Autoimmunity Centers of Excellence Progress Report 2021

Overview

The Autoimmunity Centers of Excellence (ACE) Program consists of three interrelated research components: (i) basic science projects conducted by five U19 Centers; (ii) clinical projects, i.e., clinical trials with integrated mechanistic studies, led by five UM1 Centers; and (iii) Collaborative Projects that stem from the original peer-reviewed proposals and were expanded and integrated to leverage the skills and expertise of the ACE investigators to address clinically-important, large mechanistic questions. As described below, these three components are not independent, but rather represent a coherent approach to several of the most important challenges in the autoimmunity field.

- Basic Research Program (U19)
- Clinical Research Program (UM1)
- Collaborative Projects

Contents (linked):

Basic Research Program

The basic research program of the 2019-2024 cycle of the ACE is comprised of 5 U19 awards with each award incorporating a Principal Project; a Collaborative Project; and a Pilot Project. Within each ACE U19, the individual components weave a cohesive line of basic investigation into one or more autoimmune diseases including: SLE with a major component of pediatric SLE, IgG4-RD, Systemic Sclerosis, and Celiac Disease. All three components, proposed in the corresponding peer-reviewed applications, provide a foundation for either synergistic or additive studies to those performed by other centers thus contributing both to the larger ACE Collaborative Program that is described in more detail at the end of this document and to the development of ancillary mechanistic analyses of ACE clinical trials. In addition, the ACE Network has assembled a deliberately structured Collaborative Program comprised of highly integrated projects. The Collaborative Program was designed by the combined participation of all ACE U19 and UM1 centers.

University of Chicago: Human In situ adaptive autoimmunity

The focus of the University of Chicago ACE (UCACE) is in situ human autoimmunity. During the last funding cycle, we successfully developed techniques to fully characterize the transcriptional state of single B cells and plasmablasts sorted from tissue samples and to pair this with analyses of functional immunoglobulin repertoire. Application of this approach to lupus tubulointerstitial inflammation (TII) and renal acute mixed allograft rejection (AMR) suggest that the activation state, antigenic repertoire and mechanisms of antigenic-driven B cell selection in human inflammation is fundamentally different than that typically observed in secondary lymphoid organs (Collaborative Project). In Celiac disease, B cells expressing transglutaminase 2 (TG2) specific antibodies are massively expanded in the duodenal mucosa. A highly restricted repertoire of VH genes encode these antibodies, most notably VH5-51. Remarkably, this VH gene is also over-represented in the recirculating IgA+ B cell pool which is rich in anti-microbial activity. These findings suggest a model in which microbial antigens select for pathogenic TG2 reactive antibodies in susceptible hosts (Principal Project).

Our second technical innovation is unique to the UCACE (Collaborative Project). Previous work has demonstrated that by quantifying the distance between T and B cells in multicolor confocal images (Cell Distance Mapping, CDM) we could identify competent TFH cells and functional relationships with B cells. We have now implemented a deep convolutional neural network (DCNN) that accurately identifies both cell position and shape in multicolor confocal images. In mice, analysis of the DCNN output (CDM3) indicates that T cell shape as a function of distance from dendritic cells (DCs) discriminates between cognate and noncognate T cell:DC interactions with a sensitivity and specificity approaching that of two-photon emission microscopy (TPEM). In lupus TII, CDM3 both confirmed that myeloid DCs present antigen to CD4+ T cells in situ and identified plasmacytoid DCs as an important antigen presenting cell (APC) in severe TII. Finally, in the Pilot Project, we are applying microfluidics to develop in vitro culture systems capable of studying cognate interactions between single cells. These projects demonstrate a novel pipeline of methodologies to identify in situ cell populations, characterize their function and quantify the adaptive cell networks through which they cooperate to drive local adaptive immunity and inflammation.

Emory University: Molecular Regulation of B cells and T cells in Human SLE

The overarching objective of the Emory Autoimmunity Center of Excellence (ACE) U19 is to decipher the molecular programs responsible for the aberrant effector immune responses that lead to autoimmune disease. Specifically, based on the work performed by the Emory ACE during the previous funding cycle, we postulate that epigenetic regulation of effector B cell differentiation and function is a critical pathogenic component of Systemic Lupus Erythematosus (SLE). Further, we contend that disease-related epigenetic imprinting is first established at early stages of B cell development and then maintained throughout the differentiation of naïve cells into their effector progeny upon activation by antigens and costimulatory pathways. Finally, we propose that SLE will also be characterized by abnormal regulation of other critical effector immune responses, namely CD8 T cells and in particular, the stem-like population responsible for the maintenance of antigen-specific responses in chronic viral infections and anti-tumor responses in patients treated with checkpoint inhibitors.

