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14. ABSTRACT

Women diagnosed with breast cancer within 10 years of a completed pregnancy are 2~3x more likely to develop liver metastases than never-pregnant (nulliparous) patients, even after controlling for prognostic variables. This finding suggests a unique biology in the postpartum liver, a putative pre-metastatic niche, which makes postpartum patients more susceptible to liver metastases. Here we tackle the problem of defining the liver-breast cancer 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. In rodent models, we previously reported increased liver size, hepatocyte proliferation, and anabolic metabolism during pregnancy and lactation. Within one week post-weaning, the rodent liver returned to its pre-pregnant size via a coordinated cell death and tissue remodeling process we call liver involution.

To explore a potential relationship between liver involution and liver metastasis, we utilized an immune competent murine model of postpartum breast cancer. In this model, liver metastases are induced by portal vein injection of mammary tumor cells into nulliparous or involution hosts. Using two different mammary tumor cell lines, we find that the process of involution supports overt liver metastasis. To investigate if increased metastatic burden in the involuting liver is due to increased tumor cell seeding, the number of tumor cells that extravasated into the liver parenchyma was evaluated over time. Seeding was not enhanced in involution hosts. Further, in the first 3 days following injection, tumor cells were more likely to form small clusters in nulliparous hosts yet remain as single, elongated tumor cells in the involution hosts. Time-course experiments show the metastatic advantage in the postpartum host emerges ~2 weeks after tumor cell injection. This time frame is consistent with differential tumor cell clearance via cytotoxic immunity, which we hypothesize differs between nulliparous and involution hosts. Data in support of this hypothesis includes our recent results showing impaired T naïve T cell priming in the involution host, suggestive of immune suppression in the involution liver microenvironment.

To investigate relevance of weaning-induced liver involution in women, we recruited healthy pregnant women to undergo MRI liver scans at first and third trimester, in addition to other imaging and lab tests. In a cohort of 47 women we find, overall, increased liver volume with pregnancy (average 15% increase). A subset of participants (n=17) underwent an MRI liver scan at >3 months post-weaning to ask if liver size decreases after weaning. Post-weaning liver volumes were reduced compared to third trimester liver volumes, and were similar to baseline liver sizes. These data are consistent with the occurrence of weaning-induced liver involution in women, and may shed light into the high rates of liver metastases observed in young breast cancer patients.

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1. **INTRODUCTION:**

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. This report covers accomplishments of the first two years of funding in our three year project.

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

3. **ACCOMPLISHMENTS:**

- **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: *100% complete*
Year 1, months 7-12: tissue sectioning *100% complete*
Year 2, months 1-6: Immunohistochemistry (IHC) data capture and IHC quantitation: IHC endpoint terminated due to inability to detect the variant of GFP transfected into these cells by multiple anti-GFP antibodies (as presented in Yr 1 final report). For this D2A1-GFP model, we will rely exclusively on histological evidence of tumor mass.
Year 2, manuscript preparation: *10% complete*

Liver Metastasis Model 2- Balb C mice/ D2OR-GFP mammary tumor cells (time course of 90 min, 1, 3, and 14 days post tumor cell injection)

Year 1, months 6-12: animal studies: *100% complete*
Year 2, months 1-6: tissue sectioning *100% complete*, multiplex IHC: *55% complete*
Year 2, months 7-12: IHC data capture and quantitation: *40% complete*
Year 2, manuscript preparation: *10% complete*

NEW Metastasis model (added Yr 1Q4) Model 3 – Nude mice/D2OR mammary tumor cells

Year 1, months 9-12: animal studies *100% complete*
Year 2, months 1-3: functional evidence for immune suppression-in vivo T cell activation assays: *100% complete*
Year 2-3, manuscript preparation: *10% 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: 24 cases to date [target for Q8 was 30 total cases; overall goal by study end is 40-56 total accrual]: *40% complete*
Year 2, months 1-6: First tissue batch of block sectioning- *100% complete*; multiplex IHC: 33% complete
Year 2, months 7-12: batch #1 (n=18 cases) IHC mIHC panel #1 ("Discovery Panel") data capture and quantitation: *100% complete*

