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TITLE: A Multiantigen Vaccine Targeting EMT-Associated Proteins to Prevent Recurrent Ovarian Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our hypothesis is that OVAC4, a multi-antigen, multi-epitope vaccine targeting immunogenic proteins associated with mesenchymal transition (EMT), could be an effective method to prevent the development of EMT and ovarian cancer relapse. The specific aims are to (1) determine whether vaccination with OVAC4, concurrent with cisplatin therapy, can prevent the upregulation of EMT associated proteins in the tumor, (2) evaluate the tumor response rate and overall survival of mice treated with OVAC4 and cisplatin with or without an anti-PD-1 monoclonal antibody, and (3) assess any potential short or long-term toxicity of immunization with OVAC4. Our approach focuses on determining the mechanisms by which OVAC4 induces an anti-tumor response and inhibits the development of metastases. The data generated in this proposal will serve as the basis for an Investigational New Drug (IND) application to the FDA for the human translation of OVAC4.

15. SUBJECT TERMS
None listed.

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1. **INTRODUCTION:** Our hypothesis is that OVAC4, a multi-antigen, multi-epitope vaccine targeting immunogenic proteins associated with EMT, could be an effective method to prevent the development of EMT and ovarian cancer relapse. The specific aims are to (1) determine whether vaccination with OVAC4, concurrent with cisplatin therapy, can prevent the upregulation of EMT associated proteins in the tumor, (2) evaluate the tumor response rate and overall survival of mice treated with OVAC4 and cisplatin with or without an anti-PD-1 monoclonal antibody, and (3) assess any potential short or long-term toxicity of immunization with OVAC4. Our approach focuses on determining the mechanisms by which OVAC4 induces an anti-tumor response and inhibits the development of metastases. The data generated in this proposal will serve as the basis for an Investigational New Drug (IND) application to the FDA for the human translation of OVAC4.

2. **KEYWORDS:**

Cancer, Vaccine, Immunology, mesenchymal transition (EMT)

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1: Determine whether vaccination with OVAC4, concurrent with cisplatin therapy, can prevent the upregulation of EMT associated proteins in the tumor. Milestone achieved: Final optimized candidate vaccine identified (11 mos) Milestone achieved: Determine if decreased EMT gene signature correlates with increased CD8+ TIL (19 mos) Specific Aim 2: Evaluate the tumor response rate and overall survival of mice treated with OVAC4 and cisplatin with or without an anti-PD-1 monoclonal antibody. Milestone achieved: Define the level by which the cisplatin and anti-PD1 therapy can synergize with vaccination (36 mos) Specific Aim 3: Assess any short or long-term toxicity of immunization with OVAC4. Milestone achieved: Define the safety profile of OVAC4

What was accomplished under these goals?

Specific Aim 1, Major task 1: Evaluate the use of spacers and the order of epitope expression as methods to optimize the immunogenicity of OVAC4 (100% complete).
Milestone achieved: Final optimized candidate vaccine identified.

Specific Aim 1, Major task 2: Determine whether vaccination with OVCA4 modulates cells that have undergone EMT (50% complete).
Mice were immunized with the optimized OVAC vaccine (50 ug) four times, 10-14 days apart with soluble GM-CSF as an adjuvant. Two weeks after the last vaccine, $1x10^6$ ID8 ovarian cancer cells stably expressing luciferase were injected bilaterally i.p. into C57Bl/6 mice. Bioluminescent imaging was performed two, four and six weeks after implant. Globally, tumor growth between mice immunized with OVAC was not statistically different than the control. However, the tumor volume in 26% (4/15) of the mice was less than a mean and one standard deviation of the control mouse tumor volume (Fig 1). The magnitude of the immune response generated with the same dose and regimen of OVAC in the C57BL/6 mouse strain is statistically smaller than in the FVB/n mouse strain (Fig. 2). The average precursor frequency (PF) of antigen specific T-cells in the C57BL/6 was about 1:10,000 splenocytes whereas, in the FVB/n strain, the PF was about 1:2,000.

