AWARD NUMBER: W81XWH-15-1-0101

TITLE: Phase 1B Clinical Trial of a Candidate Breast Cancer Prevention Vaccine

PRINCIPAL INVESTIGATOR: William E. Gillanders

RECIPIENT: Washington University, St. Louis, MO

REPORT DATE: July 2021

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JULY 2021	**Annual**	01Jul2020 - 30JUN2021

4. TITLE AND SUBTITLE

Phase 1B Clinical Trial of a Candidate Breast Cancer Prevention Vaccine

5a. CONTRACT NUMBER
W81XWH-15-1-0101

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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One Brookings Drive
Saint Louis MO 63130-4862

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 This project involves a phase 1b clinical trial in breast cancer patients undergoing neoadjuvant endocrine therapy. Sixty subjects with mammaglobin-A-expressing breast cancer will be randomized in a 1:1 ratio to neoadjuvant endocrine therapy alone, or neoadjuvant endocrine therapy plus mammaglobin-A DNA vaccination. The primary objective is to assess the safety of the mammaglobin-A DNA vaccine. The secondary objective is to assess the ability of the mammaglobin-A DNA vaccine to induce an immune response to mammaglobin-A. During the first year of the project most efforts focused on optimizing patient awareness and accrual. Several protocol amendments were implemented earlier in the year to improve accrual. Additionally, we implemented screening of both medical and surgical oncologists' clinic schedules, and added Dr. Bisi Ademuyiwa, a Breast Cancer Medical Oncologist, to the trial team. To increase awareness a patient information package was prepared that explains the goal and details of the clinical trial. To date, a total of 12 patients signed the screening consent. Of these, 5 patients were eligible and 4/5 were randomized to the trial.

15. SUBJECT TERMS Phase 1b, neoadjuvant, endocrine, DNA vaccine, mammaglobin-A, immune response

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	27	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

This project involves a phase 1b clinical trial in breast cancer patients undergoing neoadjuvant endocrine therapy or chemotherapy. Forty six subjects with mammaglobin-A-expressing breast cancer will be randomized to neoadjuvant endocrine therapy alone (n=8); neoadjuvant endocrine therapy plus mammaglobin-A DNA vaccination (n=15), neoadjuvant chemotherapy (n=8) or neoadjuvant chemotherapy plus mammaglobin-A DNA vaccination (n=15). The primary objective is to gain additional information about the safety of the mammaglobin-A DNA vaccine. Safety will be closely monitored after injection with eight or more clinical and laboratory assessments in the first 24 weeks of the trial. The secondary objective is to assess the ability of the mammaglobin-A DNA vaccine to induce an immune response to mammaglobin-A. The immune response will be measured in the peripheral blood (ELISPOT analysis, multi-parameter flow cytometry), and in the primary tumor (imaging mass cytometry, IHC and RT-PCR).

2. KEYWORDS:

Breast, cancer, neoadjuvant, therapy, mammaglobin-A, DNA, vaccine, endocrine, chemo, phase 1b, ELISPOT, T-cells, tumor microenvironment

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Subtask1: Manufacture mammaglobin-A DNA vaccine. Complete manufacture, product release tests, and other IND –enabling studies of the mammaglobin-A DNA vaccine.
Subtask 2: Obtain regulatory approval for phase 1b clinical trial. Obtain FDA approval, RAC approval, Institutional Biosafety Committee approval, PRMC approval and IRB approval. All approvals have been obtained.
Subtask 3: Patient enrollment. Enroll patients to the phase 1b clinical trial.
Subtask 4: Screening studies. Complete screening studies including HLA type and mammaglobin expression levels.
Subtask 5: Assessment of safety. The primary endpoint is safety of the mammaglobin-A DNA vaccine. Safety will be closely monitored after injection and toxicity will be graded according to the NCI CTCAE version 4.0.
Subtask 6: Immune monitoring. The secondary objective is immune response in the peripheral blood. PBMC will be analyzed for the presence of mammaglobin-A-specific T cells by ELISPOT and multi-parameter flow cytometry. Tetramer staining will also be combined with intracellular cytokine staining (IFNγ, TNFα).
Subtask 7: Manuscript Preparation. Safety and immune response to the mammaglobin-A DNA vaccine are the primary and secondary objectives of the trial and will be published together.

What was accomplished under these goals?

Patient Accrual and treatment: We have experienced challenges in the last year related to the COVID-19 pandemic. Although the trial has remained open here at the Siteman Cancer Center at Washington University School of Medicine, concerns about in-person visits for vaccinations has resulted in a few patient withdrawals. During the first one year No-Cost Extension (NCE) that ended this July, we received approval to open the trial at Ohio State University Comprehensive Cancer Center. A request for a second one-year NCE in which we present our plan to recruit the remaining 22 patients (10 at OSU and 12 at Siteman Cancer Center) and complete all subtasks is pending.

The clinical trial currently has four cohorts: neoadjuvant endocrine therapy alone (n=8), neoadjuvant, neoadjuvant endocrine therapy + mammaglobin-A DNA vaccine (n = 15), neoadjuvant chemotherapy alone (n = 8), and neoadjuvant chemotherapy + mammaglobin-A DNA vaccine (n=15). To date, we completed treatment in 24 patients: 8/8 patients with neoadjuvant endocrine therapy; 14/15 patients with endocrine therapy + vaccine, and 2/15 patients with chemotherapy + vaccine. **Appendix 1** presents an overview of all patients consented to date; changes from last year are highlighted and reflect a total of 21 new patients and a status update in 1 additional patient.

Safety/toxicity: In all 24 patients that completed therapy to date, toxicity was restricted to mostly grade 1/2, and none of the patients experienced toxicity that required discontinuation of treatment.

Immune monitoring: We have continued immune monitoring assays using peripheral blood samples collected at various time points to perform pre-versus post-treatment comparison of the immune response to mammaglobin-A. A library of 15-mer peptides overlapping by 11 amino acids spanning the entire mammaglobin-A protein is used to screen for initial T cell responses to mammaglobin-A by interferon-gamma (IFNγ) ELISpot assay. A total of 20 peptides were divided into 5 pools of four peptides each. Peripheral blood mononuclear cells (PBMC) collected at each time point are tested against individual peptide pools. PBMC are then harvested from the ELISpot plates and cultured in the presence of IL-2 for 12 days, followed by a repeat of the ELISpot assay.

