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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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1. **INTRODUCTION:**

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:**

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

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

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- Sept 29, 2022	**Year 2 Sept 29, 2022- Sept 29, 2023**	Year 3	Year 4
1	Assess SHP2 signaling pathways and identify cytokine subsets aberrantly regulated in lupus-prone mice.	●●●● (35% completion)	**●●●● (35% completion)**	●●●	●
2	Assess SHP2 signaling pathways and identify cytokine subsets in human SLE.		**● (15% completion)**	●●●●	●●●●
3	Investigate SHP2 inhibition as novel treatment for SLE.			●●●	●●

Yr 2 (September 29, 2021-September 29, 2023) focused on continuing completing Aim 1 by assessing SHP2 signaling pathways and identifying cytokine subsets aberrantly regulated in lupus-prone mice. We also began our work on Aim 2, beginning our procurement and preparation of human SLE samples for assessing the samples for analysis of their signaling and cytokine abnormalities in the coming year.

Previously, in year 1, we started our project by beginning the characterization of our newly generated T cell specific SHP2 deletion model in systemic lupus erythematosus (SLE) prone (FasLPR) mice, the model is summarized in Fig 1. This year, we demonstrated and validated our preliminary findings from last year that SHP2 is necessary but not sufficient for SLE development, and that deletion of SHP2 in SLE prolongs life span, decreases spleen and lymph node sizes, results in a ~80% decrease in the number of pathogenic double negative T cells, and alleviates the pathophysiology of SLE via a T cell dependent mechanism (Fig 2).

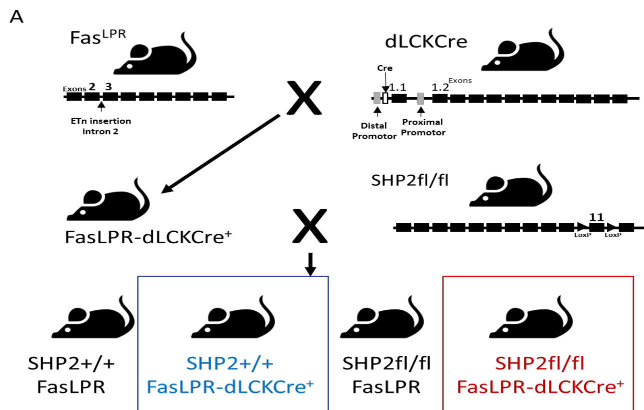
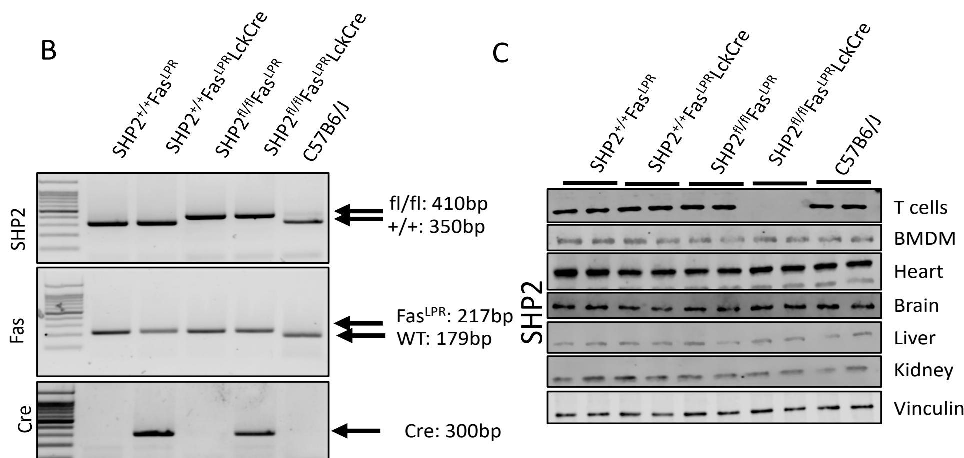


Figure 1. SHP2 is deleted specifically in T cells in SHP2fl/fl FasLPR Cre mice. (A) Standard genotyping of SHP2fl/fl FasLPR Cre mice, along with relevant strains and background C57B6/J controls. Primer sequences are detailed in table 1, product side is noted on the righthand side of DNA gels. (B) Representative immuno blots of T cells isolated from SHP2+/+ FasLPR, SHP2+/+ FasLPR Cre+, SHP2fl/fl FasLPR, SHP2fl/fl FasLPR Cre+, and C57B6/J demonstrate absence of SHP2 only in SHP2fl/fl FasLPR Cre mice. C. Comparable expression of SHP was witnessed in bone marrow derived macrophages (BMDM), heart, brain, liver and kidneys across all genotypes. Vinculin is shown as a loading control.



