Industry Collaborations

With a Network of 73 FOCIS Centers of Excellence

**National**
- Albert Einstein College of Medicine
- Baylor College of Medicine
- Beth Israel Deaconess Medical Center
- Boston Children’s Hospital
- Brigham & Women’s Hospital
- Children’s and Women’s Health Centre of British Columbia
- Cincinnati Children’s Hospital Medical Center
- Cleveland Clinic Foundation
- Columbia University
- Dartmouth Medical School
- Duke University Medical School
- Emory University
- Feinstein Institute for Medical Research
- FOCIS Center of Chicago
- FOCIS Center of Seattle
- Hospital for Special Surgery
- Massachusetts General Hospital
- Mayo Clinic
- McGill University
- Oklahoma Medical Research Foundation
- Robert Wood Johnson Medical School
- Stanford University Medical Center
- SUNY Medical University
- University of Alabama – Birmingham
- University of California, Los Angeles
- University of California, San Diego
- University of California, San Francisco
- University of Colorado Health Sciences Center
- University of Florida
- University of Iowa
- University of Miami
- University of Nebraska Medical Center
- University of Pennsylvania Medical School
- University of Pittsburgh
- University of Rochester Medical Center
- University of Southern California
- University of Toronto
- University of Western Ontario
- University Texas Southwestern Medical Center
- Washington University
- Yale University

**International**
- Assistance Publique – Hôpitaux de Paris
- Australian National University
- Charité – Universitäts Medizin Berlin
- Complejo Hospitalario Universitario de León/Universidad de León (CAULE)
- FOCIS Center of Excellence Sao Paulo
- Ghent University Hospital
- Giannina Gaslini Institute
- Immunity and Infection Center of the University Medical Center Utrecht
- Institucion Nacional de Ciencias Medicas y Nutricion Salvador Zubiran
- Institut Pasteur
- Institute Curie
- Institute of Immunology Czech Republic
- Institute of Immunology of Central University Medical School, Venezuela
- Karolinska Institute
- King’s College London
- Martin-Luther-Universität Halle-Wittenberg
- Millennium Institute on Immunology and Immunotherapy
- Nantes University
- Paris Diderot University
- Rostov-on-Don State Medical University
- Shiraz University of Medical Sciences
- Universitat Autònoma de Barcelona (UAB)-Barcelona
- Universite Libre de Bruxelles
- Hospital Erasme
- University Hospital of Freiburg
- University of Amsterdam
- University of Cambridge
- University of Florence
- University of Heidelberg
- University of Oslo
- Rikshospitalet
- University Hospital
- University of Oxford
FOCIS Centers of Excellence (FCEs)

The FCE community is a network of academic medical centers that focus on interdisciplinary and translational education and research.

The FOCIS Centers of Excellence (FCE) are organized to intensify and accelerate local multidisciplinary scientific and clinical innovation, education, and advocacy worldwide.

Partnership Benefits

- Collaborate with a worldwide network of top research institutions to advance research on immune-mediated diseases
- Leverage the unique expertise and advanced research techniques of the FCE community to help us change the face of science and medicine

Expertise in the FCE Network

Allergy • Ankylosing Spondylitis • Autoimmune and Infectious-disease Serology • Autoimmune Lymphoproliferative Syndrome • Autoimmune Thyroid Disease • Autoinflammatory Diseases • Bare Lymphocyte Syndrome • Bioinformatics and Big Data • Cellular Therapies • Chronic Granulomatous Disease • Dendritic Cells • Diabetes • Genomics • Hematopoietic Stem Cell Transplantation • Hematologic Malignancies • Immunodeficiency Diseases • Immunogenetics • Immunotherapy • Invariant NKT Cell Deficiency • In vitro study of CD4+ T cells • Kawasaki’s Disease • Leukocyte Adhesion Deficiency • Lupus • Multiple Sclerosis • Mutation Modeling in Immune Cells • Myocarditis • Oral Desensitization • Pharmacogenetics of Adverse Drug Events • Quantitative Cell Biology of Immune Cells • Recombinant Soluble HLA Molecules • Rheumatoid Arthritis • Statistical Analysis of High-dimensional Genomic Data • Structure and Ligand Binding Properties of Antibodies and T-cell Receptors • Transplantation • Vasculitis • X-Linked Disorders

