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TITLE: Impact of the SLE gene BANK1 on autophagy and plasmablast differentiation in lupus

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Seattle Children's Research Institute, 1900 Ninth Ave, Seattle WA 98101

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14. ABSTRACT
The objective of this study is to elucidate the mechanisms underlying dysregulated plasmablast and plasma cell homeostasis in SLE, resulting in production of autoantibodies that drive disease pathogenesis. Our preliminary data support a mechanistic link between SLE genetic risk variants in the *BANK1* gene with autophagy and plasmablast development. During year 1 of the grant we performed experiments to test the hypothesis that the SLE-associated risk variants in *BANK1* promote autophagy, leading to increased plasmablast differentiation and immunoglobulin secretion. Using gene editing of a B cell lymphoma cell line, we found deletion of *BANK1* resulted in increased autophagy. Expression of the *BANK1* non-risk gene in these cells restored autophagy to parental levels, while expression of the *BANK1* risk gene resulted in increased autophagy relative to the non-risk gene. Finally, we tested the prediction that increasing autophagy in B cells would promote plasmablast development. To do so, we deleted *BANK1* in primary human B cells and quantified the capacity of the edited cells to form plasmablasts/plasma cells in vitro. We detected increased plasmablast and plasma cell numbers in *BANK1* deficient primary B cells compared to *BANK1* sufficient cells, as well as increased immunoglobulin levels. Our results support the hypothesis that the SLE risk variants or deficiency in *BANK1* promotes autophagy and plasmablast development.

15. SUBJECT TERMS
B cells, autophagy, plasmablast, plasma cell, differentiation, SLE genetic variant, BANK1, gene editing

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Systemic erythematosus lupus (SLE) is a chronic autoimmune disease affecting up to 1.5 million Americans, and has an incidence of 158 cases per 100,000 Veterans. Our studies will advance the knowledge of lupus disease mechanism and will provide insight into patient heterogeneity with respect to genetic variation, advancing the goal of personalized medicine for people with SLE. The overarching challenge to be addressed is the significant gap in knowledge about the mechanisms underlying the dysregulation of plasmablast and plasma cell homeostasis in SLE that result in production of autoantibodies driving disease pathogenesis. Recent findings support a role for autophagy in the dysregulation of plasma cell homeostasis in SLE. Furthermore, our preliminary data support a mechanistic link between autophagy, plasmablast development, and the *BANK1* SLE genetic risk variants. Based on these published and preliminary studies, we propose to test the novel hypothesis that the SLE-associated risk variants in *BANK1* promote autophagy, leading to increased plasmablast differentiation and/or immunoglobulin secretion that contributes to lupus susceptibility and disease. We predict that autophagy-targeting agents will limit plasma cell differentiation in B cells and may benefit patients with SLE, especially those with the *BANK1* risk variants. Our approach will utilize primary B cells from well-characterized healthy control subjects and SLE patients carrying the *BANK1* risk and non-risk variants, and cutting-edge gene editing of primary B cells and cell lines. In Specific Aim 1, we will determine if the BANK1 risk protein increases autophagy and leads to increased plasmablast differentiation and immunoglobulin secretion. We will address this *in vitro* using gene editing to express the BANK1 risk or non-risk proteins in an isogenic background in primary human B cells and cell lines, and by comparing *BANK1* risk and non-risk primary B cells from healthy control and SLE subjects. These findings will be correlated with levels of circulating plasmablasts and autoantibodies in SLE subjects at the same time point to determine whether in vitro plasma cell differentiation corresponds to *in vivo* development. Finally, we will investigate the mechanism linking BANK1 to autophagy by carefully characterizing the role of its binding partners, ATG3 and TBC1D2B in *in vitro* plasma cell differentiation. In Specific Aim 2, we will determine if targeting autophagy with small molecule inhibitors limits plasmablast differentiation, survival, or immunoglobulin secretion. We will test the autophagy inhibitors using *BANK1* risk and non-risk B cells from genotyped healthy control and SLE subjects. These outcomes will improve the diagnosis and care of active military personnel and Veterans with lupus, and will offer insight into individuals at risk for developing SLE and possible interventions to prevent development of disease.

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

B cells, SLE genetic variant, BANK1, autophagy, plasmablast, plasma cell, differentiation, gene editing

