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TITLE: Elucidating the Functional Mechanisms by Which the Protein Tyrosine Phosphatase SHP2 Is Involved in the Pathogenesis of Systemic Lupus Erythematosus

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Elucidating the Functional Mechanisms by Which the Protein Tyrosine Phosphatase SHP2 Is Involved in the Pathogenesis of Systemic Lupus Erythematosus

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14. ABSTRACT

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder. However, how SLE occurs remains unknown. >50 patients with Noonan Syndrome (NS), a congenital disorder mediated by gain-of-function mutations in SHP2, developed SLE, suggesting correlation between phosphatase activity and systemic autoimmunity. We measured SHP2 activity in lupus-prone MRL/lpr mouse spleens, and found that phosphatase activity was significantly increased. Moreover, SHP2 activity was increased in lupus patient peripheral blood mononuclear cells (PBMCs), suggesting SHP2 activity is causal to SLE. We next utilized an SHP2 inhibitor, 11a-1, and showed that treated MRL/lpr mice and SLE patient T cells reduced proliferation of T cells and decreased production of interferon gamma (IFNγ) and interleukin 17A/F (IL17A/F). Importantly, normalized SHP2 activity in lupus-prone mice increased lifespan, suppressed glomerulonephritis, reduced spleen size, and diminished skin lesions, implicating SHP2 in lupus-associated immunopathology. How this occurs remains unclear. We hypothesize that increased SHP2 activity in SLE causes aberrant T cell signaling, inducing proliferation and production of pro-inflammatory cytokines to mediate organ damage. Our Aims will assess signaling pathways and cytokine subsets aberrantly regulated in SLE by SHP2 in 1) lupus-prone mice and 2) human SLE; and 3) investigate whether use of an allosteric SHP2 inhibitor can treat SLE patients. In Year 1, we found that T cell-specific expression of SHP2 is a critical driver of SLE pathology, specifically in the regulation of double negative (DN) T cells. These data suggest that SHP2 deletion improves physiological outcomes and slows the progression of SLE pathophysiology. Working to develop either a systemic or targeted inhibitor for SHP2 may yield a potent, novel, and specific treatment for SLE.

15. SUBJECT TERMS

NONE LISTED

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

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder. However, how SLE occurs remains unknown. >50 patients with Noonan Syndrome (NS), a congenital disorder mediated by gain-of-function mutations in SHP2, developed SLE, suggesting correlation between phosphatase activity and systemic autoimmunity. We measured SHP2 activity in lupus-prone MRL/lpr mouse spleens, and found that phosphatase activity was significantly increased. Moreover, SHP2 activity was increased in lupus patient peripheral blood mononuclear cells (PBMCs), suggesting SHP2 activity is causal to SLE. We next utilized an SHP2 inhibitor, 11a-1, and showed that treated MRL/lpr mice and SLE patient T cells reduced proliferation of T cells and decreased production of interferon gamma (IFNγ) and interleukin 17A/F (IL17A/F). Importantly, normalized SHP2 activity in lupus-prone mice increased lifespan, suppressed glomerulonephritis, reduced spleen size, and diminished skin lesions, implicating SHP2 in lupus-associated immunopathology. How this occurs remains unclear. We hypothesize that increased SHP2 activity in SLE causes aberrant T cell signaling, inducing proliferation and production of pro-inflammatory cytokines to mediate organ damage. Our Aims will assess signaling pathways and cytokine subsets aberrantly regulated in SLE by SHP2 in 1) lupus-prone mice and 2) human SLE; and 3) investigate whether use of an allosteric SHP2 inhibitor can treat SLE patients.

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

Lupus, SHP2, Noonan, phosphatase, tyrosine, SLE, signaling, inflammation, cytokines, IFNγ, IL-17, RASopathies, T cells, lupus-prone mice, glomerulonephritis, spleen, kidney, lesions

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.

Aim 1: To assess the SHP2-specific molecular signaling pathways and to identify the cytokine subsets aberrantly regulated in <u>lupus-prone mice</u>. SHP2 phosphatase activity is significantly elevated in tissue lysates isolated from lupus-prone MRL/*lpr* mice. Moreover, T cells isolated from lupus- prone mice treated with the catalytic site inhibitor for SHP2 have reduced proliferation and decreased production of at least two cytokines, INF gamma and IL17A/F, suggesting SHP2 likely plays an active role in mediating SLE pathogenesis. However, the mechanisms by which this is aberrantly regulated in SLE remain unclear. Here, we will examine the activities of SHP2-dependent downstream signaling effectors proteins in various lupus-prone mice, including further analysis of female MRL/*lpr* and its strain control Mrl/MpJ and analysis of Sle1 and Sle3 (congenic) mice. Signaling will be assayed in kidney, spleen, thymus, as well as in primary cells from these tissues, PBMCs, and isolated immune cell subsets, to elucidate SHP2 function in SLE. In addition, cytokine profile analyses from serum, immune-cell subsets and tissue derived from the various lupus-prone mice will also be conducted.

