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13. SUPPLEMENTARY NOTES

14. ABSTRACT Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. Currently, surgery plus platinum-based chemotherapy is the standard treatment. However, individual response to therapy is highly variable and unpredictable: some women experience progression-free survival (PFS) of years while others progress during treatment or shortly thereafter. Eventually, most women develop and succumb to platinum-resistant disease. While there have been attempts to improve outcomes using immunotherapies, their success to date has been limited. Current trials now focus on chemo-immunotherapy combinations, acknowledging that platinum-based therapies remain an important part of treatment. However, no methods exist to identify who will respond to or fail treatments. Moreover, there are no clinically-validated interventions to improve treatment response or outcomes. This project aims to determine whether *circulating anti-tumor antibodies (AAbs) may serve as these biomarkers*. We propose the first-ever population-based study of the association between antibody-mediated, humoral immune responses to tumor antigens, the tumor microenvironment immune phenotype, and EOC outcome. Using banked biospecimens and data on women newly diagnosed with EOC, we will assess the relationship between anti-tumor antibodies and both therapy response and survival (Aim 1) and the antibodies' relationship to the immune response in tumors (Aim 2). We will then use this information to build statistical models to predict which women will and will not respond well to therapy (Aim 3).

15. SUBJECT TERMS. Epithelial ovarian cancer, Anti-tumor antibodies, tumor microenvironment, humoral and cellular immunity, CD8, molecular epidemiology,

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1. Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy. Currently, surgery plus platinum-based chemotherapy is the standard treatment. However, individual response to therapy is highly variable and unpredictable: some women experience progression-free survival (PFS) of years while others progress during treatment or shortly thereafter. Eventually, most women develop and succumb to platinum-resistant disease. While there have been attempts to improve outcome using immunotherapies, their success to date has been limited, with less than 15% response rates. Current trials now focus on chemo-immunotherapy combinations, acknowledging that platinum-based therapies remain an important part of treatment. However, no methods exist to identify who will respond to or fail treatments. Moreover, there are no clinically-validated interventions to improve treatment response or outcomes. Biomarkers that can predict therapy response, provide early indication of efficacy, and suggest interventions to improve outcomes are urgently needed.

In this project, we posit that circulating anti-tumor antibodies (AAbs) may serve as these biomarkers. Robust anti-tumor immunity is critical for tumor eradication. However, most research has focused on cellular T-lymphocyte mediated immunity, mainly the presence of CD8+ T-cells in the tumor micro-environment (TME), which greatly influences treatment response and is strongly linked to prognosis. Much less is known about the amplitude and quality of antibody-mediated, humoral immune responses against tumor antigens in EOC patients and about their role in tumor biology and response to therapy. No studies have investigated the relationship between circulating anti-tumor humoral markers and the TME immune signature; nor have studies examined the joint associations of these factors with therapy response, prognosis, and outcome. In this study, we will examine the association between antibody-mediated, humoral immune responses to tumor antigens, the TME immune phenotype, and EOC outcome.

2. Keywords

Epithelial ovarian cancer, Anti-tumor antibodies, tumor microenvironment, humoral and cellular immunity, CD8, molecular epidemiology.

3. Accomplishments

3a. What were the major goals of the project?

Goal/Aim1: to evaluate the role of circulating antibodies to tumor antigens in predicting treatment response, resistant/refractory disease, relapse within the first year, PFS, and overall survival (OS).

Goal/Aim2: to determine the relationship between humoral immunity and the phenotype of immune cells in the TME.

Goal/Aim 3: to develop a new predictive signature for EOC therapy response that incorporates the independent and combined effects of (1) systemic markers of anti-tumor immune response, (2) TME immune cell phenotypes, and (3) standard prognostic variables.

3b. What was accomplished under these goals?

3b.1 & 3b.2. Major Activities and Specific Objectives (per the approved work statement)

Aim 1

1. Task 1: Regulatory Approval and Other Approvals – *SITE: MWRI/Inova (Modugno, Maxwell)*
   a. Obtained USAMRDC Human Research Protection Office (HRPO) approval
   b. Executed Data Usage and Material Transfer Agreements with Inova

2. Task 2: Finalize Cohort and Serum Specimens - *SITE:MWRI/MWH (Modugno, Lopa, Elishaev, Taylor)*
    a. Extracted relevant clinical, demographic, treatment, and outcome data from GCBDB data repository and electronic medical records. We are in the process of cleaning and validating the data
    b. Identified, pulled and aliquotted 250 serum samples including 5% blinded duplicate splits

Aim 1 Milestones:

