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

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14. ABSTRACT 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. Polyreactive anti-tumor antibodies are not produced when peripheral B cell tolerance mechanisms are intact. Our 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.

15. SUBJECT TERMS
Immunotherapy, anti-tumor antibody, B cell, allogeneic stem cell transplantation

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1. INTRODUCTION:

Our major objective is to develop targeted immunotherapies that 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 hemato-lymphoid and solid tumors, tumor specific and tumor associated antigen (TAA) targets are variably immunogeneic and animal vaccinations with these antigens do not readily produce therapeutically viable antibodies. We have previously demonstrated that constitutive IgG production by aberrantly activated B-cells after hematopoietic allogeneic stem cell transplantation (HCT) potentially produce antibodies that might target tumor antigens. We have also demonstrated that these B-cells are promoted by RNA-receptor (TLR7) responsive. We focus on a high order RNA-packaged vaccine vector called VRP that we showed promotes B-cell responses to pursue--our aims 1) to augment B-cell responses and 2) to harness anti-tumor antibody producing B-cells.

KEYWORDS:

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

2. ACCOMPLISHMENTS:

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.

We have systematically approached this hypothesis as outlined in our proposal and we have accomplished our major task as detailed below in the subsections under "What was accomplished under these goals?" below (major and specific objectives).

Subtask 1: Submit and obtain documents for ACURO approvals (1-3)
Task completed 100%

Subtask 2: Test antibody and anti-tumor response of adoptively transferred syngeneic B cell products (3-9)
Task completed 100%

Subtask 3: Test whether B cell response after VRP-Flt3 is TLR9 or TLR7 mediated, using TLR9 -/- donor cell (10-12)
Task is 60% completed. We have made VRP vector needed for experiments and experiments testing the roles for TLRs are underway.

Subtask 4: Elucidate molecular mechanisms induced by TLR9 agonist CpG in human B cells after auto-HCT.
Task is 75% completed. We have tested CpG and TLR7 responses of B cells from patients and determined relative diminished TLR9 responses in patients who have achieved cancer remission.

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

Major Task 1: Induce anti-tumor B cell responses in mice.

1) Major activities

During this funding period, we have completed Subtask 2. Specifically we employed VRP vaccination strategies in our leukemia models and we found that B cells are promoted and antibodies to tumor are produced. This mouse work was halted because of the COVID-19 pandemic (please see our request for no-cost extension for Subtasks 3 and 4).

2) Specific objective

During this funding period our specific objective was to enable work proposed aimed at defining a B cell and antibody response to syngeneic hematolymphoid tumors. After obtaining mouse approval (Subtask 1)), our specific objective was to produce and employ Venezuelan Replicon Particle (VRP) vaccination that resulted in specific B-cell responses in our mouse cancer models. To this end, we produced VRP-Flt3 and VRP-mock vectors and tested them.

3) Significant results

To investigate the potential use of VRP vaccines for leukemia, a C1498 mouse leukemia model was employed in our study. To better assess the efficacy of the VRP vaccine, C1498 mouse leukemia cells were transfected with plasmid vectors harboring the mouse FLT3 gene. The expression level of FLT3 on C1498-FLT3 cells was checked (S1 Fig) before the tumor cells were intravenously injected into syngeneic B6.SJL mice. VRPs were administered by footpad injection as therapeutic vaccines on days 4 and 18 after tumor challenge. With VRP-FLT3 vaccination, the number of circulating C1498-FLT3 tumor cells in the peripheral blood of tumor-bearing mice was significantly lower compared with the PBS vehicle control group on day 28 and day 35 (**Fig 1A**), and there was a significant improvement in survival in mice that received the VRP-FLT3 vaccine compared with the PBS control group (**Fig 1B**). The data indicate that VRP vaccination attenuates the expansion of leukemia *in vivo*. We also found that VRP-FLT3 vaccine was superior to VRP-Ctrl (without overexpression of the tumor associated antigen) vaccine, although complete tumor regression was not achieved.

To ensure our vaccination strategy was able to attenuate tumor development, we examined another hematolymphoid tumor model to investigate the efficacy of VRP vaccines. A20 murine B lymphoma cells were