3. **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(specified in proposal) We will determine if the BANK1 risk protein increases autophagy and leads to increased plasmablast differentiation and Ig secretion.	**Timeline**	**Site 1 Benaroya Research Institute**	**Site 2 Seattle Children's Research Institute**	**Percent completed**
Major Task 1 Obtain HRPO/ACURO Approval.	Months			
Subtask 1: Obtain local IRB approval.	3/2019	Dr. Cerosaletti	Dr. James	100%
Subtask 2: Submit IRB approval and necessary documents for HRPO review.	3/2019	Dr. Cerosaletti	Dr. James	100%
Milestone Achieved: HRPO/ACURO Approval.	3/2019			100%
Major Task 2 Determine the effect of *BANK1* risk and non-risk variants on autophagy and plasma cell differentiation in primary human B cells.				
Subtask 1: Make AAV repair templates for each full-length BANK1 variant for delivery to the *CCR5* locus.	3/2019-9/2019		Dr. James	100%
Subtask 2: Test dual editing strategy to disrupt *BANK1* while also delivering over-expressed *BANK1* variants to the CCR5 locus in primary human B cells. Measure indel and homology directed recombination frequencies. B cells will be isolated from cryopreserved PBMC samples of healthy control subjects.	9/2019-3/2020		Dr. James # of healthy human subjects=10; all samples deidentified	100%
Subtask 3: Determine the effect of *BANK1* variants on macro- and TLR-induced autophagy, and plasma cell differentiation in an isogenic background using gene editing to introduce full length risk or non-risk isoform cDNA using homology directed recombination. Experiments will be performed using primary human B cells isolated from cryopreserved PBMC samples of healthy control subjects.	3/2020-3/2021		Dr. James # of healthy human subjects=10; all samples deidentified	30%
Subtask 4: Determine the effect of *BANK1* variants on macro- and TLR-induced autophagy, and plasma cell differentiation in primary human B cells from PBMC of genotyped healthy control subjects and individuals with SLE. All PBMC are pre-existing cryopreserved samples.	9/2019-12/2020	Dr. Cerosaletti Total # of healthy human subjects=60; # SLE subjects=60; all samples de-identified		20%
Subtask 5: Determine circulating plasmablast in PBMC samples and autoantibody levels (ANA) in serum from genotyped SLE patients and correlate with in vitro plasma cell differentiation. All PBMC and sera are pre-existing cryopreserved samples.	3/2020-3/2021	Dr. Cerosaletti Total# of human SLE subjects=60 (same SLE subjects in subtask 4); all samples deidentified		0%
Milestone(s) Achieved: Impact of the *BANK1* risk locus on macro- and TLR-induced autophagy and plasma cell development in health and in lupus.	3/2021			30%

Major Task 3 Loss- and gain-of-function of BANK1 interacting proteins.				
Subtask 1 Make AAV repair templates for delivery of ATG3 and TBC1D2B to the CCR5 locus. Test ATG3 and TBC1D2B guide RNAs in cell lines.	3/2019-9/2019		Dr. James	30%
Subtask 2: Use gene editing and homology-directed repair in primary human B cells to assess loss- and gain-of-function of TBC1D2B on macro- and TLR-induced autophagy, and plasma cell differentiation. B cells will be isolated from cryopreserved PBMC of healthy control subjects.	9/2019-3/2021		Dr. James # of healthy human subjects=10; all samples deidentified	0%
Subtask 3 Use gene editing and homology-directed repair in primary human B cells to assess loss- and gain-of-function of ATG3 on macro- and TLR-induced autophagy and plasma cell differentiation. B cells will be isolated from cryopreserved PBMC of healthy control subjects.	3/2020-3/2021		Dr. James # of healthy human subjects=10; all samples deidentified	0%
Milestone(s) Achieved: Role of BANK1 interacting proteins on autophagy and plasma cell differentiation.	3/2021			10%
Specific Aim 2 (specified in proposal) We will investigate if small molecules that inhibit autophagy limit plasmablast differentiation, survival, or Ig secretion.	**Timeline**	**Site 1 Benaroya Research Institute**	**Site 2 Seattle Children's Research Institute**	
Major Task 1 Determine autophagy inhibitor concentration in lymphoma cell lines.	Months			
Subtask 1: Dose-response experiments in the HBL1 and TMD8 lymphoma cell lines to identify inhibitor concentrations that results in 90% inhibition of autophagy.	3/2019-7/2019		Dr. James	33%
Milestone(s) Achieved: Inhibitor concentrations.	7/2019			33%
Major Task 2 Assess effect of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from healthy control subjects.	Months			
Subtask 1: Test autophagy inhibitors alone for their impact on macroautophagy and plasma cell differentiation in B cells from healthy control subjects. B cells will be isolated from pre-existing cryopreserved PBMC samples.	7/2019-9/2020		Dr. James # of healthy human subjects=10; all samples deidentified	10%
Subtask 2: Test autophagy inhibitors in presence of TLR agonists for their impact on TLR induced autophagy and plasma cell differentiation in B cells from healthy control subjects. B cells will be isolated from pre-existing cryopreserved PBMC samples.	7/2019-9/2020		Dr. James # of healthy human subjects=10; all samples deidentified	0%

Milestone(s) Achieved: Impact of autophagy inhibitors on plasma cell differentiation in primary human B cells.	9/2020			5%
Major Task 3 Assess effect of autophagy inhibitors in B cells from healthy control subjects and SLE subjects carrying the *BANK1$^{R/R}$*, *BANK1$^{R/NR}$* and *BANK1$^{NR/NR}$* genotypes.	Months			
Subtask 1: Test impact of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from healthy control subjects carrying *BANK1* genotypes. B cells will be isolated from pre-existing cryopreserved PBMC samples.	3/2020-3/2021	Dr. Cerosaletti Total # of healthy human subjects=60 (overlapping with major task 2); all samples deidentified		0%
Subtask 2: Test impact of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from SLE subjects carrying *BANK1* genotypes. B cells will be isolated from pre-existing cryopreserved PBMC samples	3/2020-3/2021	Dr. Cerosaletti Total # of human SLE subjects=60 (overlapping with major task 2)		0%
Milestone(s) Achieved: Impact of autophagy inhibitors on plasma cell differentiation in healthy subjects and SLE patients in the context of the BANK1 risk variants.	3/2021			0%

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.

