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TITLE: In-Depth Analysis of Citrulline-Specific CD4 T-Cells in Rheumatoid Arthritis

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| 6. AUTHOR(S)<br>Jane Buckner, MD, Benaroya Research Institute<br>Bernard Ng, MD, Puget Sound Veterans Administration<br><br>E-Mail: jrbuckner@benaroyaresearch.org<br>Bernard.nh@va.gov                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |             |                          |                            | 5e. TASK NUMBER                              |                                            |
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| 13. SUPPLEMENTARY NOTES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |             |                          |                            |                                              |                                            |
| 14. ABSTRACT<br>The goal of this project is to test the hypothesis that cit-specific CD4 T cells present in rheumatoid arthritis (RA) patients exhibit a distinct cell surface phenotype and transcriptional signature that could be used to predict disease, response to therapy and identify novel therapeutic targets for the treatment of RA. We have accomplished our major goals for year 1. We began patient recruitment and to date have consented 70% of the subjects for the cross-sectional study (Aim 1) and 40% of the subjects for the longitudinal study (Aim 3). We also accomplished our goal to develop new tools for characterizing cit-specific T cells. We developed both a panel of HLA-DRB1*04:04 tetramers, expanded our panel of HLA-DRB1*04:01 tetramers, and developed multiplexing technology for evaluation of multiple specificities in a single assay. We also worked on optimizing the RNAseq methodology for transcriptional profiling (Aim 2). We were able to obtain high quality RNAseq data from bulk sorted Tmr+ cells with as few as 50 cells total. Given these accomplishments in Year 1, we do not anticipate any problems in the next year and are on track to achieve our Year 2 goals |             |                          |                            |                                              |                                            |
| 15. SUBJECT TERMS<br>Rheumatoid arthritis; CD4 T cells; citrulline; HLA class II tetramers; RNAseq; Transcriptional profiling                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |             |                          |                            |                                              |                                            |
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## **1. INTRODUCTION**

Rheumatoid Arthritis (RA) affects over 1.3 million Americans. It is a chronic disease, which if untreated results in pain and permanent disability. Our current approaches to treatment are expensive, lead to systemic immune suppression and do not cure the disease. It is now known that joint-associated proteins are biochemically altered by inflammation and that these alterations provoke cellular immune responses against joint tissue. In particular, T cell responses directed against the joints drive development of RA, but are not well understood. Our research group has developed the ability to identify and isolate joint specific T cells from the blood of RA patients using a tool called HLA class II tetramers. In this proposal our objective is to use this tool to better understand the unique features of joint specific T cells and how these features change with disease activity and with therapy. The information will be useful to diagnose RA earlier – which could allow for earlier intervention, decreasing the morbidity of disease. Further it may be a means to predict response to therapy very soon after the initiation of a new therapy, which would decrease the expense and exposure to drugs that are unhelpful or potentially harmful. Findings from our DoD funded work will not only enhance our scientific knowledge related to the causes of RA, but also identify new determinants which can be therapeutically targeted while protecting the remaining immune cells needed for the patient's health.

## **2. KEYWORDS**

Rheumatoid arthritis; CD4 T cells; citrulline; HLA class II tetramers; RNAseq; Transcriptional profiling

## **3. ACCOMPLISHMENTS**

### **What were the major goals of the project?**

The major goal of this project is to test the hypothesis that cit-specific CD4 T cells present in RA patients exhibit a distinct cell surface phenotype and transcriptional signature that could be used to predict disease, response to therapy and identify novel therapeutic targets for the treatment of RA. In Specific Aim 1, we will utilize HLA class II tetramers to visualize and characterize T cells that recognize citrullinated epitopes in RA patients with respect to time from diagnosis and disease activity. In Specific Aim 2, we will utilize C1 Fluidigm technology combined with RNAseq to examine the transcriptional profiles of tetramer sorted cit-specific T cells at the single cell level using samples from healthy controls and RA subjects. In Specific Aim 3, we will utilize informative cell surface markers and transcript profiles to determine the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients.

Table 1 lists the Major Tasks and Milestones associated with each Specific Aim as outlined in the approved Statement of Work (SOW). It includes both a projected timeline and actual completion dates or percent complete. In Year 1, our major goals were to begin recruitment of patients and to start ex-vivo tetramer analysis of citrulline reactive T cells (Table 1).

**TABLE 1: MAJOR GOALS, MILESTONES, TIMELINE AND COMPLETION DATES**

|                                                                                                                                                                                                                                          |                           |                                                                                                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------------------------------------------------------------------------------------------|
| <b>Specific Aim 1:</b> Utilize HLA class II tetramers to visualize and characterize T cells that recognize citrullinated epitopes in RA patients with respect to time from diagnosis and disease activity.                               | <b>Projected Timeline</b> | <b>Year 1 Progress</b>                                                                               |
| <b>Major Task 1:</b> Recruit patients and conduct studies to characterize T cells that recognize citrullinated epitopes by direct ex vivo tetramer staining.                                                                             | <b>Months</b>             | <b>Completion Dates (or % Complete)</b>                                                              |
| <b>Subtask 1:</b> Submit documents for local IRB review.                                                                                                                                                                                 | 1-2                       | <b>100% Complete</b><br>BRI IRB approved: 07/28/2014<br>VA IRB approved: 09/24/2014                  |
| <b>Subtask 2:</b> Submit IRB approval and necessary documents for HRPO review.                                                                                                                                                           | 3-4                       | <b>100% Complete</b>                                                                                 |
| <b>Milestone #1: HRPO approval received</b>                                                                                                                                                                                              | 4                         | <b>100% Complete</b><br>BRI HRPO approved: 12/24/2014<br>VA HRPO approved: 03/27/2015                |
| <b>Subtask 3:</b> Recruit at least 20 RA subjects in each of the four disease activity groups as defined by RAPID3 score as well as 20 healthy control subjects.                                                                         | 4-15                      | <b>70% Complete</b><br>BRI recruitment started: 01/21/2015<br>VA recruitment started: 08/05/2015     |
| <b>Subtask 4:</b> Ex vivo tetramer analysis of citrulline reactive T cells.                                                                                                                                                              | 4-15                      | <b>20% Complete</b><br>HLA-DRB1*04:04 TMR development ongoing; TMR multiplexing technology developed |
| <b>Milestone #2: Successful comparison of the frequency and phenotype of cit-specific T cells in RA subjects based on RAPID3 score categories. Submission of these data as an abstract at a national meeting.</b>                        | 15-16                     |                                                                                                      |
| <b>Specific Aim 2:</b> Utilize C1 Fluidigm technology combined with RNAseq to examine the transcriptional profiles of tetramer sorted cit-specific T cells at the single cell level using samples from healthy controls and RA subjects. |                           |                                                                                                      |
| <b>Major Task 2:</b> Sorting of tetramer sorted cit-specific T cells and transcript profiling.                                                                                                                                           | <b>Months</b>             | <b>Completion Dates (or % Complete)</b>                                                              |
| <b>Subtask 1:</b> Preliminary Fluidigm C1 analysis of citrulline specific CD4 T cells in 2-4 RA subjects and healthy controls known to have high T cell frequency.                                                                       | 6-15                      |                                                                                                      |
| <b>Subtask 2:</b> Confirmation of RNA seq transcript signatures using qPCR of the same amplified cDNA samples.                                                                                                                           | 16                        |                                                                                                      |
| <b>Subtask 3:</b> Further validate C1 findings on new or frozen PBMC samples using 96 well PCR analysis and/or flow cytometry.                                                                                                           | 17                        |                                                                                                      |
| <b>Subtask 4:</b> Select and re-sample (if needed) previously identified Tmr+ RA and healthy control subjects for transcript analysis.                                                                                                   | 17-20                     |                                                                                                      |
| <b>Subtask 5:</b> Transcript analysis and flow cytometric assessment of the significance of RA specific transcript markers in cit-specific T cells in population identified in Subtask 4.                                                | 20-24                     |                                                                                                      |

|                                                                                                                                                                            |               |                                         |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|-----------------------------------------|
| <b>Milestone #3: Co-author manuscript on the frequency, phenotype, and transcript profile of cit-specific T cells in RA subjects.</b>                                      | <b>24</b>     |                                         |
| <b>Major Task 3:</b> Longitudinal study of the immune phenotype of cit-specific T cells in RA patients following first administration of biologic or non-biologic therapy. | <b>Months</b> | <b>Completion Dates (or % Complete)</b> |
| <b>Subtask 1:</b> Recruitment of patients for longitudinal studies.                                                                                                        | 16-26         | <b>40% Complete</b>                     |
| <b>Subtask 2:</b> Selection of informative panel of markers for longitudinal studies.                                                                                      | 24            |                                         |
| <b>Subtask 3:</b> Longitudinal study of the immune phenotype of cit-specific T cells in RA patients.                                                                       | 24-32         |                                         |
| <b>Subtask 4:</b> Data analysis / correlation of informative phenotypic markers with response to therapy                                                                   | 32-34         |                                         |
| <b>Milestone #4: Co-author manuscript on the impact of biologic therapy on the immune phenotype of cit-specific T cells isolated from RA patients</b>                      | 34-36         |                                         |

### What was accomplished under these goals?

Patient Recruitment: In the past year, we met the patient recruitment goals for Year 1 outlined in the approved SOW. We received HRPO approval and began recruiting patients for both the cross-sectional study proposed in Specific Aim 1 and the longitudinal study in Specific Aim 3. We achieved our first SOW milestone (Table 1) when we received HRPO approval; BRI received approval on 12/24/2014 and the VA received approval on 03/27/15. Patient recruitment began shortly after HRPO approval: BRI on 01/21/2015 and VA on 08/05/2015. As of December 15, 2015, we have consented a total of 117 RA subjects (Table 2). Of these 117 subjects, 86 qualified for enrolment. The remaining 31 subjects did not qualify because they did not have the HLA-DR genotype required for our study. Although the VA started recruitment later, both the VA and BRI are consenting comparable numbers of patients per month; the VA average is 6 patients per month and the BRI average is 8 patients per month. Interestingly, the VA cohort has a larger percentage of subjects that did not qualify due to HLA-DR genotype; 44% of VA subjects compared to 22% of BRI subjects did not qualify. We are investigating this difference by examining the differences in the patient demographics between the VA and BRI. In any case we do not believe these differences will slow overall enrollment given the contribution of BRI.