1. Month 6: USAMRDC HRPO approval received - achieved
2. Month 6: MTA/DUA executed with Inova - achieved
3. Month 10: GCBDB Samples identified and shipped to lab – partially achieved (20% of Pittsburgh cohort)

Aim 2

1. Task1: Process and Ship FFPE tumor tissue - *SITE:MWRI (Modugno, Elishaev)*
    a. Obtained FFPE tumor blocks on GCBDB cases
    b. Sectioned FFPE tumor blocks for IHC and RNA assays
    c. Shipped FFPE slides to RPCI-PNSR and GRC

2. Task 2: Characterize TME using IHC - *SITE:RPCI-PNSR (Bshara)*
    a. Assessing TME using Immuno-histochemistry (IHC) markers on FFPE slides for Pittsburgh cohort
    b. Quantitating IHC marker levels for Pittsburgh cohort
    c.
3. Task 3: Characterize TME using NanoString - *SITE: GCR (Lamb)*
    a. Extracted RNA from FFPE slides (for Pittsburgh cohort)
    b. Assessing TME using NanoString Counter Cancer Immunology panel (for Pittsburgh cohort)

Aim 2 Milestones:

1. Month 15: FFPE blocks sectioned and shipped to labs – achieved this reporting period
2. Month 25: NanoString marker assessment complete – achieved ~50% this reporting period
3. Month 28: IHC marker assessment complete – in progress for Pittsburgh cohort (25% complete)

Aim 3

- no progress this reporting period

Aim 3A Milestones:

1. Month 9: Validation cohort finalized – not achieved

3b.3. Significant Results/Key Outcomes

Scientifically, we are still in data generation mode. Hence, there are no significant results or key outcomes to report.

Our major achievement this reporting period was to identify and finalize the Pittsburgh cohort, obtain biospecimens from the biorepository, section FFPE blocks for IHC and Nanostring work, and ship the tumor samples to the labs for analyses.

Table 1 shows the basic demographic and other data for the Pittsburgh cohort. We extracted these data from the parent database as well as the electronic medical record. Because our initial cleaning of the data identified several inconsistencies, we are currently in the process of validating and cleaning

these data in preparation for data analysis. Because we have as some of our endpoints disease-free survival and overall survival, we will periodically re-extract these data points and finalize them for our final analyses closer to the time the analyses will be performed.

Table 1: Basic Cohort Description (N=275)

Age, mean (SD)	63.2 (0.66)
Race N (%)	
Black	8 (2.9)
White	264 (96)
Other	3 (1.1)
Smoking N (%)	
current	49 (17.8)
former	59 (21.5)
never	130 (47.3)
unknown	37 (13.5)
Histology N (%)	
carcinosarcoma	14 (5.1)
clear cell	31 (11.3)
endometrioid	32 (11.6)
high grade serous	141 (51.27)
low grade serous	14 (5.1)
mixed cell	16 (5.8)
mucinous	18 (6.5)
other	9 (3.3)
Stage N(%)	
I-II	108 (39.3)
III-IV	124 (45.1)
unknown	16 (5.8)
Debulking status N (%)	
optimal	171 (62.2)
non-optimal	34 (12.4)
unknown	70 (25.5)
Vital Status N (%)	
Alive	182 (66.2)
Dead	93 (33.8)
Chemo type N (%)	
Adjuvant	207 (75.3)

Neo-Adjuvant	68 (24.7)
RECIST 1.1 Response	
Complete response	188 (88.4)
partial response	19 (6.9)
stable disease	9 (3.3)
progressive disease	9 (3.3)
unknown	31 (11.3)
Platinum Status N (%)	
sensitive	192 (69.8)
resistant	32 (11.6)
refractory	22 (8.0)
unknown	29 (10.5)

We are ahead of schedule for both the nanostring and IHC work. Specifically, we obtained all the tumor samples on the Pittsburgh cohort and shipped them to the labs. Dr. Bshara has already completed the staining for 5 of our markers (CD3, CD8, CD20, FOXP3, MUC1; Figure 1) and will next move to quantifying the markers. The nanostring data is also in progress. We anticipate having those data completed by the end of the next reporting period, which will enable us to begin to analyze data for Aim 2.

3b.4. Other Achievements

Because Dr. Matsuzaki moved from Roswell Park Cancer Institute to the University of Chicago, we have been delayed in running our anti-tumor antibody markers. It took several months for Dr. Matsuzaki to re-establish her lab. In addition, the University of Pittsburgh required we execute a new agreement since the University of Chicago was not named in the original grant. That paperwork took several months to be approved and was only recently approved. Accordingly, while we had our samples ready to ship, we were not able to.