The focus of Year 1 activities was on objectives in Aim 1. Specific outcomes are as follows.

1. **Aim 1: Obtain HRPO/ACURO Approval.** Local IRB approval was achieved; it was determined that the study was exempt because all human samples were collected and banked prior to the initiation of the study and will be provided to the researchers completely de-identified. Local IRB approval was submitted for HRPO review; HRPO approval took longer than expected- a total of 6 months after the grant was awarded.
2. **Aim 1: Determine the effect of *BANK1* risk and non-risk variants on autophagy and plasma cell differentiation in primary human B cells.** Repair templates and AAV vectors have been made for delivery of full-length risk and non-risk *BANK1* cDNAs into the *BANK1* locus. We decided to slightly alter our strategy by introducing the risk and non-risk versions of *BANK1* directly into the *BANK1* locus. This change enabled us to deliver the variants under control of the endogenous promoter. Using the altered strategy, we have successfully used gene editing to introduce risk and non-risk *BANK1* cDNAs coupled to cis-linked expression of mCherry into human B cells. These templates can be expressed and quantified as shown in **Figure 1.** Our initial results (**Figure 2**) show that deletion of *BANK1* resulted in increased autophagy, and expression of the *BANK1* non-risk cDNA in these cells restored autophagy to parental levels as shown by increased levels of p62 by western blot analysis (p62 is consumed during autophagy). In contrast, expression of the *BANK1* risk cDNA resulted in increased autophagy (lower p62 levels) relative to the non-risk gene. To link increased autophagy to increased plasmablast development, we deleted *BANK1* in primary human B cells and differentiated the edited cells into plasmablasts/plasma cells in vitro. We detected increased plasmablast and plasma cells numbers in *BANK1* deficient primary B cells compared to

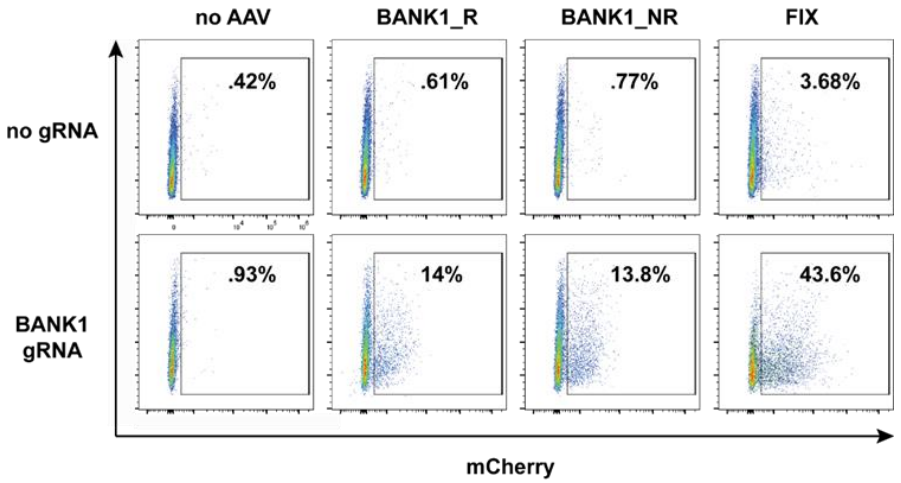


Figure 1: Introduction of BANK1 risk and non-risk sequence into B cells. We designed guide RNAs targeting exon 2 of BANK1 and corresponding repair templates to introduce full-length risk (R) or non-risk (NR) BANK1 coupled to cis-linked expression of mCherry. In the presence of the guide RNAs and either repair template, we show HDR-mediated integration of cherry into HBL1 lymphoma cells (vs. no template, left). Following cell sorting, we generated polyclonal cell lines expressing the variants and validated HDR using sequencing and digital droplet PCR (data not shown).

BANK1 sufficient cells using flow cytometry, as well as increased IgG to IgM ratio as quantified by ELISA (**Figure 3**). These results support the hypothesis that the SLE risk variants or deficiency in *BANK1* promotes autophagy and plasmablast development.

Experiments are currently underway to test autophagy and plasmablast/plasma cell differentiation in primary B cells from healthy controls and SLE subjects carrying the *BANK1* risk or non-risk variants. We have optimized in vitro differentiation culture conditions using primary human B cells to enhance plasmablast and plasma cell differentiation, and to optimize our readouts. Results from the optimal conditions show similar differentiation plasma cells in the presence or absence of TLR stimulation, but greater class switching to IgG production in the absence of TLR stimulation (**Figure 4)**. We have also validated the flow cytometric detection of autophagy as measured by LC3-B and p62. Banked PBMC samples from genotyped healthy controls and SLE subjects have been obtained for the experiments to assess the effect of the *BANK1* risk and non-risk variants on autophagy and plasmablast development **(Table I).** Note that we decided to hold genotype constant for another SLE risk gene *BLK*, based on a recent report indicating that *BLK* single nucleotide variants impair phosphorylation of BANK1 following B cell stimulation, which might contribute variability to our experiments (Jiang et al. 2019) These experiments will take place in the upcoming budget period. Progress on these cross sectional studies was dalayed by the extended period of time required for HRPO approval.