Aim 2: To assess the SHP2-dependent molecular signaling pathways and to identify the cytokine subsets affected in human SLE. Our preliminary data demonstrate that SHP2 activity is also increased in PBMCs isolated from female SLE patients. To assess whether SHP2 phosphatase activity is aberrantly regulated in human SLE, we will determine the total protein, mRNA and activity levels of SHP2 from serum and PBMCs isolated from disease active SLE patients, as well as from normal subjects. Signaling effectors of SHP2 will be measured in PBMCs and immune cell subsets isolated from these patients to determine whether SHP2 activity correlates with SLE. Finally, we will conduct cytokine analysis profile in SLE patient samples to identify cytokines involved in SHP2-dependent pathogenesis of SLE.

Aim 3: To investigate whether SHP2 inhibition can be used as novel treatment for SLE. Current therapy for SLE involves a variety of non-specific immunosuppressive agents that have significant side effects and are often ineffective. Given that our preliminary data suggest that SHP2 is integral to human SLE pathology and that its inhibition can normalize T cell activity, we propose that inhibition of SHP2 might offer a novel approach to treating SLE. Here, we will determine the potency of a novel, newly synthesized, commercially available, and orally bioavailable allosteric SHP2 inhibitor to ameliorate SLE-associated organ damage and pathogenicity. Studies will include *in vitro* analysis of tissue-specific isolated primary cells (kidney, spleen), PBMCs and immune cell subsets, as well as *in vivo* analysis of drug effects (histology, immunohistochemistry, etc.) to measure SLE onset and disease progression.

Aim	Milestone	**Year 1 Sept 29, 2021-Oct 29, 2022**	Year 2	Year 3	Year 4
1	Assess SHP2 signaling pathways and identify cytokine subsets aberrantly regulated in lupus-prone mice.	●●●● **(35% completion)**	●●●●	●●●	●
2	Assess SHP2 signaling pathways and identify cytokine subsets in human SLE.		●●●●	●●●	●●
3	Investigate SHP2 inhibition as novel treatment for SLE.			●●●	●●

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.

Current work for Yr1 (September 29, 2021-October 29, 2022) has focused on Aim 1 and the physiological and immunological phenotyping of our novel SHP2 T cell-specific deleted (Lck-Cre) mice on the C57B6 lupus-prone background. We demonstrate that, as hypothesized, SHP2 deletion within the T cell compartment significantly increases life span of female lupus mice, from a mean 38 weeks to undefined, as assessed by Kaplan-Meier with Log-Rank Mantal-cox posthoc (Figure 1A).

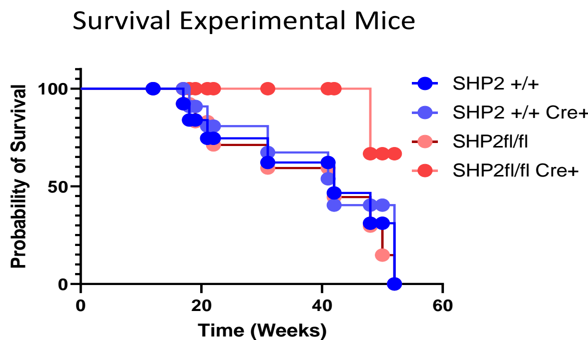

Figure 1: Deletion of SHP2 in T cells extends lifetime of both experimental, and breeding, C57B6 Lupus prone mice. A: Kaplan-Meier survival curve of female experimental lupus prone mice expressing wild type SHP2, or floxed SHP2, with and without Lck-Cre. N=10/genotype. Statistics: Kaplan-Meier survival curve w/Log-Rank Mantel-Cox posthoc.

Physiologically, T-cell SHP2 deletion mice (SHP2fl/fl Cre+) mice displayed reduced spleen to body weight ratios and lymph node (LN) to body weight ratios (Figure 2A&B). This was also accompanied by decreased blood sera IL-17A/F heterodimer expression (Figure 2C.) Furthermore, these changes were witnessed at both 32 and 52 weeks of age, confirming that SHP2 deletion not only improves physiological outcomes, but slows the progression of SLE pathology as well.

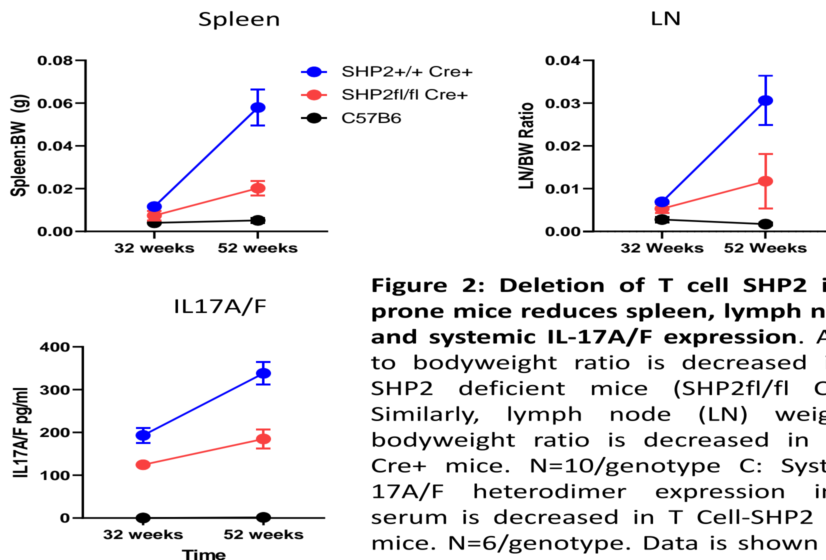

Figure 2: Deletion of T cell SHP2 in lupus-prone mice reduces spleen, lymph node size, and systemic IL-17A/F expression. A: Spleen to bodyweight ratio is decreased in T cell SHP2 deficient mice (SHP2fl/fl Cre+). B: Similarly, lymph node (LN) weight to bodyweight ratio is decreased in SHP2fl/fl Cre+ mice. N=10/genotype C: Systemic IL-17A/F heterodimer expression in blood serum is decreased in T Cell-SHP2 deficient mice. N=6/genotype. Data is shown as mean +/- SEM. Stats: 2 Way ANOVA with Tukey posthoc.