Concerned that we would not be able to use Dr. Matsuzaki, we explored several options to complete the anti-tumor antibody work.

In consultation with our team and advisors, we explored other laboratory options, including the Luminex facility and the Meso Discovery Scale (MSD) platform, both of which will allow us to plex our markers and use a much smaller specimen volume. We are currently working with MSD to develop our study-specific plex assay, which will be run in the local MSD lab. This lab has extensive experience with the MSD plex assays, and we are confident the results will be robust and reproducible. We anticipate that this panel will be ready during the next reporting period and we will then move forward with completing the anti-tumor antibody assays on the Pittsburgh cohort. We are reserving Luminex as our backup because our literature review and dialog with laboratories indicate that MSD allows for more accurate quantitation.

In consultation with our team, we made an additional change to our original plan. To ensure the conservation of precious samples, the Inova team requested that we run the serum assays first on the Pittsburgh cohort and eliminate any serum markers that were not informative. Accordingly, we moved forward with the Pittsburgh cohort only. During the next reporting period, we will work with Inova to finalize their cohort so that as soon as our marker panels are complete, we can move

forward. Note that the plex assay also addresses Inova's concern so that once we validate that assay, we can move forward with assessing the Inova cohort.

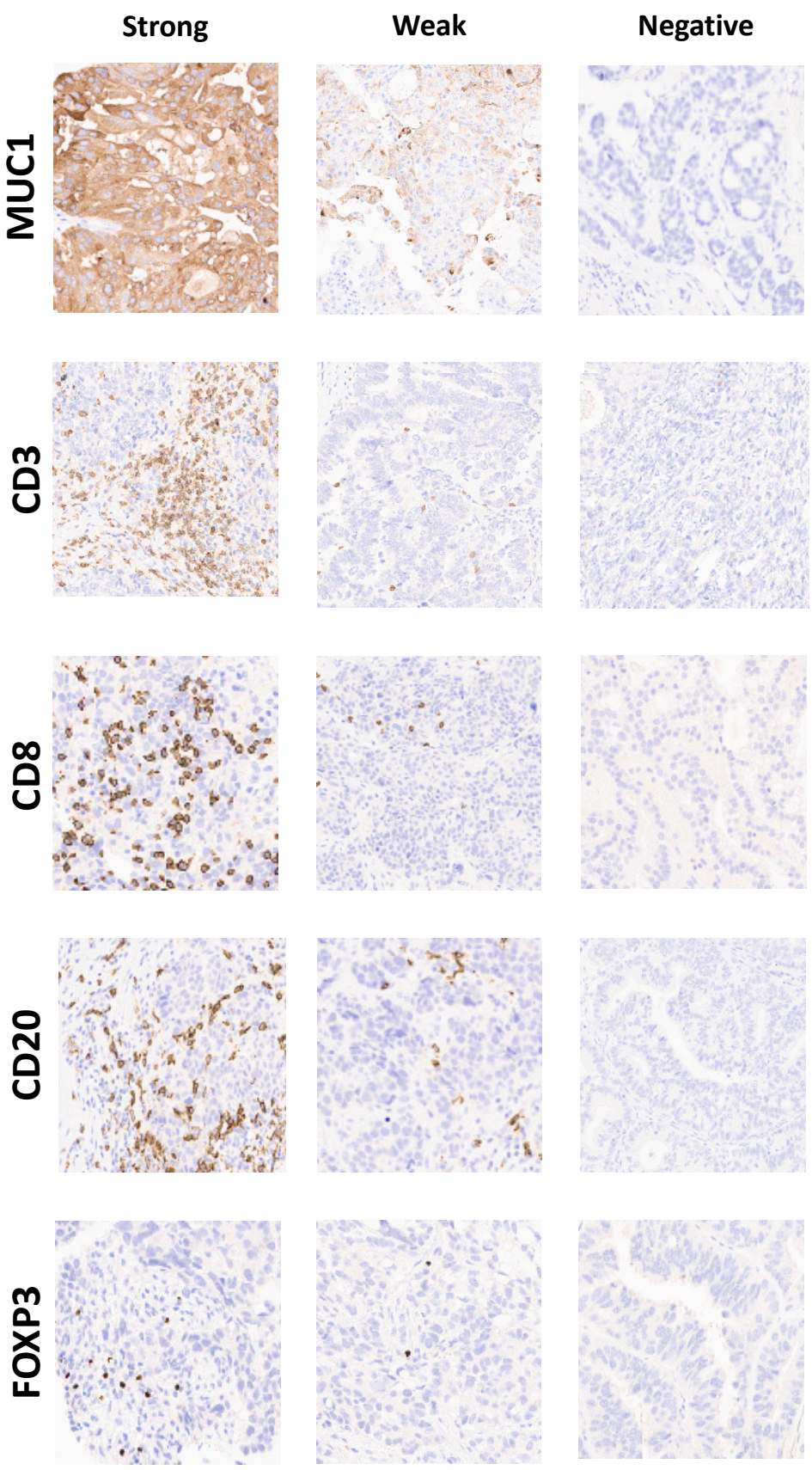

Figure 1: Strong, Weak, and Control Stains for the markers of interest.