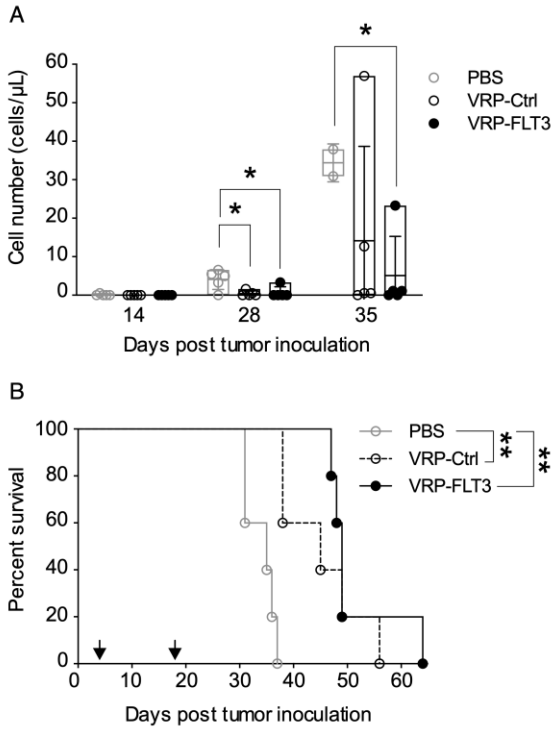

Fig 1. VRP vaccination attenuates the growth of FLT3 expressing C1498 tumor cells.
Mice intravenously inoculated with FLT3-expressing C1498 tumor cells on day 0 were vaccinated on day 4 and day 18 with 1 x 10^6 IU of VRP-Ctrl or VRP-FLT3 on the footpad. The control mice received PBS. (**A**) C1498-FLT3 tumor cells in peripheral blood were determined by Flow cytometry (y-axis). Each symbol represents an individual mouse (n=5 per group). Bars represent the mean ± SD. Statistical significance was determined by unpaired *t* test, *P<0.05. (**B**) Kaplan-Meier survival curves of tumor inoculated mice (n=5 per group). Arrows indicate the days of VRP vaccination. Statistical significance was determined by Log-rank (Mantel-Cox) test, **P<0.01. The best of two independent experiments is depicted.

transfected with FLT3, and the FLT3 expression was confirmed by flow cytometry (**data not shown**). The A20-FLT3 tumor cells were subcutaneously inoculated on the backs of syngeneic BALB/c mice so that we could measure tumor size. We found that the VRP vaccine was able to the size of A20-FLT3 tumor significantly in mice vaccinated with VRP-FLT3 compared to the mice receiving PBS or VRP-Ctrl vaccines (**Fig 2**). The VRP-FLT3 vaccine was unable to completely eliminate the tumor, although no survival benefit was observed with either VRP-FLT3 or VRP-Ctrl vaccination (**data not shown**).

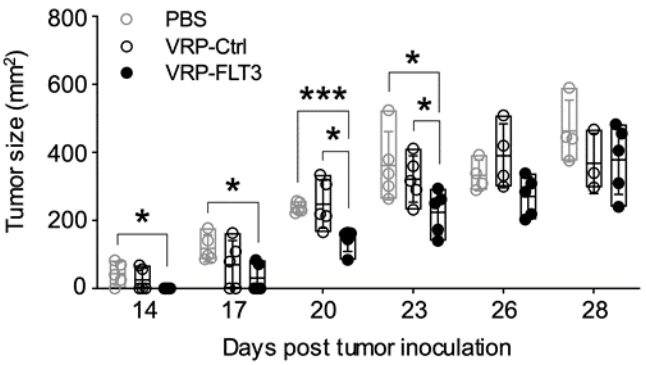

Fig 2. VRP-FLT3 vaccination inhibits the growth of FLT3-expressing A20 tumor cells.
Mice inoculated with FLT3-expressing A20 tumor cells subcutaneously on the back on day 0 were vaccinated on days 4 and 18 with 1 x 10^6 IU of VRP-Ctrl or VRP-FLT3 on the footpad. Control mice received PBS. Tumor growth curves of individual mice are depicted (n=5 per group). Statistical significance was determined by unpaired *t* test compared with PBS control, *P<0.05, ***P<0.001, or compared with VRP-Ctrl, $^{\#}$P<0.05.

Consistent with our hypothesis that B-cell responses to tumor were activated by the VRP vaccine, we found that total lymphocyte numbers and B-cell numbers (**Fig 3 A-B**) were significantly increased in vaccinated mice. To determine if tumor attenuation associated with a B cell response to self-antigen, after VRP-vaccination we further investigated antibody production in the tumor-bearing mice. Plasma samples collected on day 28 were first analyzed for total IgG concentration. We found that both VRP-FLT3 and VRP-Ctrl vaccinations significantly increased the total IgG level (**Fig 3C**). To determine whether the antibody specific to FLT3 was induced in tumor-bearing mice, ELISA using a recombinant mouse FLT3 (rFLT3) protein was employed. A significant level of plasma IgG that recognized rFLT3 was detected in mice vaccinated with VRP-FLT3, while this was not observed in VRP-Ctrl vaccinated group (**Fig 3D**). Taken together, we demonstrated that VRP-FLT3 vaccine is capable of inducing the FLT3-specific antibody in our leukemia mouse model. Thus, these data suggest that vaccination of VRP-FLT3 overcome the intrinsic B cell tolerance to self-antigen, FLT3.