Year 2, months 7-12: development of mIHC panel #2 (ECM and integrin panel) –panel optimization is *90% complete*
Year 2, months 7-12: Batch #1, mIHC panel #2-*0% complete*; *delayed due to temporary lab closure due to Covid19*
Year 2, months 7-12: Second tissue batch of three/block sectioning, multiplex IHC: *10% complete*
Year 3: (4-15-2020 through 4-14-2021)
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: *100% 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: *100% 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: *100% complete*
Years 2-3: manuscripts preparation: *80% complete*

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.*

Aim 1: Major Activities and Specific Objectives

- Mouse metastasis studies with D2OR-GFP tumor cells to investigate if the involution metastatic advantage emerges by 2 weeks post-injection, a time point after which the adaptive immune system would be activated to respond to tumor cells
- Mouse adoptive cell transfer studies to investigate if the involution liver is immune suppressed
- Mouse metastasis studies in immunocompromised hosts with D2OR tumor cells to investigate the role of T cells in establishment of overt liver metastasis nulliparous and involution hosts
- Multiplex IHC studies in liver tissues from mice injected with D2OR-GFP tumor cells to investigate how tumor cells interact with liver environment

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, and as reported in our Year 1 annual report. We subsequently pursued an alternative hypothesis-differential immune cell clearance of tumor cells between nulliparous and involution hosts accounts for the difference in overt metastases at study endpoint (5-6 weeks). Differential tumor cell clearance due to T cell activation would be expected to be manifest between 7-10 days post tumor cell injection. To investigate the role of the adaptive immune system, we extended the tumor time-course to 14 days after tumor cell injection, as this is time would capture activation of the adaptive immune system and resultant tumor cell clearance. We find significantly elevated tumor size per lesion in involution mice compared to nulliparous mice, measured from histological analyses, data consistent with suppression of immune surveillance in the involuting liver, and tumor cell outgrowth (**Figure 1**).

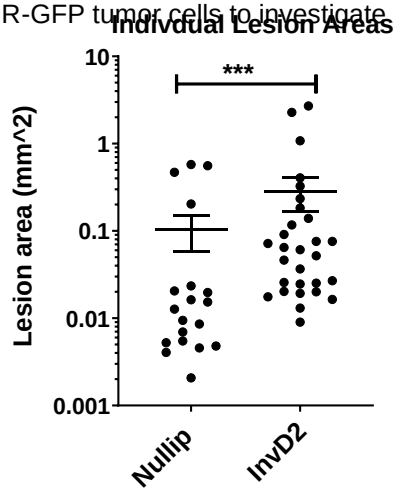

Figure 1. Area (mm^2) of individual liver metastatic lesions from mice euthanized 14 days post-tumor cell injection; p=0.006 by Mann-Whitney test.

We next directly tested the hypothesis that the involution liver is immune suppressed. Specifically, we hypothesized that antigen-specific T cells responses are inhibited in the involuting compared to nulliparous liver. To test this hypothesis we designed an in vivo assay where ovalbumin-specific CD4 T cells were delivered systemically. Subsequently, mice were intra-hepatically injected with ovalbumin (e.g. the cognate antigen) or PBS. After 5 days, sufficient time for antigen presentation, T cell priming, and T cell activation, mice were euthanized. The frequency of ovalbumin (ova) specific CD4 T cells was measured in the liver and the spleen by flow cytometry. We find significantly increased ova-specific T cells in the nulliparous host liver when antigen is given ("Nullip OVA") compared to PBS, indicating T cell activation. However, the involution group mice do not show signs of T cell activation when antigen is given as there is no difference in frequency of ova-specific T cells between PBS and OVA treated mice (**Figure 1)**. This result is supportive of the hypothesis that antigen-specific T cell responses are inhibited in the involution liver. In sum, these data are the first, to our knowledge, to demonstrate reproductive control of T cell responses in the liver, with implications for breast cancer metastasis to the liver.

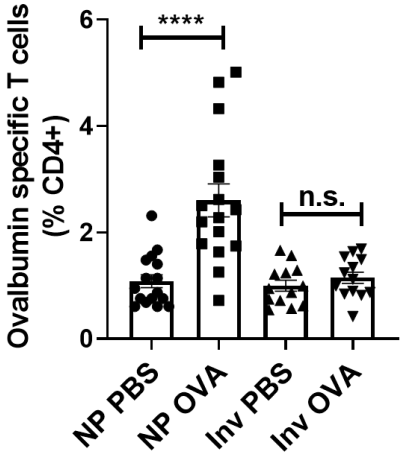

Figure 2. Frequency of ovalbumin-specific CD4 T cells in the mouse liver. Data represent 2 independent experiments; analyses One-way ANOVA with multiple comparisons, ** $p<0.01$.