ELISpot data on the first 13 patients and further experimental details are presented in **Appendix 2.** A schematic in Figure 1 illustrates the assays performed, with the summary table highlighting the calculated frequency of mammaglobin-A-specific T cells in patients at three time points, at baseline, after neoadjuvant therapy (at the time of surgery), and at 52-weeks post-surgery. The data suggest that the average number of mammaglobin-specific T cells increased in vaccinated patients compared to non-vaccinated patients. The data also suggest the increased response is reduced, but still elevated compared to baseline at one-year post surgery.

We have continued studies using imaging mass cytometry (IMC) to characterize the tumor immune microenvironment before and after treatment (see **Appendix 3**). Briefly, IMC is a state-of-the-art technique applied to tissue sections in which $\geq$30 markers can be simultaneously analyzed (Figs. 1, 2). We have performed pilot studies on tissue samples from one patient, and illustrate various parameters in Figure 3 of Appendix 3 that can be assessed in a single IMC experiment, such as cellular composition based on expression of the 30-plus markers, and "neighborhood" analysis to assess potential interactions between various cell types in the tumor. We plan to apply IMC to tissue collected before and after treatment, to assess possible treatment-related changes in the tumor microenvironment.

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

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

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

The **overall goal** is to complete the clinical trial and all proposed immune monitoring. Specifically:
Patient enrollment: Enroll patients to the phase 1b clinical trial.
Assessment of safety: Assess the safety of the mammaglobin-A DNA vaccine. Safety will be closely monitored after injection and toxicity will be graded according to the NCI CTCAE version 4.0.
Immune monitoring: Assess the immune response to mammaglobin-A in the peripheral blood. PBMC will be analyzed for the presence of mammaglobin-A-specific T cells by ELISPOT and multi-parameter flow cytometry. Tetramer staining will also be combined with intracellular cytokine staining (IFNγ, TNFα).
Study the impact of mammaglobin-A DNA vaccination on CD8 T cells in the primary tumor. Tissue from the primary breast cancer will be obtained before and after vaccination. IHC, IMC or CODEX will be used to study the CD8 T cell response in the primary breast cancer.
Study the impact of mammaglobin-A DNA vaccination on the tumor microenvironment. Tissue from the primary breast cancer will be obtained before and after vaccination. IHC, IMC or CODEX will be performed to detect changes in the tumor microenvironment.
Report/publish the study results.

4. IMPACT:

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

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

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

Please see above for discussion regarding delays, and the pending NCE request

Changes that had a significant impact on expenditures

Nothing to report – the NCE request includes a revised budget allocation due to the opening of the trial at a second site (OSU).

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers, and presentations.

Nothing to report

- **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?

William Gillanders: no change

Peter Goedegebuure: no change

Foluso Ademuyiwa: no change

Feng Gao: no change

Kathleen Harris: no change

Nancy B. Myers: no change

Rashmi Mishra: no change

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

William Gillanders

NEW AWARDS

U01CA248235 (Griffith) 8/1/2020-7/31/2023 0.3

National Institutes of Health calendar

Informatics Tools for Identification, Prioritization and Clinical Application of Neoantigens

The goal of this project is to develop a suite of tools for predicting immunogenic tumor-specific neoantigens. We will create the first open source software toolkit for comprehensive identification and characterization of all major classes of neoantigens, enhance prioritization of neoantigens by elucidating key factors that influence immunogenicity, and assess predictive value of neoantigen tools using clinical trial data. These tools will address important outstanding questions, including how to associate specific neoepitopes with specific T cell receptors, how to

predict response to checkpoint blockade therapies, how to design the optimal personalized cancer vaccine, and how neoantigens contribute to mechanisms of resistance to immunotherapies.

Specific Aims

Aim 1. Develop a software toolkit for comprehensive identification and characterization of neoantigens.

Aim 2. Enhance prioritization of neoantigens by elucidating key factors that influence immunogenicity.

Aim 3. Assess predictive value of neoantigen tools using clinical trial data.

Program Official:
Miguel Ossandon
ossandom@mail.nih.gov /

Admin Official:
Jake Pritchard
jake.pritchard@nih.gov/

UH2 CA263954 (PI: Fitzpatrick) 0.12
07/01/2021-6/30/2023 NIH/NCI calendar

Imaging the Native 3D Architecture of Pancreatic and Breast Tumor Patient Tissue at Single-Cell Resolution

The goal of this project are to quantitatively characterize the native three-dimensional architecture of human solid tumor tissue from triple negative breast cancer (TNBC) and pancreatic ductal adenocarcinoma (PDAC) patients using the state-of-the art, yet mature technologies of tissue clearing, immunofluorescence and in situ hybridization labeling, and high-resolution lightsheet fluorescence microscopy.

Specific Aims

Aim 1:Image the three-dimensional spatial distribution of tumor,immune and stromal cells in relation to vasculature, lymphaticsand the extracellular matrixinnativesolid tumor tissue from human pancreatic (PDAC) and breast (TNBC) malignancies using a combination of tissue clearing, immunofluorescence, in situ hybridizationand 3D lightsheet microscopy.

Aim 2: Develop a computational pipeline to build three-dimensional spatial maps of protein expression and RNA transcriptlocalizationin intact solid tumor tissue from human pancreatic (PDAC) and breast (TNBC) malignancies.

Program Official:
Philipp Oberdoerffer
Philipp.Oberdoerffer@nih.gov / 240-276-6250

Admin Official:
Viviana Knowles
viviana.knowles@nih.gov / 240-276-5757

EXPIRED AWARDS

CCR-14-500 (Gillanders) 1/1/2015-12/31/2020 0.3
Rising Tide Foundation
Calendar

Phase IB Clinical Trial of a Candidate Breast Cancer Prevention Vaccine

The major goal of this project is to significantly advance the clinical development of the mammaglobin-A DNA vaccine. If an antitumor immune response is observed in the primary tumor, the trial will provide strong rationale to support initiation of a phase 2 clinical trial. If no antitumor immune response is observed in the primary tumor, the trial will provide the biologic insights required to optimize vaccine efficacy, and to support a second Phase 1b clinical trial.

