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TITLE: Breaking B-Cell Tolerance to Produce Antibodies that Eradicate Leukemias and Lymphomas

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Stefanie Sarantopoulos, PhD

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Durham, NC 27708-4677

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14. ABSTRACT

Our prior work studying allo-HCT patient samples has now led to improved understanding about how B cell tolerance mechanisms dampen recognition of host/self. Cancer relapse after allo-HCT in patients who never develop chronic graft versus host disease (cGVHD) reveals how host-protective mechanisms dampen responsiveness to tumor. Our primary objective is to develop urgently needed B-cell immunotherapies for the treatment of life-threatening hematolymphoid malignancies. Rationale: Our previous grant (PRCRP CA100254, W81XWH-11-1-0537) afforded preliminary data that forms the basis of the current proposal. We now have critical insights and reagents, including a viable leukemia and lymphoma mouse tumor vaccine system that augments anti-tumor antibody production. Applying what we know about B cell activation signaling, we will devise ways to confer in vivo anti-tumor antibody responses. We hypothesize that anti-tumor B cells, capable of producing functional anti-tumor antibodies, exist in patients with hematolymphoid cancers and these B cells can be augmented and harnessed. We will address this hypothesis using mouse models and human samples.

15. SUBJECT TERMS-

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Targeted immunotherapies are urgently needed for the treatment of life-threatening leukemias and lymphomas. Tumor specific antigens are not universally present on cells and the antigen profiles of tumors change when single antigens are targeted by monoclonal antibodies. Thus, while monoclonal antibodies have proven capacity to eradicate hematolymphoid and solid tumors, tumor specific and tumor associated antigen (TAA) targets are variably immunogenic and animal vaccinations with these antigens do not readily produce therapeutically viable antibodies. Our objective is to develop strategies to improve production of anti-tumor antibodies by studying mouse models and by harnessing antibody-producing B cells from patients.

KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Leukemia, Lymphoma, anti-tumor antibodies, stem cell transplantation, B cell

2. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Specific Aim 1: Devise a strategy for safe B cell adoptive therapy that eradicates hematolymphoid tumors.
Major Task 1: Induce anti-tumor B cell responses in mice.
Subtask 1: Submit and obtain documents for ACURO approvals (1-3). Award granted on September 30, 2019 As planned, we obtained ACURO approval was granted on 12/7/19.
Subtask 2: Test antibody and anti-tumor response of adoptively transferred syngeneic B cell products (we have not completed as planned in months 3-9, but substantial progress was made as reported under accomplishments) We have produced VRP vaccine and we are prepared to test this hypothesis. We will adoptively transfer syngeneic B cells and Viral Replicon Particle (VRP) vaccine (+/- CpG) in a leukemia and lymphoma model system immediately after myeloablative syngeneic ('autologous') bone marrow transplantation in order to activate anti-tumor B cells.
Subtask 3: Test whether B cell response after VRP-Flt3 is TLR9 or TLR7 mediated, using TLR9 -/- donor cell (10-12). We have reagents on hand and we have produced VRP vaccine and we are ordering mice for these experiments now.
Subtask 4: Elucidate molecular mechanisms induced by TLR9 agonist CpG in human B cells after auto-HCT. We have reagents on hand and we have produced VRP vaccine and we are ordering mice for these experiments now.

Milestone(s) Achieved: Demonstrate feasibility and safety of inducing anti-tumor B cell responses in preclinical models. (planned for month 12). We anticipate achieving this milestone at the 18-20 month mark.

Specific Aim 2: Use activated B cells from patients after stem cell transplantation to produce broadly reactive antitumor antibodies.

Major Task 2: Produce human antibodies
Subtask 1: Obtained HRPO approval for the use of human anatomical substances (1-2) on August 12, 2019. This approval took nearly the entire duration of the first year. Thus, we were unable to work with patient samples. Instead we used tumor cell lines and healthy donor plasma and monoclonal antibodies to produce
Subtask 2: Screen for patient-specific anti-tumor antibodies (2-18). This work is now underway. We will sort purify CD27+ B cells from de-identified patients and hand off the cells to our collaborators who have a validated full-length IgG production process ready to employ.
Milestone(s) Achieved: Production of human anti-CLL antibodies (month 16). We anticipate reaching this milestone by month 24.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities: establish and test mouse models (Aim 1); establish and test system for identifying tumor-specific antibodies (Aim 2)

2) Specific objectives: Our specific objectives were as follows: 1) to obtain approval for the mouse and human work we have proposed. The mouse work was approved on 12/7/2018 by ACURO. Our work with de-identified samples was approved on 8/12/19 when our HPRO protocol was approved; 2) to produce VRP vaccine and verify the B cell effect in a leukemia and a lymphoma mouse model. This was accomplished as detailed below and as shown in Figure 1; 3) to develop assays that allow us to identify membrane protein antigen targets of plasma IgG and monoclonal IgG (produced using a high-throughput system for production of full-length IgG).