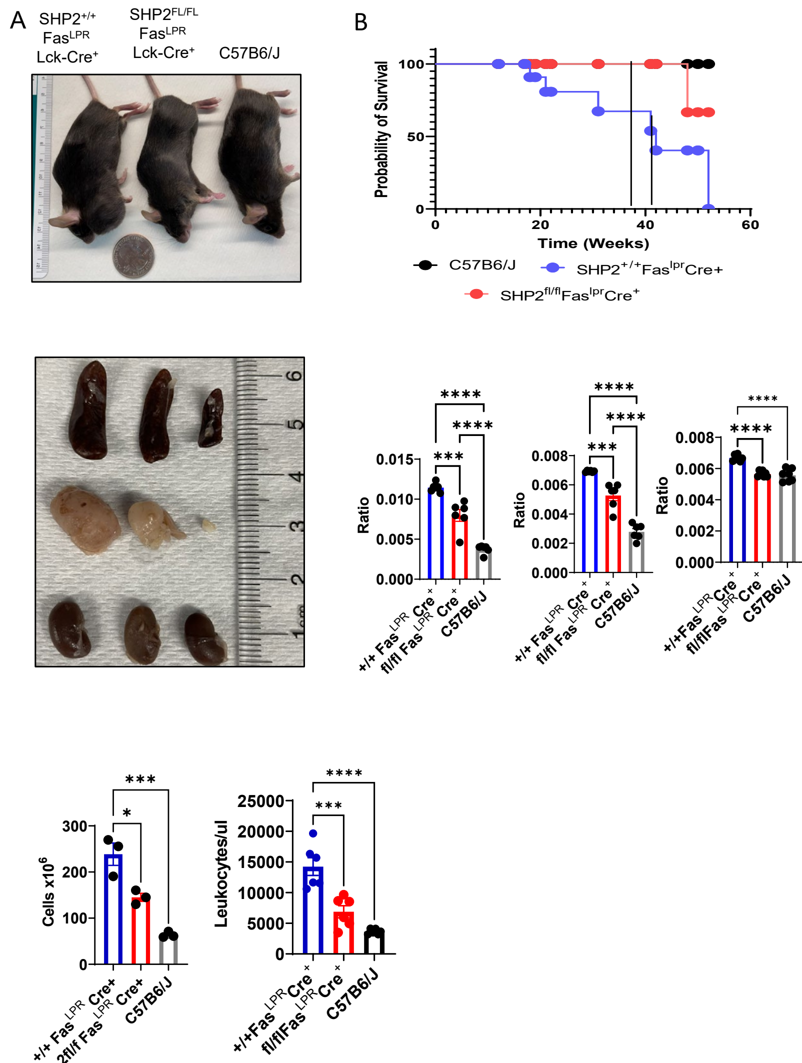


Figure 2. Deletion of SHP2 in T cells in the murine SLE model FasLPR reduces disease severity. (A) Representative photos of mice and (B) survival curve of SHP2fl/fl FasLPR Cre+ mice (blue line), SHP2+/+ FasLPR Cre+ (red line), and C57B6/J strain controls (black line), demonstrating decreased mortality and increased lfe span in SHP2fl/fl FasLPR Cre+ mice. N=10 female mice/ genotype (C) Representative photographs, and corresponding quantification, demonstrating (D) splenomegaly, (E) lymphomegaly and (F) renalmegaly in SHP2+/+ FasLPR Cre+, SHP2fl/fl FasLPR Cre+, and C57B6/J mice. N = 6 female mice / genotype; (G) Total splenocytes, n=3 female mice / genotype (H) Circulating leukocytes per ul of whole blood n=3 female mice/genotype. All mice were 32-weeks-old at sacrifice. Data represent means +/- SEM. Statistics, survival curve Kaplan-Meir, all other data was analyses with one way ANOVA with Tukey post-hoc when significant. *p<0.05, **p<0.01, *p<0.001, p<0.0001.