Structural analysis of spleens and kidneys from female 32-week-old SLE mice with either wildtype SHP2 T-cell expression (SHP2++ Cre+), T-cell specific SHP2 deletion (SHPfl/fl Cre+), or C547B6 wildtype were sectioned and stained by H&E (Figure 3). Data revealed partial correction of structure in SHP2 T-cell specific deleted tissues, as compared to wildtype. In splenic samples, partial restoration of white pulp and red pulp divide was present in SHP2fl/fl Cre+ mice, when compared to the large diffuse expansion of white pulp in wildtype SHP2+/+ Cre+ mice. Further, partial restoration of kidney structure, as shown by the absence of gross pathological immune infiltrates and preservation of tubules was also observed in the SHP2 T-cell specific deleted lupus mice.

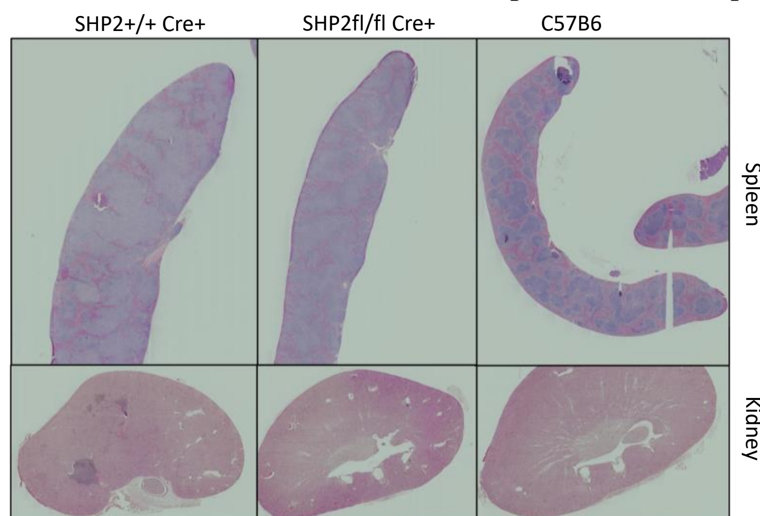

Figure 3: Deletion of SHP2 in lupus-prone mice partially restores splenic and kidney structures. Spleens and Kidneys from 32 week old female lupus prone mice containing wild type expression of T cell SHP2 (SHP2++), deleted expression of SHP2 in T cells (SHP2fl/fl), or healthy C57B6 were sectioned and stained with H&E to reveal structure.

Immunologically, flow cytometric analysis of spleens revealed a significant decrease in total number of total T cells in spleens isolated from T-cell specific SHP2 deleted animals (fl/fl Cre+/+) (Figure 4A-D). Interestingly, we found that this result was largely mediated by a remarkable decrease in DNT T cell numbers in SHP2 T-cell specific deleted lupus-prone mice, which went from an average 40% of total splenic cells to 10% of splenic cells (Figure 4D).

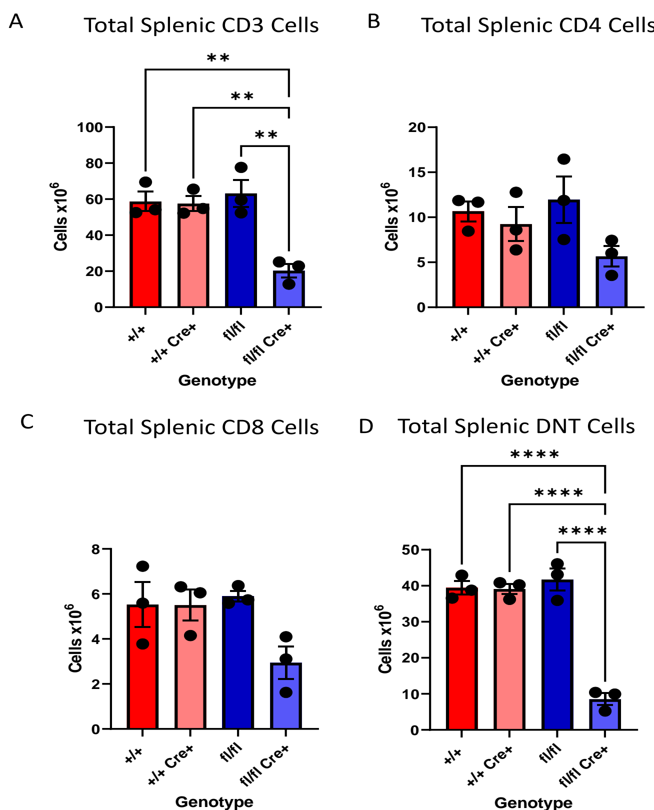

Figure 4: CD3+ T cells are decreased in the spleens of Lupus SHP2 T Cell deletion mice (fl/fl Cre+) mice, as a consequence of DN T cell decrease. A: Total number of splenic CD3+ T cell mice. B: Total number of splenic CD4+ T cells. C: Total number of splenic CD8+ T cells. D: Total number of splenic DNT cells. N=3/genotype. Total number of cells was calculated by back calculating the % of Total CD3, CD4, CD8 and CD3+CD4-CD8- (DNT cells) as determined by flow cytometry to the total number of splenic cells of each animal. Statistics: 1 way ANOVA with Tukey posthoc. * = P<0.05, ** = p<0.01, *** p<0.001, ****p<0.0001