3) Key outcomes: As detailed below, we are actively producing VRP vaccine and performing mouse experiments as proposed. I've detailed the protocol below and report that the production of sufficient vaccine to accomplished n=40 mouse experiments takes approximately 3 months which is longer than anticipated. This is because efficiency of transfection and viral packaging is lower with Flt3 (a large protein). Thus a viral particle concentration step is needed after multiple rounds of transfection and packaging as detailed below.

For Subtask 2 and 3 – We produced VRP vaccine and refined our hematolymphoid mouse models. Specifically, virus-replicon particles were assembled with or without the insertion of the Flt3 gene (VRP-Flt3 and VRP-Ctrl, respectively). For each upcoming experiment to study the effects of virus-replicon particle (VRP) vaccination on B cell responses in tumor-bearing mice, $1x10^6$ infectious units of VRP are needed per subcutaneous footpad injection, per mouse, on days 4 and 18 post-tumor challenge. For that reason, $2x10^6$ infectious units of VRP are required per mouse per experiment. The extracellular-transmembrane domain of mouse Flt3 (AA 1 to 584) was cloned into the pVKE plasmid. pVKE plasmid with or without the Flt3 transgene, as well as two helper plasmids encoding the Venezuelan equine encephalitis (VEE) capsid and glycoprotein genes were linearized by NotI digestion and used as templates for *in vitro* transcription using the T7 mMESSAGE mMACHINE® Kit (Ambion). These capped VRP transcripts were transfected into baby hamster kidney (BHK) cells by the ECM® 630 Exponential Decay Wave Electroporation System (BTX) under conditions of 1000V/100 Ω/25uF/3 pulses. The transfected cells were cultured with MEM-α medium containing 10% FBS, 2 mM L-Glutamine, 1 mM sodium pyruvate, pen/strep and 10% Tryptose Phosphate Broth (Sigma) at 37°C under 5% CO_2 for 24 hours. Culture supernatants containing the VRPs were harvested and clarified by centrifugation at 800 xg at 4°C for 10 minutes. The infectious unit titers of VRP-Flt3 and VRP-Ctrl were then determined by infecting BHK cell monolayers with diluted VRP-Flt3 or VRP-Ctrl for 24 hours at 37°C in 5% CO_2, upon

which cells were harvested, counted, and intracellularly stained for VEE-nonstructural protein (VEE-NSP). Cells that stained positive for VEE-NSP were considered to be infected. The fraction of infected cells and the volume of VRP stock used for infection was plotted, and data points in the linear range were used for titer determination. The titer of VRPs was calculated by the following equation: Titer (IU/mL) = (fraction of infected cells/100) x cell number / (volume of VRPs used for infection).

The *in vitro* transcription, electroporation, and supernatant concentration processes needed to be repeated nine times to achieve high enough titer VRP-Flt3 to be able to immunize a minimum of 20 mice. VRP-Ctrl titer was much greater than that of VRP-Flt3, so the aforementioned VRP assembly methods only needed to be repeated one time to achieve a VRP-Ctrl titer sufficient for immunizing a minimum of 20 mice. As explained above, 20 VRP-Flt3 mice and 20 VRP-Ctrl mice are needed for one experiment. VRPs were further concentrated by ultracentrifugation at 24,000 rpm/ 4°C for 3 hours through a 5 mL cushion of 20% (w/v) sucrose in PBS. VRPs were resuspended in PBS prior to aliquoting and storing them in -80°C, ready for immediate use in our mouse experiments upon thawing.

We first tested the VRP vaccine in two different tumor models (C1498 leukemia and A20 lymphoma). As shown in **Figure 1 on the next page below**, VRP-Flt3 induced a significant increase in B lymphocyte number on days 14-28 after tumor inoculation. A significant increase in IgG concentration was found after VRP vaccine (with or without Flt3 antigen). Together, data affirm that B cell responses are induced in our model system.

