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TITLE: The Role of the Interferon-Gamma-Jak/STAT Pathway in Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR: Stanley Louis Bridges, Jr., MD, PhD

CONTRACTING ORGANIZATION: University of Alabama at Birmingham

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14. ABSTRACT
This project addresses the hypothesis that elevated and/or altered IFN-γ signaling within selective subsets of mononuclear cells promotes disease severity in RA. This study developed from our novel observation that in peripheral blood the expression levels of interferon gamma receptor 1 (IFNGR1) is associated with RA and the expression levels of IFNGR2 correlates significantly with the degree of radiographic damage in RA patients. The aims of this proposal are: (1) To identify the specific circulating cell type in which IFNGR expression is elevated in RA. Using a combination of molecular biological and immunological approaches, we will analyze the expression levels of IFNGR1 and IFNGR2 in monocytes, naïve and memory B cell populations, naïve and memory T cell populations including T-follicular helper cells, Treg cells and T helper effector subpopulations (Th1, Th17 and Th17/1). (2) To determine the outcome of IFNGR signals by assaying the activation of IFN-γ induced STAT1 and changes in activation of STAT3 and STAT5 in RA versus healthy controls, at basal level and following stimulation with cytokines such as IL-2, IL6 etc. (3) To determine the molecular mechanism and outcome of attenuated IL-2 induced activation of STAT5 in specific subpopulations of T cells in RA. The information to be gained can potentially help to identify new cell signaling targets, perhaps cell-type specific, for RA and other autoimmune diseases, and perhaps malignancies. This in turn may help to develop new drugs that are more targeted, either to particular cell types or patients in whom these cell types are most important to the disease. Ultimately, this may lead to more effective, and safer drugs with fewer adverse effects.

15. SUBJECT TERMS
Rheumatoid arthritis; Autoimmunity; T lymphocyte subsets; Cell Signaling; Interferon-gamma; STAT1; STAT3; STAT5: Interleukin-2

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1. INTRODUCTION

This project addresses the role of STAT signaling in circulating immune cells on susceptibility to rheumatoid arthritis, its severity, and potentially response to treatment. This study developed from our novel observation that in peripheral blood the expression levels of interferon gamma receptor 1 (IFNGR1) is associated with RA and the expression levels of IFNGR2 correlates significantly with the degree of radiographic damage in RA patients. The aims of this proposal and our progress toward them, are laid out in Section 3 below.

2. KEYWORDS

Rheumatoid arthritis; Autoimmunity; T lymphocyte subsets; Cell Signaling; Interferon-gamma; STAT1; STAT3; STAT5: Interleukin-2

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1 (specified in proposal)	**Timeline**	**Status**
Major Task 1 - To identify the circulating cell types in which IFNGR expression is upregulated in RA and determine how it relates to disease activity.	Months	**Completed, % complete, or Future Work**
Subtask 1 – To recruit 250 participants for Major Tasks 1, 2, and 3. This includes 150 with RA (50 each with low disease activity/remission; moderate disease activity; high disease activity); 50 with multiple sclerosis; 50 healthy controls. Collect data, including disease activity, medications, demographics, etc.	Begin Month 4 (after IRB approval); end Month 27	We have screened ~203 RA participants and enrolled 138, and have enrolled ~63 participants with MS and ~30 healthy controls. We have exceeded our goals for MS, and will continue to recruitment of RA and HC.
Subtask 2 – Perform FACS and quantitative real-time PCR (qRT-PCR) to measure IFNGR1 and IFNGR2 expression in multiple T cell and B cell populations and monocytes.	Begin Month 4 and proceed in batches; end Month 27	Completed on ~60% of participants enrolled through Year 3
Subtask 3 - Assess IFN-γ receptor protein levels in cell subsets (including Th1, Th17, Th17/1, etc.) in RA with different disease activity (remission/low; moderate; high), MS, and controls.	Begin Month 4 and proceed in batches; end Month 27	Completed on ~40% of participants enrolled through Year 3.
Subtask 4 - We will compare results among patients with RA with different disease activity (remission/low; moderate; high), MS, and controls.	Begin Month 7 and proceed throughout the funding period	Comparison of results among these three groups has been completed on ~40% of participants enrolled through Year 3.
Milestone(s) Achieved		
Local IRB Approval	3	Year 3: 06-03-2019
HRPO Approval	6	Year 3: HRPO Log Number A-19648 - approved on

		06-20-2019
Present results at scientific meetings	18, 24	As reported in Year 2 progress report; none in Year 3
Publish results in scientific journals	24, 30	Manuscript in preparation
Specific Aim 2 (specified in proposal)		
Major Task 2 - To determine the effect of upregulated IFNGR expression on IFN-γ-induced activation of STAT1, STAT3, and STAT5 signaling in peripheral blood cell subsets in RA.		
Subtask 1 - Compare the level of activation of STATs (as assessed by the degree of phosphorylation) in peripheral blood naïve and memory CD4+ T cells, Th effector populations, Treg, naïve and memory B cells, and monocytes at baseline and following stimulation with IFN- γ in RA (n=150) using phospho-flow cytometry.	Begin Month 4 and proceed in batches; end Month 27	This task is ongoing. We have completed initial analysis of peripheral blood populations on about 30% of participants. In addition, we have added analysis of an existing dataset from the TETRAD Study.
Subtask 2 - Determine if altered STAT1 (or STAT3 or STAT5) activation leads to differences in nuclear localization of STAT1 (or STAT3 or STAT5) followed by changes in cellular morphology in different mononuclear subpopulations using quantitative image analysis and flow cytometry (Imagestream).	Begin Month 4 and proceed in batches; end Month 27	This subtask has not been performed because our studies are revealing that IFN-γ induced STAT1 activation in RA is lower than HC. Thus, based on our preliminary data, pursuit of this aim will not provide meaningfully to our understanding of RA.
Subtask 3 - Determine if IFN-γ signals alter the ability of other cytokines (IL-2, GM-CSF, IL-6, IL-23) to activate their respective STATs.	Begin Month 4 and proceed in batches; end Month 27	The work on IFN-γ induced STAT1 activation in HC, RA and MS is largely completed. We have not yet examined responses to IL-6, GM-CSF and IL-23 alone or in conjunction with IFN-γ. As part of this study, we identified a dataset (TETRAD) that would help address the role of cytokine-stimulated STAT activation in T and B cells in RA. See details in Major Activities below.
Present results at scientific meetings	Mos. 18, 24	None
Publish results in scientific journals	Mos. 24, 30	None, but