Because our data indicated that T cell specific deletion of SHP2 in lupus prone mice (SHP2fl/fl::fasLPR-dLCKCre+) preserved spleen, lymph nodes, and kidney structure, we next assessed effects on specific tissue function. Kidneys isolated from 36-week-old SHP2fl/flFasLPRCre+, SHP2+/+FasLPRCre+, and C57B6/J mice histologically showed that deletion of SHP2 in T cells persevered renal architecture in SHP2fl/flFasLPRCre+ mice and reduced fibrosis and immune infiltrates (Fig 3A-C). To investigate if these effects correlated to kidney function, urine albumin and creatinine was measured by ELISA, and albumin to creatinine ratios were calculated (uACR) (Fig 3D). We found that T cell specific deletion of SHP2 in SHP2fl/flfasLPRCre+ mice completely normalized uACR, preserving kidney function. To determine the effects of SHP2 deletion on kidney immune infiltrates, flow cytometric analysis in 36-week-old mouse kidneys was conducted. We found complete normalization of the immune populations within the kidneys of SHP2fl/flFasLPRCre+ mice (Fig 3E-K).

Similarly, we found that immune cell populations were normalized, and immune cell infiltrates were reduced within spleens isolated from SHP2fl/flFasLPRCre+ mice, as compared to controls (Fig 4). Specifically, deletion of SHP2 reduced the total number of CD3+ splenic T cells (Fig 4A) and decreased the number of overall double negative cytotoxic T cells in SLE (Fig 4B-E). Using an in vitro proliferation assay, we also found that, mechanistically, deletion of SHP2 reduced T cell proliferation (Fig 4F-4G) and ameliorated expression of the pro-inflammatory cytokine heterodimer IL-17A/F (Fig 4H). Consequently, deletion of SHP2 in T cells led to reduced levels of total serum IgG (Fig 4I), a hallmark indication of SLE pathology.

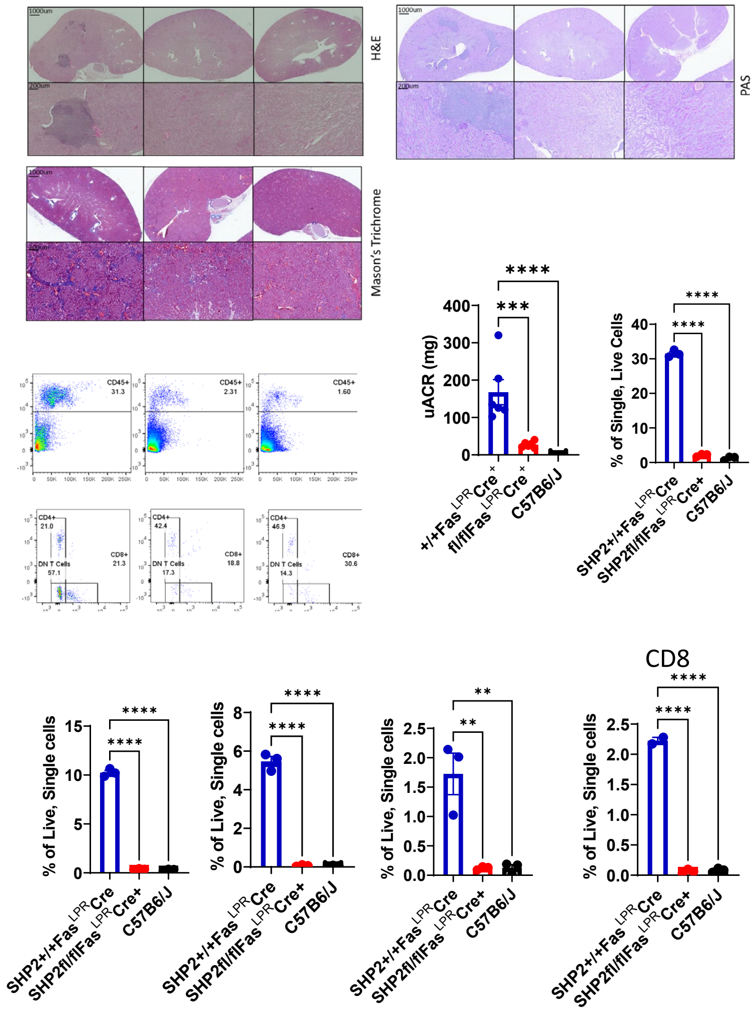


Figure 3. T cell specific deletion of SHP2 preserves kidney structure and function. Representative histological analysis of kidney tissues from SHP2+/FasLPRCre, SHP2fl/flFLPRCre, and C557B6/J mice. (A) H&E. (B) PAS. (C) Masons Trichrome. (D) ELISA analysis of urine albumin and creatinine, expressed as ratio of albumin to creatinine. (E) Flow cytometric analysis of single cell suspensions from kidneys of SHP2+/FasLPRCre, SHP2fl/flFLPRCre, and C557B6/J mice. Percentage of CD45 gated on single, live cells. (F) Representative psudocolour dot plots of CD45 BV711 verses Forward Scatter, gated on single, live cells. (G) Representative psudocolour dot plots of CD4 APC verses CD8 PerCP gated on single, live, CD3+, CD45+ cells. Percentage of T cell subsets in the single, live cell gate. (H) CD3. (I) DNT. (J) CD4. (K) CD8. N=3/genotype. Statistics, one way ANOVA with Tukey post-hoc when significant. *p<0.05, **p<0.01, *p<0.001, p<0.0001.