Table I. BANK1 risk and non-risk subjects for Aim 1.

BANK1 genotype	# healthy controls	# SLE patients
Homozygous risk	23	13
Heterozygous	23	12
Homozygous non-risk	12	0

3. **Aim 1: Loss- and gain-of-function of BANK1 interacting proteins.** AAV repair templates have been designed for ATG3 and TBC1D2B, but the vectors haven't been cloned. Guide RNAs for each gene have been designed and ordered. Completion of this subtask will happen in the upcoming budget period as

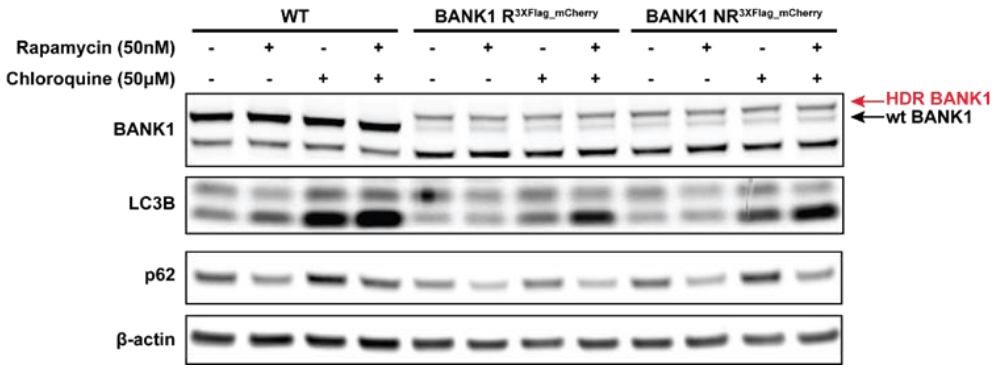
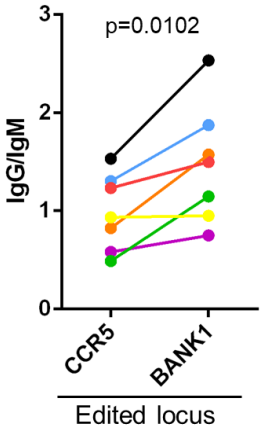


Figure 2: Effect of *BANK1* SNPs on B cell macroautophagy. HBL1 lymphoma cells stably expressing either the full-length BANK1 risk or non-risk proteins were cultured with either the autophagy activator Rapamycin or with Chloroquine. Following treatment, we lysed the cells and processed for western blot using the indicated antibodies. Note that the introduced BANK1 variants are slightly larger than the wild type form due to the inclusion of a 3X flag tag. Similar to what we previously observed in *BANK1* knockout lymphoma cells, p62 levels are decreased in BANK1 risk expressing cells relative to that observed in non-risk-expressing cells, indicating increased autophagy.

scheduled.

4. **Aim 2: Determine autophagy inhibitor concentration in lymphoma cell lines.** We have tested the dose responsiveness of Lys05 in cell lines. It effectively blocks autophagy at 10uM, a 10-fold lower dose than required for chloroquine to obtain the same level of inhibition. We expect to complete dose response testing of the autophagy inhibitors SBI-0206965 and sar405 in the next three months.
5. **Aim 2: Assess effect of autophagy inhibitors on macro- and TLR-induced autophagy and plasma cell differentiation in B cells from healthy control subjects.** We have begun to test the effect of Lys05 on plasma cell differentiation. Lys05 potently inhibited differentiation in one donor in a dose specific manner. In the upcoming budget period we will test the effect of each of the inhibitors on autophagy and plasma cell differentiation in additional healthy donors and in cohorts of *BANK1* genotyped healthy controls and SLE subjects.

Figure 3. Increased class switched plasma cell development in *BANK1* deficient B cells. Primary human B cells were edited to delete the *BANK1* locus or the *CCR5* locus as a control. Edited cells were then differentiated into plasma cells in vitro. After 14 days, IgG and IgM levels in the culture supernatant were quantified by ELISA and expressed as the ratio of IgG to IgM levels.



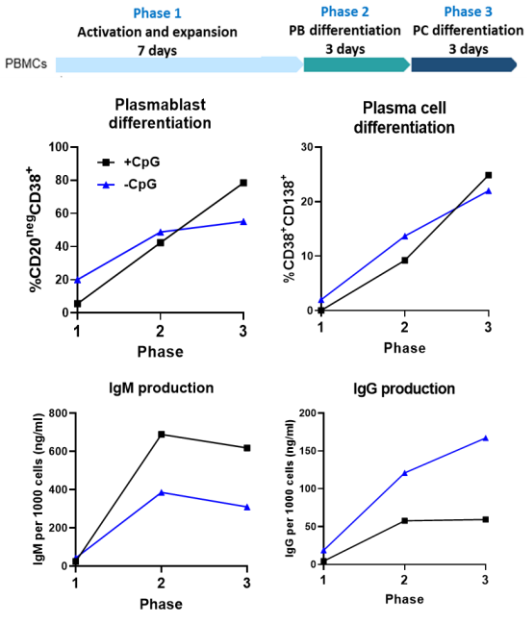


Figure 4. Plasmablast and plasma cell differentiation from naïve primary human B cells. Naïve B cells were isolated from a healthy control subject and stimulated with CD40 ligand, IL-2, IL-10, IL-15 and IL-21 +/- CpG for 7 days (phase 1), followed by differentiation for 6 days in phases 2 and 3 in the presence of IL-6, IFN alpha, and BAFF. Plasmablasts and plasma cells were quantified by flow cytometry (top panels), and IgM and IgG secretion into the culture supernatant was detected by ELISA (bottom panels).