Specific Aims

Aim 1: To gain additional information about the safety of the mammaglobin-A DNA vaccine.

Program Official:
Eveline Mumenthaler
Eveline.Mumenthaler@risingtide.ch

Admin Official:
Eveline Mumenthaler

Peter Goedegebuure

NEW AWARDS

5367 (Goedegebuure) 7/1/2020-6/30/2021 0.6
Barnes-Jewish Hospital Foundation Calendar
Breast Cancer Research
The funding will be used for salary support for Dr. Goedegebuure, who works closely with Dr. William Gillanders on the design and clinical testing of novel immune-based therapies for breast cancer.

Specific Aims
N/A

Admin Official:
Donald Buckner / Donald.Buckner@bjc.org /

not assigned (Fields) 6/1/2021-5/31/2022 0.6
Blue Cotinga, LLC Calendar
Onvansertib drug study
The primary goal of the study is to determine whether onvansertib, a Polo-like Kinase 1 (PLK1) inhibitor confers cell death in mutant KRAS colorectal (CRC) cancer cell lines but not wild type (wt) KRAS-expressing CRC.

Specific Aims
1. Testing of onvansertib in 2D monolayer tumor cell cultures using the Celltiter-Glo viability assay (the Titer-Glo 2.0 assay (Promega) measures viable cells through detection of ATP present in metabolically active cells).
2. Establishment of 3D organoid cultures.

Program Official:
not yet available

Admin Official:
not yet available

EXPIRED AWARDS

CCR-14-500 (Gillanders) 1/1/2015-12/31/2018 0.3
Rising Tide Foundation calendar
Phase IB Clinical Trial of a Candidate Breast Cancer Prevention Vaccine
The major goal of this project is to significantly advance the clinical development of the mammaglobin-A DNA vaccine. If an antitumor immune response is observed in the primary tumor, the trial will provide strong rationale to support initiation of a phase 2 clinical trial. If no antitumor immune response is observed in the primary tumor, the trial will provide the biologic insights required to optimize vaccine efficacy, and to support a second Phase 1b clinical trial.

Specific Aims
Aim 1: To gain additional information about the safety of the mammaglobin-A DNA vaccine.
Admin Official:
Eveline Mumenthaler / Eveline.Mumenthaler@risingtide.ch /

R21CA223836 (Fields) 2/1/2018-1/31/2020 1.2
National Institutes of Health calendar
Advancing Cancer Biology, Diagnostics and Therapeutics Outside of the Patient: Creation of a Novel, Autologous, Ex Vivo, Vascularized Model of the Tumor Microenvironment
The overall goal of this multi-disciplinary proposal is to create a novel, high-throughput, autologous, ex vivo model system that recapitulates cancer biology, immuno-biology, vasculature formation, and multiple aspects of the tumor microenvironment (TME).
Specific Aims
Aim 1: Create an ex vivo microphysiological platform capable of high-throughput screening to support a comprehensive tumor microenvironment using established cancer and non-cancer cell types, including fibroblasts and leukocytes.
Aim 2: Utilize the microphysiological platform to create an autologous system to model the human tumor microenvironment.
Program Official: Name:
Kuhn, Nastaran Z Phone:
Email: nas.kuhn@nih.gov

Admin Official:
Alania Foster
alania.foster@nih.go
v Phone:

Foluso Ademuyiwa

EXPIRED AWARDS
(Ademuyiwa) 8/1/2018-7/31/2020 0.12
NeoImmune Tech, Inc. Calendar
Correlative Studies of a Phase 2 Clinical Trial of Neoadjuvant Chemotherapy with Docetaxel and Carboplatin for Triple Negative Breast Cancer (TNBC)
Grant officer: Jeong Gu Kang, mkwak@neoimmunetech.com

Feng Gao

NEW AWARDS
R01CA253475 (Chang) 9/25/2020-6/30/2023 0.6
National Institutes of Health Calendar
Addressing Racial Disparities in Monoclonal Gammopathy of Undetermined Significance and Progression to Multiple Myeloma from a Prevention Perspective
This project plans to study racial differences in risk factors/biomarkers for MGUSand the progression of MGUS to MM to inform future biological mechanism studies and MGUS/MM

prevention trials with the goal of reducing MM health disparities.
Specific Aims
Aim 1. Identify whether high body mass index (BMI) and significant BMI change over the life course are risk factors for MGUS by race.
Aim 2. In the subgroup of MGUS patients diagnosed with DM, assess racial differences in:
Aim 2.1. M-protein trajectory after MGUS diagnosis in metformin versus non-metformin users
Aim 2.2. Association of metformin use with progression of MGUS to MM.
Aim 3. In the subgroup of MGUS patients without DM, assess racial differences in M-protein trajectory.

Program Official: Admin Official:
Asad Umar; ; umara@mail.nih.gov Viviana Knowles; iviana.knowles@nih.gov

EXPIRED AWARDS

R21CA234640 (Ratner) 12/10/2018-11/30/2020 0.48
National Institutes of Health Calendar
Immune Checkpoint Blockade Promotes Adult T Cell Leukemia
The goal of this proposal is to define the molecular mechanisms underlying this paradoxical effect, in order to better understand the potential benefits and risks of immune checkpoint blockade in cancer, especially T cell malignances.
Specific Aims
Aim 1. Define the effect of immune checkpoint blockade on tumor cell clonality. 1a. Genomics, 1b. Transcriptomics.
Aim 2. Define the effects of immune checkpoint blockade on T cell signaling pathways: a) Proliferation rate, b) PD-1, PD-L1 levels, c) PI3K and RAS/MAPK pathways.
Aim 3. Define the effect of immune checkpoint blockade on T cell proliferation: a) In culture and b) In NSG mice.