3c. What opportunities for training and professional development has the project provided?
Nothing to report

3d. How were the results disseminated to communities of interest?
Nothing to report

3e. What do you plan to do during the next reporting period to accomplish the goals?
We will continue to adhere to the Statement of Work with a few modifications:

1. We will continue to clean and validate data on the Pittsburgh cohort
2. We will test and finalize the MDS plex assay for our anti-tumor antibody markers. We will then run the Pittsburgh cohort on these markers
3. We will complete the IHC work with Dr. Bshara (ahead of schedule)
4. We will complete the Nanostring work (ahead of schedule)
5. We will begin preliminary analyses of the IHC and Nanostring endpoints
6. We will work with Inova to finalize their cohort.

4. IMPACT:

4a. What was the impact on the development of the principal discipline(s) of the project?
Nothing to report

4b. What was the impact on other disciplines?
Nothing to report

4c. What was the impact on technology transfer?
Nothing to report

4d. What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:

5a. Changes in approach and reasons for change
As noted above, because of Dr. Matsuzaki's new affiliation, we investigated other options for our anti-tumor antibody work. We anticipate a successful plex assay using the MSD platform as noted above.

5b. Actual or anticipated problems or delays and actions or plans to resolve them
As noted above, we were significantly delayed in getting approval to use Dr. Matsuzaki's lab. In the interim, we identified an alternative technology (MSD) that will provide the data we need using a fraction of the specimen volume. Since we started down this path prior to getting approval to use Dr. Matsuzaki's lab, we decided to assess this assay and determine if it would be a better fit for our work. During this upcoming year, we will decide which lab to use.

Also as noted above, Inova asked us to minimize the quantity of serum specimen we use in order to preserve this valuable resource. Another reason for our potentially using MSD is that for the same volume of sample, we will need to run one marker, we can run up to 6 markers. Thus, we did not finalize the Inova cohort nor obtain their specimens during this reporting period as originally planned.

5c. Changes that had a significant impact on expenditures
Ms. Laslavic, project coordinator, took another position in May. We recently hired Lauren Borho, who began in our group on September 1. This reduced salary expenditures. In addition, Dr. Modugno

spent 2022 as an IPA at the NSF. Accordingly, her salary support was shifted to Dr. Elishaev so that Dr. Elishaev could oversee the project. Drs. Modugno and Elishaev met periodically to review progress, and Dr. Modugno devoted part of her NSF IRD effort to helping with this project.

Because of the delays in the anti-tumor antibody work, we did not spend those funds. We also did not transfer funds to Inova in year 1 as originally planned due to the request to delay obtaining those samples until we determine the exact volume of serum specimen needed.

We anticipate in Year 2 we will incur all the expenses for the Pittsburgh lab work and begin to incur the Inova expenses.

5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

5e. Significant changes in use or care of human subjects
Nothing to report

5f. Significant changes in use or care of vertebrate animals.
N/A

5g. Significant changes in use of biohazards and/or select agents.
N/A

6. PRODUCTS:
Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.a What individuals have worked on the project?

Name	Francesmary Modugno
Project Role	PI
Researcher ID (eg, ORCID ID)	0000-0003-0637-1534
Nearest Person Month Worked	0.6-3.0
Contribution to Project	oversaw all work
Funding Support	n/a

Name	Esther Elishaev
Project Role	co-I; interim PI
Researcher ID (eg, ORCID ID)	0000-0002-6271-0222
Nearest Person Month Worked	0.6-2.4
Contribution to Project	Pathologist; oversite in Dr. Modugno's absence
Funding Support	n/a

Name	Anda Vlad
Project Role	co-I; immunologist
Researcher ID (eg, ORCID ID)	0000-001-5266-9695
Nearest Person Month Worked	0.6

Contribution to Project	help with selecting samples, labs, and interpreting data
Funding Support	n/a

Name	Sarah Taylor
Project Role	co-I; gynecologic oncologist
Researcher ID (eg, ORCID ID)	0000-0002-1385-1707
Nearest Person Month Worked	0.24
Contribution to Project	guidance on clinical questions, clinical significance of work
Funding Support	n/a