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.

While this study was not intended to provide training, it has nonetheless led to the development and refinement of new technologies and skill sets in the Cerosaletti and James labs. Janice Chen in the Cerosaletti lab has refined the plasmablast/plasma cell differentiation assay for use with primary human B cells, and optimized the flow cytometric readouts of differentiation and autophagy. In the James lab, Emma Suchland performed the autophagy and differentiation assays in cell lines and gene edited primary B cells. Iana Meitlis assisted with cloning and guide RNA design. Professionally, we have established a bimonthly lab meeting between the Cerosaletti and James labs to share results, which provides an opportunity for lab members to present their results in front of peers and receive feedback. We have also established a Slack channel to share lab results in real-time.

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.

The Benaroya Research Institute "BRIng It On" Newsletter for fall 2019 highlighted our grant (https://www.benaroyaresearch.org/news/newsletter-archive). A military veteran, Toni Grimes, shared her story of developing lupus while an active duty troop causing her to retire, followed by a lay description of the study an testing of potential new therapies for lupus. The newsletter was distributed to 9,500 readers in the local and regional community.

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.

Goals that will addressed in the next budget period include the following. For Aim 1 we will determine the effect of the *BANK1* risk and non-risk variants on autophagy and plasmablast/plasma cell differentiation in an isogenic background using gene edited primary human B cells (major task 1, subtask 3), and the cross-sectional cohorts of genotyped healthy controls and SLE patients (major task 1, subtask 4). We will also quantify the autoantibody levels and circulating plasmablast levels in SLE patients genotyped for *BANK1* (major task 1, subtask 5). The impact of the BANK1 interacting proteins ATG3 and TBC1D2B on autophagy and plasma cell differentiation will also be analyzed in primary human B cells using gene editing (major task 3, subtasks 2 and 3). For Aim 2, dose response testing of the autophagy inhibitors will be completed (major task 1) and then inhibitors will be tested for their impact on macroautophagy and plasma cell differentiation in B cells from healthy donors, +/- TLR stimulation (major task 2, subtasks 1 and 2). These studies will be extended to the cohorts of healthy control subjects and SLE patients carrying the *BANK1* risk or non-risk genotypes (major task 3, subtasks 1 and 2).

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).

The results from year 1 of this study have an impact on our understanding of the function of the *BANK1* gene in the autophagy pathway in B cells; genetic variants in *BANK1* that are linked to risk for SLE appear to promote this pathway. We also show that the Lys05 inhibitor is 10-fold more potent than chloroquine in inhibiting autophagy which has potential implications for treatment of SLE. The gene editing tools used in this study impact study of primary human B cells in general because they allow investigators to change a single gene within the B cells from one individual and then test the impact of that specific change while leaving the remainder of the genome the same.

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.

Our findings with BANK1 have the potential to advance our understanding of other rheumatic diseases that have been associated with genetic variants in the *BANK1* gene, including rheumatoid arthritis and scleroderma.

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.

We made a change in the selection criteria for healthy control subjects and SLE patients based on a publication showing that single nucleotide variants in the *BLK* gene impair phosphorylation of BANK1 following B cell stimulation, which might contribute variability to our experiments (Jiang et al. 2019). Thus, we made the decision to hold genotype constant for the SLE risk variant in *BLK* rs13277113 while selecting subjects based on risk and non-risk genotype at *BANK1.*

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.

The approval by HRPO to use human samples for this project took over 6 months to accomplish. As a result, we were unable to begin experiments until March 2019 and our milestones for Aim 1 major task 2, subtasks 3 and 4 are somewhat delayed.

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.

Because of the delay in HRPO human subjects approval, we were unable to begin experiments until March 2019 and our expenditures are slightly behind schedule (projected $262,500; actual $185,343).

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

Not applicable.

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.

  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.

We established a dual editing strategy to knock out the *BANK1* locus in a B cell lymphoma line and then deliver the BANK1 risk or non-risk cDNA into the *BANK1* locus using homology directed repair. This technology has been shared within our institutions.

- **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.*

We have generated several B cell lymphoma cell lines by gene editing. The HBL1 lymphoma line was edited to delete the *CCR5* locus (control) or the *BANK1* locus. We also generated HBL1 cells with dual editing to delete the *BANK1* locus and simultaneously knock-in the BANK1 risk or non-risk cDNAs into the *BANK1* locus by homology directed repair. DNA reagents generated for these experiments include *BANK1* guide RNAs, and the AAV repair templates for the *BANK1* risk or non-risk cDNA sequences.