**TABLE 2: TOTAL PATIENT RECRUITMENT AS OF 12/15/15**

| <b>SITE</b>  | <b>TOTAL CONSENTED</b> | <b>DID NOT QUALIFY*</b> | <b>TOTAL ENROLLED**</b> |
|--------------|------------------------|-------------------------|-------------------------|
| <b>BRI</b>   | 94                     | 21                      | 73                      |
| <b>VA</b>    | 23                     | 10                      | 13                      |
| <b>TOTAL</b> | <b>117</b>             | <b>31</b>               | <b>86</b>               |

\*DID NOT QUALIFY DUE TO HLA-DR GENOTYPE

\*\*HLA-DRB1\*04:01 and HLA-DRB1\*04:04

Of the 86 enrolled subjects, 56 are in the cross-sectional study and 30 in the longitudinal study. Table 3 shows recruitment numbers for each of the four disease activity groups in the cross-sectional study. We have reached 80% of our recruitment goal for both the remission group and the high severity group. For the remaining two disease activity groups, we are at 55% of our recruitment goal for the low severity group and 65% of the goal for the medium severity group. Based on these numbers, we expect to have completed patient recruitment for the cross-sectional study in the next three months, as projected in our original Statement of Work.

**TABLE 3: PATIENT ENROLMENT FOR CROSS-SECTIONAL STUDY'S DISEASE ACTIVITY GROUPS**

| SITE         | TOTAL ENROLLED<br>12/15/2015 | DISEASE ACTIVITY GROUP (RECRUITMENT GOAL) |                               |                                  |                                |
|--------------|------------------------------|-------------------------------------------|-------------------------------|----------------------------------|--------------------------------|
|              |                              | REMISSION<br>(20 SUBJECTS)                | LOW SEVERITY<br>(20 SUBJECTS) | MEDIUM SEVERITY<br>(20 SUBJECTS) | HIGH SEVERITY<br>(20 SUBJECTS) |
| BRI          | 45                           | 13                                        | 9                             | 11                               | 12                             |
| VA           | 11                           | 3                                         | 2                             | 2                                | 4                              |
| <b>TOTAL</b> | <b>56</b>                    | <b>16</b>                                 | <b>11</b>                     | <b>13</b>                        | <b>16</b>                      |

Table 4 shows current recruitment numbers for each of the treatment groups in the longitudinal study. Of the 30 subjects enrolled in the longitudinal study, 5 RA patients have completed their blood donation for the study with blood collected at initiation of treatment, and at 3 and 9 months after starting treatment. Another 18 subjects are 2/3 completed having provided blood samples for the first 2 time points. We have reached our recruitment goal for the group treated with anti-TNF biologics so in Year 2 will focus on recruiting for the other 4 treatment groups. Given our current enrollment numbers, we do not anticipate any problem completing recruitment for the longitudinal study by the end of Year 2, as we originally projected.

**TABLE 4: PATIENT ENROLMENT FOR LONGITUDINAL STUDY'S TREATMENT GROUPS**

| SITE         | TOTAL ENROLLED<br>12/15/2015 | TREATMENT GROUP (RECRUITMENT GOAL)              |                                                |                                                |                                                |                                                   |
|--------------|------------------------------|-------------------------------------------------|------------------------------------------------|------------------------------------------------|------------------------------------------------|---------------------------------------------------|
|              |                              | BIOLOGIC ANTI-TNF <sup>1</sup><br>(12 SUBJECTS) | BIOLOGIC ORENCIA <sup>2</sup><br>(12 SUBJECTS) | BIOLOGIC ACTEMRA <sup>3</sup><br>(12 SUBJECTS) | NON-BIOLOGIC MTX <sup>4</sup><br>(12 SUBJECTS) | NON-BIOLOGIC DMARDS <sup>5</sup><br>(12 SUBJECTS) |
| BRI          | 28                           | 18                                              | 4                                              | 1                                              | 3                                              | 4                                                 |
| VA           | 2*                           |                                                 |                                                |                                                |                                                |                                                   |
| <b>TOTAL</b> | <b>30</b>                    | <b>18</b>                                       | <b>4</b>                                       | <b>1</b>                                       | <b>3</b>                                       | <b>4</b>                                          |

1. Anti-TNF: Adalimumab (Humira); Etanercept (Enbrel); Golimumab (Simponi); and Infliximab (Remicade); 2. CTLA-4Ig: Abatacept; 3. Anti-IL6R: Tocilizumab; 4. Methotrexate 5. DMARDs: Leflunomide, Azathioprine, Sulfasalazine, and Hydroxychloroquine.\*As of 12/15/2015, VA subjects have not yet started treatment

*Ex vivo Tetramer Analysis of Citrulline Reactive T Cells:* In the past year, we have also made significant progress toward achieving SOW Milestones 2 through 4 (Table 1). We have developed and validated a panel of HLA-DRB1\*04:04 tetramers specific to Alpha Enolase, Alpha/beta Fibrinogen, CILP (Cartilage Intermediate Layer Protein) and Vimentin. We have extended the HLA-DRB1\*04:01 panel by determining whether citrullinated peptides derived from Aggrecan, Histone H2A, Histone H3, Histone H4, Clusterin, Insulin like growth factor 6, and Osteopontin are autoantigens detectable by tetramers in RA. We have also developed a multiplexing technology that will allow the evaluation of multiple specificities in a single assay, allowing for fewer cells per assay. These tools will be applied to characterize the phenotype of cit-specific T cells in the cross-sectional RA study (Milestone 2) and the longitudinal RA study (Milestone 4).

In the past year, we also worked on optimizing the Fluidigm C1 platform for single cell RNAseq proposed in Specific Aim 2 Milestone 3 (Table 1). In our original proposal, we anticipated that capturing single Tmr+ cells would be challenging due to their rarity in the T cell population. This was indeed the case and although we have the ability to capture single tetramer positive cells, we have found that numbers are limiting ( typically 20-40 individuals cells from a 100cc blood draw) this small number of cells leads to challenges related to the breadth of genes that can be analyzed and data analysis of this small sample size. In contrast, we were able

to obtain high quality RNAseq data from bulk sorted Tmr+ cells with as few as 50 cells total. Based on these results we will use this strategy for the transcriptional profiling proposed in Specific Aim 2.

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

Dr. Hannes Uchtenhagen is a postdoctoral fellow working on this project. He has been actively engaged in developing the multiplex technology and he has also interacted with the BRI bio-informatics group in order to analyze our RNAseq data. These opportunities are allowing him to grow as a scientist. In addition, Hannes participates regularly in presenting his data related to RA tetramer development at BRI and with our collaborating institutions. He hopes to present his data in 2016 at either the FOCIS or ACR meetings.

**How were the results disseminated to communities of interest?**

We have shared our findings and technology with our collaborators at the Karolinska University in Stockholm, Vivianne Malmstrom and Lars Klareskog, in order to help them develop tools to study their RA cohorts and synovial fluid sample set. In addition we have worked with the Accelerated Medical Partnership RA project group, using the tetramers to evaluate synovial T cells isolated from joint biopsies that are being obtained by this research group. Thus the tools that we are generating in this grant will be applied by the Rheumatology Community to questions and sample sets beyond the scope of our DOD project.

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

We plan to complete recruitment for Milestones #2 and #3 (Table 1) by the end of the next reporting period (Year 2). For Milestone #2, we will complete the cross-sectional study by comparing the frequency and phenotype of cit-specific T cells in RA subjects in each of the four disease activity groups. We plan to submit an abstract based on this work to either the Federation of Clinical Immunology Societies (FOCIS) meeting or the American College of Rheumatology annual meeting. For Milestone #3, we will begin the transcriptional profiling of cit-specific T cells, although these studies and the analysis of this data will extend into year 3 of this award. We will then submit a manuscript on the frequency, phenotype and transcriptional profile of cit-specific T cells in RA subjects in year 3 if not before.

By the end of the next reporting period, we will also have made significant progress on the longitudinal study investigating the effect of anti-TNF biologic therapies on cit-specific T cells in RA subjects. Specifically, we plan to have completed patient recruitment and selected the panel of informative phenotypic markers for the longitudinal study.

**4. IMPACT**

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

Nothing to Report

**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**

We do not believe that any significant changes have been made in our study design or in its application. We have made several minor changes in response to our findings as we develop the tetramer assay, preferring to use bulk RNAseq on low numbers of Tmr+ CD4 T cells rather than single cells RNAseq. We believe the data generated from these samples and the analysis that can be performed will be more informative than that gained from single cell data. We have also altered our definitions for patient groups that we are recruiting for the longitudinal study. In each case we have increased the scope of the treatment type we include. We have



done this as we find that recruitment is going well, and these additional subjects may yield important comparisons that will strengthen our findings.

#### **Changes in approach and reasons for change**

We have had two changes in approach. Importantly, these changes are not significant as they do not alter either the objectives or scope of the SOW. The first change relates to the transcriptional profiling proposed in Specific Aim 2. Due to challenges with single cell capture for the Fluidigm C1 platform, we now propose to perform transcriptional profiling on bulk instead of single Tmr+ cells. Given the rarity of Tmr+ cells, we anticipated this problem and proposed RNAseq on bulk sorted Tmr+ cells as an alternative strategy in the original proposal. Over the 3 years of this project, as single cell RNAseq strategies improve we hope to be able to utilize this technique to address specific hypothesis generated from our bulk RNAseq data.

The other change is related to how we group treatments in the longitudinal study in Specific Aim 3. We now propose to combine all Anti-TNF biologics into a single treatment group. In our practice the initial anti-TNF selected for a patient may vary. We are able to capture many of these individuals, and believe that it would be useful to have a larger group of anti-TNF treated subjects that represent several of these therapies. In addition we have chosen to recruit some RA subjects not on methotrexate (MTX) but who are receiving other non-biologic DMARDs including leflunamide, azathioprine and hydroxychloroquine. These non-biologic DMARDs will be in a separate group from the MTX cohort. We believe these individuals may be revealing with respect to what we see with the MTX cohort. Again, these new groupings do not significantly change the objectives or scope of the SOW.

#### **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**

Nothing to Report

#### **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**

Nothing to Report

#### **Journal publications**

Nothing to Report

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

Nothing to Report

#### **Other publications, conference papers, and presentations**

Nothing to Report

**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**

Biospecimen collections: In the past year, through recruitment of RA subjects for this study, we have made a significant contribution to the BRI Immune Mediated Disease Registry and Repository (BRI-IMDR). Specifically the number of RA subjects in the BRI-IMDR has increased from 510 to 626. The samples collected from these subjects will be first used to address questions related to the DOD project, but remaining samples will be available to other scientists for their investigation into the causes of immune-mediated disease.