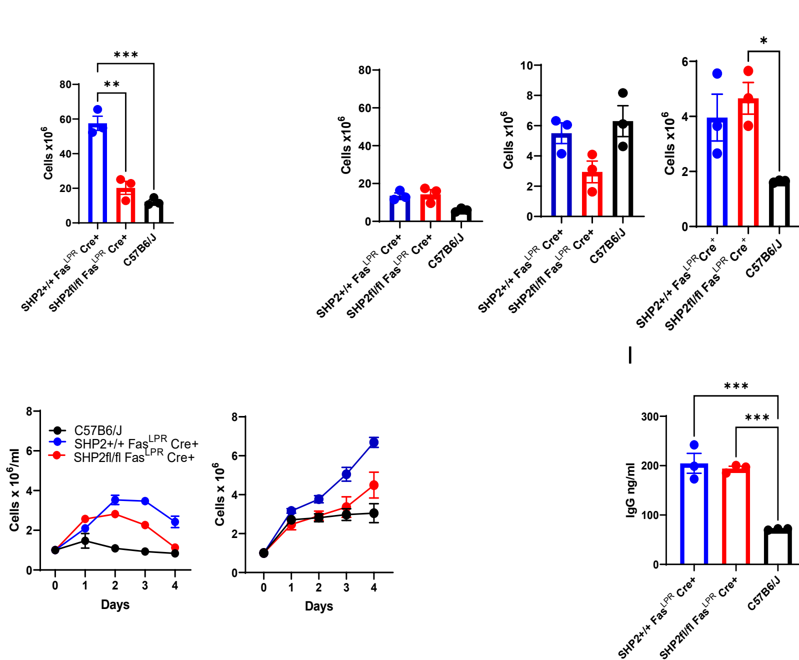


Figure 4. Reduction in CD3+ splenic T cells is due to reduction in double negative T cells. (A) Number of splenic CD3+ T cells, (B) number of DN T cells, (C) number of CD4+ T cells, (D) number of CD8+ T cells, (E) T regulatory cells. *In vitro* proliferation of (G) unstimulated isolated T cells, (G) *in vitro* proliferation of CD3/CD28 activated T cells. (H) Concentration of serum IL-17A/F, and (I) Total serum IgG. N=3/genotype parts A to G. H & F n = 5. Statistics, one way ANOVA with Tukey post-hoc when significant. *p<0.05, **p<0.01, *p<0.001, p<0.0001.

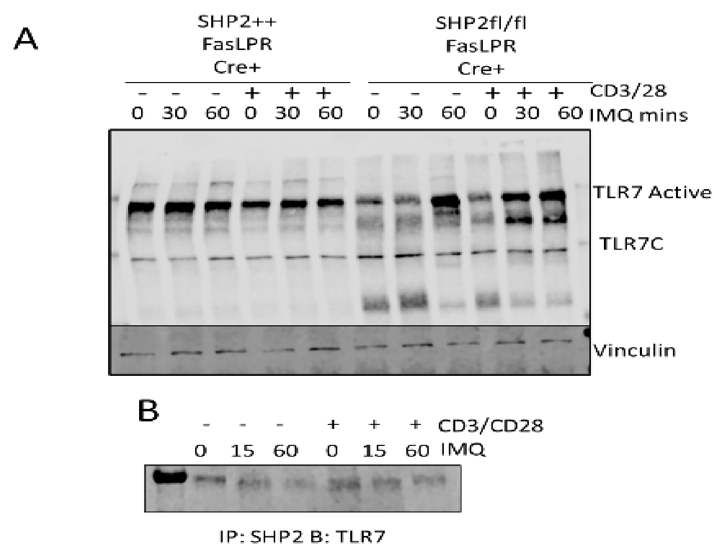


Figure 5. Full length active TLR7 is decreased in SHP2fl/flFasLPRCre+ T cells. (A) T cells were either left untouched or co cultured with anti CD3 CD28 beads and stimulated wth 20ng/ml IMQ and immunoblotted for TLR7. (B) Co-Immunoprecipitation of SHP2 from the T cells of C57B6/J mice was resolved on SDSPAGE gel and immunoblotted for TLR7. Images representative of N=3