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

1. Principal investigators:

Name: Karen Cerosaletti PhD (Benaroya Research Institute)
Project Role: Principal investigator
Researcher Identifier (ORCID ID): orcid.org/0000-0002-7403-6239
Nearest person month worked: 1
Contribution to project: Dr. Cerosaletti, in collaboration with Dr. James, has helped oversee the overall scientific direction and reporting of results for the project. She directed and analyzed experiments to optimize plasma cell differentiation and autophagy readouts in primary human B cells for Aim 1; selected the healthy control and SLE samples for Aims 1 and 2; and met with Dr. James bi-monthly to share research results and progress.
Funding support: this award

Name: Rich James PhD (Seattle Children's Research Institute)
Project Role: Co-investigator
Researcher Identifier (ORCID ID): RICKERJAMES
Nearest person month worked: 1
Contribution to project: Dr. James, in coordination with Dr. Cerosaletti, has helped oversee the overall scientific direction and reporting of results for the project. Dr. James directed the gene editing studies described in Aim 1A and 1C, including establishing plasmablast differentiation culture conditions for edited B cells, and has begun assessing dosing for drug evaluation experiments in Aim 2.
Funding support: this award

2. Other personnel:

Name: Janice Chen MS (Benaroya Research Institute)
Project Role: Research technician III
Researcher Identifier (ORCID ID): N/A
Nearest person month worked: 3
Contribution to project: Ms. Chen performed and analyzed experiments to optimize plasma cell differentiation and autophagy readouts in primary human B cells. She has met with the James bi-monthly to share experimental results and strategies.
Funding support: this award

Name: Emmaline Suchland (Seattle Children's Research Institute)
Project Role: Research scientist
Researcher Identifier (ORCID ID): N/A
Nearest person month worked: 4
Contribution to project: Ms. Suchland has executed the primary cell and cell line gene editing experiments described in Aim 1A and 1C.
Funding support: this award

Name: Iana Meitlis (Seattle Children's Research Institute)
Project Role: Research scientist
Researcher Identifier (ORCID ID): N/A
Nearest person month worked: 3
Contribution to project: Ms. Meitlis helped order reagents, clone constructs, test guide RNAs and produce AAV for the gene editing experiments described in Aim 1A and 1C.
Funding support: 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?
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.

PI: Karen Cerosaletti PhD
1. New awards:
Title: ITN EP292 TSLP-related SNP Genotyping ITN057AD / CATNIP
Percent effort: 0%
Supporting agency: NIH/NIAID 5 UM1 AI109565-06 (Nepom)
Grants officer: N/A
Performance period: 02/01/2019 – 01/31/2020
Funding amount $4,895 (total cost)
Project goals: We will address the hypothesis that genotype at TSLP impacts clinical response in the CATNIP trial. Subjects in the treatment arm of this trial received anti-TSLP neutralizing antibody as part of the allergen desensitization protocol. Participants will be genotyped for 6 SNPs in the TSLP gene that are associated with allergy, asthma, or atopic dermatitis.
Overlap: None

Title: ITN EP322: HLA typing and IL6R SNP Genotyping
Percent effort: 0.8%
Supporting agency: NIH/NIAID 5 UM1 AI109565-06 (Nepom)
Grants officer: N/A
Performance period: 06/01/2019 – 01/31/2020
Funding amount $14,300 (total cost)
Project goals: We will isolate DNA from whole blood of all EXTEND participants and contract with Thermo Fisher to run the Axiom Precision Medicine Array (Affymetrix), clean and analyze the data and impute the HLA typing data. We will then extract genotypes and HLA typing and return to ITN.
Overlap: None

Title: Single cell sequencing for islet-reactive T cell clonotype and transcript signatures
Percent effort: 5%
Supporting agency: Juvenile Diabetes Research Foundation 3-SRA-2019-793-S-B (Cerosaletti)
Grants officer: Simi Ahmed
Performance period: 06/01/2019 – 05/31/2022
Funding amount $142,466 (total cost all years)
Project goals: We will investigate the TCR expansion and transcriptional phenotype of islet specific CD8 T cells in peripheral blood and determine if a signature of islet-specific TCR sequences can be tracked in peripheral blood as a proof of concept for follow-on studies.
Overlap: None

Title: Single Cell RNA-seq analysis of islet antigen reactive CD4+ T cells during T1D progression
Percent effort: 10%
Supporting agency: American Diabetes Association 1-19-ICTS-006 (Cerosaletti)
Grants officer: Magda Galindo
Performance period: 01/01/2019 – 12/31/2022
Funding amount $545,442 (total cost all years)
Project goals: This award will use single cell molecular analysis to 1) determine if this unique subset of islet T cells appears in the very early stages of development of T1D; and 2) correlate these unique islet T cells with destruction of islets and loss of insulin production over time in newly diagnosed T1D patients.
Overlap: None

2. Completed awards:
Title: Defining the role of altered cytokine signaling pathways on autoimmunity
Percent effort: 8%
Supporting agency: NIH/NIAID, 4 U01 AI101990-05 (Buckner)
Grants officer: Thomas Esch
Performance period: 07/01/2012 – 06/30/2018
Funding amount $1,976,968 (annual directs)
Project goals: In this grant we pose the hypothesis that in autoimmune individuals, enhanced phosphorylation of STAT3 and diminished phosphorylation of STAT5 establish a functional program biasing immune responses towards a skewed, pro-autoimmune profile.
Overlap: None