To investigate the role of T cells in our liver metastasis model, we repeated the overt metastases experiments in the background of immune compromised mice, and find – surprisingly- that no liver metastases grew in nude hosts of either nulliparous (n=16) or involution (n=6) groups. Yet, our wildtype (WT) controls behaved as we expected with ~3x increased overt liver metastatic incidence in involution compared to nulliparous WT mice. Furthermore, in the nude model, we performed tumor cell injection into the mammary gland. Within the same mouse, tumor cells grew out in the mammary gland, but not in the liver, demonstrating that the tumor cells were viable. These data demonstrate that the lack of liver tumors in the nude hosts cannot be accounted for by the trivial explanation that the tumor cells were compromised. Overall, the expected liver metastases in the control WT group and observed mammary tumor growth in nude hosts help us rule out experimental confounders and add rigor. While these data support a key role for immune milieu in establishing liver metastases, the surprising, and novel findings of lack of liver metastases in the nude hosts remain outside the scope of this DOD application and will not be pursed with this grant mechanism.

Finally, we developed and optimized a multiplex immunohistochemistry staining panel to assess how infiltrating tumor cells interact with the murine liver environment. In particular, we utilized the markers CK18, CK19, and Heppar to distinguish tumor cells, bile ducts, and hepatocytes. Bile ducts are dual positive for CK18 and CK19 in the mouse, and show up as orange in the figure to the right (**Figure 3**). Tumor cells are CK19-, Heppar-, and CK18+ (green, in Figure 3). Initial experiments show an intriguing relationship between tumor cells and bile ducts, where some tumor cells seem to integrate themselves into bile duct structures (see yellow areas, **Figure 3**). This is an observation we will continue to follow up on, as it could be a novel mechanism of tumor dormancy in the rodent liver.

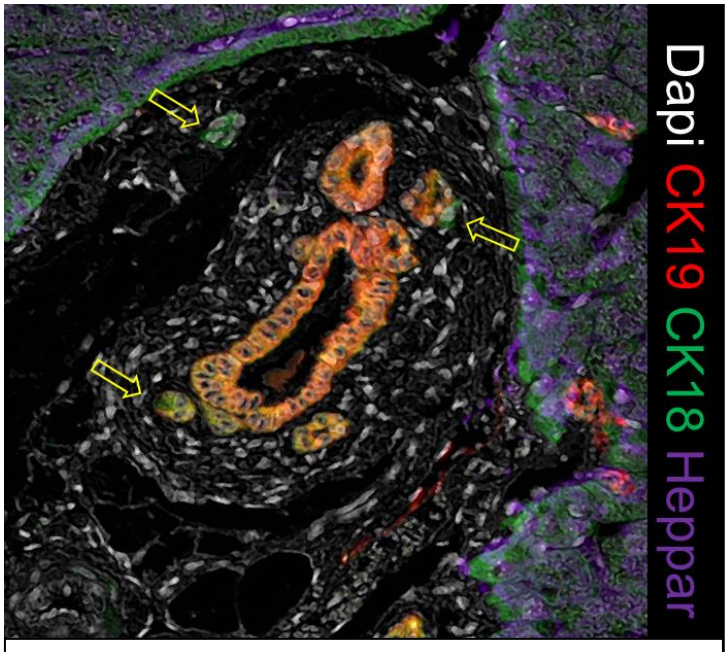

Figure 3. Multiplex immunohistochemistry image showing tumor cells integrated into bile ducts in a mouse model of PPBC.

Aim 2: Major Activities and Significant Results

In year 2, our Aim 2 IRB protocol, consent and authorization forms were approved by both OHSU's IRB office and HRPO. As per HRPO request, we have identified an independent research monitor (Sarah Ward), as our protocol is deemed greater-than-minimal risk for individuals who received research-only liver biopsies.

To date, we have received 24 cases of breast cancer liver metastases and 4 matched primary breast cancers, 21 cases from the OHSU tissue biorepository and 3 prospective cases from patients receiving a clinically indicated liver resection. No subjects have been enrolled for research-only liver biopsies.