Given a 5-fold difference in magnitude of the antigen-specific IFN-g immune response generated between the strains, we questioned if we could increase efficacy of OVAC if it were administered in the FVB/n strain. Thus, we obtained an FVB/n-derived ovarian cancer cell line (BR-luc) with combinations of genetic alterations frequently present in human high grade serous ovarian cancer (p53-/-, Brca1-/-, myc, and Akt). Several features of this model make it suitable for studying microenvironment dynamics during ovarian cancer progression: 1) intact immune system; 2) tumors form with 100% penetrance and predictable latency; 3) the main genetic and pathologic aspects of human ovarian cancer are represented; and 4) intense luc expression in BR-luc cells allows for longitudinal in vivo luciferase imaging. This ovarian cancer model recapitulates human serous histology, pattern of metastatic spread, and response to standard and targeted therapies. To optimize the dose of cells injected, we considered the tumor growth 4 weeks compared to 1 week after implant via bioluminescent imaging. We observed a significant increase in tumor volume when FVB/n mice were injected with $1x10^6$ cells bilaterally, i.p., whereas there was no difference in tumor growth when mice were injected with $0.1x10^6$ or $0.5x10^6$ Br-luc cells (Fig. 3A). We decided to inject $1x10^6$ cells bilaterally for the remaining experiments. Due to its mutations, this cell line is exquisitely sensitive to cisplatin, so we evaluated three extremely low doses in the model. Tumor volume was monitored 1 week after implant via bioluminescent imaging, then cisplatin at 0.5, 1 and 2.5 mg/kg was injected every 10 days i.p. and tumor growth was monitored again after 4 weeks. Cisplatin at any dose could significantly reduce tumor volume as compared to the untreated mice (Fig. 3B; $p<0.0001$). However, there was no difference in tumor volume among the doses. Therefore, we decided to use the lowest cisplatin dose (0.5mg/kg) to reduce potential toxicity and discomfort in the mice.

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?
If this is the final report, state "Nothing to Report."

We will evaluate if the vaccine can modulate cells that have undergone EMT in alone or in conjunction with chemotherapy in the Br-Luc-FVB/n model.

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?

Knowledge generated from this study on difference in the magnitude of the immune response generated from a multi-antigen DNA vaccine in different mouse strains can inform other cancer vaccine or infectious disease studies.

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:

Nothing to Report

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

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

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

Not Applicable

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:

- **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

Name: Mary (Nora) L. Disis MD
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID) 0000-0001-7653-4648
Nearest person month worked: 0.84

Contribution to Project: Dr. Disis oversees all aspects of the project, designing experiments in collaboration with the senior scientists, implementing study protocols, assuring completion of the scope, and preparing and submitting results.
Funding Support: (No funding support is provided from other than this award.)

Name: Denise Cecil PhD
Project Role: Senior Research Scientist
Researcher Identifier (e.g. ORCID ID) N/A
Nearest person month worked: 2.23

Contribution to Project: Dr. Cecil is responsible for leading the project, managing the research staff and performing assays. She has expertise in cellular immunology assays and bioluminescent imaging of mice.
Funding Support: (No funding support is provided from other than this award.)

Name: Erin Rodmaker
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID) N/A
Nearest person month worked: 1.89

Contribution to Project: Erin assists the Animal Core with immunizations, harvesting and the database.
Funding Support: (No funding support is provided from other than this award.)

Name: Kevin Potts
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID) N/A
Nearest person month worked: 2.88

Contribution to Project: Kevin supports the protein assays.
Funding Support: (No funding support is provided from other than this award.)

Name: Katie Hitchcock-Bernhardt
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID) N/A
Nearest person month worked: 1.40

Contribution to Project: Katie supports Dr. Cecil and managed flow cytometric analyses and protein assays.
Funding Support: (No funding support is provided from other than this award.)

Name: Danielle Rodriguez
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID) N/A
Nearest person month worked: 2.39

Contribution to Project: Danielle performs animal breeding, maintenance, PCR, tissue collections and data entry.
Funding Support: (No funding support is provided from other than this award.)

Name: June Rambousek
Project Role: Research Scientist
Researcher Identifier (e.g. ORCID ID) N/A
Nearest person month worked: 1.51

Contribution to Project: June assists the animal core with animal monitoring and preparing vaccines.
Funding Support: (No funding support is provided from other than this award.)