		manuscript in preparation.
Specific Aim 3 (specified in proposal)		
Major Task 3 - To determine the molecular mechanism and outcome of attenuated IL-2 induced activation of STAT5 in specific subpopulations of T cells in RA.		
Subtask 1 – Determine whether altered IL-2 mediated activation of STAT5 in subpopulations of T cells in RA contributes to disease pathogenesis.	Begin Month 4 and proceed in batches; end Month 27	Through Year 3, we analyzed peripheral blood from 30% of participants.
Subtask 2 – Determine the outcome of attenuated IL-2 mediated activation of STAT5 on Th effector cell and regulatory cell expansion and function.	Begin Month 4 and proceed in batches; end Month 27	We are no longer pursing this subtask as additional data from our lab suggests IFN-γ attenuated STAT-5 activation is not a consistent finding.
Present results at scientific meetings	18, 24	None
Publish results in scientific journals	24, 30	None

1. Major activities

Collection of Blood samples from RA, MS (multiple sclerosis) and healthy controls (HC).
As stated in the Table above, during Year 3, we continued to enroll RA patients, MS patients, and healthy controls.

Training of graduate students:
Two graduate students, Mr. Vishal Sharma (Ph.D. Immunology program) and Mr. Brandon Pope (MD/Ph.D. program) have continued to enhance their training in the techniques needed for these studies.

2. Specific objectives.

Aim 1. To identify the circulating cell types in which IFNGR expression is upregulated in RA and determine how it relates to disease activity.

IFNGR expression in mononuclear cell subpopulations is not different between RA and MS. Our results show no differences in expression of IFNGR1, IFNGR2, IL2RA, IL2RB, IL2RG in CD4 and CD8 naïve, effector, central memory and effector memory cells T cells and regulatory T cells between RA and MS (Fig. 1). Similarly, we found no significant differences in expression in these same analytes between RA and MS B cells and monocytes (data not shown).

IL-2 enhances IFN-γ induced STAT1 in RA CD4 populations. Based on preliminary data, we expected that IFN-γ attenuated IL-2 induced activation of STAT5. This data was not sustained. However, our results reveal a novel biology related to integration of IFN-γ and IL-2 signals. We found that IL-2 co-stimulation enhanced IFN-γ induced activation of STAT-1 in RA CD4 naïve, central memory and effector populations but not CD4 effector memory populations (Figure 4). Co-stimulation of CD4 populations from HC and MS did not increase STAT-1 activation over that if IFN-γ alone. However, co-stimulation resulted in sustained IFN-γ induced STAT1 activation in HC and MS for a period up to 4h (Figure 4). IL-2 had no effect on IFN-γ induced activation of STAT-1 in any CD8 T cell population (data not shown). IFN-γ had no effect on IL-2 induced STAT5 or STAT-3 (data not shown).

Aim 2. To determine effect of upregulated IFNGR expression on IFN-γ-induced activation of STAT1, STAT3, and STAT5 signaling in peripheral blood cell subsets in RA.

IFN-γ induced activation of STAT1 is lower in CD4 and CD8 T cells populations for RA compared to HC and MS. Our original prediction was that IFN-γ induced activation of STAT-1 will be greater in RA CD4 and CD8 T cell populations than HC. Remarkably, we find that RA T cell populations respond less efficiently to IFN-γ than both HC and MS (Figure 2 and data not shown). The lower activation of IFN-γ