Research material: We have also developed a panel of HLA-DRB\*04:04 tetramers. This new tool allows us to characterize T cell responses in patients with RA and healthy subjects with the DDRB1\*04:04 haplotypes.

**7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS****What individuals have worked on the project?**

|                                   |                                                                                                                                                                                                                               |
|-----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Jane Buckner, MD</b>                                                                                                                                                                                                       |
| Project Role:                     | Principal Investigator                                                                                                                                                                                                        |
| Research Identifier (e.g. ORCID): | JBUCKNER                                                                                                                                                                                                                      |
| Nearest person month worked:      | 1                                                                                                                                                                                                                             |
| Contribution to project:          | Dr. Buckner will direct the research, supervise the postdoctoral fellow and research technicians in this project. She will meet with all investigators on a monthly basis and be responsible for preparation of publications. |

|                                   |                                                                            |
|-----------------------------------|----------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Bernard Ng, MD</b>                                                      |
| Project Role:                     | Principal Investigator                                                     |
| Research Identifier (e.g. ORCID): | BERNARDNG                                                                  |
| Nearest person month worked:      | 1                                                                          |
| Contribution to project:          | Dr. Ng will supervise recruitment of study participants at the Seattle VA. |

|                                   |                                                                                                                                                                                                                                                  |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Eddie James, PhD</b>                                                                                                                                                                                                                          |
| Project Role:                     | Co-Investigator                                                                                                                                                                                                                                  |
| Research Identifier (e.g. ORCID): | EJAMES2                                                                                                                                                                                                                                          |
| Nearest person month worked:      | 1                                                                                                                                                                                                                                                |
| Contribution to project:          | Dr. James will work closely with Dr. Buckner and her team on Aim 1 applying the myc-tagged tetramer technology and multiparameter flow cytometry to RA samples. Dr. James will assist in analysis of these data and preparation of publications. |

|                                   |                           |
|-----------------------------------|---------------------------|
| <b>Name:</b>                      | <b>Peter Linsley, PhD</b> |
| Project Role:                     | Co-Investigator           |
| Research Identifier (e.g. ORCID): | PLINSLEY                  |

|                              |                                                                                                                                                                                        |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ORCID):                      |                                                                                                                                                                                        |
| Nearest person month worked: | 11                                                                                                                                                                                     |
| Contribution to project:     | Dr. Linsley will direct the RNAseq studies, and oversee the work of biostatisticians analyzing the data. He will also assist with data interpretation and preparation of publications. |

|                                   |                                                                                                                                                                                 |
|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Sylvia Posso</b>                                                                                                                                                             |
| Project Role:                     | Clinical Research Coordinator                                                                                                                                                   |
| Research Identifier (e.g. ORCID): |                                                                                                                                                                                 |
| Nearest person month worked:      | 2                                                                                                                                                                               |
| Contribution to project:          | Ms. Posso is the clinical research coordinator responsible for patient recruitment, maintaining IRB approval and clinical data management at BRI-Virginia Mason Medical Center. |
| Funding Support:                  | Benaroya Research Institute at Virginia Mason internal funding                                                                                                                  |

|                                   |                                                                                            |
|-----------------------------------|--------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Mohammad Pourmandi</b>                                                                  |
| Project Role:                     | Research Assistant                                                                         |
| Research Identifier (e.g. ORCID): |                                                                                            |
| Nearest person month worked:      | 2                                                                                          |
| Contribution to project:          | Mr. Pourmandi is responsible for patient recruitment at BRI-Virginia Mason Medical Center. |

|                                   |                                                                                                       |
|-----------------------------------|-------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Cliff Rims</b>                                                                                     |
| Project Role:                     | Research Technician                                                                                   |
| Research Identifier (e.g. ORCID): |                                                                                                       |
| Nearest person month worked:      | 11                                                                                                    |
| Contribution to project:          | Mr. Rims will assist Dr. James and Dr. Buckner in handling blood samples, FACs staining and analysis. |

|                                   |                                                                                                                                                            |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Kevin Criste</b>                                                                                                                                        |
| Project Role:                     | Research Assistant                                                                                                                                         |
| Research Identifier (e.g. ORCID): |                                                                                                                                                            |
| Nearest person month worked:      | 1                                                                                                                                                          |
| Contribution to project:          | Mr. Criste was responsible for patient recruitment at BRI-Virginia Mason Medical Center. He has since left the BRI and was replaced by Mohammad Pourmandi. |

|                                   |                                                                                                                                            |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                      | <b>Jeffrey Carlin, MD</b>                                                                                                                  |
| Project Role:                     | Director of the BRI Rheumatic Disease Registry                                                                                             |
| Research Identifier (e.g. ORCID): | JSCARLIN                                                                                                                                   |
| Nearest person month worked:      | 1                                                                                                                                          |
| Contribution to project:          | Dr. Carlin is the director of the rheumatic disease registry at BRI. He oversees patient recruitment at BRI-Virginia Mason Medical Center. |
| Funding Support:                  | Virginia Mason Medical Center                                                                                                              |

|                                          |                                                                                                                                                                             |
|------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Name:</b>                             | <b>Hannes Uchtenhagen</b>                                                                                                                                                   |
| <b>Project Role:</b>                     | Visiting Postdoctoral Fellow                                                                                                                                                |
| <b>Research Identifier (e.g. ORCID):</b> |                                                                                                                                                                             |
| <b>Nearest person month worked:</b>      | 6                                                                                                                                                                           |
| <b>Contribution to project:</b>          | Dr. Uchtenhagen will work with Drs. James and Linsley for the tetramer analyses and RNAseq studies.                                                                         |
| <b>Funding Support:</b>                  | National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institute of Allergy and Infectious Diseases, Benaroya Research Institute at Virginia Mason |

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

See Appendix I, Senior/Key Personnel Other Support

**What other organizations were involved as partners?**

**Organization Name:** Seattle Institute for Biomedical & Clinical Research

**Location of Organization:** 1100 Olive Way, Seattle WA 98101

**Partner's contribution to the project:** Collaboration

The Seattle Institute for Biomedical & Clinical Research receives and administers non-VA appropriated funds in support of research performed at the VA Puget Sound Health Care System. The collaborating organization is the Seattle Institute for Biomedical & Clinical Research. The performance site is the VA Puget Sound Health Care System.

**Partnering Organization Performance Site:**

Department of Veterans Affairs  
Puget Sound Health Care System  
1660 S. Columbian Way  
Seattle, WA 98108-1597

## 8. SPECIAL REPORTING REQUIREMENTS

The collaborating Principal Investigator's (Bernard Ng) technical report is a duplicate that is separately submitted.

## 9. APPENDICES

Appendix I. Senior/Key Changes in Other Support

## 9. APPENDIX I: Senior/Key Changes in Other Support

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### OTHER SUPPORT

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**Buckner, Jane H.**

#### ACTIVE

##### **(NEW)**

2-SRA-2014-150-Q-R (Bluestone, J)  
JDRF

06/01/2014 – 05/31/2016

4% effort

##### **Collaborative Center for Treg Biology (JDRF-CCTB)**

Dr. Buckner's group will examine a panel of T1D subjects with either normal or blunted IL-2/pSTAT5 responses for the ability to maintain FOXP3 expression and suppressive function after expansion. We will evaluate the impact of low STAT5 phosphorylation on the loss of FOXP3 expression and suppression by expanded Tregs (eTregs). Expansion of Treg will be performed in vitro, outcome measures will include Treg number, FOXP3 expression, TSDR methylation, IFN $\gamma$ , pSTAT5 in response to IL-2, we estimate that 20 subjects will be studied in each group. Further studies will be performed with T1D Treg that have low pSTAT5 to improve responses to IL-2 these will include extended in vitro stimulations, and inhibition of PTPN2 using small molecule inhibitors

and/ or siRNA knockdown of PTPN2.

Role: Subaward Principal Investigator

Program Officer: Simi Ahmed

##### **(NEW)**

1 UH2 AR067681-02 (Holers, VM)  
NIH-NIAID

09/24/2014 – 05/31/2016

2% effort

##### **Evolving Adaptive and Effector Mechanisms from Pre-RA through Established Disease**

The central hypothesis of this Clinical and Technology Research Site proposal focuses on Rheumatoid Arthritis (RA) is that novel disease stage-and cell lineage-specific therapeutic targets can be identified through the comprehensive evaluation of the linked adaptive and effector arms of the immune system as the disease sequentially progresses from its earliest origins through to a fully established destructive arthritis.

*Aim 1: Using a larger sample of peripheral blood and synovial-derived cells drawn from patients at multiple stages of disease evolution, develop a comprehensive data set of single cell signatures from control cells and utilize this to identify novel pathways and targets within antigen-specific B and T lymphocytes as well as FLS and osteoclast precursors*

*Aim 2: To develop a further understanding of pathogenic pathways and imprinting in FLS and monocytic osteoclast precursors.*

Role: Subaward Principal Investigator

Program Official: Susana Serrate-sztein

##### **(NEW)**

1 DP3 DK104466-01 (Buckner, JH)  
NIH/NIDDK

09/19/2014 – 08/31/2017

1.5% effort

##### **Investigating the role of IL-6 signaling in Teff resistance and T1D development**

The focus of this project is enhanced responsiveness to IL-6 and or IL-21 predates the development of T1D and contributes to the development of Teff resistance. Additionally, we will examine the hypothesis that the combination of enhanced IL-6(pSTAT3) and blunted IL-2(pSTAT5) responses result in an increase in pathogenic CD4 T cells and impaired Treg development and function resulting in the progression to T1D.

*Aim 1: We will test the hypothesis that enhanced responses to IL-6 and/or IL-21 are present prior to onset of T1D and contribute to the cascade of inflammatory events that result in beta cell destruction.*

*Aim 2: We will determine if the increase in Teff resistance seen in Ab<sup>+</sup> FDR who progress to T1D correlate with increased IL-6(pSTAT3) or IL-21(pSTAT3) responses.*

*Aim 3: We will test the hypothesis that Teff resistance will arise when Ab<sup>+</sup> individuals at risk for T1D begin to develop systemic inflammation and beta cell destruction. We propose to test this by examining basal pSTAT3, IL-*

6(pSTAT3), IL-21(pSTAT3) and Teff resistance in longitudinal samples from subjects in the TrialNet natural history study who have been followed for 2 or more years prior to diagnosis.