Since significant increases in IgG indicate that vaccination of VRP-FLT3 not only elicits and antibody response to FLT3 (Fig 2C) we examined whether VRP itself elicits antibody responses to other tumor associated antigens (TAAs) in mice inoculated with FLT3-expressing tumor. To do this, we applied another flow cytometric approach to investigate the reactivity of plasma antibody to the tumor cells. Plasma samples collected on days 35-42 after tumor challenge were diluted according to the total IgG concentration to tailor the IgG amount in each test to 1 ug before incubating with the FLT3-expressing C1498 cells (Fig 4A, right) that were the same cell line used for tumor inoculation in our leukemia mouse model. Plasma IgG that reacted with tumor cells was detected by a fluorophore-conjugated secondary antibody. The result showed that VRP-FLT3 vaccination increased the production of IgG that binds to the C1498-FLT3 cells compared with VRP-Ctrl vaccination and the PBS control (**Fig 4A, left**). Since FLT3 is only one of numerous antigens expressed on tumor cells, we also wanted to test whether VRP vaccines could elicit antibody responses against tumor antigens other than FLT3. To do so, we performed the same flow cytometric binding test with the target was changed to C1498-mock cells, C1498 cells that did not express FLT3 on the surface (**Fig 4B, right**). Plasma samples from mice that received the VRP-FLT3 vaccination had higher levels of IgG bound to the C1498-mock tumor cells than plasma samples from PBS control mice (Fig 4B, left). To verify this finding, we performed an ELISA with the membrane protein extracts of C1498-mock cells. After refining a tumor cell membrane extraction technique, we coated cell membrane on ELISA plates and included proper controls. We then tested whether IgG from plasma samples collected on days 35-42 from tumor-bearing mice could preferentially bind to tumor after vaccination with VRP. We found that mice vaccinated with VRP-FLT3 developed a significantly higher level of IgG that reacted to tumor membrane proteins other than FLT3 compared to the PBS control mice (**Fig 4C**). These results indicate that vaccination of VRP-FLT3 not only elicits and antibody response to FLT3 (**Fig 4D**) but also elicited epitope-spreading with antibody responses to other TAAs in mice inoculated with FLT3-expressing tumor.

Thus, we have completed SOW Task 1 by demonstrating that anti-tumor responses can be mounted by breaking B cell tolerance in syngeneic model system.