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

HHSN261201500036I Task: 26100 (Kennedy) 07/18 – 02/23 0.36 CAL (**Ended**)
NIH/NCI/University of Alabama
Evaluating whether a concurrent retinoid X receptor agonist can enhance the efficacy of the Her2-IGFBP2-IGF1R Vaccine in Eliminating existing DCIS and preventing progression of invasive breast cancer
Role: Principal Investigator. PoC: Greg Kennedy, gkennedy@uabmc.edu

HHSN261201500036I Task: 011 (Kennedy) 09/18 – 12/22 0.18 CAL (**Ended**)
NIH/NCI/University of Alabama
Further Development of Multi-Antigen Vaccines for the Primary and Secondary Prevention of Bladder Cancer
Role: Co-Investigator. PoC: Greg Kennedy, gkennedy@uabmc.edu

75N91019D00019 Task: F00131 (Kennedy) 09/19 – 12/22 0.72 CAL (**Ended**)
NIH/NCI/University of Alabama
Further Development of Colovac, a Multi-antigen Multi-peptide Vaccine, for Colon Cancer Prevention
Role: Co-Investigator. PoC: Greg Kennedy, gkennedy@uabmc.edu

2021.0001 04/21 – 03/23 0.60 CAL (**Ended**)
Andy Hill Cancer Research Endowment/Institute for Systems Biology
Proactive Cancer Immunotherapies for Initial and Recurrent Disease
Role: Co-Investigator. PoC: James Heath

BCRF-22-038 10/22 – 09/23 0.60 CAL (**Renewed**)
Breast Cancer Research Foundation
Augmenting the effects of chemotherapy in triple negative breast cancer with Th1 selective vaccination
Role: Principal Investigator. PoC Jamie O'Brien, jobrien@bcrf.org

PN-301-21 09/22 – 08/24 0.60 CAL (**New**)
Aston Sci
A Phase 2 Study to Evaluate the Efficacy and Safety of an Adjuvant Therapeutic Cancer Vaccine (AST-301, pNGVL3-hICD) in Patients with HER2 Low Breast Cancer (Cornerstone 001)
Role: Site Investigator. PoC: Nicole Procaccini, nicole.procacciini@catalyst.com

R21CA 273739 12/20 – 11/24 0.12 CAL (**New**)
NIH/NIBIB
Nano-bio interaction-enabled engineering of ultrafine and high efficient chemoimmunotherapeutic nanoparticles
Role: Co-Investigator. PoC Qingxin Mu, qmu@uw.edu

2022 Discovery Grants 01/23 – 12/25 0.96 CAL (**New**)
Kuni Foundation
Optimizing precision probiotics for improving cancer therapy
Role: Principal Investigator. PoC: Gretchen Schackel, retchen@kunifoundation.org

FIA-22-001-01-FIA 04/22 – 03/26 0.36 CAL (**New**)
American Cancer Society
Fostering Innovation Award (FIA) with Meharry Medical College
Role: Co-Investigator. PoC: Kim Smith, kim.a.smith@cancer.org

R37CA277812 09/22 – 08/26 0.60 CAL (**New**)
NIH/NCI/Rutgers
Screening and confirmatory machine learning for explainable modeling of non-cancer (Subaward, University of Washington)
Role: Principal Investigator. PoC: Lanjing Zhang, lanjing.zhang@rutgers.edu

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

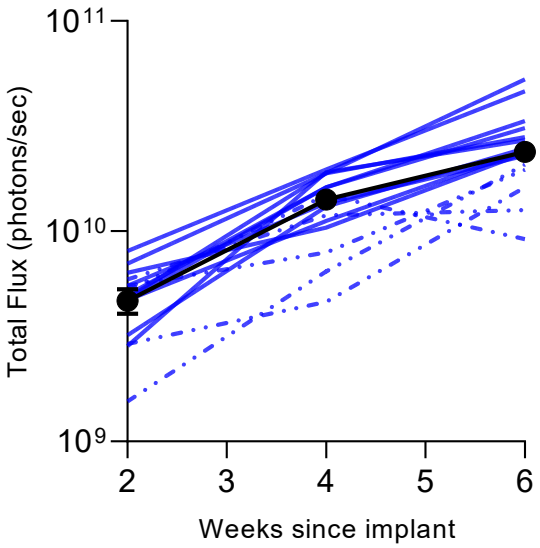

Figure 1. Ovarian cancer growth is inhibited after immunization with OVAC in some mice. (A) Total flux (photons/sec) for mice immunized with adjuvant alone (mean ± SEM; black line) or OVAC (blue lines; all mice). Tumors less than the mean and one standard deviation of the control are in dashed blue lines. n=15 mice/group.