Table 1: Discovery mIHC Panel

Biomarker	**Target**
Gata3	Breast cancer cells
CK5	Basal breast cancer cells
CK7	Luminal breast cancer cells
CK18	Breast cells, hepatocytes
Heppar	Hepatocytes
ER	Estrogen receptor expressing cells
D240	Lymphatic vessels
Aqp1	Blood vessels (Aqp1: aquaporin)
Col IV	Basement membrane
Ki67	Proliferating cells

We completed multiplex immunohistochemical (mIHC) staining for 18 of the liver metastasis cases with 10 distinct cancer biomarkers (Table 1, "Discovery mIHC Panel"). We also optimized a second mIHC panel to assess extracellular matrix and integrin proteins in liver metastases, and are preparing to stain the first cohort of cases (contingent on lab re-openings at OHSU).

Data capture is complete and data analysis is ongoing for this first batch of staining with the "Discovery Panel", which is designed to investigate biologic subtype features of breast cancer metastases in the liver. We are also able to determine what liver anatomical structures the metastases interact with, such as the venous blood supply, bile ducts, and/or hepatic sinusoids.

Initial data assessment reveals that biomarkers are relatively homogenous within a case yet heterogeneous across cases. We took representative intra-tumoral areas from each case and created a qualitative heatmap of staining intensity where each row represents one case of liver metastasis (**Figure 4**). Note that the data shown in the heatmap only represents the internal tumor areas and does not include tumor border or adjacent normal liver areas. Across cases, we find a range of expression for cytokeratins with a luminal type (CK18+, CK7) being most common and almost no basal type (CK5+). Further, we find low signal for estrogen receptor, which is intriguing since the majority of primary tumors were estrogen receptor positive. We are continuing these analyses with quantitative approaches for each biomarker.

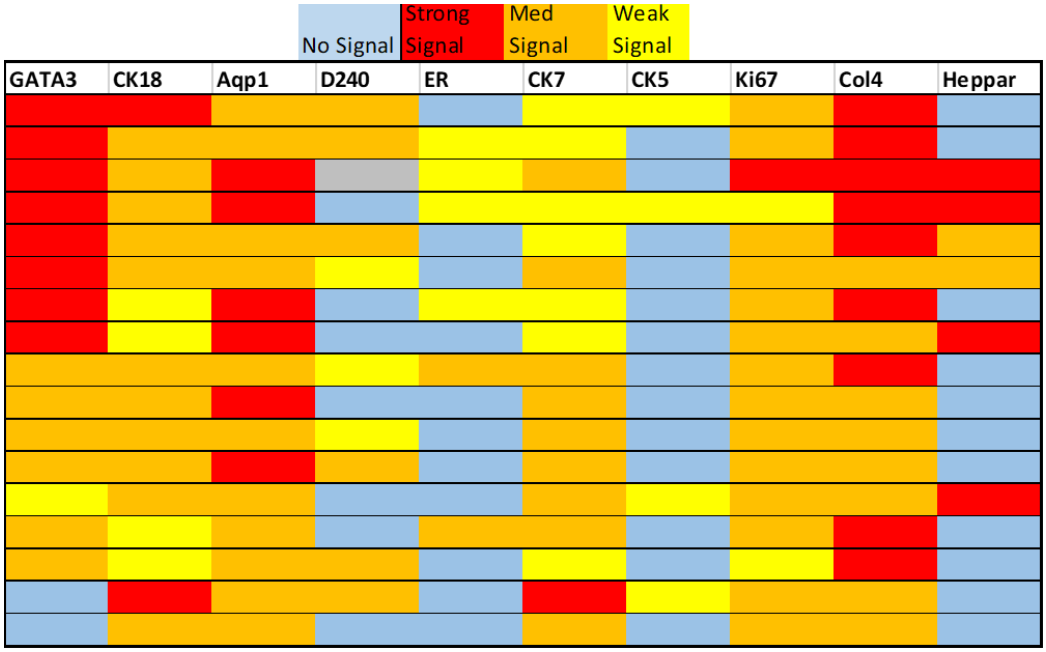

Figure 4. Heatmap of qualitative assessment of staining intensity for Discovery Panel biomarkers based on intra-tumoral regions. Every row represents one liver metastasis case.