To begin to assess functional consequences of our phenotype, we determined effects of SHP2 deletion on downstream signaling pathways. TLR7 is known to play a role in pathogenesis of SLE, whereby in SLE mice and humans, TLR7 activity is increased. Therefore, we next investigated TLR7 signaling in our T-cell specific SHP2 deleted FasLPR model. Isolated splenic T cells were co-cultured with anti CD3 anti CD28 beads to mimic natural antigen presentation and then stimulated with imiquimod (IMQ), a TLR7 agonist. We observed constitutive presence of the full length, active, TLR7 receptor in FasLPR mice with SHP2 (SHP2+/+FasLPRCre+), which was reduced in resting T cells without IMQ (Fig 5A). Even following IMQ stimulation, full length active membrane TLR7 was still reduced. To confirm if TLR7 is a SHP2 substrate, using T cells isolated from SHP2fl/flFasLPRCre+ which express SHP2, we immunoprecipitated SHP2 and blotted for TLR7. Indeed, we found that TLR7 and SHP2 directly interact, and that T cell activation increases this interaction (Fig 5B).

Phospho-tyrosine blotting of C57B6/J, SHP2+/+FasLPRCre+ and SHP2fl/flFasLPRCre+ splenic T cells revealed a stark decrease in phosphorylation in SHP2fl/flFasLPRCre+ mice (Fig. 6). This is paradoxical given that SHP2 is a tyrosine phosphatase; however, low weight molecular hyperphosphorylation was detected in SHP2fl/flFasLPRCre+. These small molecular weight proteins (10-20kD) correspond to the molecular weight of multiple inhibitor chains within the T cell receptor, for example the CD3 Zeta chain, which is tyrosine phosphorylated at inhibitory residues to prevent T cell receptor signaling – this line of inquiry will be further investigated within the next year to assess mechanism of regulation of SHP2 in SLE.

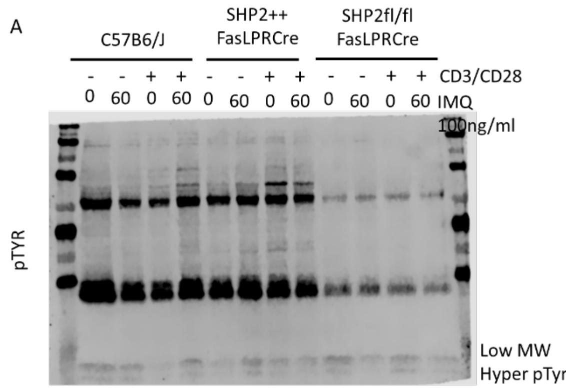


Fig 6. Phosphotyrosine blot of T cells stimulated with TLR7 agonist IMQ. (A) T cells from C57B6/J, SHPR++FasLPRCre+ or SHP2fl/flFasLPRCre+ mice were cocultured with activation beads or left untouched, then stimulated with IMQ. Protein lysates were resolved on SDSPAGE gel and blotted for phosphotyrosine.

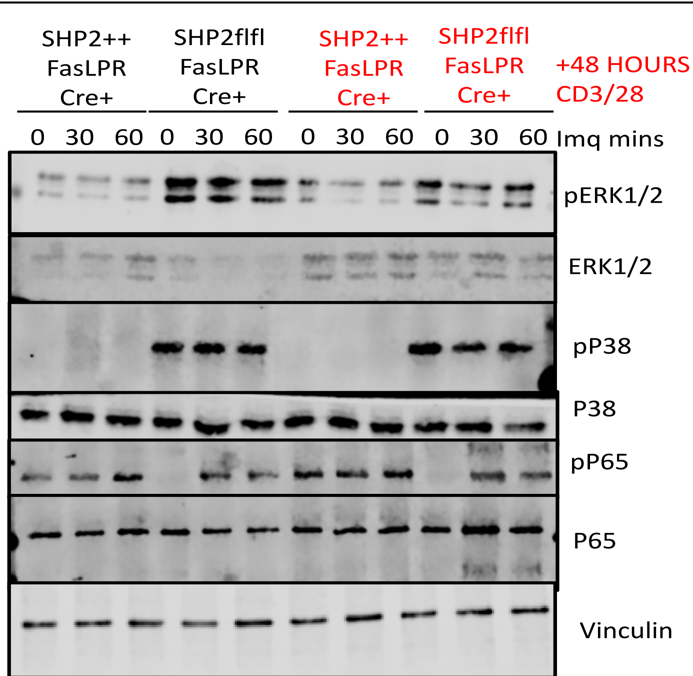


Figure 7. P65 phosphorylation is decreased in SHP2 deletion, meanwhile P38 phosphorylation is increased in a phosphatase dependent manner. (A) T cells were cultured with and without CD3 CD28 activation beads then stimulated with IMQ. Immunoblots for pP65, total P65, pAKT Serine, total AKT, total ERK1/2, pP38, total P38 and vinculin were conducted. Blots are representative of n=3