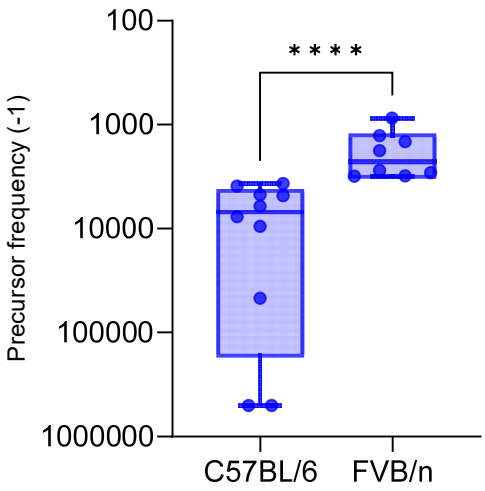

Figure 2. More T-cells are OVAC-specific after immunization in the FVB/n mouse strain as compared to the C57BL/6 mouse strain. Precursor frequency of antigen-specific IFN-g secreting T-cells in the indicated mouse strain after immunization with the same dose of OVAC; n=10 mice/group; ****p<0.0001.

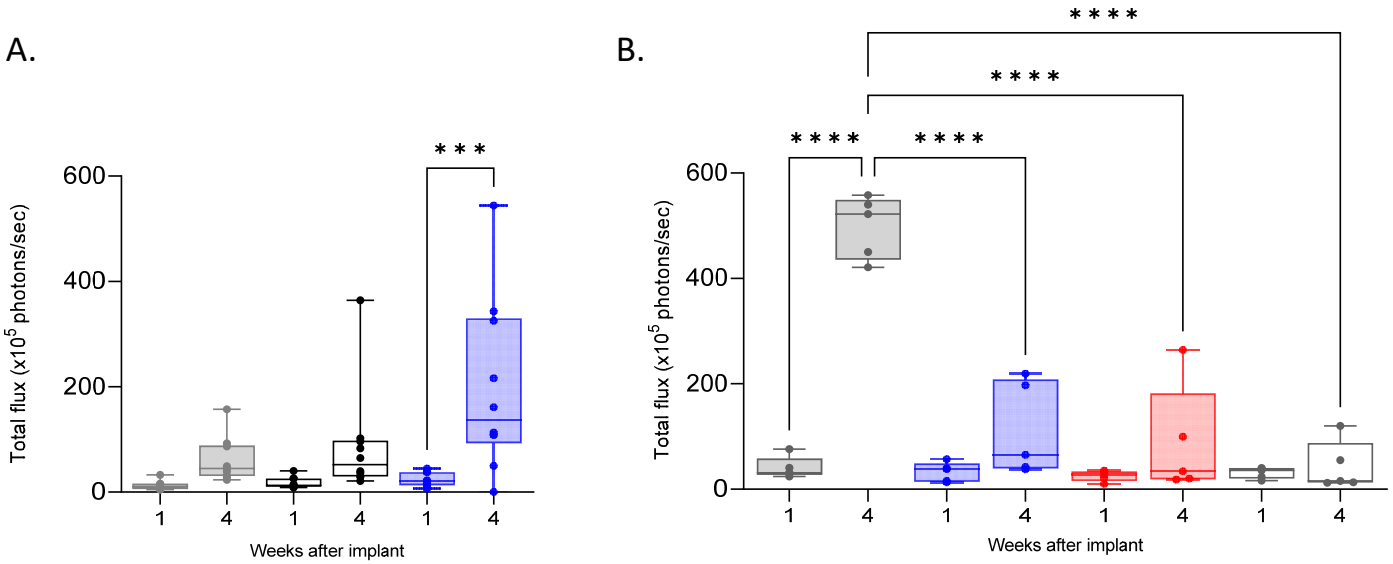

Figure 3. Doses of Br-luc cells and cisplatin are optimized. (A) Total flux (photons/sec) for mice implanted with 0.1 x10^6 (gray bars), 0.5 x10^6 (white bars) or 1 x10^6 (blue bars) cells. n=9-10 mice/group; ****p<0.0001. (B) Total flux (photons/sec) for control mice (gray bars) or mice treated with cisplatin at 0.5 mg/kg (blue bars),1 mg/kg (red bars) or 2 mg/kg (white bars). n=5 mice/group; ****p<0.0001.