*Aim 4:* We pose the hypothesis that enhanced IL-6(pSTAT3) and blunted IL-2(pSTAT5) responses in combination will differentiate FDR who progress to T1D from Ab- FDR in a more selective manner than either immune phenotype alone and may be a marker of more rapid progression to disease.

Role: Principal Investigator

Program Official: Lisa M. Spain

**(NEW)**

5 UM1 AI109565-02 (Nepom, G)

02/01/2015 – 01/31/2016

5% effort

NIH/NIAID

**ITN PROJECT: Impact of costimulatory blockade on myeloid and lymphocyte subset phenotype and function in ACCLAIM**

Dr. Buckner is Co-Protocol Chair of this trial with Dr. Carla Greenbaum. ITN Clinical Trial Protocol Chairs have overall responsibility for the conduct of the study, including oversight of all scientific, reporting, and financial matters. They will oversee recruitment activities, patient care management, and participate in monthly study teleconferences. Under the guidance of the ITN members, Protocol Chairs also interact with regulatory agencies, as needed, for matters regarding this clinical trial.

Role: Project Principal Investigator

Program Officer: Leighton A. Thomas

**(NEW)**

1 R01 AR065952-01A1 (Hawkins, RD)

03/01/2015 – 01/31/2016

2% effort

NIH/NIAMS

**Functional validation of Rheumatoid Arthritis-associated distal related regulatory SNPs i**

This proposal is to functionally validate distal regulatory SNPs associated with rheumatoid arthritis. Dr. Buckner will collaborate on data analysis and interpretation with regards to the way distal rSNPs alter enhancer function/TF binding and the relationship to gene expression and disease.

Role: Subaward Principal Investigator

Program Official: Yan Z. Wang

**(NEW)**

1 UC4 DK097835-01 (Krischer, JP)

05/01/2015 – 08/31/2016

1.5% effort

NIH/NIDDK

**NIDDK T1D TrialNet Data Coordinating Center | TrialNet Core Biomarkers and Mechanisms Panel (BMP)**

Members of the TrialNet Core BMP are charged with development and execution of a strategic plan for mechanistic and biomarkers research within TrialNet towards its mission of Type 1 Diabetes prevention.

*Aim 1:* To provide oversight of the TN bioresource by reviewing proposals for access to samples or patients and working with TrialNet Coordinating Center (TNCC) on appropriate sample allocation.

*Aim 2:* Review data emerging from TN ancillary studies

*Aim 3:* Provide leadership on new mechanistic studies commissioned and run by TN and performed on existing studies

*Aim 4:* Provide leadership on new protocols for mechanistic studies commissioned by TN as stand-alone studies,

Program Official: Ellen W. Leschek

**(NEW)**

Collaborative Research Agreement

01/09/2014 – 01/08/2016

Confidentiality Agreement in Place

5% effort

1 DP3 DK097672-01 (Buckner, JH)

09/15/2012 – 09/14/2016

10% effort

NIH/NIDDK

**Defining the functional impact of T1D genes in mouse and man: a unified strategy**

It is clear that genes play a significant role in the development of T1D. Progress has been made but several hurdles have impeded progress in defining the functional consequences of the genes associated with T1D risk. These include the

modest risk associated with individual genomic regions and uncertainty as to which of the SNPs in these regions are causative limits mechanistic studies relating genotype to phenotype. The multiplicity of genes and the heterogeneity among individuals poses further complexity to the study of the functional consequences of variation in genes in the human subjects, while murine models may not reflect the impact of a gene on the human immune response. To overcome each of these hurdles we propose a novel strategy to define the functional significance of novel genetic variants in T1D by integrating three different approaches:

*Aim 1:* We will utilize a novel genetic approach to identify causative genetic loci in T1D thus identifying variants that can be studied in a targeted manner in mouse and man.

*Aim 2:* We will model causative variants in mice allowing us to study the impact of the genetic variants on immune development, in multiple cell population and in response to in vivo antigen exposure.

*Aim 3:* We will define the functional phenotypes related to these causative genetic variants in humans with and without T1D. The studies will be in part driven by findings in murine models while they will also determine the relevance of findings from these models to human subjects and T1D.

Role: Principal Investigator

Program Officer: Beena Akolkar, PhD

1 DP3 DK098217-01 (Greenbaum, C)

05/15/2013 – 04/30/2016

2.5% effort

NIH/NIDDK

### **In vivo assessment of T cell kinetics in individuals at risk for Type 1 diabetes**

In this proposal, we aim to evaluate *in vivo* T cell kinetics in individuals at risk for type 1 diabetes identified by Diabetes TrialNet and healthy control subjects.

*Aim 1:* To determine whether altered T cell kinetics is present prior to diagnosis in individuals at risk for type 1 diabetes.

*Aim 2:* To determine the changes in T cell kinetics over time.

Role: Co-Investigator

Program Official: Lisa M. Spain

5 U01 AI101990-04 (Buckner, JH)

07/01/2012 – 06/30/2017

16% effort

NIH/NIAID

### **Defining the role of altered cytokine signaling pathways on autoimmunity**

In this proposal, we will determine whether alterations in cytokine-mediated STAT phosphorylation lead to an imbalance between Treg and Th17 cells, and thus enhance the resistance of effector T cells (Teff) to suppression, in T1D, MS, and CD. We will then determine the molecular mechanisms that lead to these alterations, using disease-associated genetic polymorphism as a guidepost. Finally we will assess how therapeutic interventions targeting these pathways influence the balance between regulation and inflammation in disease.

*Aim 1:* We will address the hypothesis that blunted STAT5 signaling in response to IL-2 results in a diminished induction, function and/or stability of Treg in T1D, CD and MS. We will examine this question in the context of blood and then extend these studies to the tissue in the setting of CD. We will then determine the biochemical and genetic mechanisms that lead to the defect in IL-2R signaling in T1D, MS and IBD.

*Aim 2:* We will address the hypothesis that enhanced phosphorylation of STAT3 in response to IL-6 leads to the development and persistence of pathogenic T cells in autoimmunity, by promoting the expression of ROR $\gamma$ t and/or enhancing the resistance of effector T cells to suppression by Treg. We will examine this question using genotyped controls to address molecular mechanism and T1D, MS and CD patients to address disease phenotypes.

*Aim 3:* We will test the hypothesis that enhanced phosphorylation of STAT3 and diminished phosphorylation of STAT5 in combination reverses the balance of Treg and Teff functional profiles resulting in a persistent proinflammatory response.

Award includes an administrative supplement in support of the Cooperative Study Group for Autoimmune Disease Prevention (CSGADP). The CSGADP goal is to halt the development of autoimmune disease prior to clinical onset by means other than global immunosuppression, the CSGADP will support collaborative projects, innovative pilot and feasibility projects, and development of reagents and resources. The Infrastructure and Opportunities Fund (IOF) of the CSGADP will facilitate the mission.

Role: Principal Investigator

Program Official: Thomas R. Esch, PhD

5 U01 AI101981-03 (Holers, VM)  
NIH/NIAID

07/01/2012 – 06/30/2017

5% effort

**Prevention Center U01: Early targets for antigen-specific tolerance induction in Preclinical Rheumatoid Arthritis**

Identify novel synovial T cell epitopes in RA, and develop HLA class II tetramers to detect autoreactive T cells. The data generated from these studies will be vital in the development of tools to predict the development of RA and will also yield information that will assist in the development of treatments to prevent the development of RA.

*Aim 1:* Identify the innate and adaptive immune response modifications that are causally associated with the break in tolerance to citrullinated autoantigens and progression to active disease.

Role: Subaward Principal Investigator

Program Official: Thomas R. Esch, PhD

**COMPLETED RESEARCH SUPPORT**

**(PREVIOUS)**

4-2011-248 (Bluestone, J)  
JDRF

11/01/2010 – 10/31/2013

**JDRF Collaborative Center for Cell Therapy**

Using a novel inter-institutional, interdisciplinary collaborative program to make substantial advances in the understanding of Tregs in humans and rapidly translate the basic science into clinical application.

*Aim 1:* Determine the relationship between pSTAT5 and ability to expand "stable" Tregs from patients with T1D.

*Aim 2:* Develop and test new IL-2 mutants) that will selectively target the signaling pathways critical for Treg activation.

*Aim 3:* Manipulation of FOXP3 expression and function to better understand Treg stability, expand Tregs more effectively and create a platform for drug development.

Role: Subaward Principal Investigator

Program Officer: Teodora Staeva, PhD

**(PREVIOUS)**

N01-AI-15416 (Bluestone, J)  
NIH/NIAID

12/01/2012 – 04/30/2014

**The Collaborative Network for Clinical Research in Immune Tolerance: Protocol Chair** (Greenbaum, C)

Mechanistic Outcomes Committee, for which Drs. Greenbaum and Buckner serve as a protocol co-chairs for the Immune Tolerance Network (ITN).

*Aims:* Not Applicable

Role: Subaward Co-Investigator

Program Officer: Jim McNamara, PhD

**(PREVIOUS)**

1 DP3 DK085678-01 (Concannon, P)  
NIH/NIDDK

03/01/2012 – 06/24/2014

**Expression and proteomic characterization of risk loci for type 1 diabetes**

The goal of this project is to determine the how genotypes influence phenotypes and disease susceptibility specifically looking at risk alleles in the genes that encode proteins involved in antigen receptor and cytokine signaling including PTPN22.

*Aim 1:* Characterization of transcriptional regulation of genes identified as associated with T1D risk

*Aim 2:* Proteomic characterization of these genes

*Aim 3:* Characterization of the transcriptome in MHC discordant sibling pairs with T1D

Role: Subaward Principal Investigator

Program Officer: Beena Akolkar, PhD

**(PREVIOUS)**

245284 (Buckner, JH)  
Alliance for Lupus Research

08/01/2012 – 07/31/2014



### **The impact of genetic variants on B cell development and function in SLE**

We hypothesize that the genetic variants in *BLK* and *BANK1* differentially impact B cell signaling and development, leading to a failure of B cell tolerance induction which defines one of the mechanisms that contribute to B cell autoreactivity in SLE.

*Aim 1:* We will determine the effect of the SLE risk variants on expression of *BLK* and *BANK1* in B cell subsets of genotyped healthy controls and SLE subjects.

*Aim 2:* We will evaluate the impact of each *BLK* and *BANK1* variant, individually and in combination, on B cell signaling over a range of BCR and/or CD40 stimulation conditions.