To investigate the pathways downstream of TLR7, T cells were either left untouched, or co-cultured with CD3/CD28 beads and either unstimulated, or unstimulated (Fig. 7). While AKT phosphorylation was unchanged, we observed normalization of ERK1/2 expression in SHP2fl/flFasLPRCre+ mice. Interestingly, phosphorylation of P38 was increased at all time points in both nascent T cells and inT cells co-cultured with CD3 CD28 activation beads, indicating that this is independent of T cell activation or TLR7 stimulation and is dependent upon SHP2 activity. We also found that p65 phosphorylation was decreased in SHP2fl/flFasLPRCre+ mice compared to SHP2+/+FasLPRCre+.

We are now entering a stage where we are beginning to elucidate the mechanism by which SHP2 is regulating pathophysiology of SLE. In the next year, we will finalize the characterization and identification of these molecular mechanisms of SHP2 in SLE T cells, and more specifically, understand its role in response to TCR activation and TLR augmentation. We plan to conduct additional co-IP of SHP2 and blotting for components of the TCR pathway such as the accessory zeta, gamma, and delta chains to identify direct interaction will take place, along with co-IP to determine SHP2:TLR7 interactivity. Western blotting will be used to assess temporal and amplitude roles of SHP2 in this dynamic signaling pathways, namely, the MAPKs ERK1/2, JNK, and P38, AMPK which is reported to regulate P65 activity, and components of the PI3K-AKT-mTOR pathway which is critical for cellular growth in T cells.

Finally, we have been collecting human SLE patient samples this year and expect to similarly assess and validate our mouse findings in human SLE in the coming year. Specifically, we will begin by assessing SHP2 signaling pathways and identify cytokine subsets in patient SLE.

What opportunities for training and professional development has the project provided?

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

1) Research –Our project has focused on how SHP2 is involved in development of SLE. Inhibition may serve as a valid target for the treatment of this disorder. Dr. Samantha Le Sommer and Mr. Levi Legler have been working to develop their skills and knowledge in immunology, cell biology, molecular biology, and protein biochemistry and have thus far worked successfully to complete the project. They have the opportunity to work in and understand the molecular pathways that influence phosphatase-mediated functions in the immune system and have gained knowledge that will allow them 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 have been networking and collaborating with lab members and other PIs at MMRI, as well as with our collaborators 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 autoimmune, neurological and cardiovascular disease. MMRI has a 65-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 informally 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 attended national meetings this past year, providing them opportunities 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.
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.

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 continue to 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.

4) Networking – In addition to presenting at conferences, Samantha and Levi participate in organizational committees within national organizations, including the Lupus Research Alliance (LRA). This is an enriching experience that has provided them (and MMRI) visibility and enabled 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?

Like last year, this year too 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. We hosted an event in honor of Lupus Awareness Month in May and invited their board and honored the research embarked at MMRI in large part due to their continued support of our mission. We have generated 4 additional SLE projects focused on understanding mechanisms and targeted therapeutics.

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.

Finally, both Dr. Le Sommer and Mr. Legler attended and presented posters on their work at the FASEB Summer Phosphatase meeting this year; they received great feedback on their current projects, to understand the effects of SHP2, a phosphatase, on the development of SLE. Dr. Le Sommer also attended the American College of Rheumatology Conference, where she learned and participated in seminars and sessions on SLE and novel findings.

What do you plan to do during the next reporting period to accomplish the goals?

We are currently on task with the goals we set out for our project. In Year 3 of our project, we will nearly complete 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. Similarly, we will start our final aim, to utilize an SHP2 inhibitor to see if we can reverse/ameliorate the progression of SLE.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Thus far, 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. Mechanistically, we suggest SHP2 functions through activation of TLR7 to modulate proliferation of T cells and induction of cytotoxic cytokines. Our data suggest that SHP2 deletion in T cells 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?

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?

Nothing to report as yet, 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?

Nothing to report as yet, but we expect that the outcomes of this project will lead to new drug development and therapeutics for SLE treatment.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

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:

- **Publications, conference papers, and presentations**

Journal publications.

All publications below have acknowledged federal support for the work.