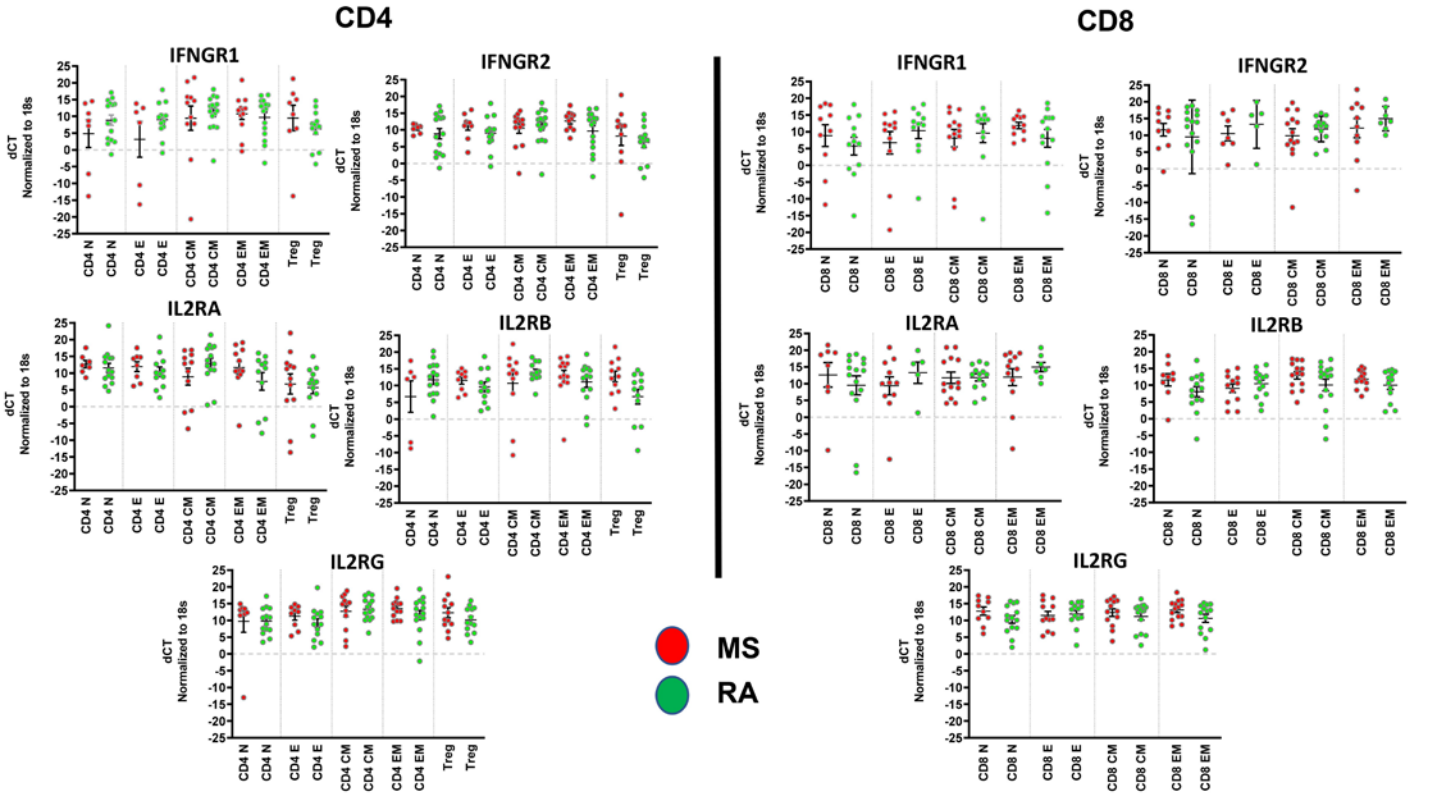

Figure 1. Expression of IFNGR and IL2 receptor on CD4 and CD8 T cell populations is not different between RA and MS. The expression of IFNGR1, IFNGR2, IL2RA, IL2RB and IL2RG was determined in CD4 and CD8 T cell populations (N = naïve; E = Effector; CM = Central Memory; EM = Effector Memory). and Treg cells as indicated. The results show no difference in expression of transcripts to any of the receptor chains between MS and RA.

induced activation of STAT1 in RA was observed for all time points of stimulations from 15 mins to 4 h. The lower response in RA compared to MS is not due to levels of receptor expression (Figure 1).

Higher IFN-γ induced activation of STAT1 in RA patients with active disease. We examined IFN-γ induced activation of STAT1 after stratifying patients into remission RA (CDAI <2.8) and active RA (CDAI>2.9). Overall, the activation of IFN-γ induced STAT1 was slightly higher in CD4 and CD8 T cell populations patients with active disease, but the difference was not significant (Figure 3 and data not shown). IFN-γ efficiently stimulated STAT1 in both remission and active RA patients.

Associations of cytokine-induced STAT activation in RA susceptibility, severity, and treatment response. As part of the current DoD PRMRP project, we gained access to a wealth of data to analyze through the TETRAD (Treatment Efficacy and Toxicity in Rheumatoid Arthritis Database and repository) cohort– see Appendix for details. We studied functional immune signaling capacity in multiple pathways to examine disease mechanism and treatment response in RA using single-cell network profiling (SCNP).

We used existing data from this innovative systems immunology approach to help

address the hypotheses proposed in this CDMRP grant. We assessed existing data obtained simultaneously on signaling nodes (Jak/STAT signaling readouts modulated by cytokines and other stimuli) in 21 immune cell subsets. We studied data from 194 RA patients and 41 controls, including 146 well-characterized RA patients prior to, and 6 months after, initiation of methotrexate or biologic agents. Our analyses found strikingly attenuated signaling capacity in RA patients in IFNα stimulation followed by measurement of phosphorylated STAT1 [IFNα stimulation using phosphorylated STAT1 as a readout] in six immune cell subsets (see Appendix 1 for details). Multiple nodes showed negative association with disease activity, including IFNα→STAT5 signaling in naive and memory B cells. In contrast, IL-6-induced STAT1 and STAT3 activation in central memory CD4-negative T cells showed a positive association with disease activity. Multiple nodes were associated with treatment response, including IFNα→STAT1 in monocytes and IL-6→STAT3 in CD4+ naive T cells. These findings demonstrate that IFN stimulated STAT activation in immune cells plays an important role in RA, a key hypothesis of this CDMRP grant. This manuscript is under review.

Aim 3. To determine the molecular mechanism and outcome of attenuated IL-2 induced activation of STAT5 in specific subpopulations of T cells in RA

IL-2 induce activation of STAT5 is equivalent in HC and RA. The results above show that IL-2 enhanced IFN-γ induced STAT-1 in RA, but not in HC and MS. We therefore asked if there were differences in activation of STAT-5, the major STAT activated by IL-2. Our results show that in both CD4 and CD8 T cells, the activation of STAT5 (pY694) is equivalent in HC and RA for all time points (15 min to 4h). Remarkably, IL-2 induced activation of STAT-5 was much greater in MS than either HC or RA. This result indicates to us that the enhancement of IFN-γ induced STAT-1 by IL-2 co-stimulation could represent a biology associated with RA disease.