The fundamental goals of the Emory ACE are: 1) to understand B cells and CD8 T cells dysregulation in human SLE; and 2) to assemble a scientific and technological platform that engages other ACE U19 and UM1 Centers to perform similar studies in other immune cells and autoimmune disorders. The specific aims of the Emory ACE U19 are: Aim 1: To establish an Administrative Core (I. Sanz, Core Director) for the successful operation of the ACE U19 Scientific Program and its interaction with the ACE Network; Aim 2: To develop a highly integrated Emory ACE U19 Scientific Program comprised of the following components: Principal Project (Sanz, PI): Mechanisms of B cell dysregulation in human SLE; Collaborative Project (Boss, PI): Epigenetic regulation of autoimmune responses; Pilot Project (Ahmed, PI): Characterization of stem-like CD8 T cells in SLE.

Since the beginning of the current funding cycle, we have optimized all the required technologies and incorporated new ones. Notably, we have developed updated multidimensional flow cytometry protocols using multi-spectral technology; new in vitro culture systems to differentiate and sustain survival of human plasma cells; comprehensive single cell multi-omics for integrated analysis of RNAseq, ATAC-seq, VDJ BCR repertoire and surface expression of over 120 markers. These markers include multiple SARS-CoV2 antigens for the detection and analysis of antigen-specific cells in COVID-19 vaccination and infections.

To date and from the start of this funding cycle, our labs have used multi-dimensional flow cytometry and single cell analysis as well as paired RNA-seq, ATAC-seq and BCR repertoire of bulk-sorted B cell and PC subsets to: 1) ascertain the heterogeneity and molecular programs of peripheral B cells and PC in SLE; these studies demonstrate substantial heterogeneity within these populations with distinct transcriptional and epigenetic programs between populations and relative to other autoimmune diseases and HC. In the process, to the best of our knowledge, we have provided the first description of disease-associated B cell abnormalities in RA, scleroderma, AAV, pemphigus, dermatomyositis and Sjogren's syndrome; 2) elucidated at the single cell level the regulatory developmental programs of bone marrow SLE B cells; these studies have defined additional developmental subsets of pre-B and B cells in the human bone marrow and pinpointed the regulatory abnormalities in early SLE development. Through the identification of subsets and individual cells bearing hallmarks of BCR activation, we will use these data to reconstruct the antigenic history of autoimmune B cells and determine the nature of SLE triggering autoantigens and the differential programming and fate of these cells. As also indicated in the corresponding section, through the Collaborative Agenda, we are working with multiple ACE centers to understand the epigenetic regulation of B cells in additional autoimmune conditions and in several clinical protocols

In addition, since the onset of the COVID-19 pandemic, the Emory ACE has very actively participated in the study of infection and vaccination through both, our own Center-specific administrative supplements and AE network initiatives including: MISC immunological studies within the PRISM network; B cell and plasma cell contributions to COVID-19 infection and vaccination in the general population and in SLE; and vaccination studies in autoimmune patients studies either under standard-of-care or in the context of modification of their baseline immunosuppression (ACV01 and ACV02 protocols).

Our initial studies of COVID-19 infection demonstrated that severe infection was strongly associated with effector extrafollicular B cell responses similar to those observed during acute flares of severe SLE. Moreover, such responses were associated with enhanced autoreactivity and a broad breach of tolerance appears to be associated with severe COVID infection. Current studies are focused on further understanding the cellular basis of these observations, and the short-term and long-term regulation of early tolerance breakdown and its implications for the development of MISC and long-haul syndrome (PASC).

Feinstein Institute for Medical Research: Heterogeneous pathways to autoantibody production: implications for prognosis and therapeutic targeting

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease in which autoantibodies to ubiquitous nuclear antigens cause inflammation and tissue damage in multiple organs. The treatment of SLE has improved considerably over the past 30 years, but these advances have relied on existing medications with insufficient efficacy and significant toxicities. Although several new therapeutics are advancing to Phase 2