1: Cardio-rheumatology: the cardiovascular, pharmacological, and surgical risks associated with rheumatological diseases in women; Samantha Le Sommer and Maria I Kontaridis; *Under Review*.

2: Detection and eradication of a Demodex Musculi infestation in specific pathogen free high barrier laboratory mouse facility housing immunocompromised animals;
Samantha Le Sommer, Yan Sun, Levi Legler, Katherine Nelson, Laura Coon, Damian Bohler, and Maria I Kontaridis; *Under Review*.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

2022: Invited Inaugural Speaker: "The Road to Scientific Success: From Phosphatases to Leadership," Kranias Symposium on Early Career Mentorship, University of Cincinnati, Department of Pharmacology & Systems Physiology, Cincinnati, OH

2022: Invited Speaker and Chair: "The Differential Role of PTPN11 Loss-of-Function and Gain-of-Function Mutations in Myeloid Immunology," Federation of American Societies for Experimental Biology (FASEB) and Japanese Association or Protein Phosphatase Research (JAPPR) Conference on Protein Phosphatases in Heart, Lung, and Blood Biology Session Palm Springs, CA

2022: Press Release and Invited Speaker: "Lupus Research at MMRI: SHP2 and beyond," Lupus Awareness Month. Lupus and Allied Diseases Association, Inc., MMRI, Utica, NY.

2022: 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)**

https://www.mmri.edu/category/news-2022/
Website link for press release for MMRI on Lupus and Allied Diseases, Inc support of 4 separate projects at MMRI.

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

Nothing to report.

- **Inventions, patent applications, and/or licenses**

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- Nothing to report.

- **Other Products**

Development of the SHP2 T-cell specific deletion lupus-prone mice: SHP2fl/flLckCre/+- C57BL/6lpr/lpr mice

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Maria Kontaridis
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 at BIDMC.

Name: Samantha LeSommer
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.

Name: Vasileios Kyttaris, M.D.
Project Role: Co-Investigator
Research Identifier: 0000-0001-7652-3826
Nearest person month worked: 0.636
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.

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

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

Title: Splenic Modulation of SHP-2 Activity as a Therapeutic Option for Systemic Lupus Erythematosus
Time Commitments: 0.6 calendar
Supporting Agency: NIH/NIAMS R21AR081535/McCarthy
Address: NIAMS 1 AMS Circle, Bethesda, MD 20892-3675
Contracting/Grants Officer: Marie Mancini
Performance period: 03/01/2023 – 02/28/2024
Level of funding:
Project Goal: In this proposal, we will investigate a novel paradigm for the treatment of lupus, whereby modulators of the protein tyrosine phosphatase SHP-2 are delivered directly to the spleen with the goal of eliciting a systemic therapeutic effect.
Specific Aims: Aim 1. To generate and characterize eSENT-based nanomaterials for the modulation of SHP-2 activity in the spleen. SHP-2 is integrally involved in the pathology of SLE. We thus propose to use our drug delivery platform designed specifically for splenic accumulation to localize modulators of this important pathway to one of the organs central to this disease. In this Aim we will generate nanoparticles containing a small molecule inhibitor, ASO, or siRNA for SHP-2, and coat them with our biomimetic lipids. These materials will each be fully characterized for size, colloidal stability, zeta potential, and drug release profiles. Modulatory efficacy will be investigated in vitro using relevant cell types.
Aim 2. Examination of spleen-targeted therapeutics as a novel option in the treatment of SLE. Using a murine model of SLE (MRL/*lpr*), we will investigate the therapeutic efficacy of the splenic modulation of SHP-2 activity. In these experiments, cohorts of mice will be administered the spleen-targeted materials or respective controls, and therapeutic efficacy will be determined longitudinally. Analyses will include targeting ability, dosing, and inhibitory efficacy, with the latter being determined by gross physiological changes (i.e., splenomegaly,
nephritis, etc.), and changes in inflammatory cytokines. These results will be correlated with both histological and molecular-based assays of the splenic tissues.
Overlap: None

Title: Atrial Remodeling Precedes Ventricular Dysfunction in Proteotoxic Cardiac Disease
Time Commitments: 0.24 calendar
Supporting Agency: AHA/23TPA1065811/University of Alabama at Birmingham (PI - Namakkal-Soorappan, R)
Address: 7272 Greenville Avenue, Dallas, TX
Contracting/Grants Officer: Michael Matthews
Performance period: 07/01/2023 – 06/30/2026
Level of funding:

Project Goal: We propose to determine whether proteotoxicity impairs electrical signaling and induces atrial remodeling and if enhancing autophagy-dependent PQC preserves electrical function and prevents atrio-ventricular remodeling. The outcome of this transformative award will enable us to systematically plan on translational research to explore whether premature atrial remodeling that precedes ventricular dysfunction over time.