Culture of isolated CD3+ T cells from lymph nodes revealed that reduction in DNT T cell number witnessed in the spleen is not dependent upon T cell receptor ligation. In vitro, isolated T cells were stimulated with antiCD3 and anti-CD28 beads to mimic natural antigen presentation-based activation (the usual mechanism by which T cells are activated in the lymph nodes and spleens) or PMA/Ionomycin activation (which bypasses the TCR and activates cells through calcium ion influx). These data revealed that the reduction of DNT is preserved after both methods of activation, despite decreased proliferation (data not shown). Thus, decreased proliferation is not TCR dependent, likely suggesting that SHP2 has an intrinsic role in the regulation of T cell proliferation (Figure 5).

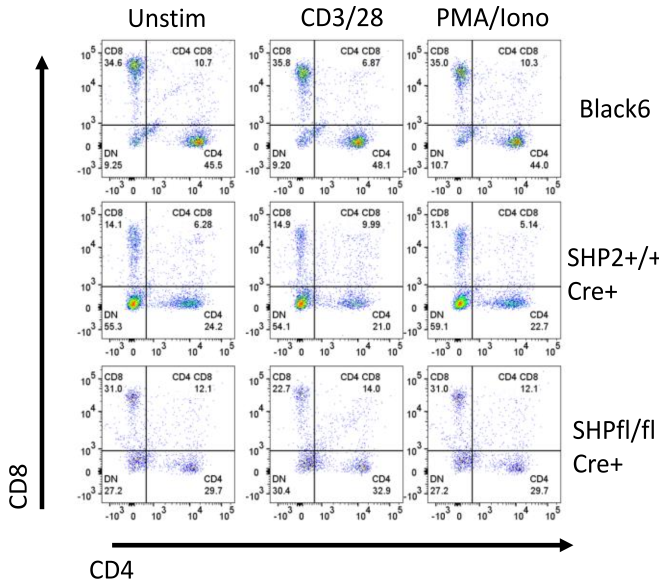

Figure 5: Reduction in DNT cells in vitro is TCR independent. Isolated splenic CD3+ T cells were cultured in vitro for 24 hours and the proportion of CD4+, CD8+ and CD4-CD8- (DN) T cells were assessed by flow cytometry. DNT cell number were decreased in SHP2fl/fl Cre+ lupus mice, this reduction was not specific to CD3/28 activated T cells (TCR dependent) or PMA/Ionomycin activated T cells (TCR independent.)

Furthermore, decreased total IL-17 expression was recapitulated in DNT in vitro post anti CD3-CD28 and PMA/Ionomycin activation (Figure 6). Interestingly, in wildtype SHP2 expressing lupus DNT cells (SHP2++ Cre+), IL-17 expression was independent of TCR stimulation, while in SHP2 deleted DNT IL-17, expression was only decreased following TCR stimulation. Hence, it is likely that IL-17 production in DNT is partially reliant on SHP2 TCR mediated cellular signaling. Further mechanistic work will be conducted to investigate this more thoroughly.

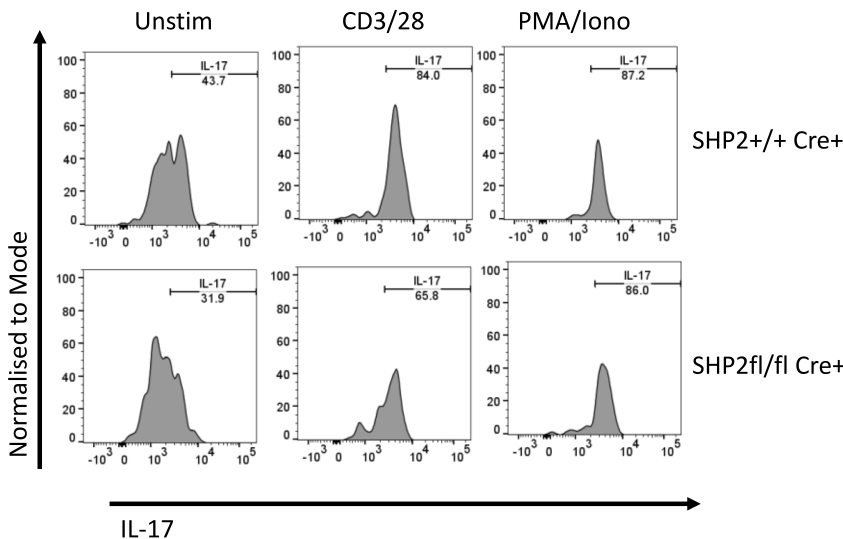

Figure 6: The % of total IL-17 DNT Cells is decreased in SHP2fl/fl Cre+ lupus mice in vitro. Intracellular IL-17 within the DNT cell compartment (Live, Single, CD3+ CD4- CD8- cells) was decreased in SHP2 deficient DNT cells.