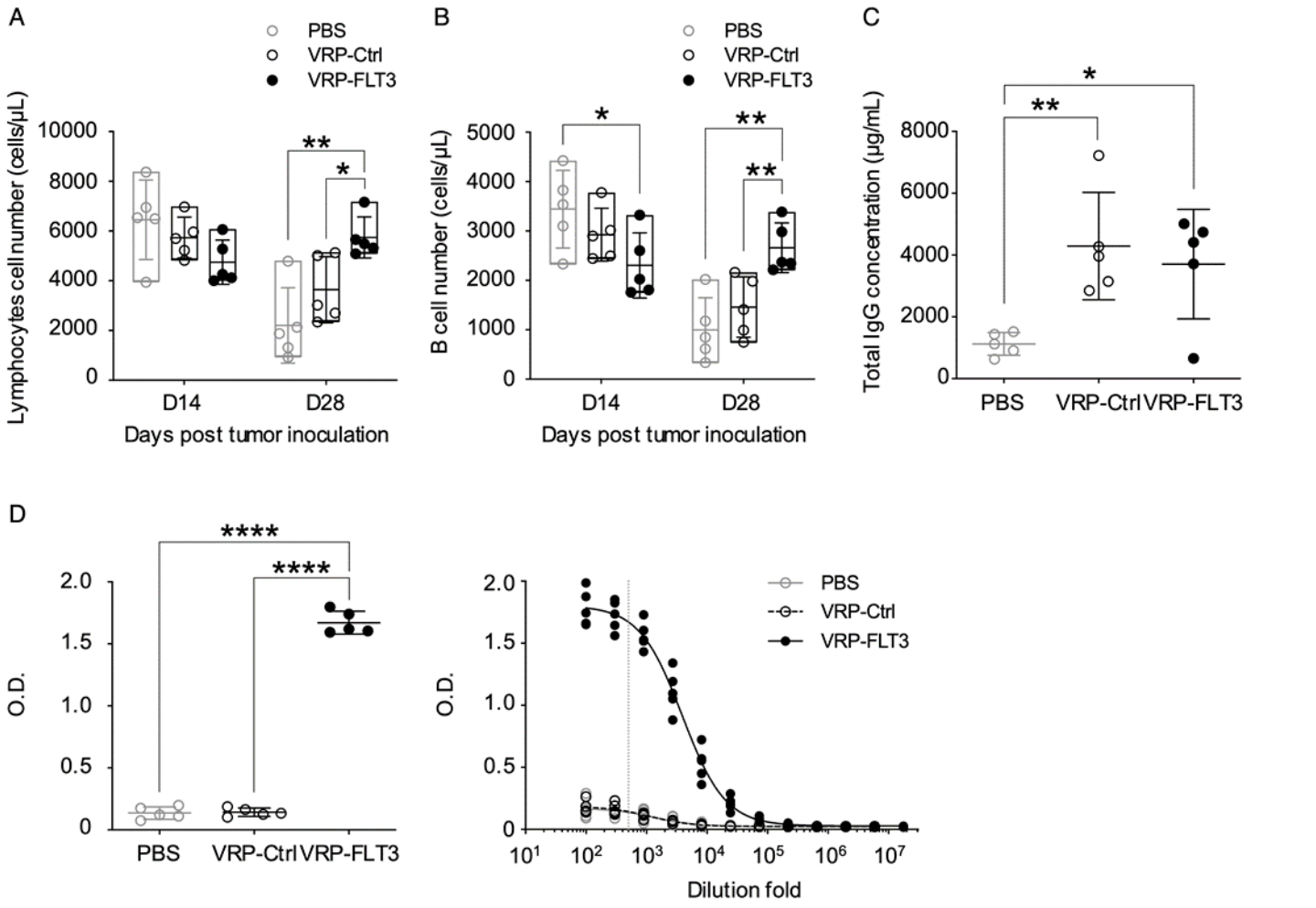

Fig 3. VRP-FLT3 vaccination elicits a FLT3-specific antibody response in a mouse leukemia model. Inoculation of FLT3-expressing C1498 tumor cells and the vaccination schedule are as described in Fig 1. (**A**, **B**) Blood samples harvested on days 14 and 28 were analyzed by flow cytometry for the numbers of (**A**) lymphocytes and (**B**) $CD19^+$ B cells. (**C**) Concentrations of total IgG in plasma on day 28 were determined by ELISA. (**D**) Levels of FLT3-specific plasma IgG on day 28 were determined by ELISA. Titration curves (left) and O.D. value at 1:500 dilution are shown (right). Each symbol represents an individual mouse (n=5 per group). Bars represent the mean ± SD. Statistical significance were determined by unpaired *t* test, *P<0.05, **P<0.01, ****P<0.0001. Data represents one of two independent experiments.