We also assessed the pattern in which breast cancer cells grew within the liver. For example, we were interested to see if breast cancer liver metastases showed signs of desmoplasia or epithelial-to-mesenchymal transition. Intriguingly, we mostly observed growth patterns that resemble normal mammary gland structures or well-differentiated breast cancers. Shown in **Figure 5** is a representative image of breast tumor cells (in green/yellow) that are arranged in a ductal/alveolar-

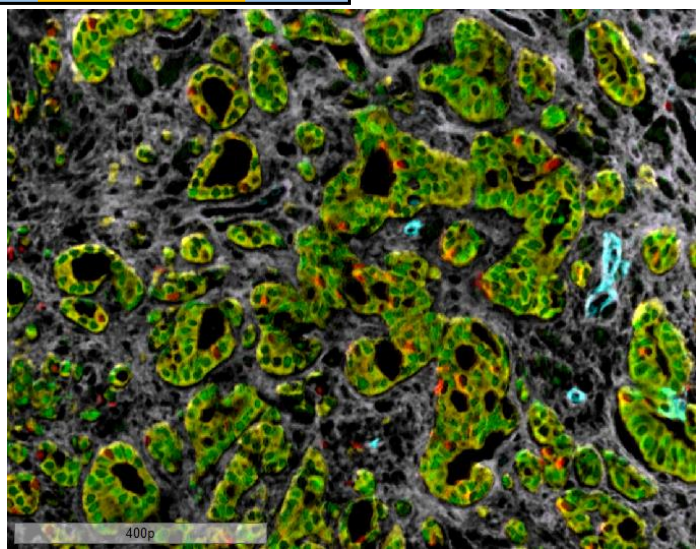

Figure 5. Representative mIHC image from a breast cancer liver metastasis showing ductal-alveolar growth pattern. Green: Gata3 (tumor); Yellow: CK18; Red: Ki67; Cyan: Aqp1 (blood vessels).

pattern. We find no evidence of EMT or desmoplasia, except in the treated cases with residual disease.

Aim 3: Major Activities

- Completed pregnancy visits for n=45 participants
- Completed post-wean study visits for all enrolled participants (n=17 participants).
- Performed correlative analysis of liver volumetric data with clinical parameters to determine what variables, if any, correlate with liver size during pregnancy and post-wean
- Completed full draft of study manuscript and figures

Aim 3: Significant Results and Key Conclusions

Pregnancy Data: Firstly, we find that the liver volume in the first trimester is highly correlated to a women's body size overall. 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. Secondly, we find that the majority of women gain liver volume between their first and third trimesters. However, surprisingly, third trimester body size did not correlated well with liver size, indicating that the mechanism by which liver size changes during pregnancy is unlinked to overall body size.

Using insight gleaned from rodent studies, demonstrating a role for bile acid pools in determining liver growth, we developed a robust assay to measure bile acids in human serum using LC-MS/MS methods, with data collection complete. Intriguingly, we find the rate of bile acid synthesis, measured via a byproduct of the synthetic reaction 7alphahydroxy-4cholesten-3-one, increases between early and late pregnancy but only in women who have an increase in liver volume during pregnancy (**Figure 6**). These data, combined with a known role for bile acids in liver regeneration, suggests that bile acid biology may play a role in pregnancy-induced liver volume increase.

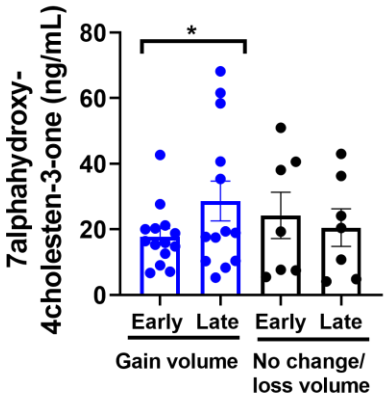

Figure 6. 7alphahydroxy-4cholesten-3-one concentrations at early and late pregnancy in women who gained liver volume with pregnancy (n = 14) and women who did not (n = 7). Boxes represent mean and whiskers represent SEM; Paired t-test