Title: An integrated strategy to define the functional and synergistic impact of T1D causal variants
Percent effort: 10%
Supporting agency: NIH/NIDDK, DP3 DK111802 (Rawlings)
Grants officer: Beena Alkolar
Performance period: 12/01/2016 – 06/30/2018
Funding amount $360,000 (annual directs)
Project goals: In this grant, we propose to investigate how genes associated with T1D function individually and in combination to impair immune programs that contribute to T1D. Specifically we will study the T1D risk variants in PTPN22, SH2B3, TYK2 individually and in combination on the adaptive and innate immune responses
Overlap: None

Title: Single Cell Transcriptome Analysis of Islet Antigen Reactive Memory CD4+ T Cells in Established TlD
Percent effort: 5.16%
Supporting agency: NIH/NIDDK, 1DP3DK110867-01 (Linsley)
Grants officer: Lisa Spain
Performance period: 07/01/2016 – 07/31/2018
Funding amount $297,376 (annual directs)
Project goals: We are using cutting edge now cytometry and systems biology approaches to identify single cell transcriptome signatures in islet antigen reactive memory CD4+ (IARM-CD4) T cells from subjects with established TlD and healthy controls. We anticipate discovering unique, data driven insights into immunological

aspects of T1D progression. This study will also provide information regarding the feasibility,cost, effect size and variability needed to design appropriate follow on studies to rigorously evaluate
IARM-CD4 T cells as new biomarkers and therapeutic targets
Overlap: None

Title: ITN EP202: CD154 Assay in T1DAL Responders and Non-responders
Percent effort: 5%
Supporting agency: JDRF, 1-PAR-2017-427-S-N (Nepom)
Grants officer: N/A
Performance period: 07/15/2017 – 07/14/2018
Funding amount $158,477 (annual directs)
Project goals: This proposal will use single cell RNA sequencing to profile islet autoreactive CD4 T cells in peripheral blood from T1D subjects before and after immunotherapy to determine if the frequency or phenotype of these cells change in relation to response to therapy. The results will expand the understanding of the mechanism of response to therapy and determine if islet T cell signatures can be used as a biomarker for response to therapy.
Overlap: None

Title: Mechanisms of IL-6 mediated T cell pathogenesis in autoimmunity
Percent effort: 5%
Supporting agency: NIH/NIDDK, 1 DP3 DK104466-01 (Buckner)
Grants officer: Todd Le
Performance period: 09/14/2014 – 08/31/2018
Funding amount $171,701 (annual directs)
Project goals: In this grant, we propose to use samples from individuals prior to the development of diabetes in order to dissect the role of CD4 T cell response to the cytokines IL-6 and IL-21 in the development of disease. We then plan to integrate our findings with our other studies of these samples to understand their role in altered tolerance and to establish a timeline of immune alteration in the context of beta cell destruction and clinical disease.
Overlap: None

Title: Immune effector and regulatory balance as a predictor for preserved beta cell function in subjects with established T1D
Percent effort: 10%
Supporting agency: JDRF, 3-SRA-2014-315-M-R (James)
Grants officer: Simi Ahmed
Performance period: 09/01/2014 – 12/31/2018
Funding amount $417,332 (annual directs)
Project goals: The objective of this project is to gain an understanding of the mechanisms that lead to loss of residual insulin production by examining various factors, including ratios between regulatory cells and cytotoxic cells, the breadth numbers of self-proteins, the detailed phenotype of immune cells, the degree of immune focusing of the cytotoxic response, and the number of genetic risk alleles present in subjects with rapid vs. slow C-peptide decline.
Overlap: None

Title: ITN EP246: CD154 Assay Testing in UST1D Samples
Percent effort: 5%
Supporting agency: NIH/NIAID, 5UM1AI109565-04 (Nepom)
Grants officer: Leighton Thomas
Performance period: 02/01/2018 – 01/31/2019
Funding amount $17,784 (annual directs)
Project goals: This proposal will use the CD154 activation assay to quantitate and phenotype islet autoreactive CD4 T cells in peripheral blood from T1D subjects before and after immunotherapy with ustekinumab (anti-p40, UST1D trial) to determine if the frequency or phenotype of these cells change in relation to response to therapy. The results will expand the understanding of the mechanism of response to therapy with ustekinumab

and determine if the CD154 assay of islet CD4 T cells is a useful bioassay for response to treatment in the follow-on clinical trial UST1D2.
Overlap: None

Co-investigator Richard James PhD

1. New awards:
Title: Role of Dock8 in Mucosal Immunity
Percent effort: 11.52%
Supporting agency: NIH/NIAID 1R01AI140626-02 (James/Piliponsky- PI change from Oukka)
Grants officer: N/A
Performance period: 07/01/2018 – 06/30/2023
Funding amount $1,687,816 (total cost all years)
Project goals: Th17 cells have emerged as an important subset of T helper cells involved in protective immunity against various pathogens, however, the mechanisms by which Th17 cells fulfill their functions remain elusive. Our study is aimed at understanding the molecular mechanisms by which Dock8 regulates the differentiation of Th17 cells during the immune response directed against bacterial infection.
Overlap: None