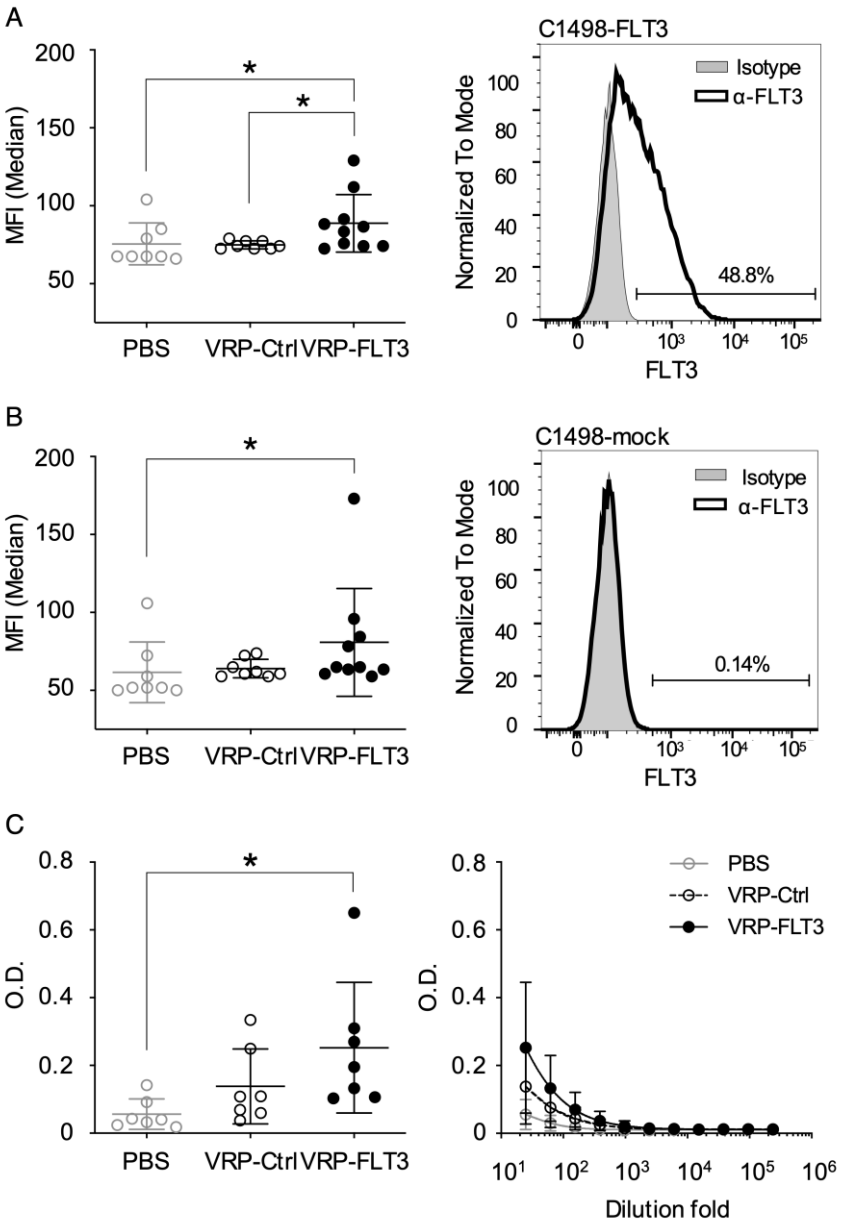

Fig 4. VRP-FLT3 vaccination induces IgG responses to other cell surface antigens on hematolymphoid tumors. Mice with C1498 FLT3 tumor were vaccinated on days 4,18,21 with 2.5 x 10E6 IU VRP-Ctfl or VRP-Flt3 via footpad injection. Control mice received PBS alone. Plasma samples were collected on days 35-42 and tested by flow cytometry or by direct binding ELISA on tumor plasma membrane coated ELISA plates. (A) C1498 Flt3 cells or (B) C1498-mock cells were incubated with diluted plasma containing 1ug total IgG, and the tumor cell bound IgG was detected by flow cytometry with fluorescent secondary antibody to mouse IgG. Representative Flt3 expression level on the target tumor cell is shown in the right panels and the MFI of total IgG binding to the Flt3 expressing or non-expressing cells is shown on the left. Each symbol represents an individual mouse plasma sample (n=8 or 10 per group). (C) IgG from plasma taken on days 35-43 bound to C1498 mock membrane extracts measured by direct binding ELISA. On left, OD values for each individual plasma sample (all at 1:25 dilution) and on the right titration curves. Statistical significance was determined by Mann-Whitney test or unpaired t test, $p<0.05$.

Summary of **Specific Aim 1, Major Task 1.** We have found that after vaccination, antibodies to leukemia cells that over express FLT-3 are produced. Importantly, antibodies from these mice also bind to leukemia cells that do not overexpress FLT3, demonstrating that other antigens are targeted. Our data suggest that after vaccination with a same species self antigen using the VRP vector can 'break B-cell tolerance' and result in anti-self antigen and anti-leukemia antigen targeting by B-cells. The manuscript describing these results is in the final writing stages and was submitted for publication. We can now test the role of nucleic acid-sensing TLRs in anti-tumor B cell responses because we have now produced additional VRP vaccine needed to perform work as proposed in SOW.

Milestone(s) Achieved in Major Task 1: We have demonstrate feasibility and safety of inducing anti-tumor B cell responses in preclinical models.

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
This task is 85% completed. We have produced IgG from cloned B cells from patients after transplantation and we have devised an antigen capture system for screening. We are now cloning B cells from CLL patients for production of full length anti-tumor antibodies.

Subtask 1: Obtain local IRB and DoD HRPO approval for the use of human anatomical substances (1-2)
Grant started September 2018.
This task is 100% complete. The approval for this work was August 12, 2019

Subtask 2: Screen for patient-specific anti-tumor antibodies (2-18)
This subtask is 70% completed. We have developed a work flow for antibody production and screening as detailed below. We have dissected and worked on each piece of the flowchart. With each step now worked out in the laboratory with test patient samples and we are now prepared to screen antibodies produced.

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

We have developed a tumor cell-binding assay that affords maintenance of the 3D antigen integrity on the tumor cells needed for Task 2. Mattia Bonsignori from the Hayne's group is performing high-throughput single cell B cell isolation/cloning and full-length antibody production. During this funding period, we have devised and tested our antibody production strategy using patient samples. While this work was stalled because of the COVID-19 pandemic (please see our request for no-cost extension), we have made significant progress toward completing the milestones in the SOW.

2) Specific objective
Our objective during this funding period was to isolate anti-tumor antibodies from single, activated B cells from patients who demonstrate reduced B-cell tolerance after transplantation and to screen these antibodies for their ability to bind and kill CLL cells. Our strategy is depicted in **Figure 5** below.