Name	Riyue Bao
Project Role	co-I; bioinformatician
Researcher ID (eg, ORCID ID)	0000-0002-6105-1704
Nearest Person Month Worked	1.2
Contribution to Project	selection of samples, support for data extraction; data analysis and interpretation
Funding Support	n/a

Name	Samia Lopa
Project Role	co-I; biostatician
Researcher ID (eg, ORCID ID)	0000-0002-8475-9262
Nearest Person Month Worked	0.6
Contribution to Project	help with sample selection; data analysis and interpretation
Funding Support	n/a

Name	Angela Laslavic
Project Role	Project coordinator
Researcher ID (eg, ORCID ID)	n/a
Nearest Person Month Worked	1.2
Contribution to Project	MTA execution, sample identification, data extraction, shipping specimens, daily study operations
Funding Support	n/a

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

Yes, Dr. Modugno has received additional funding. She received a grant from the NIH examining the role of the microbiome in ovarian cancer.

7.c What other organizations were involved as partners?

- **Organization Name:** Roswell Park Cancer Center
- **Location of Organization:** Buffalo, NY

- **Partner's contribution to the projec**t *(identify one or more)*
    - **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)*;
    - **Other:** Dr. Bshara is conducting the IHC staining and quantitation of the TME

8. **Special Reporting Requirements**
   N/A

9. **Appendices**

A copy of the executed MTA with Inova is attached

COLLABORATIVE MATERIAL TRANSFER AND DATA USE AGREEMENT

This collaborative material transfer and data use agreement ("Agreement"), entered into on the last date of authorized signature on this Agreement (the "Effective Date"), is between **The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.**, having an address at 6720-A Rockledge Drive, Suite 100, Bethesda, MD 20817 ("HJF"), **Inova Health Care Services**, on behalf of its Office of Research at Inova, a Virginia non-stock, nonprofit corporation, located at 3300 Gallows Road, Falls Church, VA 22042 ("Inova") and **the University of Pittsburgh – of the commonwealth system of higher education (**"UPITT"**)**, located at 3420 Forbes Ave, 300 MURDC, Pittsburgh, Pennsylvania 15260 ("UPitt"), each of which is a "Party" and together are the "Parties." "Provider" refers to any Party when acting as a provider of Research Resources (as defined below) or confidential information (as defined in Paragraph 5) under this Agreement and "Recipient" refers to any Party when acting as a recipient of same. Researchers of a Party may also be referred to as "Recipient Investigator" or "Provider Investigator" as applicable.

BACKGROUND

HJF supports the Women's Health Integrated Research Center ("WHIRC"), located at 3289 Woodburn Road, Suites 030, 370, 375, and 390, Annandale, VA 22003, a program of the Gynecologic Cancer Center of Excellence ("GYN-COE Program"), under the terms of Cooperative Agreements with the Uniformed Services University of the Health Sciences, and Inova. For the purposes of this Agreement, HJF and Inova will jointly act as Provider or Recipient on behalf of the GYN-COE Program and WHIRC.

Under this Agreement, the Parties may exchange research resources including research material(s) with associated data ("Material"), and/or Limited Data Sets ("LDS") (collectively the "Research Resources") as defined below along with scientific/technical expertise for collaborative research as described in **Appendix A** ("Research Plan").

The Parties agree as follows:

1. **Research Resources**: The following Research Resources will be transferred:

   a. **Material**:

   HJF/Inova will transfer following Material to UPitt: 200 (20-50 µl each) de-identified serum samples and archival formalin-fixed and paraffin-embedded tumor tissue sections from women with a primary invasive epithelial ovarian cancer collected under WCG Institutional Review Board (IRB) approved protocol in accordance with U.S. federal guidelines for "Protection of Human Subjects": TDAN eProtocol 15-1813 / WCG IRB Protocol number 20110222 in the study entitled "Tissue and Data Acquisition for the Study of Gynecologic Disease (TDAN)", and distributed for use for collaborative research with the GYN-COE under the use protocol 14-1679 entitled " Integrated Molecular Analysis of Endometrial Cancer, Ovarian Cancer and Other Medical Conditions to Identify and Validate Clinically-Informative Biomarkers and Factors (Integromics)".

   b. **LDS**: LDS means a limited set of identifiable data obtained from participants enrolled in the IRB approved protocol, which may be disclosed for research, public health, and health care operations as set forth in 45 CFR 164.514(e)(2) and **Appendix B**.

   HJF/Inova will transfer the following LDS to UPitt. LDS derived from Protocol number 15-1813 "TDAN" and distributed for use under Protocol number 14-1679 "Integromics". The Provider will