Specific Aims: Aim 1: Determine whether proteotoxicity impairs electrical signaling and induces atrial remodeling. *We hypothesize that aggregation of proteins, if unresolved, leads to sequestration of CaMKII into aggregates, and thereby impairs HCN/RyR2 signaling, and promotes atrial remodeling and dysfunction*. We will assess (a) whether atria are susceptible (more than ventricle) to a proteotoxic insult, (b) whether uncontrolled oxidation of CaMKII results in its sequestration into protein aggregates, thereby impairing RyR2/HCN signaling and (c) what is the subsequent impact on Ca^{2+} transients in atrial myocytes using *IonOptics* technology [23-26].

Aim 2: Determine whether enhancing autophagy-dependent PQC preserves electrical function and prevents atrio-ventricular remodeling. *We hypothesize that an early activation of autophagy will prevent proteotoxicity, thereby preserving electrical signaling and atrial function*. We will investigate whether (a) augmentation of ATG5 or P62 could prevent proteotoxicity, improve PQC and prevents atrial remodeling at early age (6 & 12 weeks) in the hR120GCryAB (TG) mice, and (b) preserving atrial structure and function prevents the onset of ventricular remodeling and dysfunction in TG mice.

Overlap: None

Closed Grants:

Title: Role of RhoA in the Molcular Pathogenesis of Heart Disease
Time Commitments: 0.60 calendar
Supporting Agency: NHLBI
Address: NHLBI Bldg 31, 31 Center Drive, Bethesda, MD 20892
Contracting/Grants Officer: Bishow B. Adhikari
Performance period: 04/15/2010 – 12/31/2022
Level of funding:
Project Goals: In this project, we will 1) examine RhoA-mediated paracrine signals that drive myofibroblast transformation and activation; 2) determine if RhoA signaling is necessary and sufficient for direct myofibroblast function both in vitro and in vivo using a novel inducible, fibroblast-specific transgenic approach; 3) utilize novel nanoparticle targeting technology to deliver cell specific inhibitors of RhoA effectors to ameliorate fibrosis and to prevent cardiac disease progression.
Specific Aims:
Overlap: None

What other organizations were involved as partners?

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

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: *N/A*

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.

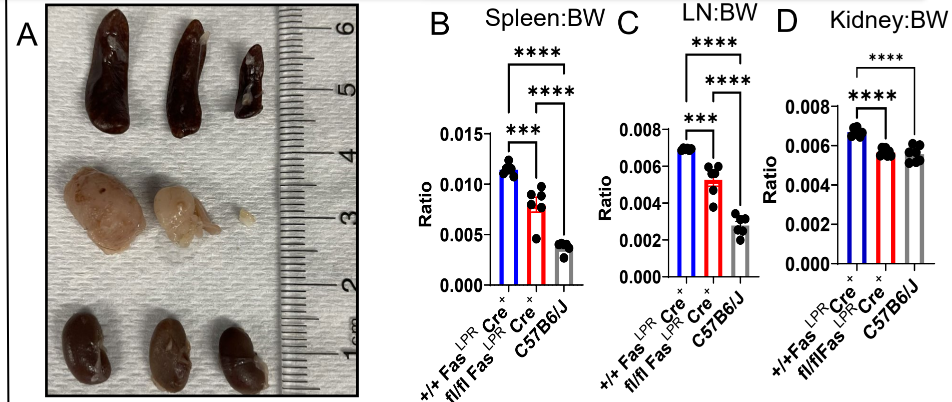

Figure: Deletion of SHP2 in T cells in SLE FasLPR mice reduces, but does not ameliorate, SLE disease severity. (A) Representative photographs and corresponding quantification of (B) splenomegaly, (C) lymphomegaly and (D) renalmegaly in SHP2+/+ FasLPR Cre+, SHP2fl/fl FasLPR Cre+, and C57B6/J mice. N = 6 female mice / genotype. Data represent means +/- SEM, with one way ANOVA and Tukey post-hoc when significant. *p<0.05, **p<0.01, *p<0.001, p<0.0001.

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: 10/30/2023

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.

☑ We have started this validation in 2023 and expect to complete the studies in 2024.

CY24-25 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

- None at this time; we are on track to complete the project on time.

Budget Expenditure to Date

Projected Expenditure: $375,000
Actual Expenditure: $364,692

9. APPENDICES:

N/A