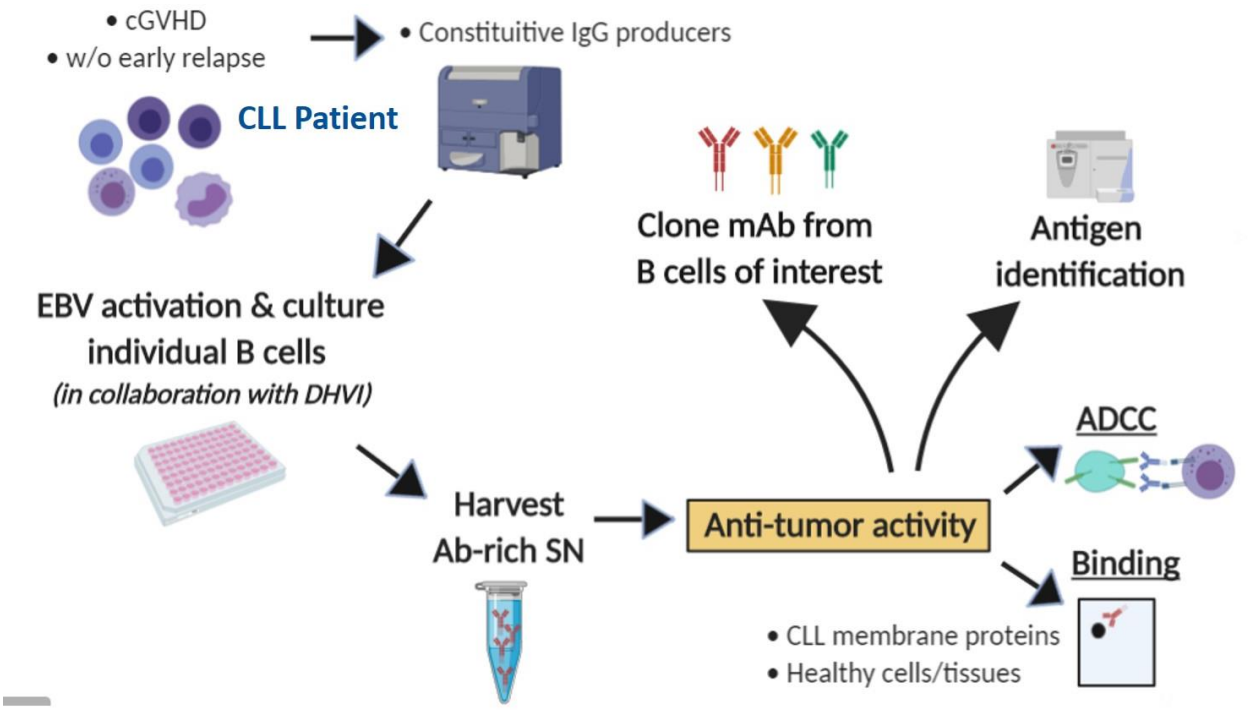

Fig 5. Method for cloning human anti-tumor (chronic lymphocytic leukemia, CLL) antibodies from patients. B cell subsets known to be activated and able to constitutively produce IgG will be single cell cloned and activated either with ebstein-barr virus (EBV) or cytokine cocktails. IgG from the supernatants (SN) will be tested for IgG production and used in functional antibody-dependent cellular cytotoxicity screening assays that identify anti-tumor antibodies. The full length antibodies will then be produced and using these antibodies, tumors can be eradicated and novel antigen targets can be identified.

3) Significant results

During this funding period we have developed a high-throughput method that allows for production of monoclonal antibodies from cancer patients who have achieved a remission related to loss of B-cell tolerance. As shown in **Fig 6**, we are able to clone and perpetuate high IgG-producing B cells using our method.

Cloning Plate 4: Healthy CD19+ CD38 lo IgD lo, Day 27 (previously harvested at Day 23, 4 days of IgG production)

Plate #4	1	2	3	4	5	6	7	8	9	10	11	12	
A	?????	2788.428	?????	1407.515	148035.461	1981.1	936.118	18683.284	170.998	570.57	68252.353	1701.873	7
B	767.93	?????	4422.249	?????	101385.521	695.533	6964.893	96880.296	936.118	170.998	?????	?????	4
C	180227.191	4021.183	69541.821	1304.559	?????	767.93	?????	66274.182	847.864	?????	100	66986.794	5
D	51407.906	3502.097	570.57	19142.059	94830.015	2684.508	742.997	820.335	37497.969	1577.384	39557.774	589.717	8
E	121151.478	?????	?????	?????	742.997	73755.187	136134.578	1209.133	?????	718.873	?????	570.57	4
F	4150.658	589.717	1836.186	?????	1078.921	?????	609.507	?????	?????	?????	?????	1038.711	4
G	?????	81193.225	136697.327	852.18	1697.765	8585.163	2416.179	125.85	?????	125.85	?????	3616.378	6
H	4715.205	500	852.18	169579.137	235126.129	5757.712	17237.769	2077.064	199.324	?????	?????	158.382	6