Program Official: Admin Official:
not yet available not yet available

UM1CA186704 (Abbruzzese/Dees/Wang-Gillam) 1.2
3/1/2015-2/28/2020 National Institutes of Health Calendar
Duke-UNC-Wash U Partnership for Early Phase Clinical Trials in Cancer
The ET-CTN goal is to provide break-through advances in management of cancer. The Duke-UNC-Wash U
Partnership will help achieve ET-CTN goals through the following specific steps; (1) engaging world class disease and translational teams that are collaborative and team-science oriented; (2) Developing innovative strategies and approaches for development of novel anti-cancer agents and combination regimens and participating in ET-CTN project teams to generate drug development plans for novel approaches; (3) Efficiently developing high-quality formal proposals and protocols in response to requests from the NCI's Cancer Therapy Evaluation Program (CTEP); (4) Effectively managing trials and accruing rapidly to all ET-CTN trials; (5) Translating information gained from the trials and correlative studies, both bench-to-bedside and bedside-to-bench; (6) Mentoring new investigators and fellows in oncology drug development.

To match our clinical and scientific strengths, particular emphasis will be placed upon novel therapies and combinations that target specific genetic alterations, altered signaling pathways, host immune responses, and angiogenesis and other stromal responses.

Specific Aims

Aim 1. Establishing a team-science oriented partnership with Duke & UNC to conduct innovative early phase studies through the CTEP- ET-CTN (experimental therapeutics clinical trials network).
Aim 2. Expanded access to novel clinical trials being conducted within the ER-CTN.
Aim 3. Developing innovative strategies and approaches for the development of novel anti-cancer agents and combination regimens.
Aim 4. Developing and conducting studies within the DUKE-UNC-Wash U Partnership in an efficient manner.
Aim 5. Support for the career development of junior faculty interested in early phase cancer drug development.

Program Official: not yet available
Admin Official: not yet available

R01DA044254 (Lian) 1.2
National Institutes of Health Calendar
Multilevel Interplays in the Development of Tobacco Dependence
Although the impact of neighborhood environments on smoking initiation and sustainment has been recognized, little is known about the role of neighborhood exposures in the development of tobacco dependence and its interactions with genetic susceptibility. Building on a 20-year followup study of adolescents and young adults, we will fill this important gap in our knowledge. The study will refine our understanding of the etiology of tobacco dependence and suggest the targets for multi-level interventions to reduce tobacco dependence in adolescents and young adults.

Specific Aims

Aim 1: Longitudinally assess the impacts of neighborhood conditions on the development of tobacco dependence among adolescents and young adults.
Aim 2: Characterize the geographic pattern of within-subject changes in neighborhood exposures and its effect on the development of tobacco dependence among adolescents and young adults.
Aim 3: Prospectively examine the interactive effects of neighborhood exposures and genetic predisposition on the risk for tobacco dependence.

Program Official: Heather L Kimmel, heather.kimmel@nih.gov,
Admin Official: Jennifer Schermerhorn, schermerhornj@mail.nih.gov,

BC170644P1 (Weber/Ma) 3/1/2018-2/28/2021 0.3
Department of Defense Calendar
Targeting Drivers of Aggressive Triple Negative Breast Cancer in African Americans
The major goals of this project are to 1) establish a spontaneous TNBC mouse model, 2) analyze 525 primary TNBC tumors and 30 PDX TNBC tumors, 3) determine whether TNBC cells are sensitive to JAK1 and CDK4 inhibition, and 4) determine whether TNBC tumors in mice are

sensitive to JAK1 and CDK4 inhibitors.

Specific Aims
Aim 1: Establish a spontaneous TNBC mouse model.
Aim 2: Analyze 525 primary TNBC tumors and 30 PDX TNBC tumors.
Aim 3: Determine whether TNBC cells are sensitive to JAK1 and CDK4 inhibition.
Aim 4: Determine whether TNBC tumors in mice are sensitive to JAK1 and CDK4 inhibitors.

Program Official:	Admin Official:
not yet available	not yet available

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

Appendix 1: Overview of trial patients through June 30, 2021

Patient ID	Eligible	Treatment Arm	Treatment Status
WU-001	No (MGB negative)		
WU-002	Yes	Endocrine+vaccine	discontinued
WU-003	No (MGB negative)		
WU-004	No (BMI)		
WU-005	No (Ki-67 too high)		
WU-006	No (MGB negative)		
WU-007	No (MGB negative)		
WU-008	No (MGB negative)		
WU-009	Yes	Other*	
WU-010	No (ineligible for neo-adj therapy)		
WU-011	No (MGB negative)		
WU-012	No (MGB negative)		
WU-013	No (MGB negative)		
WU-014	No (MGB negative)		
WU-015	Yes	Endocrine	completed
WU-016	Yes	Endocrine+vaccine	completed
WU-017	No (Ki67 too high)		
WU-018	No (MGB negative)		
WU-019	No (MGB negative)		
WU-020	Yes	Other*	
WU-021	Yes	Endocrine	completed
WU-022	Yes	Endocrine	completed
WU-023	No (Ki67 too high)		
WU-024	No (patient not compliant)		
WU-025	No (patient chose alternate trial)		
WU-026	No (patient chose surgery)		
WU-027	Yes	Endocrine	completed
WU-028	No (patient chose surgery)		
WU-029	Yes	Endocrine+vaccine	completed
WU-030	Yes	Other*	
WU-031	No (MGB negative)		
WU-032	Yes	Endocrine+vaccine	completed
WU-033	Yes	Endocrine	completed
WU-034	No (MGB positive, Ki67 not tested)	Other*	
WU-035	No (patient chose surgery)		
WU-036	No (MGB negative)		
WU-037	No (MGB negative)		
WU-038	No (MGB negative)		
WU-039	No*		
WU-040	No (MGB negative)		
WU-041	No (MGB negative)		
WU-042	Yes	Endocrine+vaccine	completed
WU-043	No (MGB negative)		
WU-044	No (MGB negative)		
WU-045	No (Ki67 too high)		

