AWARD NUMBER: W81XWH-21-1-0338

TITLE: Impact of Humoral and Cellular Immunity on Ovarian Cancer Outcomes

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CONTRACTING ORGANIZATION: University of Pittsburgh, Pittsburgh, PA

REPORT DATE: October 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2023	Annual	30Sep2022-29Sep2023

4. TITLE AND SUBTITLE

Impact of Humoral and Cellular Immunity on Ovarian Cancer Outcomes

5a. CONTRACT NUMBER
W81XWH-21-1-0338

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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8. PERFORMING ORGANIZATION REPORT NUMBER

004514360

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

U.S. Army Medical Research and Development 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 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,

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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. All approvals, including approval for working with our new immunology lab (Dana Farber – Dr. Fichorova) have been received.

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.
    b. Identified, pulled and aliquotted Pittsburgh cohort serum samples including 5% blinded duplicate splits
    c. Shipped a subset of serum aliquots to University of Chicago (new lab) and Dana Farber (new lab)
    d. Updated cohort outcomes data - We continue cleaning and validating the data on the Pittsburgh cohort

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 (83% of Pittsburgh cohort; the remainder of the Pittsburgh cohort will be shipped with the validation set to increase the size of that set)
4. Month 21: Data on samples updated – achieved and will continue to do so over the next period
5. Month 20: Anti-tumor antibodies measured in serum – 223 samples are at the labs being analyzed; remaining Pittsburgh cohort will ship with the validation 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

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 – 83% has been achieved; remaining have been sectioned and will be shipped to lab next month.
2. Month 25: NanoString marker assessment complete – achieved 83% this reporting period; remaining have been sectioned and will be shipped to lab next month
3. Month 28: IHC marker assessment complete – in progress for Pittsburgh cohort (100% complete on 80% of Pittsburgh cohort)

Aim 3

1. Task 1: Finalize Validation Cohort– *SITE:Inova (Maxwell, Modugno,Lopa, Taylor)*
    a. Months 6-12: Extract, clean and validate relevant clinical, demographic, treatment, and outcome data from Inova data repository and EMRs for 150

Aim 3A Milestones:

    b. Month 9: Validation cohort finalized - identified 125 cases that met inclusion criteria; initial data extracted and will be cleaned and validated during the upcoming year.

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 finalize the validation cohort and begin to obtain their data and biospecimens. As their cohort is spread among 4 institutions and required approval from each institution, this has proven more time consuming than initially anticipated. In addition, because their cohort fell short of the goal of 150 cases by 25 cases, we expanded the Pittsburgh cohort to 287 cases, which will provide sufficient cases to meet our analysis criteria.

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 validated and cleaned these data in preparation for data analysis and dropped cases that did not complete an entire course of chemotherapy. 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=287)

Age, mean (SD)	63.0 (11.0)
Race N (%)	
Black	6 (2.09)
White	279 (97.2)
Other	2 (0.7)
Smoking N (%)	
current	49 (17.07)
former	62 (21.6)
never	150 (52.26)
unknown	26 (9.06)
Histology N (%)	
carcinosarcoma	17 (5.9)
clear cell	30 (10.5)
endometrioid	28 (9.8)
high grade serous	163 (56.8)
low grade serous	16 (5.6)
mixed cell	15 (5.23)
mucinous	12 (4.2)
other	5 (1.8)
Stage N(%)	
I-II	87 (34.5)
III-IV	174 (60.6)
unknown	6 (2.09)

Debulking status N (%)	
optimal	202 (70.4)
non-optimal	33 (11.5)
unknown	52 (18.1)
Vital Status N (%)	
Alive	172 (59.9)
Dead	115 (40.1)
Chemo type N (%)	
Adjuvant	207 (75.3)
Neo-Adjuvant	68 (24.7)
RECIST 1.1 Response	
Complete response	217 (75.6)
partial response	30 (10.5)
stable disease	7 (2.4)
progressive disease	33 (11.5)
Platinum Status N (%)	
sensitive	219 (76.3)
resistant	33 (11.5)
refractory	34 (11.9)
unknown	1 (0.4)

Table 2 shows the basic descriptives of our validation cohort. These are summary statistics based on a first pass of the data extraction. We will be doing a more comprehensive data extraction to clean and validate the data.

Table 2: Validation Cohort Description N=125		
Race	N=	(%)
Non- Hispanic White	111	88.80
Non- Hispanic Black	3	2.40
Asian or Pacific Islander including Native Hawaiian	8	6.40
Hispanic	3	2.40
Age		
40-49 y/o	14	11.20
50-59 y/o	29	23.20
60-69 y/o	55	44.00
70-79 y/o	21	16.80
80+ y/o	6	4.80
Smoking		
Current	6	4.80

Former	36	28.80
Never	82	65.50
Unknown	1	0.80
Histology		
Carcinosarcoma	4	3.20
Clear Cell	13	10.40
Adenocarcinoma Unspecified	5	4.00
Endometrioid	14	11.20
HG Serous	61	48.80
LG Serous	10	8.00
Mixed Cell	12	9.60
Mucinous	4	3.20
Other	2	1.60
Stage		
I-II	44	35.20
III-IV	81	64.80
Unknown	0	
Debulking Status		
Optimal	72	57.60
Non-Optimal	38	30.40
Unknown	15	12.00
Vital Status		
Alive	81	64.80
Dead	44	35.20
Chemo Type		
NACT	17	13.60
ACT	107	85.60
Not Reported	1	0.80
RECIST 1.1 Response		
Complete Response	73	58.4
Partial Response	3	2.40
Stable Disease	1	0.80
Progressive Disease	2	1.60
Pending (Need further QC)	46	36.8
Platinum Status		
Sensitive	63	50.40
Resistant	16	12.80
Refractory	39	31.20
Pending (Need further QC)	7	5.60

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 approved at the end of the last grant year and samples were subsequently shipped to Dr. Matsuzuki.