We have 53.34 x 10^6 infectious units of VRP-Flt3, and 137.38 x 10^6 infectious units of VRP-Ctrl on hand, enough for injecting 26 and 68 mice, respectively. Production of more VRP-Flt3 and VRP-Ctrl is ongoing. The next experiment planned will study TLR adjuvant effects on tumor clearance, by injecting mice with both VRP and CpG, or with VRP only after leukemia tumor challenge. Treatment groups will be as follows: VRP-Ctrl (n=10)**,** VRP-Flt3 (n=10)**,** VRP-Flt3 + adjuvant CpG (n=10), VRP-Ctrl + adjuvant CpG only (n=10). The next experiment planned follows a similar set-up, except instead of studying the effects of adjuvant CpG in addition to VRP vaccination, we will be studying the influence of a syngeneic B cell boost in combination with VRP vaccination. Based on the treatment groups described above, 40x10^6 infectious units of VRP-Ctrl and 40x10^6 infectious units of VRP-Flt3 will be required per experiment.

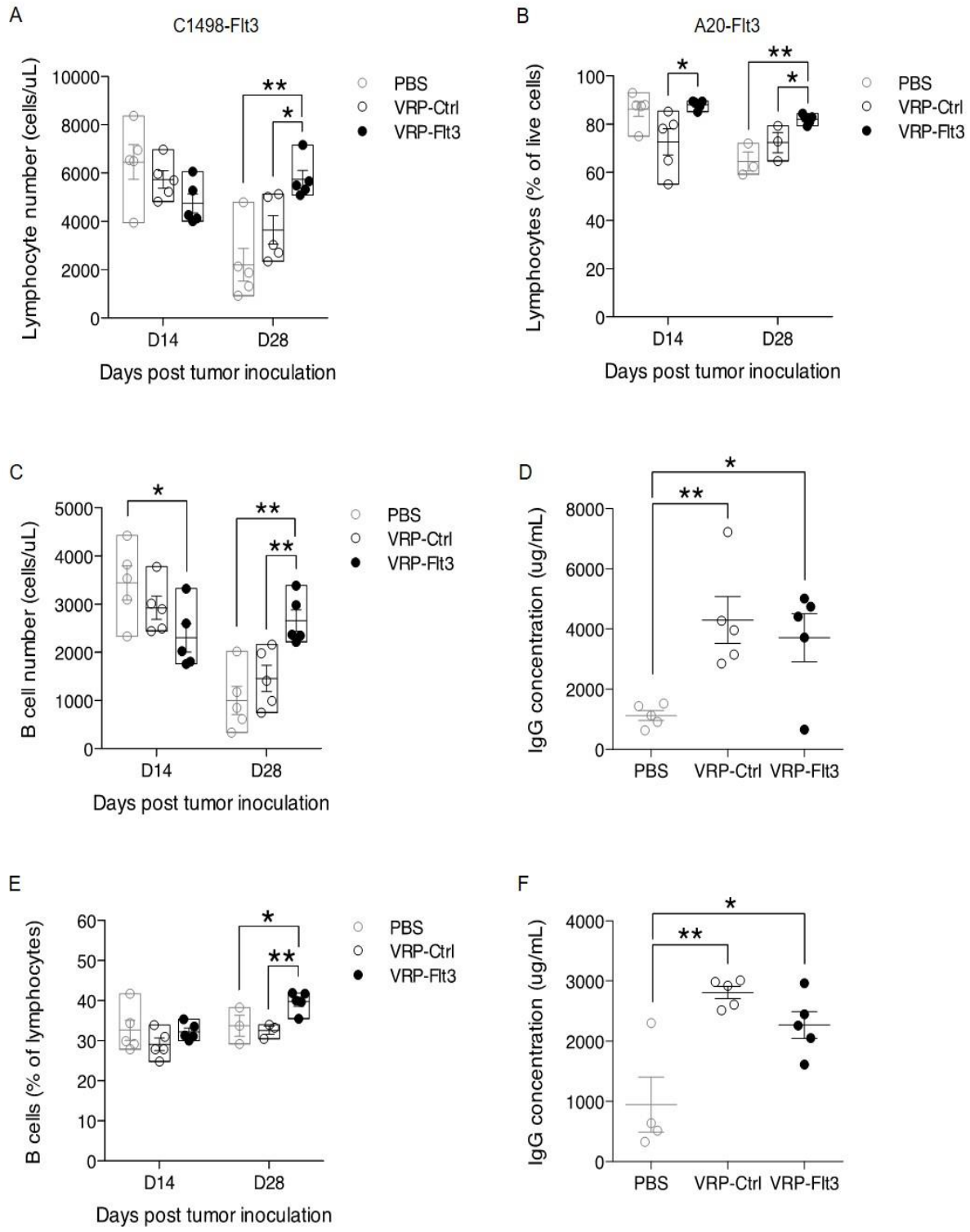

FIGURE 1. VRP treatment induces B cell response in tumor-bearing mice. C1498-Flt3 tumor cells inoculated B6.SJL mice or A20-Flt3 tumor cells inoculated BALB/c mice received different VRP vaccination on days 4 and 18 post tumor challenge. Lymphocytes or B cells in the peripheral blood were analyzed by FACS on days 14 and 28. IgG level in the plasma collected on day 28 post tumor challenge were analyzed by ELISA. **A,** Lymphocyte number of C1498-Flt3 tumor-bearing B6.SJL mice (n=5). **B,** Lymphocyte percentage of A20-Flt3 tumor-bearing BALB/c mice (n=5). **C,** B cell number (n=5) and **D,** IgG concentration of C1498-Flt3 