*Aim 3:* We will analyze the impact of the *BLK* and *BANK1* variants on the development of B cell tolerance and maturation by evaluating the frequency of transitional and anergic BND B cell subsets, the BCR-driven apoptotic response of transitional and naïve B cells, and the induction of switched memory and plasma lineage B cells in genotyped healthy controls and SLE subjects.

Role: Principal Investigator

Program Officer: Diomaris Gonzalezg

### **(PREVIOUS)**

5 R01 AI083455-03 (Buckner, JH)

08/15/2011 – 08/31/2015

NIH/NIAID

### **Impact of the autoimmunity associated PTPN22 1858T variant on the human immune response**

In this proposal we will address the hypothesis that the impaired T cell receptor signaling that results from the PTPN22 1858T variant shapes CD4 T cell function in a manner which favors the development of strong proinflammatory responses, which can contribute to the development of autoimmunity.

*Aim 1:* We will investigate the impact of impaired TCR signaling associated with the PTPN22 1858T allele on the commitment of naïve T cells and the function and survival of memory T cells *in vitro*.

a. We will examine function and lineage commitment of CD4 T cells *in vitro* using measures of cell survival, proliferation and cytokine production to define the functional phenotype associated with PTPN22 1858T.

b. We will investigate the mechanisms by which the Lyp620W variant skews the character of the T cell response through the use of biochemical assessment of LypR620W on the TCR signaling pathway.

*Aim 2:* We will test the hypothesis that individuals who possess the PTPN22 1858T allele develop an enhanced proinflammatory memory T cell response *in vivo*, following immunization with the polyvalent pneumococcal vaccine conjugated to the CRM197 carrier protein.

*Aim 3:* We will examine how phenotypes associated with the LypR620W variant are expressed in the context of autoimmune diabetes. In T1D subjects where multiple genetic variants with potential immune impact are present, we will address T cell function with respect to PTPN22 1858T genotype both *in vivo*, and *in vitro*.

Role: Principal Investigator

Program Officer: Kasia Bourcier

### **(PREVIOUS)**

5 U01 DK061034-12 (Greenbaum, C)

09/30/2009 – 04/30/2014

NIH/NIDDK

### **Northwest Clinical Center for Type 1 Diabetes - TrialNet**

This project is a nationwide, multi-center consortium to conduct type 1 diabetes clinical trials, including the Diabetes Prevention Trials Type-1 (DPT-1).

*Aims:* Not Applicable

Role: Co-Investigator

Program Officer: Ellen W. Leschek

### **(PREVIOUS)**

### **Collaborative Research Agreement**

11/05/2012 – 11/05/2013

Bristol-Myers Squibb

*Due to confidentiality terms in the agreement, we are unable to disclose information on this project.* Program Officer: Steven Nadler

**(PREVIOUS)**

**Collaborative Research Agreement**

11/12/2013 – 11/12/2015

Pfizer

*Due to confidentiality terms in the agreement, we are unable to disclose information on this project.* Program Officer: Li-Fen Lee

1 RC4 DK090814-01 (Rawlings, D)

09/30/2010 – 08/31/2013

NIH/NIDDK

**B cell function and phenotype as predictors of therapeutic response to rituximab in T1D**

The Buckner lab will examine the B cell populations for their signaling characteristics upon activation utilizing flow cytometric analysis in the BRI Imaging core. Additionally, if snp typing is required, DNA will be isolated from the non-B cell population obtained and sent to the BRI Genotyping core for SNP analysis for PTPN22 1858T, and if appropriate other variants known to be involved in BCR signaling and associated with T1D.

Role: Subaward Principal Investigator

2087550 (Nepom, G)

07/14/2008 – 07/13/2013

Washington State Life Sciences Discovery Fund

**Program for Autoimmune Disease Intervention**

The aims of this program are to: 1. Develop and apply novel strategies for evaluating patients with diabetes, multiple sclerosis, and lupus, based on immunologic and genetic profiling; 2. Develop new biologically based therapeutics using our expertise in single chain fragment technologies and inhibitory monoclonal antibodies, specifically targeting a key step shared in common by multiple autoimmune disease pathways, dendritic cell-T cell interactions; and 3. Expand access to the Clinical Research Core program for patients with diabetes, multiple sclerosis, and lupus throughout Washington State, integrating research and treatment.

Role: Co-Investigator

4-2007-1058 (Nepom, G)

01/01/2008 – 12/31/2012

JDRF

**JDRF-BRI Center for Translational Research**

This project addresses the hypothesis that B cell selection and maturation are defective in T1D resulting in a loss of B cell tolerance. In this grant we propose to examine the peripheral B cell compartment in T1D subjects and specifically examine the impact of PTPN221858T mutation on the development of B cell tolerance, examine the BCR signaling defects in T1D.

Role: Principal Investigator - Project 2; Co-Investigator - Project 1, Project 3 and Core A

1 RC4 DK090796-01 (Buckner, JH)

09/30/2010 – 08/31/2012

NIH/NIDDK

**Understanding regulatory defects T1D: natural history and impact of therapy**

In this grant we propose to examine samples obtained by TrialNet to assess when Teff resistance to Treg or impaired IL-2R signaling occurs during the course of disease from pre-clinical through clinical diagnosis and whether these defects predict subsequent disease course.

Role: Principal Investigator

5 U19 AI050864-10 (Eisenbarth, G)

09/01/2010 – 08/31/2012

NIH/NIAID

**BRI/UCDHSC Autoimmune Cooperative Study: Pilot - Defining common mechanisms of autoimmunity through genotype-phenotype analysis** (Buckner, J)

We will test the hypothesis that phenotypes defined by genetic variants associated with autoimmunity define common mechanisms that underlie the diseases associated with that gene. Specifically we will: 1) examine the TCR and BCR signaling signatures and the composition of the B and T cell compartments of subjects with diseases associated with the PTPN22 variant: T1D, SLE, RPC compared to subjects with MS and IBD, diseases not associated with the PTPN22 variant. 2) We will characterize IL-2 signaling and the expression of FOXP3 in the presence and absence of IL-2 in subjects with diseases associated with variants in either CD25 or PTPN2: MS, T1D and IBD, compared to subjects with SLE, a disease not associated with genes in the IL-2R pathway.

Role: Pilot Principal Investigator

1 R56 AI083455-01A1 (Buckner, J)  
NIH/NIAID

09/01/2010 – 08/31/2012

**Impact of the autoimmunity associated PTPN22 1858T variant on the human immune response**

In this proposal we will address the hypothesis that the impaired T cell receptor signaling that results from the PTPN22 1858T variant shapes CD4 T cell function in a manner which favors the development of strong proinflammatory responses, which can contribute to the development of autoimmunity.

5 R01 AR051394-05 (Holers, M/Norris, J)  
NIH/NIAMS

07/15/2005 – 06/30/2012

**RA-Related Autoantibodies in Healthy FDR of RA Patients: Rheumatoid Arthritis First Degree Relative Study (SERA)**

This project aims to establish BRI as a rheumatoid arthritis first degree relative study site.

Role: Project Leader

W81XWH-10-1-0149 (Nepom, G)  
DOD/CDMRP

04/01/2010 – 03/14/2012

**Cytoprotection: immune and matrix modulation of tissue repair – Project 1-C**

This interdisciplinary, targeted research program is designed to develop a selective anti-inflammatory strategy, which will become a valuable asset to the therapeutic approach to tissue replacement and healing. The anticipated outcome of Aim 1.C. is a set of validated molecular pathway targets for modifying Treg function in association with our engineered tissue implants.

Role: Co-Investigator Aim 1-C

5 U19 AI050864-09 (Eisenbarth, G)  
NIH/NIAID

12/01/2008 – 08/31/2011

**BRI/UCDHC Autoimmune Cooperative Study: Pilot - Citrullinated synovial antigens as T cell epitopes: developing a biomarker for RA (Buckner, J)**

This grant is requested as supplemental funding to develop a new approach to identify and screen candidate citrulline peptides in order to examine T cell responses in RA synovial fluid.

Role: Pilot Principal Investigator

33-2008-398 (Buckner, JH)  
JDRF

03/01/2008 – 08/31/2011

**Functional and Genetic Analysis of Shared Regulatory Defects in T1D and MS**

The goals of this project are to: (1) localize the defects in regulation at the  $T_R$ :  $T_E$  interface, in both MS and T1D, then address the correlation between these defects and the presence of disease associated IL-2RA and IL-7R variants.

Role: Principal Investigator

5 R03 DA027013-02 (Buckner, JH)  
NIH/NIDA

09/30/2009 – 08/31/2011

**Linking Genetic Variation in the PTPN2 Gene to Autoimmune Disease Susceptibility**

In this grant we examine the hypothesis that genetic variants in the PTPN2 gene result in altered IL-2 signal transduction affecting differentiation and function of T cells, predisposing an individual to develop autoimmunity.

Role: Principal Investigator

N01-AI-15416 (Bluestone, J)  
NIH/NIAID

12/01/2010 – 07/31/2011

**The Collaborative Network for Clinical Research in Immune Tolerance: IL-2 Rapa Mechanistic Study**

The ongoing study of IL-2/rapamycin treatment in individuals with T1D is an opportunity to examine the *in vivo* impact of this therapy on the number and function of Treg.

Role: Project Principal Investigator

Alliance for Lupus Research

**The Impact of Genetic Variants on BCR Signal Transduction in SLE**

In this proposal we will investigate the hypothesis that each of these genetic variants modulates BCR signaling and that these alterations in BCR signaling will reveal mechanisms by which B cell tolerance is lost in SLE.

Role: Principal Investigator

**OVERLAP**

There is no scientific or budget overlap between these projects. If pending grants are awarded, the percent/calendar month effort will be adjusted accordingly.

*All funding levels are annual direct costs.*

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## OTHER SUPPORT

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Ng, Bernard

*No changes in other support*

### ACTIVE

NONE

### COMPLETED RESEARCH SUPPORT

Grant # VA.SCV.1010./000-00.B\_N

10/01/2010 – 09/30/2012

South Central VA Health Care Network Research Grants Program

#### **Evaluating the Optimal Use of Traditional Disease-Modifying Drugs of Rheumatic Diseases in the Biologic Era**

Brief summary – Since the advent of biological DMARDs, there has been reduced interest in the use of traditional DMARDs. Most recent literature, especially those funded by large pharmaceuticals, focuses on new and expensive drugs. There is little funding and interest to look at optimizing MTX use and the use of triple traditional DMARDs RA therapy. Knowing the trends of prescription behavior of DMARDs will be important to make cost-effective recommendations regarding traditional and biological DMARDs.