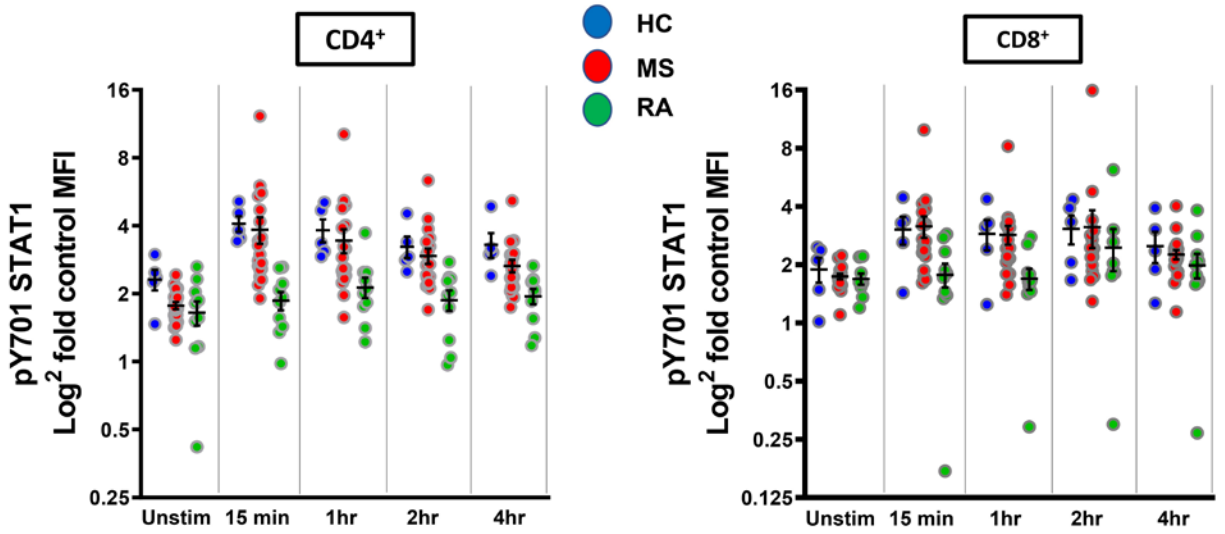

Figure 2. IFN-γ induced activation in RA is lower in CD4 and CD8 T cells from RA compared to HC and MS. PBMC were unstimulated or stimulated with 50 ng/ml of IFN-γ for time periods as indicated. The levels of pSTAT1 (pY701) in CD4 and CD8 T cells (data shown) and sub populations (naïve, memory, effector, Treg – not shown) was determined. Each dot represents an individual patient. Data is mean±SEM.

3. Significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

Major findings:

1. IFNγ receptor expression in CD4 and CD8 T subsets in RA and MS are not different.
2. IFN-γ induced activation of STAT-1 in RA CD4 T cell populations is lower than HC and RA.
3. IFN-γ induced activation of STAT-1 in RA may be different between patients with active disease and those in remission. The difference may become more evident following using unbiased computational analysis such as T-SNE and FlowSOM (see example in Figure 5 below).
4. IL-2 co-stimulation enhances IFN-γ induced activation of STAT1 in CD4 T cell populations from RA but not HC and MS.
5. In HC and MS, IL-2 co-stimulation contributed to sustained activation of IFN-γ induced activation of STAT-1.
6. Conventional analysis of flow cytometry data limits the ability to focus on heterogeneity of expression of markers common in human. This is because the analyses reflect analysis of expression of two markers at a time with sequential gating. Advanced computational analyses favor un-biased and favor visualization of multi-dimension marker expression profile. These include t-SNE (t-distributed scholastic neighbor embedding) and FLOW-SOM, an algorithm that clusters with Self-Organizing-Maps. We have begun to use these for analysis data obtained from the experiments performed in this proposal, an example of which is shown in Figure 6. As the representative analysis shows, t-SNE clustering reveals similarities and differences between HC and MS that would have been missed by standard flow cytometry analysis. Similarly, FLOW-SOM identified striking differences between HC and MS with respect to nodes that are activated by IL-2. We will be performing such analyses for all of the data from this study.
7. There is strikingly attenuated signaling capacity in RA patients in IFNα stimulation followed by measurement of phosphorylated STAT1 [IFNα stimulation using phosphorylated STAT1 as a readout] in six immune cell subsets (see Appendix 1 for details).
8. Multiple nodes showed negative association with RA disease activity, including IFNα→STAT5 signaling in naive and memory B cells. In contrast, IL-6-induced STAT1 and STAT3 activation in central memory CD4-negative T cells showed a positive association with RA disease activity.
9. Multiple nodes were associated with treatment response, including IFNα→STAT1 in monocytes and IL-6→STAT3 in CD4+ naive T cells.

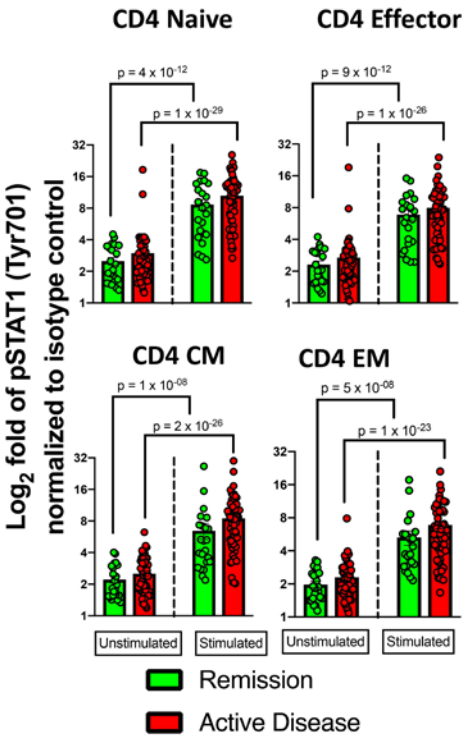

Figure 3. IFN-γ induced activation in CD4 T cells populations for remission and active disease RA patients. RA patients were stratified into remission (CDAI,2.8 and active