tumor-bearing mice (n=5). **E,** B cell percentage (n=5) and **F,** IgG concentration of A20-Flt3 tumor-bearing mice (n=5). Open gray circles represent PBS control group; open black circles represent VRP-Ctrl vaccination group; solid black circles represent VRP-Flt3 vaccination group. Bars the represent mean ± SEM. Unpaired t test, *P< 0.05; **P< 0.01.

While awaiting HRPO approval, we focused efforts on refining a method for tumor cell membrane protein purification. For this, we used cell lines. We used monoclonal antibodies to known antigens to verify that we were purifying membrane (not nuclear and not cytoplasmic proteins). We then ensured we were maintaining protein quality with our storage methods. After obtaining HRPO permission, we began testing human patient plasma on tumor cell line membranes.We also met with the Haynes group in order to finalize our immediate plan for single cell B cell isolation/cloning and full-length antibody production. While the use of IgG from plasma yields high background in these assays, monoclonal antibodies to known cell surface antigens showed a viable/significant signal. Thus, we conclude that full length monoclonal antibodies produced from single B-cells from patients (as proposed) will be informative. We now have the systems in place (Figure 2) to test this hypothesis. As shown in our original proposal dot blot assays can also be used to identify specific antibodies and we have a membrane protein dot blot assay that can be employed for large scale screening of monoclonal antibodies produced in 96 well plates (as in our expansion grant proposal).