Patient ID	Eligible	Treatment Arm	Treatment Status
WU-046	Yes	Endocrine	completed
WU-047	No (MGB negative)		
WU-048	Yes	Endocrine	completed
WU-049	Yes	Endocrine	completed
WU-050	Yes	Other*	
WU-051	No (MGB negative)		
WU-052	No (MGB negative)		
WU-053	No*		
WU-054	Yes	Other*	
WU-055	No*		
WU-056	Yes	Endocrine+vaccine	completed
WU-057	Yes	Other*	
WU-058	No (MGB negative)		
WU-059	No (Ki67 too high)		
WU-060	No (MGB negative)		
WU-061	Yes	Endocrine+vaccine	completed
WU-062	No	Other*	
WU-063	No (MGB negative)		
WU-064	Yes	Other*	
WU-065	Yes	Endocrine+vaccine	completed
WU-066	No	Other*	
WU-067	No	Other*	
WU-068	Yes	Other*	
WU-069	Yes	Endocrine+vaccine	completed
WU-070	No (MGB negative)		
WU-071	Yes	Endocrine+vaccine	completed
WU-072	No (MGB negative)		
WU-073	Yes	Endocrine+vaccine	completed
WU-074	Yes	Endocrine+vaccine	completed
WU-075	Yes	Chemo + vaccine	completed
WU-076	No*		
WU-077	No (MGB negative)		
WU-078	Yes	Endocrine+vaccine	completed
WU-079	Yes	Other*	
WU-080	No*		
WU-081	No (MGB negative)		
WU-082	No*		
WU-083	Yes	Endocrine+vaccine	completed
WU-084	Yes	Other*	
WU-085	No (MGB negative)		
WU-086	No (MGB negative)		
WU-087	Yes	Chemo + vaccine	completed
WU-088	No (MGB negative)		
WU-089	Yes	Other*	
WU-090	No (MGB negative)		
WU-091	No (MGB negative)		
WU-092	No (MGB negative)		

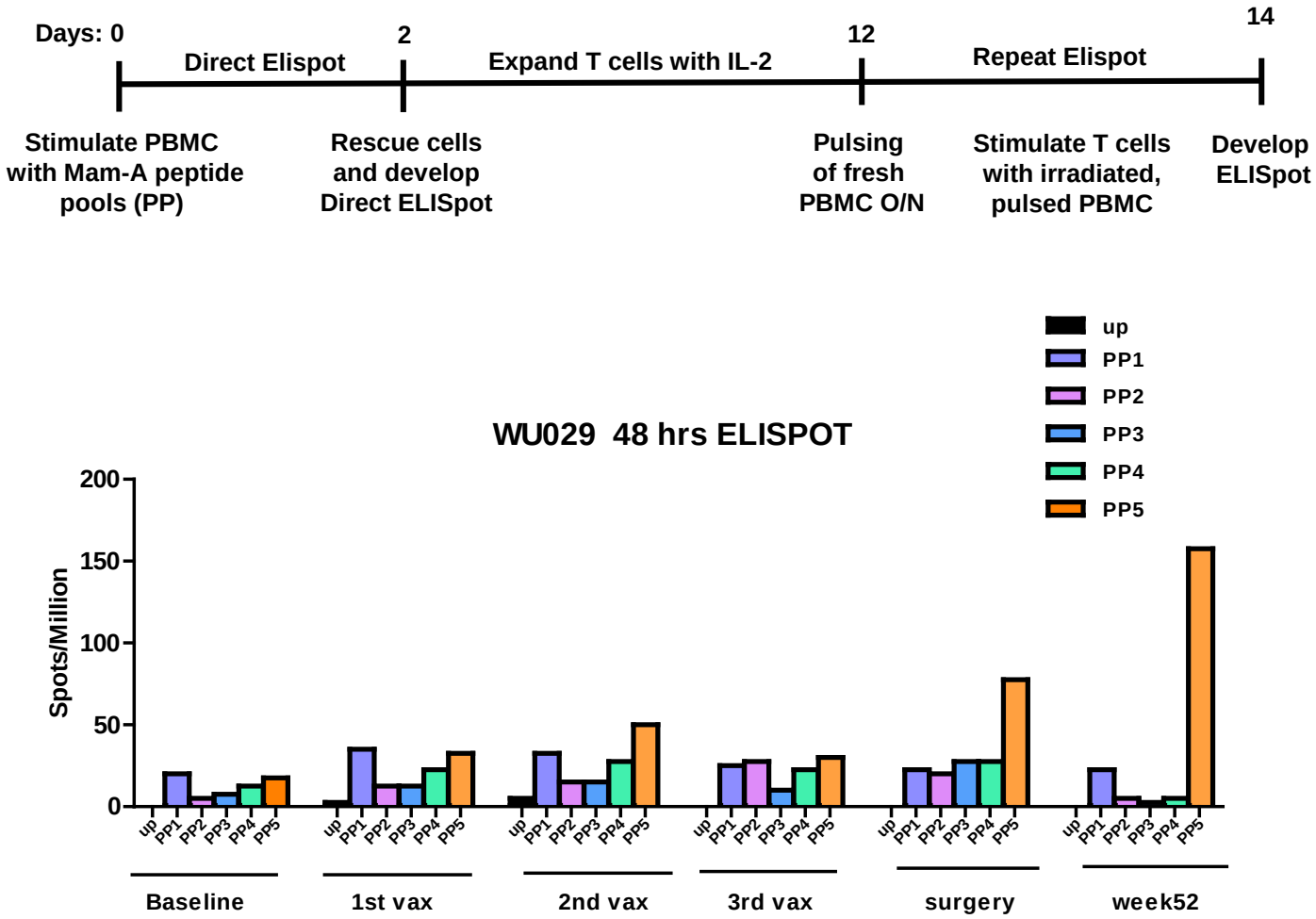
Patient ID	Eligible	Treatment Arm	Treatment Status
WU-093	No (medical screen fail)		
WU-094	Yes	Other*	
WU-095	No (HER2 POS)		
WU-096	Yes	Other*	
WU-097	Yes	Other*	
WU-098	No (MGB negative)		
WU-099	No (MGB negative)		
WU-100	No (MGB negative)		
WU-101	No (MGB negative)		
WU-102	Yes	Pending	
WU-103	Yes	Pending	
WU-104	Other*		
WU-105	Yes	Pending	
WU-106	No (MGB negative)		