Role: Principal Investigator

Grant # CF.HQU.LFP.0110.000-00.B\_N10/01/2009 09/30/2010

Houston VA Health Services Research and Development Center of Excellence

#### **Is low or moderate alcohol consumption safe in patients with Rheumatoid Arthritis (RA) on Methotrexate**

Brief summary- Methotrexate (MTX) has been used extensively in the treatment of RA since the early 1980s. It is a drug with a high benefit-to-risk ratio compared to many traditional disease-modifying anti-rheumatic drugs (DMARDs). In addition, low cost, convenient weekly dosing and wide availability make MTX a preferred drug over several other DMARDs. It is usually recommended that one should abstain from alcohol while taking MTX because of the fear of liver toxicities. This is a conservative recommendation that is based solely on expert opinions because there is no clinical data about the quantity of alcohol that can be safely consumed with MTX. A study commented that more patients refused Leflunomide, another DMARD which abstinence from alcohol is required, when told that they needed to abstain from alcohol. Though it is not unreasonable to assume that alcohol will adversely affect MTX compliance, there are no well-established studies in this area unlike the treatment of HIV and diabetes. If low or moderate alcohol consumption is found to be safe when taking MTX, the compliance for MTX may be improved when patients are told that complete abstinence from alcohol is not required.

Role: Principal Investigator

Grant # (Huston, D.P.)

07/01/2002 – 06/30/2003

GlaxoSmithKline/American Academy of Allergy Asthma and Immunology

#### **Atomic Delineation of the $\beta$ c Receptor Binding Domain of IL-5**

Brief summary –The grant funded a study that tested the hypothesis that the IL-5 domain that engages and/or activates the  $\beta$ c subunit of the IL-5 receptor involves a specific charge field around the Glu13 residue. The corollary hypothesis is that a charge alteration at this site will result in an IL-5 molecule still capable of binding the IL-5R $\alpha$  subunit but unable to transduce and/or recruit an agonistic signal through the  $\beta$ c subunit. Such molecules should have the potential to be a therapeutic molecular antagonist of IL-5-mediated eosinophilic inflammation.

Role: Research/Clinical Fellow

### OVERLAP

There is no scientific or budget overlap between these projects. If pending grants are awarded, the percent/calendar month effort will be adjusted accordingly.

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## OTHER SUPPORT

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James, Eddie A.

### ACTIVE RESEARCH SUPPORT

(NEW)

1 DP3 DK106909-01 (Kwok, W)

09/01/2015 – 08/31/2018

13% effort

NIH/NIDDK

#### **Phenotypic analysis of islet antigen-specific effector T cells in pre-diabetic subjects**

The aims are to test the following hypotheses: 1. that an increase in the frequency of recently activated auto-reactive CD4+ and CD8+ T cells precedes the onset of clinical diabetes; 2. that prediabetic subjects that progress to T1D acquire an expanded TCR repertoire of islet antigen specific T cells and that those T cells exhibit a distinct transcript signature; and 3. that progression toward T1D is accompanied by an imbalance between Treg function and effector T cell responsiveness and by periods of active beta cell destruction.

Role: Co-Investigator

Program Officer: Lisa M. Spain

(NEW)

2-SRA-2015-107-Q-R (James, E)

08/01/2015 – 07/31/2017

8% effort

Juvenile Diabetes Research Foundation

#### **Validation of an improved HLA class I Combinatorial Multimer Assay**

The goal of this project is to validate an improved combinatorial T cell assay as an effective biomarker to predict risk of imminent loss of residual insulin secretion in subjects with type 1 diabetes.

(NEW)

1 R56 AI108883-01A1 (Kwok, W)

07/15/2014 – 06/30/2016

5% effort

NIH-NIAID

#### **Mechanistic Study of Peanut-specific T cells Pre and Post Oral Immunotherapy**

This project is a multifaceted investigation utilizing unique tools and assays and unique human samples to test important new hypotheses. The cumulative result will provide practical diagnostic tools and novel mechanistic insights about peanut allergy.

*Aim 1:* Characterize peanut reactive T cells in allergic, sensitized but tolerant, and non-allergic subjects.

*Aim 2:* Compare the molecular signature of peanut reactive T cells in allergic and non-allergic subjects

*Aim 3:* Characterize changes in the phenotype, frequency and molecular signature of peanut reactive T cells in response to peanut oral immunotherapy (OIT).

Role: Co-Investigator

Program Official: Wendy F. Davidson

(NEW)

5 U01 AI101990-03 (Buckner, JH)

03/01/2015 – 02/29/2016

10% effort

NIH-NIAID

#### **CSGADP Pilot: Selective Antibody and T cell Recognition of Citrullinated MBP in Rheumatoid Arthritis Patients**

The goal of this project is to investigate responses to citrullinated MBP in RA patients and to generate novel assays that can be used to study RA subjects, both in early pathogenesis and following disease onset.

*Aim 1:* Characterize responses to citrullinated MBP in PBMC and serum samples from RA patients and HLA matched controls

*Aim 2:* Characterize domain specific responses to citrullinated MBP in PBMC and serum samples from ACPA+ and ACPA- RA patients

Role: Pilot Principal Investigator

Program Official: Thomas R. Esch, PhD

**(NEW)**

2-SRA-2014-297-Q-R (James, E)

10/01/2014 – 09/30/2016

10% effort

Juvenile Diabetes Research Foundation

**T cell recognition of modified epitopes as a mechanistic contributor and biomarker of progression in type 1 diabetes**

We hypothesize that CD4+ T cells that recognize PTM epitopes are an important component of autoimmune responses that target pancreatic beta cells and that monitoring these responses can target a useful biomarker. With recently developed panels of epitopes within beta cell antigens, such as GAD65, IA2, and IAPP, we will utilize established ex vivo assays to measure the frequency of CD4+ T cells that recognize modified epitopes from beta cell antigens.

*Aim 1:* Demonstrate that CD4+ T cells that recognize PTM epitopes occur at elevated frequencies and exhibit a distinct inflammatory phenotype in subjects with T1D

*Aim 2:* Test the hypothesis that the frequency and phenotype of CD4 T cells that recognize PTM epitopes differs based on residual Beta cell function in subjects with T1D

*Aim 3:* Demonstrate that recognition of PTM epitopes by CD4+ T cells is an important immunologic checkpoint during the development of human T1D

Role: Principal Investigator

**(NEW)**

HHSN272201400049C (Koelle, D)

09/30/2014 – 09/29/2016

2% effort

NIH/BAA/NIAID/DAIT

**Large Scale T Cell Epitope Discovery**

The goal is to map T cell epitopes in a HLA restricted fashion for Vaccinia virus and T cell epitopes in a HLA restricted fashion for Varicella virus. All the epitopes identified will be verified by ex vivo tetramer staining and examined for the frequency and phenotype of T cells of recent both Vaccinia vaccinees and subjects that received small pox vaccines 20 years ago. Lastly, we will examine the cross reactivity between varicella and HSV-1/2 reactive T cells.

*Aim 1:* We will map T cell epitopes in a HLA restricted fashion for Vaccinia virus. We will identify epitopes for 3 different proteins for each HLA study for a total of 6 different HLA. T cell epitopes that are of 20 aa in length will be identified. We expect to identify a total of at least 60 different epitopes.

*Aim 2:* We will map T cell epitopes in a HLA restricted fashion for Varicella virus. We will identify epitopes for 3 different proteins for each HLA study for a total of 6 different HLA. T cell epitopes that are of 20 aa in length will be identified. We expect to identify a total of at least 60 different epitopes.

*Aim 3:* All the epitopes identified will be verified by ex vivo tetramer staining.

*Aim 4:* We will examine the frequency and phenotype of T cells of recent vaccinia vaccinees and subjects that receive small pox vaccines 20 years ago.

*Aim 5:* We will examine the cross reactivity between varicella and HSV-1/2 reactive T cells.

Role: Subaward Co-Investigator

**(NEW)**

1 UH2 AR067681-01 (Holers, VM)

09/24/2014 – 05/31/2016

5% effort

NIH-NIAMS

**Evolving Adaptive and Effector Mechanisms from Pre-RA through Established Disease**

The central hypothesis of this Clinical and Technology Research Site proposal focuses on Rheumatoid Arthritis (RA) is that novel disease stage-and cell lineage-specific therapeutic targets can be identified through the comprehensive evaluation of the linked adaptive and effector arms of the immune system as the disease sequentially progresses from its earliest origins through to a fully established destructive arthritis.

*Aim 1:* Using a larger sample of peripheral blood and synovial-derived cells drawn from patients at multiple stages of disease evolution, develop a comprehensive data set of single cell signatures from control cells and utilize this to identify novel pathways and targets within antigen-specific B and T lymphocytes as well as FLS and osteoclast precursors

*Aim 2:* To develop a further understanding of pathogenic pathways and imprinting in FLS and monocytic osteoclast precursors.

Role: Subaward Principal Investigator

**(NEW)**

3-SRA-2014-315-M-R (James, E)

09/01/2014 – 08/31/2017

10% effort

Juvenile Diabetes Research Foundation

**Immune effector and regulatory balance as a predictor for preserved beta cell function in subjects with established T1D**

This project will investigate the central hypothesis that T cell effector and regulatory balance represents a key mechanism that determines the persistence of C-peptide in established T1D.

*Aim 1.* We will test the hypothesis that an increased ratio of Treg/autoreactive CD8 Teff cells correlates with preservation of residual C-peptide in subjects with established diabetes.

*Aim 2.* We will test the hypothesis that determinant spreading (i.e., acquisition of additional CD8 Teff cell specificities for islet antigens) correlates with poor prognosis and with loss of residual C-peptide.

*Aim 3.* We will test the hypothesis that acquisition of a distinct functional and phenotypic profile among autoreactive CD8 Teff cells is an indicator of beta cell autoimmunity, reflecting loss of residual C peptide in subjects with established diabetes.

*Aim 4.* We will test the hypothesis that a restricted CD8 Teff TCR repertoire with expanded clonotypes is an indicator of chronic antigenic stimulation and activation, reflecting loss of residual C-peptide in subjects with established diabetes.

*Aim 5.* We will test the hypothesis that having a high number of T1D susceptibility risk alleles is a covariate that increases the likelihood of loss of residual C-peptide prior to 5 years post-diagnosis.