Title: New approaches for the identification of critical signals for mast cell function
Percent effort: 5%
Supporting agency: NIH/NIAID 1R21AI144231-01 (Piliponsky)
Grants officer: N/A
Performance period: 02/01/2019 – 01/31/2021
Funding amount $517,825 (total cost all years)
Project goals: The proposed studies are focused on the characterization of mast cell surface proteomes. This information will lay the groundwork for future projects aimed at understanding how the genetic manipulation of novel surface proteins will impact the response of mast cells to infection and chronic inflammatory disorders in which these cells play an active role.
Overlap: None

Title: Engineering B-Cells to Produce Proteins
Percent effort: 19.22%
Supporting agency: Seattle Children's Research Institute Internal Research Project
Grants officer: N/A
Performance period: 03/01/2019 – 02/28/2021
Funding amount $1,700,000 (total cost all years)
Project goals: We propose to develop a therapy to produce protein drugs using antibody-secreting B cells, or drug-secreting B cells. The investment focus on improving manufacturing methods for drug-secreting B cells. We will do experiments to improve: 1) the in vitro expansion and differentiation of drug-secreting B cells, 2) the per-cell protein production of drug-secreting B cells, 3) engraftment of drug-secreting B cells and 4) the development of lead protein drugs for pre-clinical evaluation. Collectively, we envision that these studies will provide proof-of-concept data necessary for an investigational new drug designation for drug-secreting B cells for delivery of recombinant proteins that may eventually lead to a Phase I/II clinical trial.
Overlap: None

Title: Engraftability of Engineered B-Cells to produce Mabs
Percent effort: 18.72%
Supporting agency: Bill and Melinda Gates Foundation (James, no number assigned)
Grants officer: N/A
Performance period: 07/01/2019 – 06/30/2021
Funding amount $1,928,397 (total cost all years)
Project goals The Goal of the B-cell Program is to develop and test a platform for engineered B-cells that produce durable levels of defined antibodies to protect against infection. Projects directed by the James lab will be focused on determining the conditions by which ex vivo differentiated murine and/or non-human primate B-cells will effectively engraft into immune-competent recipients. Additional aims will be to query whether

modifying MHC class I and/or phagocytic regulators can confer the ability of plasma cells to stably engraft in allogeneic settings. The overall goal of the B cell platform is to generate technical proof-of-concept of this overall approach in animal models and defining a path to clinical evaluation in the next 2-years.
Overlap: None

3. Completed awards:
Title: Effector Function of Regulatory T Cells in EAE
Percent effort: 5%
Supporting agency: NIH/NIAID 1R21AI136572-01 (Oukka)
Grants officer: N/A
Performance period: 06/01/2018 – 04/17/2019
Funding amount $206,411 (total cost all years)
Project goals Regulatory T cells play an important role in suppressing Autoimmunity. However, the mechanisms by which Regulatory T cells migrate to the tissues remain elusive. Our study is aimed at understanding the molecular mechanisms by which Regulatory T cells migrate to the Central nervous system and how they regulate autoimmunity such as Multiple Sclerosis
Overlap: None

Title: Development of a Cell Therapy that uses B Cells to Produce Exogenous Therapeutic Proteins
Percent effort: 57.6%
Supporting agency: Sponsored Research Project, Vir Biotechnology (Rawlings/James)
Grants officer: N/A
Performance period: 10/01/2018 – 04/30/2019
Funding amount $$850,000 (total cost all years)
Project goals Development of a Cell Therapy that uses B Cells to Produce Exogenous Therapeutic Proteins
We are working on four sub-projects focused on developing B cell products that can be used to deliver therapeutic proteins: 1) Improving in vitro cell expansion and the B cell editing platform; 2) Improving total and per-cell protein production in edited B cells; 3) Improving engraftment of protein-producing B cells; and 4) Integration and development of a pre-clinical product. The goal of these studies is to generate proof-of-concept data for an investigative new drug designation for this novel cell therapy.
Overlap: None

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.*

Organization name: Seattle Children's Research Institute
Location: 1900 Ninth Ave, Seattle, WA 98101
Contribution: Collaboration (Richard James PhD is a co-investigator on this award)

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.*

- Attached file QuadChart LR170026 Cerosaletti_Year 1

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.*

- Literature cited:
  Jiang, SH, Athanasopoulos V, Ellyard JI, Chuah A, Capello J, Cook A, Prabhu SB, Cardenas J, Gu J, Stanely M, Roco JA, Papa I, Yabas M, Walters GD, Burgio G, McKeon K, Byers JM, Burrin C, Enders A, Miosge LA, Canete PF, Jelusic M, Tasic V, Lungu AC, Alexander SI, Kitching AR, Fulcher DA, Shen N, Arsov T, Gatenby PA, Babon JJ, Mallon DF, de Lucas Collantes C, Stone EA, Wu P, Field MA, Andrews TD, Cho E, Pascual V, Cook MC, Vinuesa CG. 2019. Funtional rare and low frequency variants in BLK and BANK1 contribute to human lupus. Nature Comm 10(1):2201 PMID: 31101814

- Attachment:
  QuadChart LR170026 Cerosaletti_Year 1