To learn more about partnering with the FOCIS Centers of Excellence, contact Timothy Niewold, MD, Mayo Clinic, Niewold.Timothy@mayo.edu
FOCIS Centers of Excellence Case Study

Oklahoma Autoimmune Disease Institute
Oklahoma Medical Research Foundation; University of Oklahoma Health Sciences Center

Extensive Regional well characterized patient collections in an environment focused on the continuum of research focused on mechanisms of disease, matched with novel clinical trial designs and samples collected/processed/stored to enable more extensive follow-up research questions.

1. Patient/Subject Collections with associated KOLs/trial design:
   - Oklahoma Lupus Cohort (over 600 well characterized, actively seen lupus patients involved in various clinical trials).
   - Oklahoma Multiple Sclerosis Center of Excellence Cohort (OMRF ADI-MSCE) (over 2500 active MS patients seen in regional clinic that are involved in various clinical trials).
   - Other local clinics focusing on Rheumatoid Arthritis, Sjogren’s Syndrome, Diabetes
   - Oklahoma Immune Cohort (over 1000 screened healthy controls available for re-contact for research studies)

2. CAP compliant (CAP certification expected July 2016) Sample & Clinical Data Biorepository available for Clinical Trial sample & clinical trial data management.
   - Over 3 decades of autoimmune diseases sample collections
   - Extensive capacity for additional sample processing/collection/archival
   - Full range of sample types and processed derivatives to enable detailed genetic variant discovery/confirmation, serum/plasma/urine/saliva available for biomarker/proteomic/metabolomic studies, viable frozen blood cells and transformed cell lines from most disease cases and controls enable functional genomic and disease mechanistic study experimental designs.
   - iPSC generation from primary blood/tissue cells and long-term cell lines for in vitro disease model development and genome engineering approaches (CRISPR/Cas9, Talen).
   - Lupus Multiplex Registry and Repository (cross-sectional Lupus repository, >3,500 lupus cases, over 12,000 total subjects including family members and controls. Extensive genetic and other experimental biomarker data in addition to clinical classification data)
   - MS Registry and Repository (longitudinal, >2,500 MS and NMO patients from OMRF ADI-MSCE)
   - OLC (longitudinal lupus cohort from OMRF clinics, >400 subjects)
   - SS Registry and Repository (cross-sectional SS repository, >1,500 subjects)
     i. Various Native American Autoimmune sample collections, serum only, cross-sectional cohort, >250 subjects.

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3. Phenotyping Core (available for add-on functional studies for Clinical Trials and Mechanistic Studies)
   - Serum proteomics
     i. Bioplex2200 ANA and other research-based multiplex testing using clinical assays.
     ii. Bioplex200 custom biomarker testing (cytokines/chemokines/autoantibodies, etc.)
     iii. Protein microarray processing/scanning
     iv. Peptide based epitope mapping/discovery
     v. Human mAb generation
   - Genetics
     i. Extensive Next Generation Sequencing Core for all types of genotyping, variant discovery (SNP exome sequencing, whole genome sequencing, deep resequencing, CNV, etc.)
   - Genomics (Transcriptomics)
     i. High throughput RT-qPCR – Fluidigm Biomark HD systems
     ii. Microarray based whole genome expression
     iii. RNA-Seq using Illumina NGS methods (whole blood, isolated cells, miRNA, exosome, etc.)
     iv. Single-Cell RNA-Seq
        1. Fluidigm C1-based Integrated Fluidics Cell Methods
        2. Sorted cell populations-based RNA-Seq
        3. Plate-based sorted single-cell RNA-Seq
   - Epigenetics
     i. Microarray-based methylation analyses
     ii. NGS based ATAC-Seq, Methyl-Seq, ChIP-Seq etc. on sorted bulk cell populations (from fresh or frozen viable cell sources.).

