation between BMI and liver volume at early pregnancy.

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

Alex Quackenbush: Ms. Quackenbush is a PhD candidate who studies are funded by this award. As such, this project has provided substantial training and professional development activities for Ms. Quackenbush. Specifically, she has:

- Developed bench skills in multiplex IHC experimental design, staining, and analysis
- Learned about and implemented IRB and DoD regulatory requirements, including document drafting and revision
- Gained experience in phase 0 clinical trial execution and data analysis (per Aim 3)
- Engaged in bi-weekly mentor meetings consisting of focused one-on-one work relevant to all three aims of this project
- Honed data presentation skills across monthly presentations either in lab meetings, departmental seminars, poster sessions, or lay public forums
- Developed professional network and awareness of state of the field at international conference on metastasis (Metastasis Research Society 2018 meeting, Princeton, New Jersey)
- Gained mentoring experience as a preceptor for undergraduate summer research intern, Ms. Churchill and as a primary technical trainer for Mr. Klug, an animal husbandry expert in the lab, whose skill set has been expanded by this award to include the liver portal vein breast cancer metastasis model.

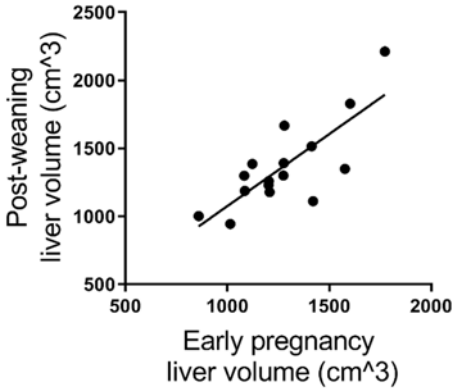

Figure 6. Correlation of liver volume at early pregnancy and post-weaning; N=16, linear regression, R^2=0.617, p=0.0003

Beth Churchill: Ms. Churchill is an undergraduate summer intern from Michigan State University, who worked with Ms. Quackenbush to carry out multiplex immunohistochemical staining for the animal studies described in Aim 1. As part of her training, Ms. Churchill was able to:

- Engage with primary literature relevant to the project
- Learn to perform multiplex IHC staining and software-based analysis for quantification of multiplex IHC staining
- Gain experience in an established breast cancer research laboratory, including participation in weekly lab meetings and several one-on-one meetings with the principle investigator

- Create a poster describing her research project and present poster at public forum

Alex Klug: Mr. Klug is a research associate who has worked with Ms. Quackenbush on all of the animal studies for this project. In this capacity he has learned our novel portal vein model of liver metastasis, as well as multiplex immunohistochemistry.

How were the results disseminated to communities of interest?

Both Dr. Schedin and Ms. Quackenbush have presented work on this project at scientific meetings whose audiences are interested in the subject matter

- Schedin:
  - Postpartum Tissue Remodeling Drives Breast Cancer Metastasis, Major Symposia: Metastatic Microenvironment Dictates Progression and Therapy Responses, AACR Annual Meeting, Chicago, Illinois, April 15, 2018
  - Mucosal Biology & Tissue Involution Cooperate to Drive Breast Cancer Metastasis: Cancer Biology Pathways Lecture, Siteman Cancer Center, Washington University, St. Louis, MO, May 22, 2018
  - Keynote, Follow the data; challenging established paradigms in young women's breast cancer. 14th Annual Baylor College of Medicine Breast Center Retreat, Houston, TX, September 6, 2018.
  - Collagen as a Target for NSAID-based Breast Cancer Prevention: A Tribute to Patricia Keely. American Society for Matrix Biology 2018 Biennial Meeting, Las Vegas, NC, October 15, 2018
  - Challenging Established Paradigms in Young Women's Breast Cancer. University of Chicago, School of Medicine, Comprehensive Cancer Center, Chicago, Illinois, October 18, 2018
  - Education Symposium, Insights & Controversies in Metastasis Biology, Breast cancer's metastatic moment: How postpartum tissue involution facilitates progression, San Antonio Breast Cancer Symposium, San Antonio, Texas, December 4, 2018
  - Challenging Established Paradigms in Young Women's Breast Cancer, Louisiana Cancer Research Center, Tulane University School of Medicine, New Orleans, LA, February 11, 2019
  - Young Women's Breast Cancer. What is the role of pregnancy? Susan G. Komen Oregon & SW Washington, 2019 Regional Breast Cancer Issues Conference, Portland, Oregon, March 16, 2019
  - Common Perceptions & Misconceptions of Young Women's Breast Cancer, Education Session: Update on Young Women's Breast Cancer, AACR Annual Meeting 2019, Atlanta, Georgia, March 30, 2019
- Quackenbush:
  - Poster Presenter, Investigating tumor promotion in the postpartum liver metastatic niche. Awarded 3rd place. 17th Biennial Congress of the Metastasis Research Society, Princeton, New Jersey, August 3, 2018.
  - Quackenbush: Poster Presenter, 2018 ARCS (Achievement Rewards for College Scientists) Foundation Oregon Luncheon. Portland, Oregon, October 30, 2018.