Cloning Plate 8: cGVHD (MW) CD19+ CD38 lo IgD lo, Day 27 (previously harvested at Day 23, 4 days of production)

Plate #8	1	2	3	4	5	6	7	8	9	10	11	12	
A	?????	40918.932	1387.73	1025.526	397.299	1254.639	10987.768	10732.569	22604.161	1534.94	144015.151	12952.491	10
B	199.324	948.078	125.85	142026.267	618.863	39482.971	15930.849	98981.61	4342.905	250.848	18019.48	199.324	6
C	948.078	?????	?????	124449.551	556.266	158.382	111734.46	54632.409	64473.467	94889.738	38097.403	4715.205	7
D	18560.217	141533.353	688.505	96443.125	4000	397.299	765.983	125.85	22828.041	120615.865	55526.766	102248.882	7
E	?????	463.112	55880.925	184.618	253017.344	9792.956	158.382	13652.584	4440.036	259743.228	1189.207	17922.343	8
F	?????	8035.853	?????	1856.85	31030.75	?????	211953.794	1681.793	53859.824	20438.413	463.112	72730.319	8
G	?????	160918.072	1379.634	12429.771	?????	?????	59813.547	3201.108	397.299	195911.249	340.838	22981.947	7
H	158.382	397.299	?????	59983.282	713435.145	?????	12905.173	116.565	637488.957	?????	154708.406	105124.038	6
												total=	59

Fig 6. High through-put human B cell cloning and IgG production. IgG measurements by sandwich ELISA with supernatants from single cell cloning B cells from healthy donor (top) and from patient whose cancer is in remission who has loss of B cell tolerance (cGVHD). Yellow boxes highlight wells with high IgG production amount. RNA from cells is being stored for future production of full length antibody.

Production of all of these into full-length antibody is not feasible. Thus as proposed in our SOW, we aimed to identify antibodies that bind tumor that are functionally relevant. To this end, we developed a screening strategy that uses an ADCC Reporter Bioassay. This assay uses an engineered Jurkat cells stably expressing the FcγRIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase as effector cells. Antibody biological activity in ADCC mechanism of action (MOA) is quantified through the luciferase produced as a result of NFAT pathway activation; luciferase activity in the effector cell is quantified with luminescence readout. During the funding period, we tested, then established and validated a method for screening patient-specific anti-tumor antibodies.

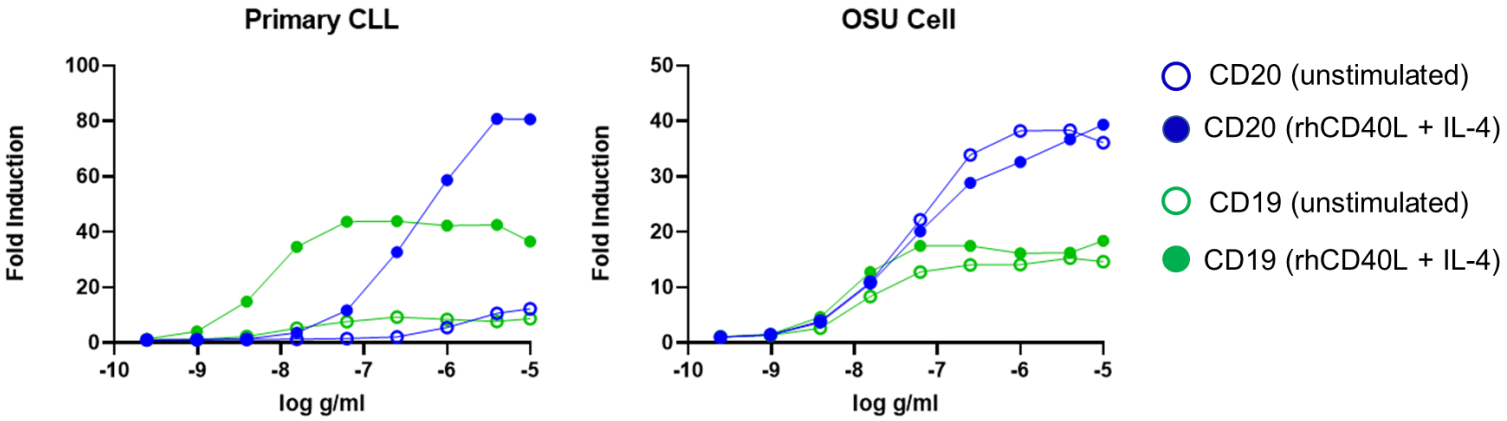

Fig 7. CD40 ligand/IL-4 co-stimulation enhanced the ADCC activity of anti-CD20 (Rituximab) and anti-CD19 (Inebilizumab) antibodies to the primary CLL tumor cells. Primary CLL cells and OSU-CLL line cells were stimulated with 100 ng/mL rhCD40L and 5 ng/mL rhIL-4 at 37°C for 24 hours. Cells were then harvested, washed, and incubated with various concentrations of anti-CD20 or anti-CD19 antibodies and with the effector cells for a 6-hr induction according to the instruction of Promega ADCC Reporter Bioassay. The effector: target cell ratio was optimized to 1:1 according to previous experiments. The ADCC activity was shown as fold of induction using the following formula: RLU (induced) / RLU (no antibody control).