4. Data Analytics
   a. Clinical Data/Biostatistical Analyses
   b. Clinical Epidemiological Analyses
   c. Statistical Genetics Analysis/Bioinformatics
   d. Translational Informatics
FOCiS Centers of Excellence Proposal  
Assessing Steroid Resistant Immune Cells in the LUCiN Rayos Trial

Summary
Corticosteroids have been a mainstay in the treatment of autoimmune disease for more than 50 years. Despite the impressive efficacy of corticosteroids, there are some patients who do not respond as expected, or who require doses which are higher than other patients. Recent studies have identified populations of pro-inflammatory immune cells which are resistant to corticosteroids, and likely contribute to incomplete response or greater steroid requirements. The persistence of these cells may be able to explain some of the variability in steroid response between individuals, and could be useful in predicting responses and doses of steroid for a given patient. The Federation of Clinical Immunology Societies (FOCiS) Centers of Excellence represent more than 70 major academic institutions from the US and around the world dedicated to human immunology. There are 27 FOCiS Centers of Excellence (FCEs) that are participating in the LUCiN trials network, and we propose a mechanistic study to be performed with the LUCiN Rayos study by the FCEs which would assess steroid resistant cells before and after treatment with Rayos. Our FCEs have worked extensively to standardize flow cytometry methods between sites [1], and we could apply both standardized flow cytometry as well as cutting edge CyTOF mass cytometry to assess steroid resistant cells in the subjects participating in the Rayos trial. These data could be analyzed in the context of response/non-response and effective dose of Rayos. This could allow for more personalized treatment in the short term, and in the longer term could suggest add-on therapies which may enhance the activity of Rayos by targeting and neutralizing steroid resistant cells.

Steroid Resistant Cells
Studies of steroid resistant immune cell populations have strongly implicated T cells of the T helper cell 17 (Th17) lineage [2, 3]. Studies have shown that Th17 cells within mixed T cell populations are more resistant to suppression by steroids than other T cells present. Two recent studies attempt to further characterize resistant Th17 T cells in patients with colitis and asthma and are summarized briefly below.

Ramesh et al studied steroid resistance in patients with colitis [2]. They used flow cytometry to study T cell subsets after steroid treatment. Total CD4+ T cells when cultured for 12 days with anti-CD3/CD28 showed an increase in the percentage Th17 cells in cultures containing corticosteroids. These steroid resistant cells expressed greater amounts of IL23R and demonstrated a pro-inflammatory gene expression profile despite being culture with corticosteroids. Schewitz-Bowers et al studied T cells of asthmatic patients and divided the patients into two groups, those that respond to steroids and those that do not [3]. They could identify steroid-resistant Th17 lineage cells in both groups of patients, and were able to document pro-inflammatory gene expression in these resistant cells. Interestingly, this population of cells was sensitive to treatment with cyclosporine, suggesting a potential adjunct treatment which could be helpful for patients who have a large number of steroid resistant cells.

METHODS
Standardized Multicenter Flow Cytometry at FCE sites
The FOCiS Centers of Excellence have pioneered the standardization of multi-site flow cytometry with human blood samples, with the goal of using this method for multi-site human immunomonitoring in the context of clinical studies. The HIP-C consortium developed panels of flow cytometry fluorophores and antibodies that have been operationalized as a standardize lyophilized plate which allows for stability of reagents and ease of use. A flow chart showing how this panel of flow cytometry markers can distinguish T cell populations is shown in Figure 1 below, with Th17 cells indicated by the blue arrow.
Assessing Steroid Resistant Immune Cells in the LUCiN Rayos Trial

Study Aim 1: We propose to use the standardized operating procedures (SOPs) and lyophilized reagent plates developed by the HIP-C consortium to assess T cell subsets and numbers of Th17 cells in the subjects in the Rayos trial at time zero and at the date at which the clinical outcome measure is to be determined. Th17 cell numbers will be correlated with response vs. non-response as well as steroid dose required for clinical response.