*Patient declined to be on trial

Changes from the previous Annual Report are highlighted

Appendix 2

Fig 1: IFN-γ production by T cells in response to Mam-A overlapping peptides in patient sample WU029 6/23/2021- 6/252021



Appendix 2

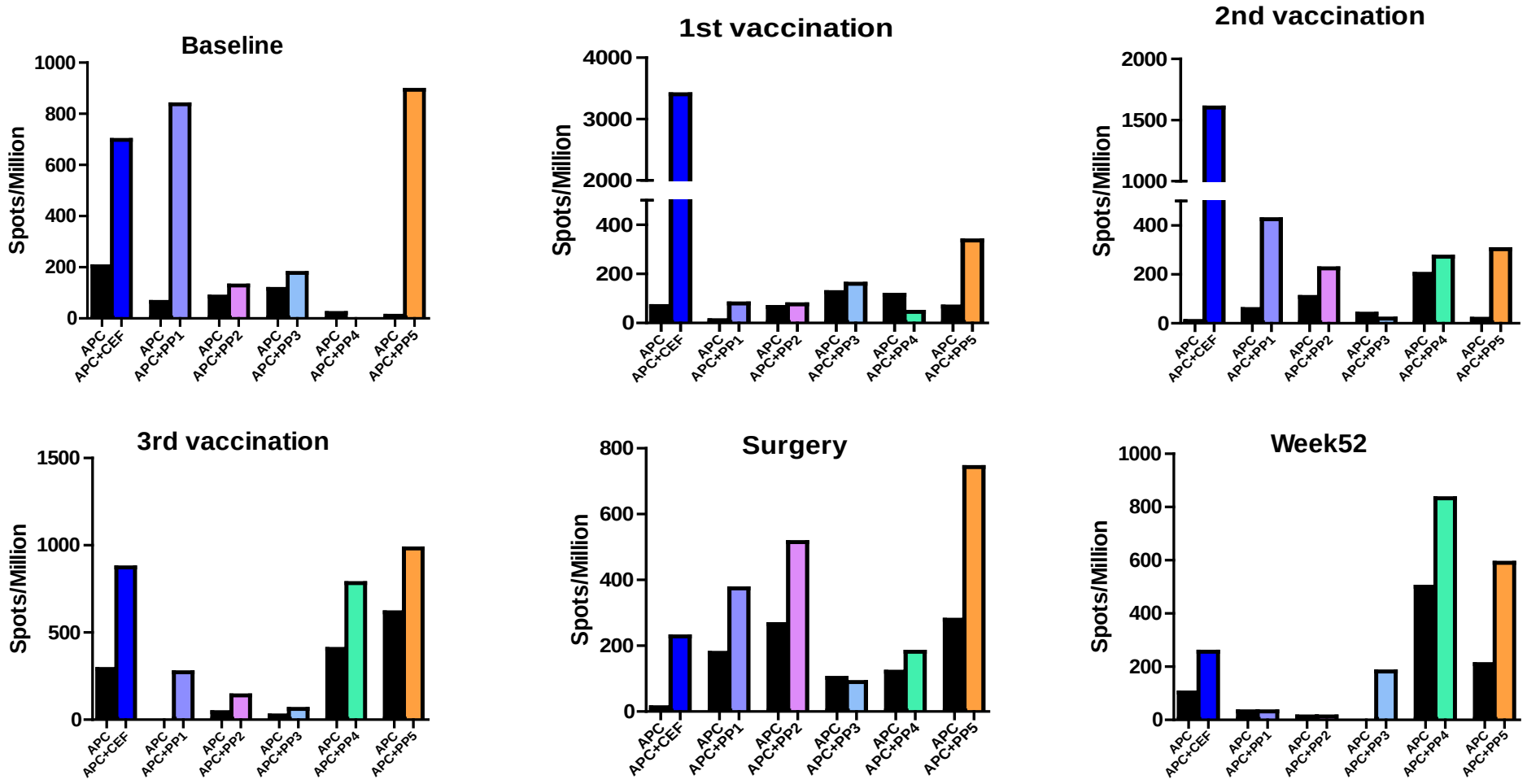
Data from direct ELISpot assay. For each patient, the cumulative number of spots against each peptide pool minus background was calculated for each time point.

MGB = mammaglobin-A
*Not included

Treatment	Patient ID	% MGB	Baseline SFU/10^6	Surgery SFU/10^6	Week 52 SFU/10^6
Endocrine	WU-015	15	10	3	3
	WU-021	70	13	0	118
	WU-022	10	567	0	40
	WU-027	40	3	268	213
	WU-033	80	5	23	15
	WU-046	40	28	23	18
	WU-048	55	128	428	4158*
	WU-049	15	17	57	22
		Avg 41	**Total: 771 Avg 96**	**Total: 802 Avg 100**	**Total: 429 Avg 61**
Endocrine + vaccine	WU-016	8	200	685	337
	WU-029	20	63	175	193
	WU-042	90	283	450	225
	WU-056	90	138	78	153
	WU-061	50	110	65	65
		Avg 52	**Total: 794 Avg 159**	**Total: 1453 Avg 291**	**Total: 973 Avg 195**