- **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 plan to continue following the SOW for year 2.*

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."*
    - ***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."*
    - ***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.
    - The development of the bile acid and 7alphaC4 assays in human plasma using LC-MS/MS methods are broadly applicable to all diseases with a bile acid component. Further, this method has already been highlighted in an OHSU SOM Lunch and Learn lecture series, which is designed to introduce OHSU faculty and staff to cutting edge advances made by SoM Core labs.
- **What was the impact on technology transfer?**
  - *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
    - ***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;*
      - ***Nothing to Report***
    - *instances where the research has led to the initiation of a start-up company; or*
      - ***Nothing to Report***
    - *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."*
    - ***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;*
    - ***Nothing to Report***
- *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 Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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:*
    - ***Nothing to Report***

- **Changes in approach and reasons for change**
    - *Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*
        - **Nothing to Report**
- **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.*
        - **Nothing to Report**
- **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.*
        - **A revised budget was submitted on January 8, 2018, and we are nearing completion of all of the required documentation required to initiate the revised new budget.**
- **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**
    - **<u>Nothing to Report</u>**

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.
        - **<u>Nothing to Report in all categories-conference presentations listed above.</u>**
        - **Journal publications.**
        - **Books or other non-periodical, one-time publications.**
        - **Other publications, conference papers, and presentations.**
    - **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.
        - **<u>Nothing to Report</u>**
    - **Technologies or techniques**
      Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.
        - **<u>Nothing to Report</u>**
    - **Inventions, patent applications, and/or licenses**
      Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. 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.
        - **<u>Nothing to Report</u>**
    - **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;*
        - *biospecimen 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."*

8. Name:	Pepper Schedin
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-4244-987X
Nearest person month worked:	1.68 calendar/no change
Contribution to Project:	Dr. Schedin led all aspects of the project, including scientific focus, experimental design, data analysis, data integrity, budget management, human and animal regulatory aspects, and manuscript writing.
Funding Support:	W81XWH-18-1-0086
Name:	Jonathan Purnell
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-5505-6333
Nearest person month worked:	0.12/no change

Contribution to Project:	Dr. Purnell is co-lead on Aim 3 designed to obtain first-of-kind evidence for weaning-induced liver involution in women via a serial MRI imaging study of livers in healthy women across pregnancy and weaning.
Funding Support:	W81XWH-18-1-0086
Name:	Zahi Mitri
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-8765-7723
Nearest person month worked:	No salary support as of 4/9/2019, as the aim Dr. Mitri is a co-investigator on has yet been initiated. This is because we are awaiting final approval of our IRB documentation from DOD.
Contribution to Project:	Dr. Mitri is a medical oncologist who is involved in Aim 2: to explore the liver metastatic niche in breast cancer patients utilizing tumor and adjacent normal liver tissue obtained from breast cancer patients with liver metastases.
Funding Support:	NA
Name:	Skye Mayo
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-1631-9855
Nearest person month worked:	No salary support as of 4/9/2019, as the aim Dr. Mayo is a co-investigator on has yet been initiated. This is because we are awaiting final approval of our IRB documentation from DOD.
Contribution to Project:	Dr. Mayo is an oncologic surgeon who is involved in Aim 2: to explore the liver metastatic niche in breast cancer patients utilizing tumor and adjacent normal liver tissue obtained from breast cancer patients with liver metastases.
Funding Support:	NA
Name:	Andrea Calhoun
Project Role:	Histotechnician