Role: Principal Investigator

5 U01 AI101981-04 (Holers, VM)

07/01/2012 – 06/30/2017

5% effort

NIH/NIAID

**Prevention Center U01: Early targets for antigen-specific tolerance induction in Preclinical Rheumatoid Arthritis**

Identify novel synovial T cell epitopes in RA, and develop HLA class II tetramers to detect autoreactive T cells. The data generated from these studies will be vital in the development of tools to predict the development of RA and will also yield information that will assist in the development of treatments to prevent the development of RA.

*Aim 1:* Identify the innate and adaptive immune response modifications that are causally associated with the break in tolerance to citrullinated autoantigens and progression to active disease.

Role: Subaward Co-Investigator

Program Officer: Thomas R. Esch, PhD

5 U19 AI100275-04 (Sette, A)

04/01/2012 – 03/31/2017

3.0% effort

NIH/NIAID

**LIAI Epitope Validation Center: Characterization of Allergen specific T cells**

**Core B: Reagent Development**

Benaroya Research Institute (BRI) will construct expression vectors for expression of different DP molecules in S2 cells. BRI will also purify DP molecules from transfected S2 cells, and assemble allergen specific DP tetramers.

*Aim 1 (Project 1):* To test the hypothesis that different TH subsets (TH2, TH1, TH17, and Tr1) are differentially associated with i) different allergic diseases and severity (allergic rhinitis, mild, moderate, and severe asthma) or ii) different allergen exposures (perennial vs seasonal, in/out of season).

*Aim 2 (Project 2):* To test the hypothesis that clinical efficacy as a result of SIT treatment is associated with differences in the quality and specificity of responses in terms of different TH subsets (TH2, TH1, TH17, Tfh, and Tr1).

*Aim 3 (Project 3):* To identify epigenetic signatures that correlate with asthma development and severity, by comparing histone modifications and DNA methylation/hydroxymethylation patterns.

*Aim 4 (Cores A, B, C, and D):* To catalyze and ensure progress for all projects.

Role: Research Scientist

Project Officer: Michael Minnicozzi

**PENDING**

BAA-NIAID-DAIT-NIHAI2013167 (Kwok, W)

10/01/2014 – 09/30/2019

25% effort

NIH/NIAID/DAIT



**CD4+ T cell Epitope Identification for high priority complex pathogens: Bordetella pertussis, Staphylococcus aureus, Clostridium difficile and Toxoplasma gondii**

The major objective for this proposal is to identify HLA class II restricted CD4+ T cell epitopes for Bordetella pertussis, Staphylococcus aureus, Clostridium difficile and Toxoplasma gondii using the Tetramer Guided Epitope Mapping approach.

Role: Project Manager

1 DP3 DK097653-01 (Kwok, W)

09/15/2012 – 09/14/2016

20% effort

NIH/ NIDDK

**Mechanisms for HLA-DQ mediated disease protection and susceptibility**

We propose to directly examine the role of DQ restricted T cells in T1D pathogenesis and protection from disease. This work will apply unique reagents and new technologies and extensive collections of DQ-restricted T cell clones and DQ-transfected cell lines.

*Aim 1:* To delineate the functional mechanisms of DQ mediated diabetes susceptibility.

*Aim 2:* To delineate the functional mechanisms of DQ mediated diabetes protection.

*Aim 3:* To directly examine the role of DQ restricted T cells in the pathogenesis of T1D.

Role: Co-Investigator

Grants Officer: Beena Akolkar, PhD

**COMPLETED RESEARCH SUPPORT**

**(PREVIOUS)**

HHSN272200900043C (Kwok, W)

09/30/2009 – 09/29/2014

NIH/NIAID

**Identifying epitopes recognized by influenza and Flavivirus responsive CD4+ T cells following vaccination or natural infection**

Class II tetramers will be used to identify and characterize CD4+T cells specific for influenza A, West Nile, Yellow fever, Dengue and Japanese Encephalitis viruses. The three major specific aims are:

*Aim 1:* To identify CD4+ T cell epitopes for seasonal influenza A proteins, including Neuraminidase (NA), Acidic Polymerase (PA), Basic Polymerase 1 and 2 (PB1 and PB2), Non-structural protein 1 (NS1) and Integral Membrane Protein (M2) of seasonal influenza A using the TGEM approach. CD4+ T cell epitopes restricted by at least 6 different DR alleles for all of the viral proteins indicated above will be identified.

*Aim 2:* To identify CD4+ T cell epitopes for avian influenza proteins, including Hemagglutinin (HA) of the H2, H7 and H9 subtypes, and NA T cell epitopes of N3 and N7 subtypes. CD4+ T cell epitopes for at least 5 class II alleles will be identified.

*Aim 3:* To identify CD4+ T cell epitopes within the Capsid (C) Protein, Precursor of M (PrM) protein, Envelope (E) protein, and Non-structural Proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 of four different species of Flavivirus using the TGEM approach. These will include: Yellow Fever Virus, West Nile Virus, Dengue Virus and Japanese Encephalitis Viruses. CD4+ T cell epitopes restricted by at least 6 different DR alleles will be identified for all of these proteins for the four different viruses indicated above.

Role: Project Manager

Program Officer: Timothy A. Gondre-Lewis

**(PREVIOUS)**

5 U01 AI01990-02 (Buckner, JH)

01/01/2014 – 12/31/2014

NIH/NIAID

**Cooperative Study Group for Autoimmune Disease Prevention Pilot: Changes in T cell recognition and transcript profiles in MS due to smoking (James, E)**

This project will assess the role of cigarette smoking in promoting T cell responses against neural antigens. We hypothesize that cigarette smoking up-regulates expression of the PAD enzymes and other pro-inflammatory mediators and co-stimulatory molecules, thereby inducing an inflammatory milieu. Synergistically, citrullination of proteins at key positions alters protein conformation and increases the processing, presentation, and recognition of altered peptides by CD4+ T cells.

Role: Principal Investigator

17-2012-121 (James, E)

06/01/2012 – 05/31/2014

JDRF

**Identifying and Monitoring T cell Responses to Modified Neo-epitopes in T1D**

We propose to investigate the role that PTM of beta cell antigens play in promoting islet-specific immune responses in T1D. We will identify specific PTM that generate neo-epitopes within beta cell antigens. We hypothesize that the PTM of key beta cell antigens leads to the generation and presentation of neo-epitopes that activate immune responses in the periphery.

*Aim 1:* Investigate the hypothesis that PTM increases immune recognition of islet antigens by generating neo-epitopes.

*Aim 2:* Investigate the hypothesis that subjects with T1D have elevated responses to islet-derived epitopes with PTM.

*Aim 3:* Investigate the hypothesis that T cell responses to modified epitopes are a meaningful biomarker in T1D.

Role: Principal Investigator

Grants Officer: Kathryn Leslie

Innovative Grant/Pilot (James, E)

03/01/2014 – 02/28/2015

JDRF

**Comparing responses to modified and unmodified T cell epitopes in subjects with T1D**

We propose to further investigate the importance of T cell responses against PTM of beta cell epitopes in T1D. Utilizing panels of epitopes within beta cell antigens such as GAD65, IA2, and IAPP, we will utilize established in vitro and ex vivo assays to compare the prevalence, frequency, phenotype and transcript profiles of CD4+ T cells that recognize modified or unmodified epitopes from beta cell antigens in subjects with T1D.

Role: Principal Investigator

Innovative Grant/Pilot (James, E)

03/01/2014 – 02/28/2015

JDRF

**Developing a combinatorial class II multimer assay for Type 1 Diabetes monitoring**

This research tools project will develop a combinatorial class II multimer assay and assess its capacity to monitor CD4+ T cell responses in patients with type 1 diabetes (T1D). We will evaluate the sensitivity and specificity of this new assay and assess its compatibility with a recently described combinatorial class I multimer assay.

Role: Principal Investigator

17-2012-559 (James, E)

10/01/2012 – 09/30/2015

JDRF

**IDS-TCW HLA Class I combinatorial multimer study**

This collaborative project, organized under the auspices of the IDS T cell Workshop, seeks to validate the reproducibility, sensitivity and specificity of combinatorial HLA-A2 multimer assays; and to subsequently test their suitability for monitoring changes in immunological status following immunotherapy. The work is divided into 3 phases, corresponding to the following aims:

*Aim 1:* To validate the standard operating procedure for the combinatorial HLA-A2 multimer assay in each participating center

*Aim 2:* To validate the reproducibility, sensitivity and specificity of the combinatorial HLAA2 multimer assay across participating centers

*Aim 3:* To evaluate the suitability of the combinatorial HLA-A2 multimer assay to monitor responses to immunotherapy

Role: Principal Investigator

Grants Officer: Kathryn Leslie

HHSN272200700046C (Kwok, W)

09/28/2007 – 09/30/2013

NIH/NIAID

**Allergen and T cell reagent resources for the study of allergic diseases**

The overall objective of this proposal is to develop class II tetramer reagents for the identification of HLA class II restricted CD4+ T cell epitopes from known allergens derived from Timothy grass, Bermuda grass, birch, alder, cat dander, peanut, fungi and yellow jacket venom proteins.

Role: Project Manager

**HPV-Specific, immune suppression in patients with RRP**

The major goal of this project is to provide tetramers in order for T cell epitope identification and tracking HPV T cells in peripheral blood.

Role: Research Associate

**OVERLAP**

There is no scientific or budgetary overlap with pending support. If any overlap occurs as pending grants are awarded, PI effort will be adjusted in accordance with Sponsor regulation and Institutional policy.

*All funding levels are annual direct costs.*

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## OTHER SUPPORT

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**Linsley, Peter**

### **ACTIVE RESEARCH SUPPORT**

5 P01 DE021954-05 (Rose, T) 05/01/2013 – 04/30/2016 1% effort  
NIH/NIDCR

#### **Oral Pathogenesis and Host Interactions of KSHV Infection – *Supplement***

Dr. Linsley will be responsible for overseeing the RNAseq analysis of KSHV-infected patient samples in Dr. Chaussabel's laboratory. Dr. Linsley will participate in the analysis of RNAseq experiments and help direct the activities of Bioinformaticians needed for advanced statistical analysis, and in systems analysis.

*Aim 1:* Project 1 will study the role of cell-cell transmission of KSHV during acquisition in oral tissues and dissemination to peripheral tissues.