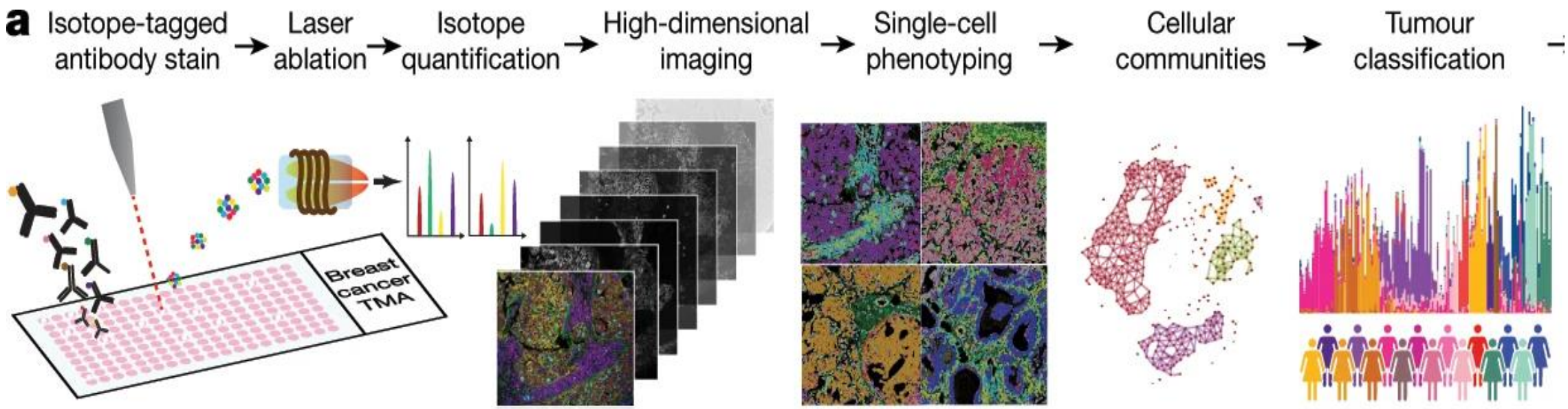
Appendix 2

Figure 2: IFN-γ production by T cells in response to Mam-A overlapping peptide pools in patient sample WU029 after 12 days of culture 07072021



Appendix 3

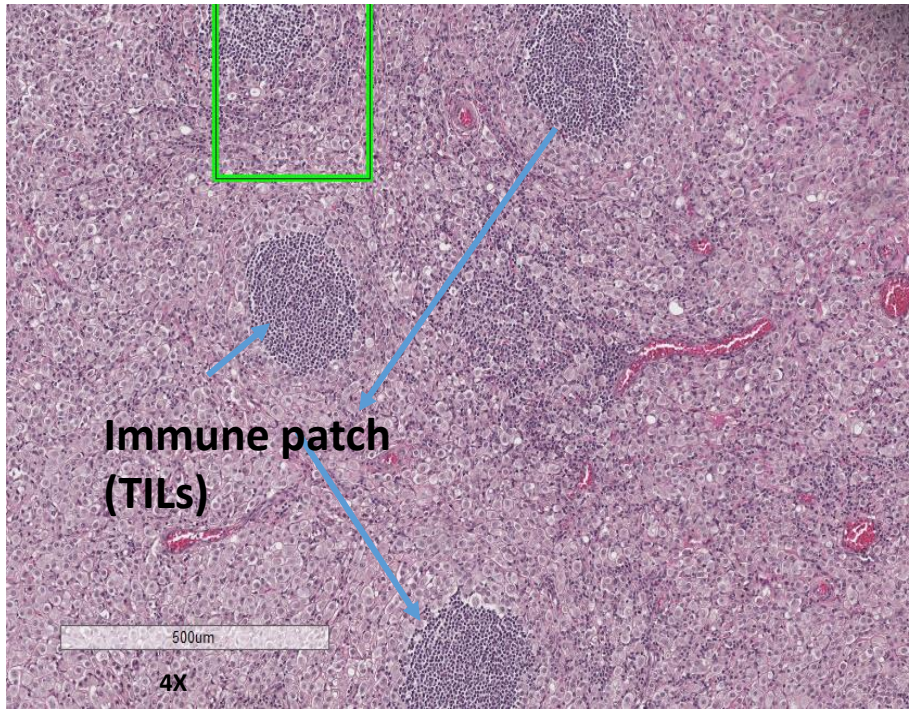
Fig 1 - Schematic representation of IMC acquisition of multiplexed Image of breast cancer