Developing this assay for use on primary tumor cells required an activation step of the primary CLL cells. Our development of this screening assay is a major advancement that will allow us to screen for functional antibodies. We have also developed a flow cytometric based screening assay for detecting the CLL tumor targeting antibodies that will provide the first level screening of low prevelance antibodies that bind to cell surface antigens of interest. We will then expand B-cell clones of interest and purify the IgG prior to use in our ADCC assay.

Milestone Major Task 2: We have now developed a method for B cell cloning and IgG production as well as a functional assay to screen for antibodies that bind to tumor cells.

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.

A primary goal of my academic medicine laboratory is to train promising young physician-scientists. This grant is not intended to train investigators, but the work proposed in this innovative project have afforded opportunities for training. During this funding period, I was fortunately able to recruit Sonali Bracken MD PhD. Dr Bracken is a trainee in the laboratory who has salary coverage by a training grant from an R38 NIH grant (See below). I provide 1:1 mentoring regarding pursuit of experiments on this DoD expansion grant, and I also implement a professional development plan with this trainee per Dr. Bracken's R38 grant. Dr. Bracken was recently admitted to our Duke Rheumatology fellowship program and I will continue to mentor her.

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.

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.

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."

The work has implications in the solid tumor oncology field. It also has implications for understanding loss of B-cell immune tolerance that is relevant in bone marrow transplantation/Graft versus Host disease and in de novo rheumatological disorders (in the Rheumatology field).

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.

As proposed our work pertains to the field (oncology and hematopoietic stem cell transplantation). The work also has potential implications for other fields. If we are able to identify anti-tumor antibodies, we may also be able to identify antibodies important in chronic graft versus host disease. Thus, our work impacts the immunology and rheumatology fields.

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.

Nothing to report

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.

The COVID-19 pandemic impacted our ability to complete work within the time-constraints of the grant as planned. Thus, work is being completed via a no-cost extension.

Update: We are now actively cloning B cells in the lab and testing for IgG production.

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.

  Being submitted, in progress.

  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).*

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.*

R38 executive committee presentation by Dr. Sonali Bracken February 24, 2020 "Harnessing B-cells to Eradicate Tumors"

Update: Dr. Bracken was accepted into Duke's Endocrinology Fellowship program and I will continue to mentor her on this project in the lab.

- **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.

  none

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

  none

- **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.

  none

- **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; n/a*
  - *physical collections; n/a*
  - *audio or video products; n/a*
  - *software; n/a*

- *models; n/a*
- *educational aids or curricula; n/a*
- *instruments or equipment; n/a*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models); n/a*
- *clinical interventions; n/a*
- *new business creation; and n/a*
- *other. n/a*

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:	*Stefanie Sarantopoulos*
Project Role:	*Primary Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4551-6687*
Nearest person month worked:	*5 (calendar months 3.0)- 20% effort*
Contribution to Project:	*Dr. Sarantopoulos is the PI of the grant and is directly supervising all work performed.*

Name:	*Sonali Bracken*
Project Role:	*Post-doctoral fellow, MD/PhD R38 trainee*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-1814-4099*
Nearest person month worked:	*5 (calendar months 6.0) – 50% effort*
Contribution to Project:	*Dr. Bracken has performed work on developing an assay to identify tumor-specific antibodies and she has successfully started sorting and cloning B cells for high-throughput antibody-production and screening* *Dr. Bracken is completing a manuscript that demonstrates the role of TLR7 in chronic GVHD—while not part of the SOW, this work is important related work and the paper is going to be submitted to Blood in September (it is in final editing stage)*
Funding Support:	*R38 NIH physician scientist training grant;*

We received a grant for 25K from the R38 parent to support part of Hsuan Su's effort.

Name:	*Hsuan Su*
Project Role:	*research technician, MS*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*6 (calendar months 6.0) – 50% effort*
Contribution to Project:	*Hsuan Su, MS has performed mouse studies, compiled data, produced comprehensive figures and helped write a manuscript*

	that is currently being submitted to PLoS ONE by the end of August.
Funding Support:	*This CMDRP grant and Sarantopoulos lab funds from Duke*

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.

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.*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A
9. APPENDICES: N/A