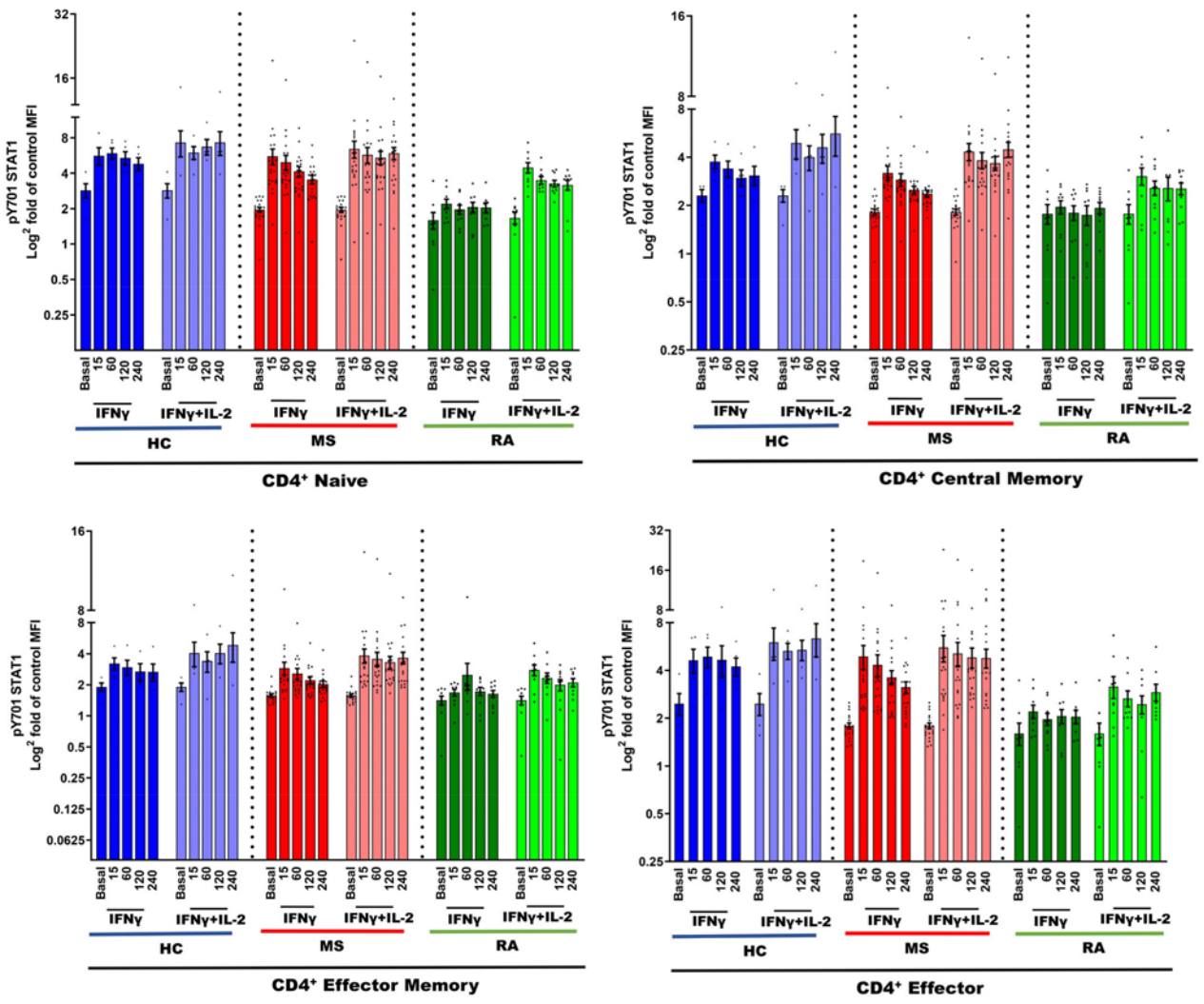

Figure 4. IL2 enhanced IFN-γ induced STAT-1 activation in select CD4 population from RA but not in HC and MS. PBMC were unstimulated of stimulated with 50 ng/ml IFN-γ in presence or absence of 10 ng/ml IL-2 for the indicated times. The activation of STAT-1 in gated CD4 populations was determined. Results show that IL-2 enhanced IFN-γ induced activation of STAT-1 in RA CD4 naïve, central memory and effector populations. In HC and MS, co-stimulation with IL-2 did not enhance IFN-γ induced STAT-1 activation but promoted its sustained activation over four hours. Each dot represents an individual patient.