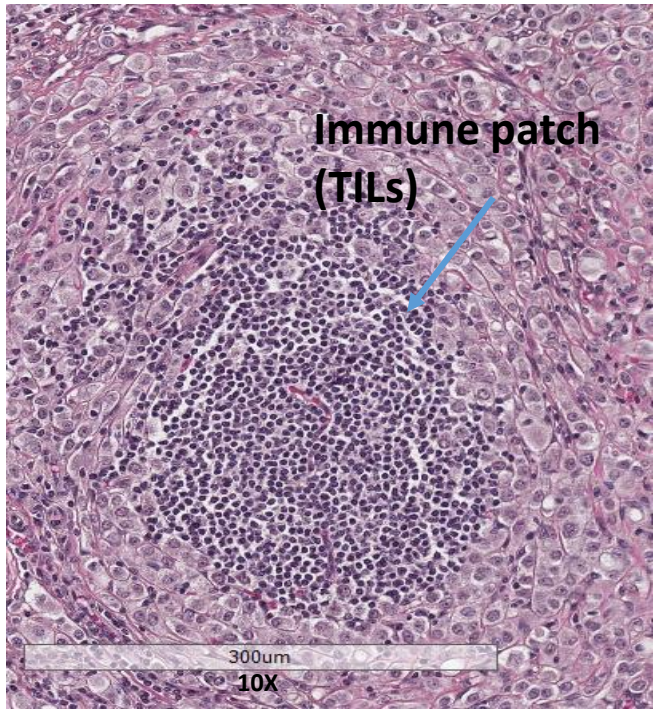


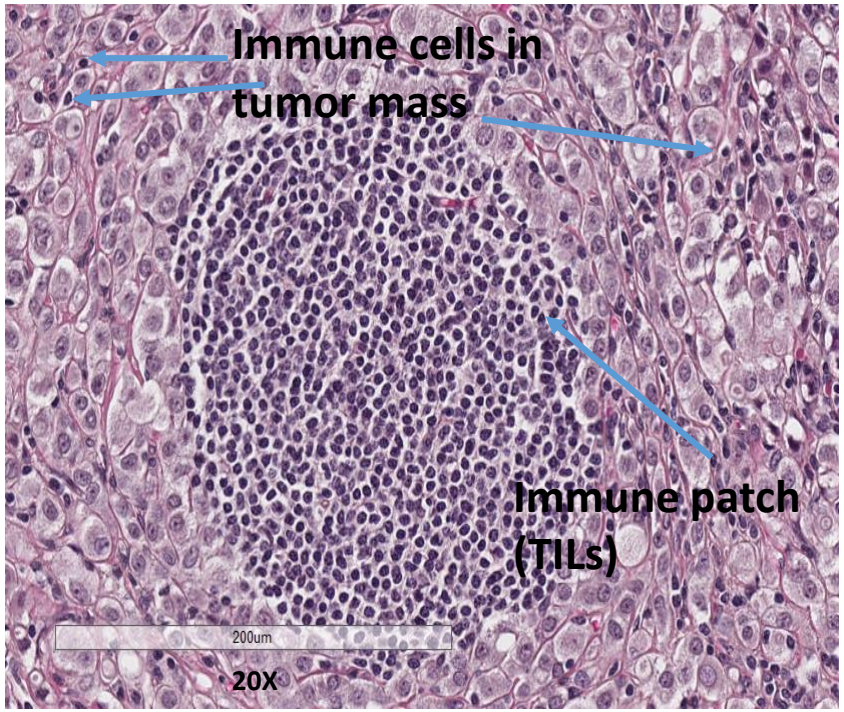
Adopted from Jackson et al, 2020

Appendix 3

Fig 2 - H&E staining of surgery sample (Endocrine therapy treated sample, WU-022)



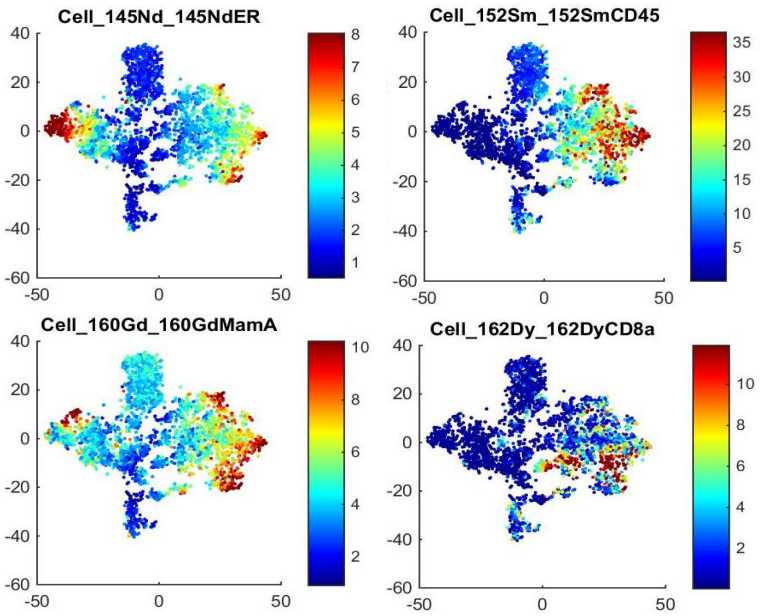




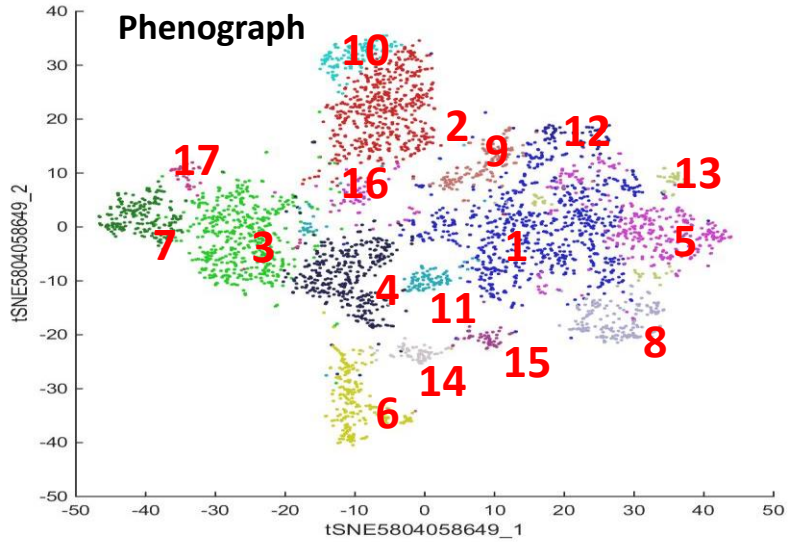
Appendix 3

Figure 3: Illustration of IMC output parameters

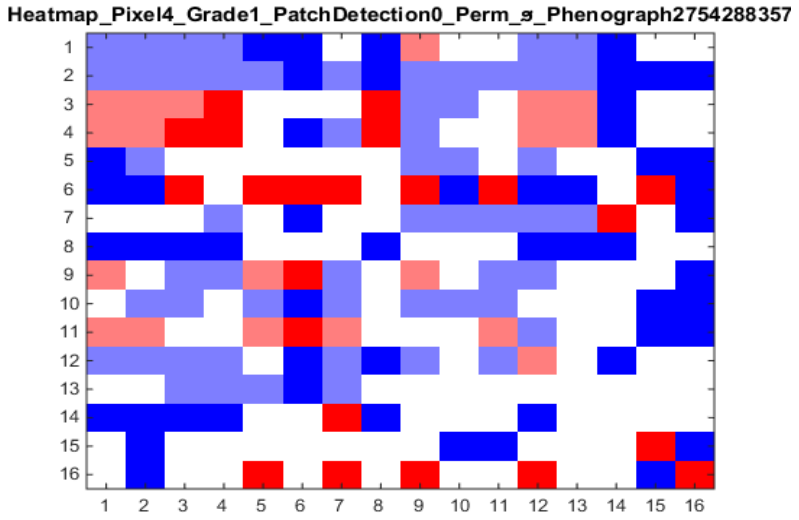
tSNE Plots



Phenograph



Neighborhood analysis and dendrogram



Cells of the cluster neighbor each other more frequently.

Cells of the cluster neighbor each other less frequently.

Cells of the cluster neighbor each other rarely or never.

Heatmap