Researcher Identifier (e.g. ORCID ID):	Does not have an ORCID ID
Nearest person month worked:	3.92 calendar/no change
Contribution to Project:	Ms. Calhoun is responsible for tissue fixation, processing, sectioning and staining related to Aim 1 of our application: Use liver metastasis mouse models to decipher the post-intravasation steps of the metastatic cascade that are supported by the involuting liver.
Funding Support:	W81XWH-18-1-0086
Name:	Sonali Jindal
Project Role:	Pathologist
Researcher Identifier (e.g. ORCID ID):	0000-0002-3911-6815
Nearest person month worked:	0.94 calendar/no change
Contribution to Project:	Dr. Jindal is an MD trained pathologist responsible for oversight of our histology lab, including personnel and the mIHC pipeline, and performs pathologic assessment of all tissues collected from both rodent and human based studies. …
Funding Support:	W81XWH-18-1-0086
Name:	Alex Klug
Project Role:	Animal Husbandry
Researcher Identifier (e.g. ORCID ID):	0000-0003-4958-1961
Nearest person month worked:	3.04 calendar/no change
Contribution to Project:	Mr. Klug is responsible for all animal husbandry associated with Aim 1 of this grant. Mr Klug is also trained in the portal vein injection surgeries, and works as part of a two member team with Ms Quackenbush, to perform all of the

	surgeries described in Aim 1. Mr Klug is also gaining expertise in mIHC and is contributing to Aim 1 objectives related to mIHC.
Funding Support:	W81XWH-18-1-0086
Name:	Alexandra Quackenbush
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0001-7912-6084
Nearest person month worked:	12.00 calendar/no change
Contribution to Project:	Ms. Quackenbush is deeply involved in all three aims of this grant. She is responsible for all of the preclinical model studies outlined in Aim 1, and in this capacity is also responsible for managing Mr. Klug's animal husbandry activities and for his training in the liver metastasis model. For Aim 2, Ms Quackenbush has been instrumental in establishing our IRB approved protocol to obtain research only liver biopsies from women with breast cancer metastatic to the liver. For Aim 3, Ms Quackenbush is lead on data acquisition, coordinating research efforts with KPNW including data transfers, and for manuscript preparation.
Funding Support:	OHSU Knight Cancer Biology Program

- **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?**
    - **No change, and nothing to report**
    - *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 projec**t *(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.**

9. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating 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.*
10. **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. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES***

BC170206: Novel Postpartum Liver Biology Has Implications for Breast Cancer Liver Metastasis

PI: Pepper Schedin, OHSU, Oregon **Budget:** $1,147,431

Topic Area: Metastasis **Mechanism:** FY17, BCRP, Breakthrough Award-Funding Level 2

Research Area(s): SCS Coding **Award Status:** 3/15/2018 – 3/14/2021

Study Goals:

We identify the lack of understanding of postpartum liver biology as a major obstacle to identifying therapeutic targets aimed at destabilizing the liver metastatic niche in postpartum breast cancer patients. The goals of these studies are to:

1) provide mechanistic understanding of how liver metastasis is promoted in the postpartum host;

2) perform discovery studies of the human liver metastatic niche through use of human specimens from patients with breast cancer metastasis to the liver;

3) build strong translational rationale by studying how healthy women's livers change with pregnancy and after weaning.

Specific Aims:

Aim 1). Use liver metastasis mouse models to decipher the post-intravasation steps of the metastatic cascade that are supported by the involuting liver. **Aim 2)** Explore the liver metastatic niche in breast cancer patients utilizing tumor and adjacent normal liver tissue obtained from breast cancer patients with liver metastases. **Aim 3)** Obtain first-of-kind evidence for weaning-induced liver involution in women via a serial MRI imaging study of livers in healthy women across pregnancy and weaning

Key Accomplishments and Outcomes:

1. Significant progress on Aim 1 rodent metastasis studies, including development of revised hypothesis; 2) have IRB for Aim 2 approved by OHSU; awaiting final approval from DoD HRPO; and, 2) completed all regulatory requirements from Aim 3, completed enrollment, 85% (16/19) of participants have completed all clinical visits, and we have advanced to analysis phase.

Presentations: Pepper Schedin: Education Symposium, Insights & Controversies in Metastasis Biology, Breast cancer's metastatic moment: How postpartum tissue involution facilitates progression, San Antonio Breast Cancer Symposium, San Antonio, TX, 12/4/18. AACR Education Session: Update on Young Women's Breast Cancer, 2019 AACR annual Meeting, Atlanta GA, March 30, 2019

Patents: none to date

Funding Obtained: We applied for and received $5,000 in funding from OHSU's School of Medicine Dean's office to investigate a new hypothesis related to Aim 3. We hypothesize that liver growth during pregnancy and subsequent reduction in liver size post-weaning is driven by shifts in the circulating bile acid pool. This funding allows us to partner with a Pharmacokinetics research core at OHSU to profile serum bile acids in our study participants, as well as develop a new assay to investigate the rate of bile acid synthesis.