Role: Staff Scientist

Program Officer: Isaac R. Rodrigues-Chavez

#### **(NEW)**

5 U01 AI082110-06 Rev (Linsley, P) 08/18/2009 – 07/31/2016 15% effort  
NIH/NIAID

#### **Blood transcriptional biomarker profiles for category B pathogens**

The major goals of this project are to identify and validate molecular biomarker signatures for category B pathogens by creating a consortium of worldwide investigators working in areas where pathogens are causing substantial morbidity and mortality.

Role: Principal Investigator

Program Official: Robert H. Hall

#### **(NEW)**

5 R01 AI108839-02 (Wambre, E) 07/01/2014 – 06/30/2019 1% effort  
NIH/NIAID

#### **Induction and signature of pathogenic T cells in allergy**

The purpose of this study is to identify a CD4+T cell signature for allergic diseases resulting from a comprehensive understanding of the mechanisms associated with the pathogenesis of allergic disease and peripheral tolerance to allergens.

*Aim 1:* To test the hypothesis that allergen-specific TH2 cells represent a stable and distinct TH2 subset unique to atopic subjects.

*Aim 2:* Determine the molecular mechanisms that defined pathogenic effector TH2 cells involved in allergic diseases.

*Aim 3:* To test the hypothesis that the allergic T cell signature (TH2A cells) can be used as a clinically meaningful biomarker in allergies.

Role: Co-Investigator

Program Official: Wendy F. Davison

#### **(NEW)**

1 R56 AI108883 - 01A1 (Kwok, W) 07/15/2014 – 06/30/2016 4% effort  
NIH/NIAID

#### **Mechanistic Study of Peanut-specific T cells Pre and Post Oral Immunotherapy**

This project is a multifaceted investigation utilizing unique tools and assays and unique human samples to test important new hypotheses. The cumulative result will provide practical diagnostic tools and novel mechanistic insights about peanut allergy.

*Aim 1:* Characterize peanut reactive T cells in allergic, sensitized but tolerant, and non-allergic subjects.

*Aim 2:* Compare the molecular signature of peanut reactive T cells in allergic and non-allergic subjects

*Aim 3:* Characterize changes in the phenotype, frequency and molecular signature of peanut reactive T

cells in response to peanut oral immunotherapy (OIT).

Role: Co-Investigator

Program Official: Wendy F. Davidson

**(NEW)**

1 DP3 DK104465-01 (Linsley, P)

09/25/2014 – 08/31/2016

30% effort

NIH/NIDDK

**Determining the molecular basis for different rates of T1D progression**

Our goal is to identify molecular and/or cellular signatures in whole blood that characterize non-progressor responses to different therapies and during natural history, and to determine whether these signatures are unique or treatment-specific. From these signatures, we anticipate discovering unique, data-driven insights into immunological aspects of T1D progression.

*Aim 1:* Identify unique and shared molecular/cellular signatures distinguishing T1D non-progressors from progressors following treatment.

*Aim 2:* Identify molecular/cellular signatures distinguishing T1D non-progressors from progressors during the natural history of T1D after diagnosis.

*Aim 3:* Identify cell types accumulating in non-progressor teplizumab-treated patients from the AbATE study and generate hypotheses as to their function.

Role: Principal Investigator

Program Official: Lisa M. Spain

**(NEW)**

3-SRA-2014-315-M-R (James, E)

09/01/2014 – 08/31/2017

10% effort

JDRF

**Immune effector and regulatory balance as a predictor for preserved beta cell function in subjects with established T1D**

This project will investigate the central hypothesis that T cell effector and regulatory balance represents a key mechanism that determines the persistence of C-peptide in established T1D.

*Aim 1.* We will test the hypothesis that an increased ratio of Treg/autoreactive CD8 Teff cells correlates with preservation of residual C-peptide in subjects with established diabetes.

*Aim 2.* We will test the hypothesis that determinant spreading (i.e., acquisition of additional CD8 Teff cell specificities for islet antigens) correlates with poor prognosis and with loss of residual C-peptide.

*Aim 3.* We will test the hypothesis that acquisition of a distinct functional and phenotypic profile among autoreactive CD8 Teff cells is an indicator of beta cell autoimmunity, reflecting loss of residual C peptide in subjects with established diabetes.

*Aim 4.* We will test the hypothesis that a restricted CD8 Teff TCR repertoire with expanded clonotypes is an indicator of chronic antigenic stimulation and activation, reflecting loss of residual C-peptide in subjects with established diabetes.

*Aim 5.* We will test the hypothesis that having a high number of T1D susceptibility risk alleles is a covariate that increases the likelihood of loss of residual C-peptide prior to 5 years post-diagnosis.

Role: Co-Investigator

**(NEW)**

5 UH2 AR067681-02 (Holers, VM)

09/24/2014 – 05/31/2016

3% effort

NIH/NIAMS

**Evolving Adaptive and Effector Mechanisms from Pre-RA through Established Disease**

The central hypothesis of this Clinical and Technology Research Site proposal focuses on Rheumatoid Arthritis (RA) is that novel disease stage-and cell lineage-specific therapeutic targets can be identified through the comprehensive evaluation of the linked adaptive and effector arms of the immune system as the disease sequentially progresses from its earliest origins through to a fully established destructive arthritis.

*Aim 1:* Using a larger sample of peripheral blood and synovial-derived cells drawn from patients at multiple stages of disease evolution, develop a comprehensive data set of single cell signatures from control cells and utilize this to identify novel pathways and targets within antigen-specific B and T lymphocytes as well as FLS and osteoclast precursors

*Aim 2:* To develop a further understanding of pathogenic pathways and imprinting in FLS and monocytic osteoclast precursors.

Role: Subaward Co-Investigator  
Program Official: Susana Serrate-sztein

**(NEW)**

5 UM 1AI109565-02 (Nepom, G)

02/01/15 – 01/31/16

9% effort

NIH/NIAID

**AbATE (ITN027AI) CD8/eomes signature in AbATE Responders**

Our aims are to establish resting and stimulation conditions for optimal CD154 upregulation, cytokine secretion, and gene expression in CD4 T cells from banked PBMC samples using foreign and islet peptides. Define conditions for tetramer staining and stimulation of banked PBMC to detect optimal numbers of tetramer positive CD4 T cells and robust gene expression using foreign and islet peptides. Compare C1 single cell RNAseq profiles from foreign and islet stimulated CD4 T cells isolated using CD154 up-regulation, CD154 and cytokine capture, and tetramer staining. Confirm the antigen specificity of expanded TCR clonotypes from foreign and islet stimulated/tetramer stained CD4 T cells. Determine the optimal assay(s) for detection and single cell RNAseq of islet antigen specific CD4 T cells from banked PBMC samples.

Role: Project Principal Investigator

Program Officer: Leighton A. Thomas

**(NEW)**

2-PAR-2015-123-Q-R (Nepom, G)

04/01/2015 – 03/31/2017

10% effort

JDRF-ITN Partnership

**Profiling immune subsets for biomarker assessments**

There is a critical need to predict, at the time of diagnosis of T1D, the rate of decline in beta cell function that will occur in the first few years of disease. The goal of this study to define blood transcriptome signatures from blood samples collected within six months of enrollment in recent onset T1D clinical trials that will predict the change in MMTT-stimulated c-peptide production at 2 years compared to baseline. Our primary aim is to define which sets of transcripts, either by their baseline level or as they change over time, correlate with rate of c-peptide decline.

Role: Co-Investigator

**(NEW)**

5 UM 1AI109565-02 (Nepom, G)

09/01/2015 – 01/31/2016

10% effort

NIH/NIAID

**Optimizing protocols for isolation of antigen reactive CD4 T cells and single cell RNAseq for use with banked PBMC samples**

The objective of this research is to identify conditions for one CD154-based assay and for a tetramer assay that: 1) have a high signal to noise ratio, 2) isolate the maximum number of flu reactive cells, and 3) induce easily detectable gene expression.

Role: Project Principal Investigator

Program Officer: Leighton A. Thomas

**COMPLETED RESEARCH SUPPORT**

**(PREVIOUS)**

9-2012-22 (Nepom, G)

01/01/2012 – 12/31/2015

JDRF

**JDRF/ITN Partnership in Immune Tolerance**

The mission of the JDRF-Immune Tolerance Network (ITN) Partnership in Immune Tolerance is to encourage and support early stage development of tolerogenic clinical protocols in T1D. The specific objective is to build clinical efforts by supporting phase I safety and phase IIa proof-of-principle trials. Examples include, but are not limited to: combination therapy trials, such as those involving antigen-specific and immunomodulatory approaches; and islet antigen-specific monotherapy trials with or without the use of tolerogenic adjuvants.

*Aim 1:* Discovery and development of antigen-specific immunotherapies;

*Aim 2:* Characterization of the roles of innate and adaptive immune responses in the immunopathogenesis of human type 1 diabetes and discovery of biomarkers for monitoring disease progression and responses to therapies in humans; and

*Aim 3: Preclinical studies, with a focus on mechanism and translatability, to support combination therapies in clinical trials.*

Role: Staff Scientist (AbATE Mechanistic Study)

Program Officer: Dr. Teodora Staeva PhD

**(PREVIOUS)**

17-2013-316 (Nepom, G)

01/01/2013 – 12/31/2015

JDRF

**T1D Biomarker Core Assay and Validation Center**

This project proposes to establish a core assay and validation center (CAV) based at the Benaroya Research Institute at Virginia Mason for the JDRF Biomarkers consortium. This CAV has 2 goals: to provide multi-assay assessment of biological samples from well-characterized patients and to coordinate data and sample sharing for consortium pilot studies that utilize distinct cutting edge technologies.

*Aim 1: Optimizing finger stick blood sample collection procedures:* We will test to what extent blood volume can be minimized without affecting reproducibility.

*Aim 2: Weekly monitoring of blood transcriptome fingerprints of T1D subjects:* We will establish in-home sample collection. We will generate data using a targeted high throughput PCR assay. Changes measured in T1D and controls will be compared.

*Aim 3: Develop companion analytic software:* We will deliver web-based analysis software to enable interactive exploration of the results. This software will also constitute a resource that can be leveraged in future studies.

Role: Staff Scientist

Program Officer: Simi T. Ahmed, PhD

**(PREVIOUS)**

Collaborative Research Agreement

11/05/2012 – 11/05/2013

Bristol-Myers Squibb

*Due to confidentiality terms in the agreement, we are unable to disclose information on this project.*

Role: Staff Scientist

Program Officer: Steven Nadler

**OVERLAP**

There is no scientific or budget overlap between these projects. If pending grants are awarded, the percent/calendar month effort will be adjusted accordingly.

*All funding levels are annual direct costs.*