Postpartum Data: We find the participants who gained liver volume during pregnancy 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. We delineated the post-wean cohort into two groups using our rodent work as a guide: women with the anticipated pattern of increase in liver volume during pregnancy followed by a decrease after weaning ("gain-loss"), and those that did not display this expected increase/decrease pattern ("not gain-loss"). Nine of the 17 women had liver volume changes that mirrored what we observe in the healthy rodents, i.e. liver gain during pregnancy and loss post-wean (n =9) (**Figure 7**). Comparatively, the group that did not follow the gain-loss pattern included 3 women who lost liver volume during pregnancy, with re-gain by post wean, 3 women with no liver volume changes at either pregnancy or post wean time points, and one woman each showing either continuous liver size loss or liver size gain across the study timeline (**Figure 8**). Interestingly, the group of women with the "not gain-loss" liver patterns were also enriched for cases of hypertension compared to the "gain-loss" group. Combined, our rat and human data support the hypothesis that one component of a healthy reproductive cycle is the ability of the liver to increase in functional size with pregnancy and return to baseline post wean.

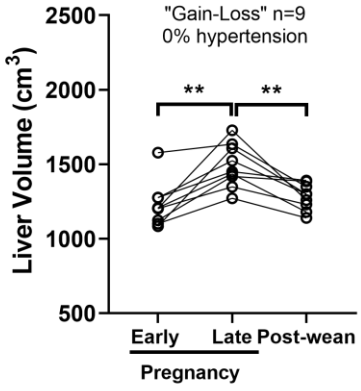

Figure 7. Plot showing dominant pattern (n = 9) of liver volume gain with pregnancy and liver volume loss post-wean. Lines connect liver volume measurements from a single case; Paired T-test

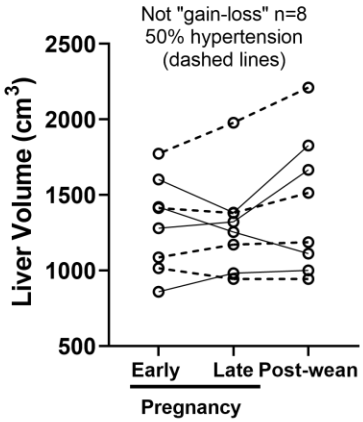

Figure 8. Plot showing sub-dominant patterns (n = 8) of liver volume change during pregnancy and post-wean. All lines connect liver volume measurements from a single case. Dashed lines show cases diagnosed with gestational hypertension (n = 4).

- **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
  - Increased proficiency in mouse models, including utilizing antibody-mediated cell depletion strategies
  - Learned about and implemented IRB and DoD regulatory requirements, including document drafting and revision
  - Gained experience in phase 0, non-intervention 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
  - Gained mentoring experience as a preceptor for undergraduate summer research intern, Ms. Fecker and a new graduate student in my lab

  Adeline Fecker: Ms. Fecker is an undergraduate summer intern from University of Oregon, 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. Fecker 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

- **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:

  Challenging Established Paradigms in Young Women's' Breast Cancer, University of Minnesota Medical School, Grand Rounds, Minnesota, MN, May 8, 2019

  Mucosal Biology and Tissue Involution Cooperate to Drive Breast Cancer Progression, Mammary Gland Biology Gordon Research Conference, Newry Maine, June 11, 2019

  Postpartum Breast Cancer: Population science to bench and bedside and back again. University of Colorado Cancer Center, Cancer Prevention and Control Seminar Series, Anschutz Medical Campus, Aurora, CO, November 13, 2019

  Postpartum Breast Cancer. Breast Cancer Think Tank 30, Isla Bella Resort, Marathon FL, January 13, 2020

  Postpartum Breast Cancer. 21st Laura Evans Memorial Breast Cancer Symposium, Sun Valley Idaho, March 6, 2020

  Prevention of postpartum breast cancer-are we close? Transdisciplinary Cancer Interception, a nature conference, Salt Lake City, Utah, March 9, 2020

  Quackenbush:

  Oral presentation, "Reproductive-Dependent Biology Drives Liver Metastasis in Young Women's Breast Cancer", Cell, Developmental, and Cancer Biology Annual Retreat, Portland, Oregon September 4, 2019.

  Abstract accepted for poster session, "Does reproductive biology drive liver metastasis in Young Women's Breast Cancer?", AACR Annual Meeting, April 24,2020* cancelled to due Covid-19

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

  We plan to continue following the SOW for year 3.