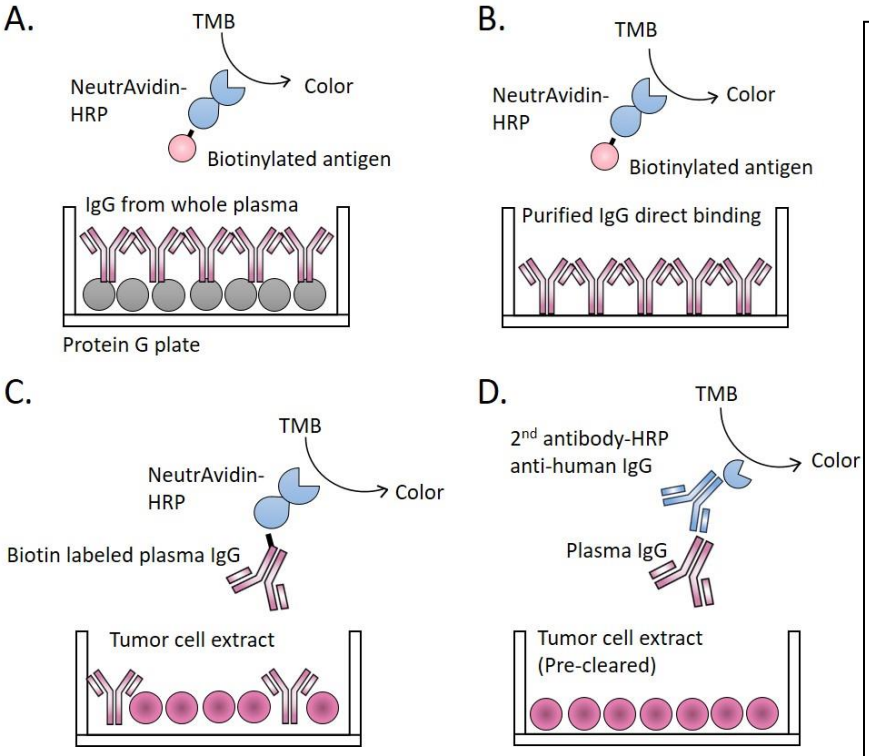

Figure 2. Strategies for detection of tumor-reactive antibodies in CLL patient plasma. (A) and **(B)**: IgG from healthy donor or patient whole plasma is isolated onto Protein G-coated ELISA plates (A), or Melon Gel-purified IgG from healthy donor plasma is isolated directly onto ELISA plates (B), followed by incubation with biotinylated MEC-1 or OSU-CLL tumor cell plasma membrane extracts, and detection of bound antigens by NeutrAvidin-HRP secondary reagent and TMB colorimetric substrate. **(C)** and **(D)**: MEC-1 or OSU-CLL tumor cell plasma membrane extracts (unmanipulated as in (C), or pre-cleared of any IgG using Protein G as in (D) are isolated onto ELISA plates and then bound tumor-specific antibodies are detected by incubation with biotinylated IgG purified from healthy donor plasma followed by detection with NeutrAvidin-HRP secondary reagent and TMB colorimetric substrate (C), or by unlabeled anonymous donor plasma IgG followed by detection with anti-human IgG-HRP secondary reagent and TMB colorimetric substrate (D).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Rachel Dicioccio, BS has been working as a post-bacclalaureate research associate on this project. Rachel is receiving 1:1 mentorship from me and from the senior research associate in the lab. Our goal is to train her and enable her to submit applications for further training in an MD/PhD program.

Sonali Bracken MD PhD is currently a resident in Internal Medicine at Duke. Using funding from another source (NIH, F38 grant, PI Permar), Dr. Bracken is receiving 1:1 mentorship from me on this project, and she is formally being trained via assigned seminars and mentoring group meetings. On this DOD project, I am formally training her to become an independent physician-scientist.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the first funding year, we obtained necessary approvals. We also developed tools and received samples. The goal in the second year of funding is to complete the mouse studies in Aim 1 as below:

Specific Aim 1: Devise a strategy for safe B cell adoptive therapy that eradicates hematolymphoid tumors.
Major Task 1: Induce anti-tumor B cell responses in mice.
Subtask 2: Test antibody and anti-tumor response of adoptively transferred syngeneic B cell products (3-9)
As above, we have produced functional VRP vaccine and we are prepared to test this hypothesis. We will adoptively transfer syngeneic B cells and Viral Replicon Particle (VRP) vaccine (+/- CpG) in a leukemia and lymphoma model system immediately after myeloablative syngeneic ('autologous') bone marrow transplantation in order to activate anti-tumor B cells.
Subtask 3: Test whether B cell response after VRP-Flt3 is TLR9 or TLR7 mediated, using TLR9 -/- donor cell (10-12). We have produced VRP vaccine and we are ordering mice for these experiments now.
Subtask 4: Elucidate molecular mechanisms induced by TLR9 agonist CpG in human B cells after auto-HCT.

We have samples on hand in the lab to perform single cell sorting of CD27+ B cells for production of full-length IgG for testing on tumor membrane preparations.
Specific Aim 2: Use activated B cells from patients after stem cell transplantation to produce broadly reactive antitumor antibodies.
Major Task 2: Produce human antibodies
Subtask 1: The approval for this work was August 12, 2019. This approval took nearly the entire duration of the first year. Thus, we were unable to work with patient samples. Instead we used tumor cell lines and healthy donor plasma and monoclonal antibodies to produce
Subtask 2: Screen for patient-specific anti-tumor antibodies (2-18). This work is now underway. We are working with David Easteroff PhD in Bart Haynes group and we plan to have plates of monoclonal antibodies for screening in the next 3-4 months.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Resolved delay: There was a delay obtaining approval for the human sample work related to templates used for our protocol describing use of de-identified samples. This was resolved after what we are doing was better communicated to the DoD regulatory group. This delay did not stop us from progress working out how best to prepare membrane protein preparations from cell lines.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None.

Significant changes in use or care of vertebrate animals

None.

Significant changes in use of biohazards and/or select agents

None.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Nothing to report.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Rachel DiCioccio*
Project Role:	*Research Technician, pre-medical post-baccalorreate student*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*11*
Contribution to Project:	*Mrs. DiCioccio performed work producing VRP vector and developing protocols so that membrane protein from tumor (cell lines) can be used to capture antibodies.*
Funding Support:	

Name:	*Sonali Bracken, MD PhD*
Project Role:	*Internal Medicine Resident at Duke*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Bracken just joined our research group and she helped*
Funding Support:	*NIH R38 HL143612*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

No change, Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *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);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.