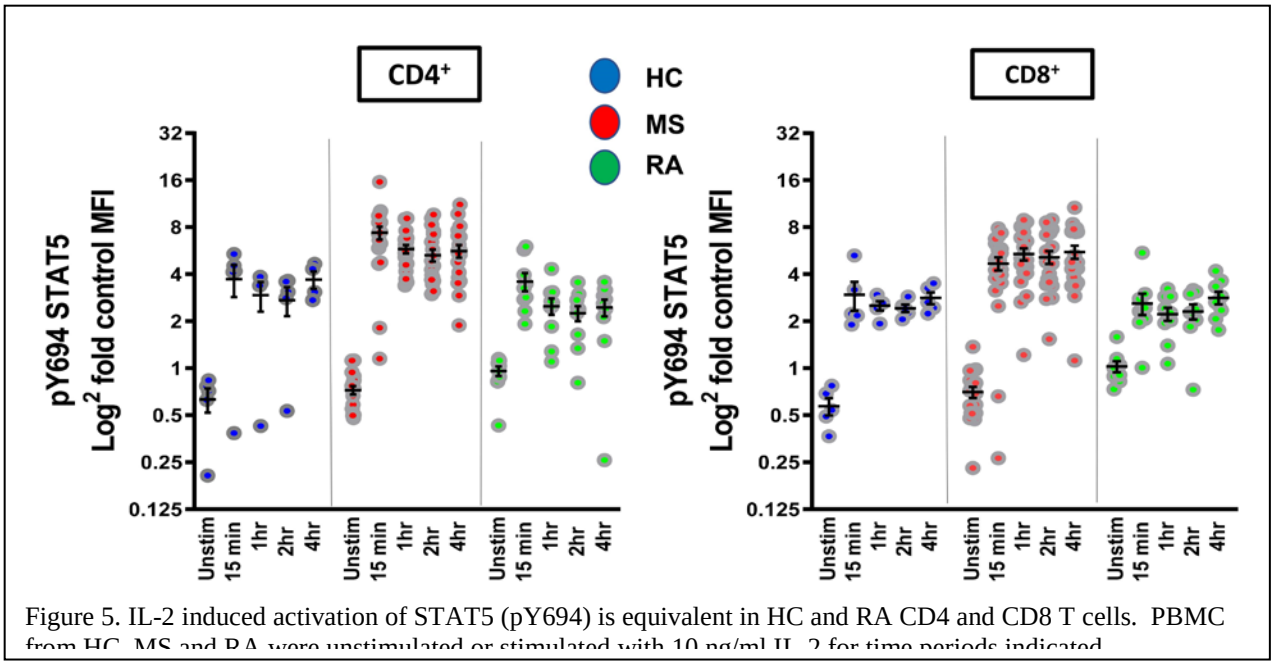

Figure 5. IL-2 induced activation of STAT5 (pY694) is equivalent in HC and RA CD4 and CD8 T cells. PBMC from HC, MS and RA were unstimulated or stimulated with 10 ng/ml IL-2 for time periods indicated

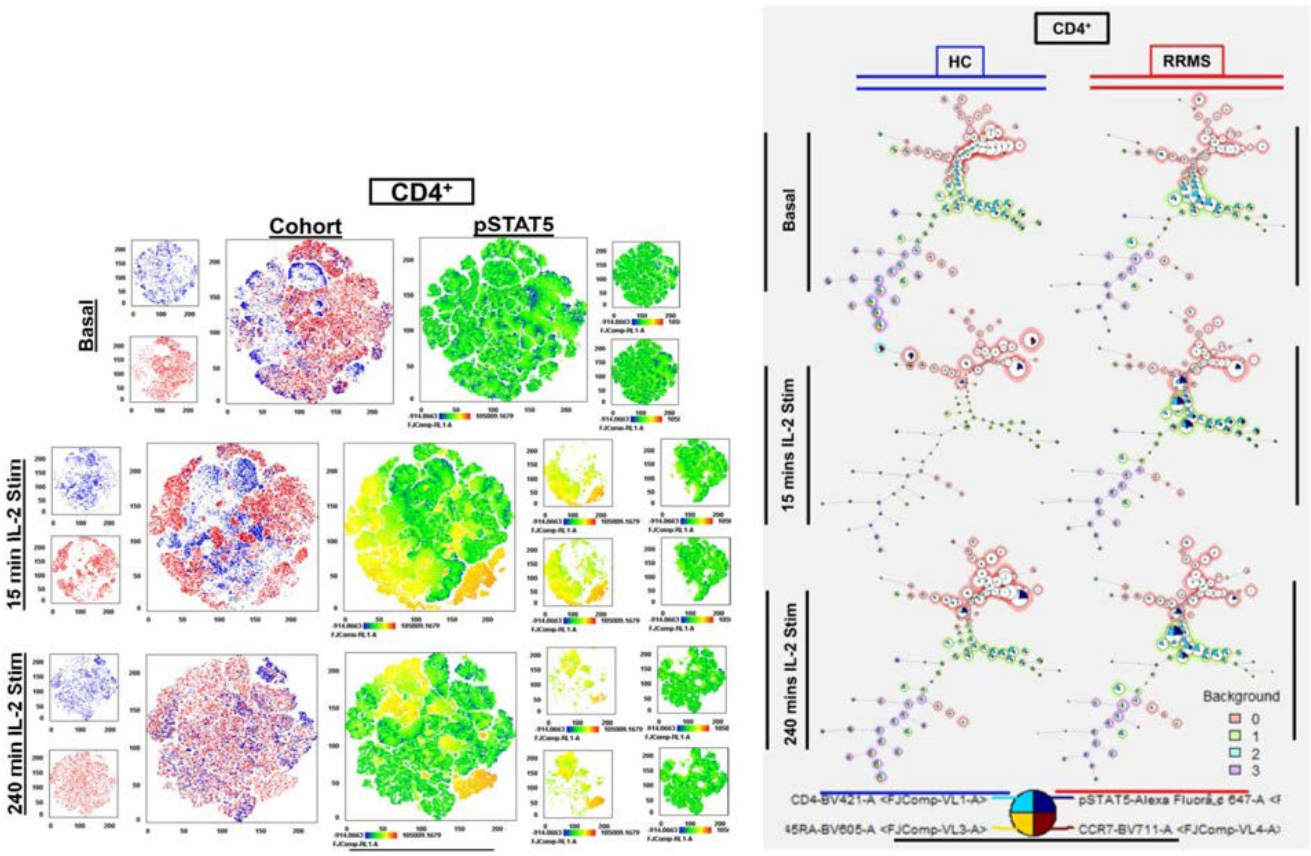

Figure 6. T-distributed stochastic neighbor embedding (t-SNE) and Flow self-organizing map analysis of unbiased analysis of cytometry data. T-SNE (upper panel) of HC (blue) and RRMS (red) at basal and after stimulation with IL-2. For these analyses, data from >15 HC and RRMS patients each were concatenated. The analysis reveals striking differences in clustering of populations and activation of STAT5 between HC and RRMS. FLOW-SOM (lower panel) is a clustering spanning tree analysis. The distance between nodes reflects dissimilarity. Each major node is colored similarly. The size f sphere reflects number of events. HC and RRMS were concatenated and simultaneously analyzed. Here again the data reveals striking differences between HC and RRMS. Such differences were not apparent by standard flow cytometry analysis. For both t-SNE and FLOW-SOM analyses, the number of events per individual were normalized such that there is no overrepresentation. This will be performed for all data in this study.

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

While this project was not intended to provide training and professional development opportunities, it has been an excellent training vehicle for several learners. This project provided opportunities for training for two PhD students, Vishal Sharma, and Brandon Pope. These trainees have benefited from mentorship from Drs. Raman and Bridges.