Concerned that we would not be able to use Dr. Matsuzaki, in consultation with our team and advisors, we explored other laboratory options, including the Meso Discovery Scale (MSD) platform, which will allow us to plex our markers and use a much smaller specimen volume. We worked with MSD to develop our study-specific plex assay, which will be run in the Dana Farber MSD lab. This lab has extensive experience with the MSD plex assays, and we are confident the results will be robust and reproducible. Therefore, in consultation with our team, we decided to complete the set of additional markers using this new technology, which also addresses concerns about sample volume. We were delayed in getting the assays run because of the additional MTA and contract agreements. Those were approved and the samples have now been shipped to the Dana Farber lab.

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. This reporting period, we worked 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. While Inova originally committed 150 samples to this project, after reviewing their pathology and treatment data, 25 cases were eliminated.

In addition, after consulting our team, we decided to eliminate cases that did not complete a full course of platinum-based therapy because these women would be at a survival disadvantage. We then re-queried our database and identified an additional 54 eligible cases. Data on those cases has been cleaned (as of September 2023), FFPE blocks have been pulled and were sent to our histology lab to be sectioned and bloods have been aliquoted. As of last week, these are ready to send to the labs. Our Pittsburgh cohort is now 287 cases, which addresses the shortfall in the Inova cohort (125 cases), bringing our total above the 400 cases originally proposed, allowing for dropping of observations that may not yield accurate lab results.

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. Complete the MDS markers (Dr. Fichorova) and other anti-tumor antibody markers (Dr. Matsuzaki)
2. We will work with Inova ship data to Pittsburgh and biospecimens to the labs.
3. Complete all assays

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. As Dr. Matsuzaki had 3 markers that have been heavily validated by her lab, we decided to continue with her work for those markers. Dr. Fichorova had several of our other markers validated in her lab, so we will be using that lab for the remaining markers.

All lab problems have been resolved.

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 obtain the Inova specimens during this reporting period as originally planned. We are working with Inova now to clean their data set and obtain all biospecimens.

Finally, two members of our team resigned from their academic position: Statistician Dr. Lopa and Immunologist Dr. Vlad. Dr. Modugno has assumed Dr. Lopa's role in cleaning data sets. Once the data sets are cleaned, we will identify another biostatistician to assume the final analyses. As the University of Pittsburgh Hillman Cancer Center recently received an NCI Ovarian Cancer SPORE grant, we will take advantage of the biostatistics core for completing that work. Notably, Dr. Bao, the bioinformatician on this project is the SPORE biostats core leader; thus we are confident we will have a successful analysis of our data. While the loss of Dr. Vlad is disappointing, Dr. Raina Fichorova is an expert in immunology and her lab is now running the majority of our assays. Thus, Dr. Vlad's efforts are assumed in Dr. Fichorova's work. In addition, Dr. Vlad has agreed to stay on the project as an unpaid consultant to troubleshoot any problems and to help with data interpretation and publications.

5c. Changes that had a significant impact on expenditures
Ms. Laslavic, project coordinator, took another position last May. We hired Lauren Borho, who began in our group on September 1, 2022. This reduced salary expenditures for a few months.

Because of the delays in the anti-tumor antibody work and the lab work for the validation cohort, we did not spend those funds. We also did not transfer funds to Inova as originally planned due to the request to delay obtaining those samples until we determine the exact volume of serum specimen needed. The funds for Dr. Lopa were also underspent as she resigned her position early in this reporting period. Dr. Vlad's resignation was effective October 1, 2023 and did not impact project expenditure.

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; assuming data cleaning role
Funding Support	n/a

Name	Esther Elishaev
Project Role	co-I;
Researcher ID (eg, ORCID ID)	0000-0002-6271-0222
Nearest Person Month Worked	0.6-2.4
Contribution to Project	Pathologist
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;
Funding Support	n/a

Name	Lauren Borho
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 is part of the newly-funded NCI Ovarian Cancer SPORE. She receives 20% salary support to run SPORE cores (pathology, CEP, DRP). Dr. Bao is also funded on that grant to run the Bioinformatics/Biospecimen Core. Dr. Elishaev co-runs the pathology Core with Dr. Modugno. Dr. Taylor has a scientific project (unrelated to the current project) in the SPORE. There is no scientific overlap.

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

- **Organization Name:** University of Chicago
- **Location of Organization:** Chicago, IL
- **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. Matsuzuki is quantifying 3 markers: NYES0, MAGEA3, P53

- **Organization Name:** Dana Farber
- **Location of Organization:** Boston, MA
- **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. Fichorova is quantifying 7 markers: Anti-Ca125, Anti-CA15.3, Anti-HE4, Anti-Folate receptor alpha, Anti- Mesothelin, Anti-Survivin and Anti-WT1

- **Organization Name:** Inova
- **Location of Organization:** Fairfax, Va
- **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:** Providing validation cohort

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

9. **Appendices**
   None