It is known, and we have previously shown, that paradoxical decreases in splenic ERK 1/2 expression are observed in SLE, and indeed, we too see the same phenomenon in our wildtype SHP2+/+ Cre+ lupus prone mice (Figure 7A). However, deletion of T cell specific SHP2 expression in lupus-prone mice partially restores ERK 1/2 expression and thereby further decreases ERK1/2 activity ratios (Figure 7B&C). This is remarkable considering that protein lysates used in this experiment are from whole spleen, and therefore contain a panoply of immune cells that still contain wildtype SHP2 expression. Therefore, taken together, these data confirm our previous work and hypothesis that T cell targeted SHP2 may be a viable target to improving systemic lupus pathophysiology.

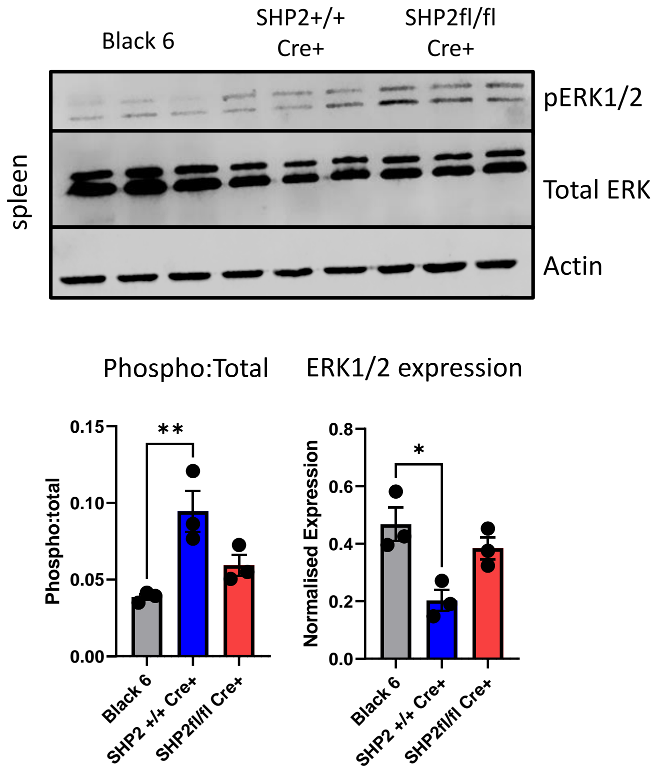

Figure 7: Deletion of SHP2 in Lupus prone C57B6 mice partially restores splenic ERK expression. A: ERK phosphorylation at Thr202/Tyr204, total ERK, and Actin loading control, were assessed by immunoblot. Quantification of immunoblots was performed and ratio of B: phosphorylated to total ERK, and C: ERK1/2 expression to Actin was calculated. Deletion of SHP2 partially restores ERK1/2 phosphorylation and expression. N=3/genotype. Data is shown as Mean+/- SEM. Stats: 1 way ANOVA with Tukey posthoc. *$p<0.05$ **$p<0.01$

Our data this year show that T cell specific SHP2 activity is a critical driver of SLE pathology. However, work to identify the molecular mechanism is still ongoing. Next steps involve 1 – further identification of the molecular pathways broadly altered in whole spleen and kidneys between wildtype SHP2 T cell mice and T cell SHP2 deficient mice through western blotting; 2- gene expression changes by qPCR; and 3 –characterization of SHP2 regulation of TCR signaling in isolated T cells. These experiments will be conducted in Year 2 of this grant.

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.

I have developed a training plan to develop my fellow and research assistant's capabilities, tailoring to their specific career goals. The plan involves training activities in the following areas: 1) research; 2) mentorship and management; 3) written and oral communication; and 4) networking.

1) Research –Our project focuses on how SHP2 is involved in development of SLE. Inhibition of this effector could potentially prove to be valid target for the treatment of this autoimmune disorder. Dr. Samantha Le Sommer and Mr. Levi Legler are developing the skills and knowledge in immunology, cell biology, molecular biology, and protein biochemistry to successfully complete the project. Working on this project provides them the opportunity to work in and understand the molecular pathways that influence phosphatase-mediated functions in the immune system and provides them the working knowledge to continue their careers in signaling in autoimmunity. Both Samantha and Levi have full access to reagents, technology, and/or equipment needed to complete the work. They also have access to the full range of core resources (including cold room, tissue culture, microscopy, animal facility, etc.) as well as central research cores at MMRI, performing a broad range of services from FACS sorting to imaging. They also will be learning networking and collaboration skills, working with our collaborator at BIDMC to complete the studies herein. Their time (>90%) is also devoted to conducting research, with the remainder of time dedicated to additional research activity, including training, mentoring, and teaching.

2) Mentorship and Management – Both Samantha and Levi have become integral parts of a vibrant scientific community at MMRI. We have a diverse group of researchers working on a myriad of biological questions related to cardiovascular disease. MMRI has a 64-year history in studying cardiovascular health and encompasses several active research programs from 8 independent research investigators, hosts monthly seminar symposia from internationally acclaimed scientists in our field, and provides educational opportunities that are open to anyone working here. Samantha and Levi will have full access to attend any relevant sessions and programs in which they may be interested. Therefore, the MMRI provides them unparalleled opportunities to benefit from scientific interactions, educational activities, and shared facilities in a cutting edge, technologically forward research institute.