Mr. Sharma and Pope presented their studies at the American Association of Immunologists annual meeting (May 4-8, 2018, Austin TX) and the Southeastern Immunology Conference, June 17-18, 2018 (UAB, Birmingham). Mr. Pope was awarded a poster award for his abstract at the AAI annual meeting.

5. Describe how the results were disseminated to communities of interest.

As noted above, we have analyzed existing data from the TETRAD cohort as part of Specific aim 2. This was uploaded for public distribution through biorxiv, a pre-print server (see Appendix 1)).

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

We will continue to enroll as many patients as possible during the no-cost extension. In addition, we will refine the analyses and prepare for manuscript submission, the data shown in Figures 1 through 6. In addition, we will continue to further study the incredible wealth of existing data from the TETRAD study. We anticipate that the preprint uploaded to bioxriv will be published in a peer reviewed journal soon.

4. IMPACT

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

We have identified potential biomarkers of RA disease activity or treatment response.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

There were no significant changes to the approach during this reporting period.

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

Enrollment has been slower than anticipated. We have nearly reached our recruitment goals, which we are confident will met during the no-cost extension.

Changes that had a significant impact on expenditures

There were no changes during the reporting period that had a significant impact on expenditures.

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

No significant changes.

Significant changes in use or care of vertebrate animals.

No significant changes.

Significant changes in use of biohazards and/or select agents

No significant changes.

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

Journal publications.
No peer reviewed publications to report.
See Appendix 1 for biorxiv preprint which will be submitted for publication.

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers, and presentations.

Presentations between Sep 1 2018 and Aug 31 2019 are shown below.

Sharma, V, Pope, B., Boland, M., Reynolds, R., Sun, D, Bridges, S. L. and Raman, C. Enhanced interferon gamma response contributes to disease remission in Rheumatoid *Presented at the 7th Annual Southeastern Immunology Symposium; June 16h to 18, 2018, Birmingham, AL.*

Pope, B., Sharma, V., Boland, M., Meador, W., Reynolds, R., Bridges, S.L., and Raman, C. IL-2 enhances IFNγ signals in subpopulations of T and B lymphocytes from treatment naïve relapsing remitting multiple sclerosis (RRMS) patients. *Presented at the 7th Annual Southeastern Immunology Symposium; June 16h to 18, 2018, Birmingham, AL.*

Pope, B., Sharma, V., Boland, M., Meador, W., Reynolds, R., Bridges, S.L., and Raman, C. IL-2 enhances IFNγ signals in subpopulations of T and B lymphocytes from treatment naïve relapsing remitting multiple sclerosis (RRMS) patients. *Presented at the* Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) 2019 Forum*; February 28 – March 2, 2019, Dallas, TX*.

Pope, B., Sharma, V., Bridges, S.L., and Raman, C., Meador, W. IL-2 enhances IFNγ signals in subpopulations of T and B lymphocytes from treatment, naïve relapsing remitting multiple sclerosis (RRMS) patients. *Presented at the Society for Neuroscience Annual Meeting; October 19 - 23, 2019, Chicago, IL.*

- Website(s) or other Internet site(s)
  Nothing to report.

- Technologies or techniques
  Nothing to report.

- Inventions, patent applications, and/or licenses
  Nothing to report.

- Other Products
  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*S. Louis Bridges, Jr., MD, PhD*
Project Role:	*Principal Investigator*
Researcher Identifier:	*ORCID ID: 0000-0003-3785-1389*
Nearest person month worked:	*1.2 calendar months*
Contribution to Project:	*Dr. Bridges has provided overall guidance for this project. He leads the effort to identify patients to be enrolled, oversees all studies in Aim 1 and works closely with Dr. Raman on all lab-based studies in the project. He supervises the Laboratory Manager (Mr. Wanzeck) and all non-lab-ased study personnel. He oversees the collection of clinical data, processing of blood samples, and all data management aspects of the project.*

Name:	*Chander Raman, PhD*
Project Role:	*Co-Investigator*
Nearest person month worked:	*4.8 calendar months*
Contribution to Project:	*Dr. Raman is critical to the success of this project. He directs and oversee the functional/mechanistic studies. He directly oversees all lab-based research personnel except for Mr. Wanzeck. Dr. Raman works closely with the PI on all three Aims of this project and is key to data analysis, manuscript preparation and submission.*

Name:	*Richard Reynolds, PhD*
Project Role:	*Co-Investigator*
Nearest person month worked:	*1.2 calendar months*
Contribution to Project:	*Dr. Reynolds has provided direct into the study design,*

	overall analysis plan, and statistical analyses for the project.
Name:	*Keith Wanzeck, BS*
Project Role:	*Laboratory Manager Nearest person month worked: 1.2 calendar months*
Contribution to Project:	*Mr. Wanzeck is be responsible for coordination of blood draws, and processing and routing of blood samples and biospecimens. He serves as a liaison between Dr. Bridges' lab and Dr. Raman's lab.*
Name:	*Stephanie Ledbetter, MS*
Project Role:	*Program Manager*
Nearest person month worked:	*1.2 calendar months*
Contribution to Project:	*Ms. Ledbetter is responsible for all regulatory issues, including the UAB IRB submissions and renewals, as well as HRPO issues. She also coordinates other aspects of the study such as laboratory meetings, and other logistic issues.*
Name:	*Vishal Sharma*
Project Role:	*Graduate student*
Nearest person month worked:	*4 calendar months*
Contribution to Project:	*As part of his PhD studies, Mr. Sharma is performing dissertation research on this project, focusing on RA. He works on performing the assays, data analysis, and presentation of results from this project, and beginning to plan follow up studies.*
Name:	*Brandon Pope*
Project Role:	*MD/PhD student*
Nearest person month worked:	*4 calendar months*
Contribution to Project:	*As part of his PhD studies, Mr. Pope is performing dissertation research on this project, focusing on MS. He works on performing the assays, data analysis, and presentation of results from this project, and beginning to plan follow up studies.*

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

There were changes in support for Dr. Bridges as outlined below. There were no significant changes to report for other investigators.