CyTOF as an alternative to identify cell populations in responder/non-responder subsets

The laboratory of Garry Nolan at Stanford has pioneered the use of CyTOF technology for comprehensive investigation of the human immune system[4-7]. They are currently using the CyTOF to generate 30- to 40-parameter flow cytometry data on cryopreserved PBMC and whole blood samples from various studies. In so doing, they are able to generate much more complete phenotypic data on the major blood cell subsets involved in disease processes and with a smaller number of cells.

Mass cytometry is based on the concept of using heavy-metal isotopes to label antibodies for flow cytometry, rather than fluorescent tags[8, 9]. The labeled cells are introduced sequentially into a mass spectrometer for quantitative detection of the metal labels associated with each cell. As seen in Fig. 2, this affords the ability to combine many more antibody specificities in a single experiment, without significant spillover between detector channels.

Figure 2. Comparison of emission spectra of conventional fluorophores (left) with detection of metal ions by ICP-MS (right).

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FOCiS Centers of Excellence Proposal (continued)
Assessing Steroid Resistant Immune Cells in the LUCIN Rayos Trial

Scientists at Stanford have developed a CyTOF panel for probing 8 phosphorylated signaling proteins in combination with 22 cell-surface markers, to interrogate cell signaling in both major and minor leukocyte lineages. Based on already standardized protocols, whole blood can be stimulated with a panel of activators, tentatively listed in Fig 3, using a standardized system known as Smart Tubes (Smart Tube Inc.). These tubes contain pre-configured, lyophilized stimuli to which whole blood is added. The tubes are incubated in a Smart Tube instrument, which times the activation and then releases an RBC lysis/fixative buffer at the end of the incubation period, mixes, and alerts the user to remove the tubes to a -80C freezer, where they can be stably held for months prior to batch analysis, e.g., by CyTOF. This system provides for a highly standardized stimulation workflow, ensuring maximum reproducibility. At the same time, it allows for activation of whole blood immediately after draw, ensuring that all potential responses and cell types are optimally measured.

FCS files generated from the CyTOF acquisition are analyzed in FlowJo software using a standard template. This generates numerical information on the abundance of 32 major and minor cell subsets. More comprehensive and unbiased analysis is also possible using SPADE[10], for which Stanford can provide guidance in its use. SPADE is a free algorithm developed in the Nolan lab for clustering and visualization of highly multidimensional data such as that generated by CyTOF.

Thus, CyTOF provides a comprehensive view of the circulating immune system, and would be a fascinating complementary technique along with standard flow cytometry as outlined in Aim 1 above. In the Rayos trial, CyTOF data from responders versus non-responders could be compared, these readouts would all be tested via ANOVA for significant differences between these two groups, with post-hoc testing to define the readouts with differences at P<0.05 (corrected for multiple comparisons). Alternately, analysis could be done via unsupervised clustering, using, for example, Citrus (8). This algorithm would test for differences between each cluster’s proportion and/or median intensity between the two outcome groups, allowing one to find the clusters with significant differences. Comparison of this to the supervised analysis should hopefully provide confidence in differences that are detected in both methods.

**Study Aim 2:** Use CyTOF profiling to assess the circulating immune response in the Rayos trial before and after treatment, including Th17 as well as other immune cell subtypes to define populations of cells associated with response vs. non-response to Rayos. A smaller subset of FCE sites with CyTOF capability would participate in this Aim.

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FOCiS Centers of Excellence Proposal (continued)
Assessing Steroid Resistant Immune Cells in the LUCiN Rayos Trial

Significance, Expected Outcomes, and Deliverables
Using the cutting-edge and standardized methods outlined above, we would be able to comprehensively assess the immune response prior to and after Rayos treatment in the subjects enrolled at FCE sites involved in the LUCiN network Rayos trial. This could provide mechanistic insight into response and non-response, and in the case of steroid-resistant Th17 cells, there are already studies suggesting that alternate medications might directly address these cells and augment Rayos treatment in those with incomplete responses. These data would be shared with Rayos, and we would plan to publish the findings in the scientific literature. This work could allow for improved implementation of Rayos clinically.

References

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