In addition to regular lab meetings, Dr. Le Sommer, Mr. Legler and I meet formally one-on-one every two weeks to review the progress of this proposal. More formally, they meet and present with other faculty at MMRI as well, where they present their work as part of a works-in-progress seminar. Finally, both Samantha and Levi have the ability to attend at national meeting, to provide them the chance to network and get feedback on their work. In further support of their career development, Samantha and Levi participate in a series of seminars offered by MMRI on Career Development, grant writing and grants management, as well as the institution's training program in Human Studies and Animal Experimentation, and the Responsible Conduct of Research. They are also annually trained in Biosafety, Conflicts of Interest, Working with Mice in Research, criteria for determination of authorship, data management, human subjects and animal use, research misconduct and research ethics.

3) Written and oral communication – Dr. Le Sommer, Mr. Legler and I are working on further developing the project ideas, writing, and revising of manuscripts and future grant proposals. I will provide guidance, feedback, and edits. As mentioned, both Samantha and Levi will be given opportunities to present at lab group meetings, as well as to at least one scientific conference each year,

to gain exposure and confidence in their presentation skills and in their scientific thought processes. Finally, MMRI participates in an Interdisciplinary Educational Seminar Series, which provides Samantha and Levi additional opportunities for learning skills in grant writing, leadership, lab management, and novel laboratory technologies.

4) Networking – In addition to presenting at conferences, I will assist and support Samantha and Levi in participating in organizational committees within national organizations, including the Lupus Research Alliance (LRA). This is an enriching experience that will provide them (and MMRI) visibility and enable them to develop meaningful collegial relationships with leaders in the field. As well, the MMRI is in the heart of NY state, with several academic institutions, including Syracuse University, SUNY upstate, SUNY Polytechnic, Utica College, Hamilton College, Columbia, and Cornell nearby, with scientists and research expertise available to help when needed. MMRI provides opportunities to benefit from scientific interactions, collaborations, educational activities, and shared facilities. As well, its diverse group of top-notch faculty all avail themselves to junior scientists looking for discussion, support and career advice. Finally, we have a once-a-month Research Seminar, where an outside speaker of national acclaim is invited, bringing with them additional research advice, collaboration, and networking opportunities.

In summary, this training plan is designed to ensure that all members of my lab, specifically Dr. Le Sommer and Mr. Legler here, succeed in their career paths and/or transition to independent academic researchers. Training activities have been carefully constructed to develop skills in research, mentorship and management, oral and written communication, and networking that are essential for success in academic research. Importantly, the plan as outlined includes appropriate oversight and a trajectory of success.

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.

This year we had the opportunity to work closely with the Lupus and Allied Diseases Association, Inc, which is located nearby in Verona, NY. This is a national volunteer organization dedicated to improving quality of life for those affected by lupus and other diseases, fostering collaboration among stakeholders, and using the patient voice as a catalyst to advance advocacy, education, awareness, and research initiatives (and I believe the group that heavily advocated for the Lupus Impact Award from the DOD). This was an amazing opportunity for us. Their board visited the MMRI on two separate occasions, whereby we had the opportunity to present our current work, along with 4 additional new projects focused on lupus mechanisms and targeted therapeutics. This amazing patient advocacy group enjoyed our seminars, appreciated, and supported our projects focused on novel therapeutics to target SLE.

In addition, I was also an Invited Speaker to present on this project to the Mohawk Valley Institute for Learning in Retirement at SUNY Poly, here in Utica, NY. This is a group of retired physicians looking to understand the most cutting-edge research going on here in our region. They were very impressed with our work and look forward to continued success of this potential drug targeting project.

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.

We are currently on task with the goals we set out for our project. In Year 2 of our project, we will continue our efforts to understand the signaling pathways and cytokine subsets aberrantly regulated in SLE by SHP2 in our lupus-prone mice and begin our validation and assessment of the same in human SLE patient peripheral blood mononuclear cells.

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).

Thus far in Year 1, we are confident in stating that T cell-specific expression of SHP2 is a critical driver of SLE pathology, specifically in the regulation of double negative (DN) T cells. These data suggest that SHP2 deletion improves physiological outcomes and slows the progression of SLE pathophysiology. Working to develop either a systemic or targeted inhibitor for SHP2 may likely yield a potent, novel and specific treatment for SLE.

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.

Results from our findings may have significant impact on mechanisms associated with causing other autoimmune diseases (Type I Diabetes, Rheumatoid Arthritis, Psoriasis, etc), particularly where DN T cells are generated, and suggest that use of an SHP2 inhibitor in those diseases could similarly be used to ameliorate disease pathogenicity.

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 at end of Year 1, but we expect that the outcomes of this project will lead to new drug development and therapeutics for SLE treatment.

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 at end of Year 1, but we expect that the outcomes of this project will lead to new drug development and therapeutics for SLE treatment.

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.

Nothing to report.

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

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *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.*

2021 Invited Speaker: "Lupus Research at MMRI: SHP2 and beyond," Lupus and Allied Diseases Association, Inc., MMRI, Utica, NY.

2021 Invited Speaker: "Congenital Heart Disease and Lupus: The Connection," Mohawk Valley Institute for Learning in Retirement, SUNY Poly, Utica, NY.