4. **IMPACT:**

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

  Nothing to report: pending publication

- **What was the impact on other disciplines?**

  Nothing to report: pending publication

- **What was the impact on technology transfer?**

  Nothing to report: pending publication

- **What was the impact on society beyond science and technology?**

  Nothing to report: pending publication

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:*

   - **Changes in approach and reasons for change**

     Nothing to report for year 2, however for year 3, our work has already been slowed by the need to shut down the lab and clinical research due to COVID19. As of 6-15-2020, we are opening the labs at 30% capacity, and the duration of this limited work capacity is currently unknown. In addition, if Oregon experiences a second peak, we will be required to close the labs again.

   - **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.*

       For Aim 2, during COVID19, we are beginning to explore accessing existing tissue banks to obtain human tissues required to complete this aim, rather than prospectively obtaining liver biopsies of metastatic breast cancer as originally proposed.

       We are prioritizing mIHC studies because these studies are amenable to shift work as well as to being ‘shut-down’ on short notice.

   - **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.*

       Cost of shifting lab based work to exclusively off site/remote work: downtime with restructuring goals, shut down of technical work.

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

     Nothing to report

   - **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.**

       - Betts, CB, Quackenbush A, Anderson W, and Schedin, PS. (2020). Mucosal immunity and liver metabolism in the complex condition of lactation insufficiency. *Journal of Human Lactation. In press.*

     - **Books or other non-periodical, one-time publications.**

     - **Other publications, conference papers, and presentations.**

       Nothing to Report in all categories-conference presentations listed above.

   - **Website(s) 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?**

     - *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."*

Name:	Pepper Schedin
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-4244-987X
Nearest person month	1.67

worked:	
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:	*(Complete only if the funding support is provided from other than this award).*
Name:	Jonathan Purnell
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-5505-6333
Nearest person month worked:	0.29
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:	
Name:	Zahi Mitri
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-8765-7723
Nearest person month worked:	0.26
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:	
Name:	Skye Mayo
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0002-1631-9855
Nearest person month worked:	0.26

Contribution to Project:	Surgeon providing liver biopsies for metastatic BrCa patients, involved in liver biopsy IRB development
Funding Support:	
Name:	Alex Quackenbush
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	0000-0001-7912-6084
Nearest person month worked:	12
Contribution to Project:	Ms. Quackenbush carried out the animal studies, worked with other team members to develop IRB protocols and other participant documents for human research protocols, organized tissue collection and multiplex IHC protocol development for Aim 2, and has performed liver volume analysis, data interpretation, and manuscript writing for Aim 3.
Funding Support:	
Name:	Alex Klug
Project Role:	Animal husbandry
Researcher Identifier (e.g. ORCID ID):	0000-0003-4958-1961
Nearest person month worked:	7.89
Contribution to Project:	Mr. Klug worked on all animal studies.
Funding Support:	
Name:	Andrea Calhoun
Project Role:	Histotechnician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5.19
Contribution to Project:	Multiplex IHC development, staining, and data capture for Aim 2.

Funding Support:	
Name:	Jayasri Narasimhan
Project Role:	Histotechnician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	7.31
Contribution to Project:	Tissue sectioning and multliplex IHC development, staining, and data capture for Aim 2.
Funding Support:	
Name:	Sonali Jindal
Project Role:	Pathologist
Researcher Identifier (e.g. ORCID ID):	0000-0002-3911-6815
Nearest person month worked:	2.55
Contribution to Project:	Lab oversight on IRB submissions and maintenance. IHC oversight and QA. Evaluation of multiplex IHC results for Aim 2.
Funding Support:	

- **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."*
        - 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:** Kaiser Permanente Pacific Northwest

- **Location of Organization:** *(if foreign location list country):* Portland, Oregon
- **Partner's contribution to the projec**t *(identify one or more)*
  - **Financial support; NA**
  - **In-kind support** *(e.g., partner makes software, computers, equipment, etc., available to project staff)*; NA
  - **Facilities** *(e.g., project staff use the partner's facilities for project activities)*; yes, Dr. Kim Vesco and her research team of KPNW are established and prior approved collaborators on this grant. However, this grant is not a collaborative grant.
  - **Collaboration** *(e.g., partner's staff work with project staff on the project)*; yes, Dr. Kim Vesco and her research team of KPNW are established and prior approved collaborators on this grant.
  - **Personnel exchanges** *(e.g., project staff and/or partner's staff use each other's facilities, work at each other's site)*; and
  - **Other.**

8. **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://ebrap.org 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. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***