<u>Changes from Year 2 to Year 3 for Dr. Bridges</u>

The following support ended:

P30 AR048311 (PI: Mountz, JD) 09/28/01 - 08/31/19 1.2 CM
NIH/NIAMS No Cost Extension
Rheumatic Diseases Core Centers: Administrative Core
Role: Associate Director of the RDCC

P60 AR064172 (Contact PI: Bridges, SL Jr.; PI: Saag, KG) 09/16/13 - 07/31/19 2.4 CM
NIH/NIAMS UAB Multidisciplinary Clinical Research Center

The following funding was added:

U19 AI142737 (PI: FE Lund) 04/01/19 – 03/31/24 0.6 CM
NIH/NIAID
Cooperative Centers on Human Immunology: Tissue and organ specific human B cell immunity (Lund). Core B: The Human Tissue Collection, Processing and Repository Core (Nellore and Bridges, Co-Core Leads). The major goals of this U19 Core is to advance understanding of humoral immunity by evaluating B cells in eight different human tissues. Aims are: 1. To collect tissues from deceased organ donors via the Alabama Organ Center; 2. To isolate and cryopreserve leukocytes from the tissues; 3. To establish a searchable biorepository of these samples with which to support the research objectives of the U19. The goals of Project 1: Development and Maintenance of Human Glycan and Phospholipid Antibody Repertoires are to define mechanisms controlling maturation of the human natural B lymphocyte repertoire and its tissue distribution. We will achieve this goal by completing a targeted analysis of B cells reactive with conserved carbohydrate and phospholipid T lymphocyte-independent antigens associated with clinically relevant bacteria and xenoantigens.

U19 AI142737 (Kearney) 04/01/19 – 03/31/24 0.48 CM
NIH/NIAID
Cooperative Centers on Human Immunology: Tissue and organ specific human B cell immunity (Lund): Project 1: Development and Maintenance of Human Glycan and Phospholipid Antibody Repertoires (Kearney and King, Co-Project Lead; Bridges – Co-Investigator). The major goals of this U19 Core is to advance understanding of humoral immunity by evaluating B cells in eight different human tissues. Aims are: 1. To collect tissues from deceased organ donors via the Alabama Organ Center; 2. To isolate and cryopreserve leukocytes from the tissues; 3. To establish a searchable biorepository of these samples with which to support the research objectives of the U19. The goals of Project 1: Development and Maintenance of Human Glycan and Phospholipid Antibody Repertoires are to define mechanisms controlling maturation of the human natural B lymphocyte repertoire and its tissue distribution. We will achieve this goal by completing a targeted analysis of B cells reactive with conserved carbohydrate and phospholipid T lymphocyte-independent antigens associated with clinically relevant bacteria and xenoantigens.

R56 AI143994 (Bridges; Ippolito [Multiple PI]) 04/01/19 – 03/31/20 0.96 CM
NIH/NIAID
Molecular, Functional and Structural Analyses of Anti-PAD Antibodies in Rheumatoid Arthritis
The major goals of this project are: 1. To identify and quantify the molecular species of anti-PAD IgG and IgA antibodies circulating in serum of RA patients and to identify the B-cell subset of origin. 2. To define the molecular correlates of anti-PAD binding and modulation of PAD4 activity. 3. To determine the structure of PAD-containing immune complexes and the mechanisms by which anti-PAD4/3 cross-reactive antibodies mediate PAD4 activation.

NCATS CD2H Idea Challenge (Bridges) 06/01/19 – 05/31/20 0.12 CM
Oregon Health and Science University/NIH/NCATS
Automated Scoring of Radiographic Damage in Rheumatoid Arthritis
The major goal of this project is to develop an automated method to quickly and accurately quantify the degree of joint damage associated with rheumatoid arthritis (RA). Based on radiographs of the hands and feet, a novel, automated scoring method could be applied broadly for patient care and research. We

challenge participants to develop algorithms to automatically assess joint space narrowing and erosions using a large set of existing radiographs with damage scores generated by visual assessment of images by trained readers using standard protocols.

RA2 DREAM Challenge (Bridges) 07/01/19 – 06/33/20 0.12 CM
BMS Foundation
Automated Scoring of Radiographic Damage in Rheumatoid Arthritis
The major goal of this project is to develop an automated method to quickly and accurately quantify the degree of joint damage associated with rheumatoid arthritis (RA). Based on radiographs of the hands and feet, a novel, automated scoring method could be applied broadly for patient care and research. We challenge participants to develop algorithms to automatically assess joint space narrowing and erosions using a large set of existing radiographs with damage scores generated by visual assessment of images by trained readers using standard protocols.

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Not applicable.

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.

Appendix 1. Pre-print manuscript uploaded to biorxiv.

A Systems Immunology Approach Identifies Cytokine-Induced STAT Signaling Pathways Critical to Rheumatoid Arthritis Disease Activity and Treatment Response.
Jason Ptacek, Rachael E. Hawtin, Dongmei Sun, Brent Louie, Erik Evensen, Barbara Mittleman, Alessandra Cesano, Guy Cavet, Clifton O. Bingham III, Stacey S. Cofield, Jeffrey R. Curtis, Maria I. Danila, Chander Raman, Richard Furie, Mark C. Genovese, William H. Robinson, Marc C. Levesque, Larry W. Moreland, Peter A. Nigrovic, Nancy A. Shadick, James R. O'Dell, Geoffrey M. Thiele, E. William St Clair, Christopher C. Striebich, Matthew B. Hale, Houman Khalili, Franak Batliwalla, Cynthia Aranow, Meggan Mackay, Betty Diamond, Garry P. Nolan, Peter K. Gregersen, S. Louis Bridges Jr. doi: https://doi.org/10.1101/691865