- **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.

https://www.mmri.edu/mmri-gets-750000-to-research-lupus-find-avenues-for-treatment/
Website link to MMRI that describes award provided by DOD to do research herein.

https://www.mmri.edu/lupus/
Website link that describes Lupus research in general at MMRI

https://www.mmri.edu/project/mkontaridis/
PI website for research on Lupus

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

Nothing to report.

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

Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Development of the SHP2 T-cell specific deletion lupus-prone mice:
$SHP2^{fl/fl}Lck^{Cre/+}$- $C57BL/6^{lpr/lpr}$ mice

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.)*

Personnel:

Name: Maria Kontaridis, Ph.D.
Project Role: Principal Investigator
Research Identifier: 0000-0002-6121-0533
Nearest person month worked: 0.6
Contribution to Project: Dr. Kontaridis is responsible for the overall direction of the project, is managing the experiments proposed herein, collecting and assisting in the analysis of the data, and supervising Dr. Le Sommer and Mr. Legler on the project and directly collaborating/communicating with Dr. Kyttaris and Research Coordinator Ms. Krishfield.

Name: Samantha Le Sommer, Ph.D.
Project Role: Postdoctoral Research Fellow
Research Identifier: 0000-0003-3301-7397
Nearest person month worked: 6.0
Contribution to Project: Dr. Le Sommer is responsible for and/or assisting with all the experiments proposed in Specific Aims 1-3, together with Dr. Kontaridis. Dr. Le Sommer will conduct the molecular biology experiments required to complete this project for Years 1-4.

Name: Levi Legler
Project Role: Research Assistant
Research Identifier: n/a
Nearest person month worked: 6.0
Contribution to Project: Mr. Legler is working on the mouse breeding, genotyping, and molecular biology techniques. He is also assisting Dr. Le Sommer with the biochemical and molecular studies in the proposal for Specific Aims 2-3 in Years 1-4.

Personnel:
Name: Vasileios Kyttaris, M.D.
Project Role: Co-Investigator
Research Identifier: 0000-0001-7652-3826
Nearest person month worked: 0.6
Contribution to Project: Dr. Kyttaris is providing us access to his human SLE samples, as well as assisting us with data analysis. Dr. Kyttaris collects and distributes "deidentified" human SLE patient blood and cell samples (classified as non-human research). These samples and data will be used for the completion of Specific Aim 2 in this study.

Name: Suzanne Krishfield
Project Role: Research Coordinator
Research Identifier: n/a
Nearest person month worked: 0.6
Contribution to Project: Ms. Krishfield is responsible for consenting research subjects and collecting peripheral blood samples. She distributes "deidentified" human patient blood and cell samples. These samples and data will be used for the completion of Specific Aim 2 in this study.

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.

Kontaridis, Maria I: Changes to Other Support
New Active Grants:

1) Title: Use of cardiosphere-derived human exosomes as therapeutic agents in SLE
Time Commitments: 0.6 calendar
Supporting Agency: Lupus and Allied Diseases Association, Inc
Address: Lupus and Allied Diseases Association, Inc, P.O. Box 170, Verona, NY 13478
Contracting/Grants Officer: Kathleen Arnsten
Performance Period: 02/01/2022 – 01/31/2023
Level of Funding: $50,000
Project Goal: The project will highlight the importance of EVs in treatment of SLE and will identify exosomes as a potential new therapeutic approach to treating lupus patients.
Specific Aims: Aim 1: Determine whether, and how, CD-EVs contribute to tissue protection in lupus. Aim 2: Assess the contribution of specific microRNA and short non-coding RNA species within CD-EVs to the tissue protective effects in lupus.
Overlap: None

Kontaridis, Maria I: Changes to Other Support (cont.)
2) Title: Gain-of-function mutations in SHP2 enhance inflammatory macrophage (Mφ) activation in SLE.
Time Commitments: 0.6 calendar
Supporting Agency: Lupus and Allied Diseases Association, Inc
Address: Lupus and Allied Diseases Association, Inc, P.O. Box 170, Verona, NY 13478
Contracting/Grants Officer: Kathleen Arnsten
Performance Period: 02/01/2022 – 01/31/2023
Level of Funding: $50,000
Project Goal: This project will determine whether SHP2 differentially regulates the pathogenesis of SLE through its regulation of the JAK-STAT and PI3K-AKT signaling pathways, inducing activation of Mφ and mediating production of cytotoxic cytokines, respectively.
Specific Aims: Aim 1: Determine whether SHP2 activity is required to induce Mφ maturation and activation in SLE. Aim 2: Assess whether SHP2 activity modulates JAK-STAT and PI3K-AKT signaling in SLE macrophage cells.
Overlap: None

Closed Grants:

1) Title: Role of melanoma-PD-1 in cancer progression
Time Commitments: 0.6 calendar
Supporting Agency: NIH/NCI, 1R01CA190838-01A1/Brigham & Women's Hospital
Address: NIH/NCI, P.O. Box 30105, Bethesda, MD 20824-0105
Contracting/Grants Officer: Alison J. Lin
Performance period: 9/13/2017 – 7/31/2022
Level of funding: $71,600
Project Goals: The major goal is to determine the functional effects of PD-1 in cancer as a consequence of SHP2 activity and downstream signaling.
Specific Aims: Aim 1: Define melanoma cell-intrinsic PD-1 signaling networks required for cancer progression. Aim 2: Examine the utility of melanoma-PD-1 signaling mediators as biomarkers of clinical response to anti-PD-1 therapy.
Overlap: None

2- Title: Treatment of cardiac hypertrophy using RAF1 inhibitors
Time Commitments: 0.48 calendar
Supporting Agency: Onconova Therapeutics, AGR-19094A1
Address: Onconova Therapeutics, 12 Penns Trail, Newtown, PA 18940
Contracting/Grants Officer: Ann Rucker
Performance period: 1/1/2020 – 07/31/2022
Level of funding: $119,584
Project Goals: The major goal is to determine the functional effects of PD-1 in cancer as a consequence of SHP2 activity and downstream signaling.
Specific Aims: Aim 1: Assess rigosertib treatment in mouse RASopathy models. Aim 2: Assess rigosertib in a mouse RASopathy model.
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: Beth Israel Deaconess Medical Center
Location of Organization: Boston, MA
Partner's contribution to the project: Collaboration with Vasileios Kyttaris, M.D. (Co-Investigator) and Suzanne Krishfield (Research Coordinator).

Organization Name: Lupus and Allied Diseases Association, Inc.
Location of Organization: Verona, NY
Partner's contribution to the project: Financial support ($25,000)

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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

N/A

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

Phases/Tasks of the Project and Duration (on task for completion):

Elucidating the functional mechanisms by which the protein tyrosine phosphatase SHP2 is involved in the pathogenesis of Systemic Lupus Erythematosus

Log Number: LR200032

Award Number W81XWH-21-1-0784

PI: Maria Kontaridis, PhD **Org:** Masonic Medical Research Institute **Award Amount:** $750,000

Study/Product Aim(s)

- Aim 1: To assess the SHP2-specific molecular signaling pathways and to identify the cytokine subsets aberrantly regulated in lupus-prone mice.
- Aim 2: To assess the SHP2-dependent molecular signaling pathways and to identify the cytokine subsets affected in human SLE.
- Aim 3: To investigate whether SHP2 inhibition can be used as novel treatment for SLE.

Approach

Systemic lupus erythematosus (SLE) is an inflammatory autoimmune disorder. However, how SLE occurs remains unknown. We hypothesize that increased SHP2 activity in SLE causes aberrant T cell signaling, inducing proliferation and production of pro-inflammatory cytokines to mediate organ damage.

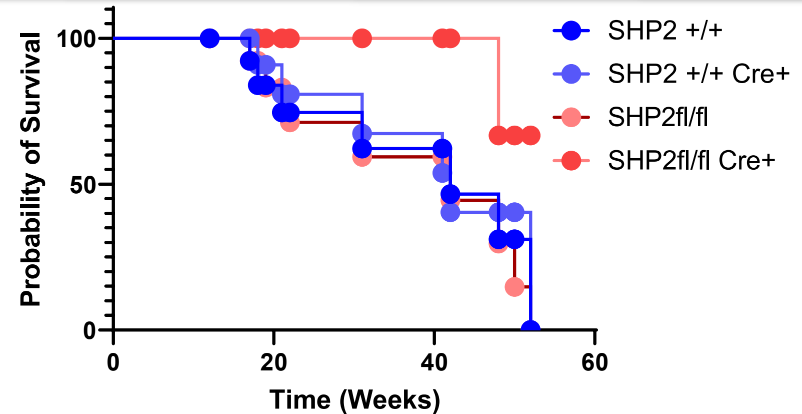

Figure: Deletion of SHP2 in T cells extends life in C57B6 Lupus prone mice. A: Kaplan-Meier survival curve of female lupus prone mice expressing wild type SHP2 or floxed SHP2, with and without Lck-Cre to delete specifically in T cells in background of lpr/lpr lupus prone C57Bl6 mice. N=10/genotype. Statistics: Kaplan-Meier survival curve w/Log-Rank Mantel-Cox posthoc.

Timeline and Cost

Activities CY	21-22	22-23	23-24	24-25
Assess SHP2-specific signaling and identify cytokine subsets in lupus-prone mice.				
Assess SHP2-dependent signaling and identify cytokine subsets in human SLE				
Investigate SHP2 inhibition as novel treatment for SLE				
Estimated Budget ($K)	**$145**	**$215**	**$215**	**$175**

Updated: 10/30/2022

Goals/Milestones

CY21-22 Goal –

☑ Develop and characterize mouse model of T-cell specific deletion of SHP2 in lupus prone lpr/lpr C57Bl6 background.

☐ Identify immune cell subsets and signaling pathways affected by SHP2 in lupus

CY22-25 Goal–

☐ Validate and verify the immune cell subsets and signaling pathways affected in SLE using human SLE patient PBMCs.

CY23-35 Goal –

☐ Investigate whether SHP2 inhibitor can be used as novel therapy for SLE using global inhibition and/or targeted nanoparticle inhibition

Comments/Challenges/Issues/Concerns

- Making the mice took longer than expected, so costs were lower than expected this year. We anticipate making up for the supplies expenses in the coming fiscal year, as we ramp up experiments.

Budget Expenditure to Date

Projected Expenditure: $ $187,500

Actual Expenditure: $144,683.92

9. **APPENDICES:** *Attach all*

N/A