and 3 trials, only one modestly effective biologic is currently FDA approved for treating active SLE. It is imperative, therefore, that advances in immunologic knowledge be applied to improve the treatment and quality of life of SLE patients. Autoantibody production is central to the pathogenesis of SLE but many questions remain about the origins of the plasma cells that produce them. It is clear that autoantibody production can precede disease onset by many years, and that flares of SLE are often associated with a new wave of plasma cell proliferation. Our Autoimmunity Center of Excellence proposal is centered on the hypothesis that an improved understanding of the mechanisms of induction and source of autoantibodies in individual patients will allow us to define the spectrum of dysregulated mechanisms responsible for loss of tolerance in human SLE and will form the basis for appropriate patient stratification and selection for clinical trials. To this end, we will apply new technologies that allow meaningful study of small numbers of human cells in a native repertoire to revisit basic questions about the origin and regulation of autoantibodies in SLE. A crucial tool is a new fluorescent nuclear antigen preparation, developed by the Diamond lab that can be used to identify and isolate autoreactive B cells that represent only a small fraction of the total B cell population. This will facilitate the use next generation sequencing of immunoglobulin genes to analyze the repertoire specifically of autoreactive B cells. The Principal Project will characterize autoreactive plasma cells from patients with SLE and ask about their origins and transcriptional profile, with a view to identifying distinct pathways of activation in individual patients that may be specific to autoreactive B cells, compared with cells that protect against microbial antigens. The Collaborative Project will examine mechanisms for the initiation of lupus-related autoimmunity in patients being treated with TNF inhibitors for inflammatory arthritis. The Pilot Project will address the transcriptional, metabolic and functional diversity of circulating T follicular helper cells in patients with SLE and ask whether it is possible to restore normal B cell helper function of these cells by altering T cell metabolism. Our proposal is bolstered by close scientific interactions among the three lead investigators, by collaborations with experts in next generation sequencing methods and data analysis and by a robust clinical infrastructure that will add clinical depth and ensure timely recruitment of patients for all three studies.

Massachusetts General Hospital: An Autoimmune Center of Excellence for the Study of IgG4-Related Disease

This project focuses on the underlying basis of autoimmune fibrotic disorders, focusing primarily on IgG4-related disease. We have used, and currently continue to use multi-color multispectral imaging of disease tissues, cell distance mapping as well as single-cell transcriptomics, TCR sequencing and BCR sequencing to interrogate tissues from IgG4-related disease. In particular we wish to identify and quantitate CD4+cytotoxic T cells and activated B cell subsets and better understand whether they directly contribute to cell death in tissues and thus to fibrosis, or if they primarily secrete fibrogenic products. Since B cell depletion resolves IgG4-related disease one focus is on identifying a key “culprit” tissue infiltrating B cell population. Such a population could be a key target for therapy in fibrotic diseases that go beyond IgG4-related disease. The studies of the MGH Basic ACE during the pandemic extended to studies in COVID-19 as well and we described major alterations in B cell populations and B cell activation that are seen both in COVID-19 and in IgG4-RD. We described a unique stage of B cell activation and these activated B cells are found both in IgG4-RD tissue lesions and in the lungs of COVID-19 patients. We are also involved in studies analyzing the molecular basis by which CD4+cytotoxic T cells differentiate. We continue to test two competing but mutually nonexclusive hypotheses - one that CD4+T cells, with the help of activated B cells drive tissue cell death as a prelude to fibrosis, and the other that the cytokines and enzymes secreted by activated T cells and disease-related B cells are causal for fibrosis. We will also examine the interactions between adaptive and innate immune cells in disease and identify antigens that trigger clonally expanded B and T cells in patients. We will also examine whether intestinal microbial gene expression reveals differences between subsets of disease or explains responsiveness to therapy. In systemic sclerosis, our pilot studies will focus on a search for T cell antigens. The possible contribution of microbial metabolites to immune cell differentiation will be examined and microbial antigens that activate host T cells will also be identified.

Weill-Cornell: Immune Cells and Secretory Pathways Leading to Human Systemic Autoimmunity

The Autoimmunity Center of Excellence based at the Drukier Institute for Children's Health Research at Weill Cornell Medicine (WCM) in New York, NY aims at 1) advancing the knowledge of pathways and mechanisms that contribute to the development and amplification of Human Systemic Autoimmune Diseases (SADs); 2) developing tools and identifying biomarkers to monitor these dysfunctional pathways. Ultimately, we aim to be able to stratify patients towards personalized approaches to treatment.

The Center will employ ex vivo and in vitro high throughput technologies and immune profiling to gain insight into two major and complementary compartments contributing to SADs: Immune Cells and Extra-Cellular Nanoparticles. The appropriate infrastructure is in place to support patient-oriented studies, including established pediatric SLE cohorts followed by experienced clinicians with an exceptional record of participation in translational research. While the initial focus will be the study of children with Systemic Lupus Erythematosus (SLE), extrapolation of the Center findings to adult SLE as well as other SAD scenarios will be pursued, particularly in the context of the ACE Collaborative efforts.

The Drukier Institute for Children's Health Research at Weill Cornell Medicine has gathered a multidisciplinary team of pediatric basic and patient-oriented investigators with expertise in immunology, autoimmunity, cancer biology, molecular biology, bioinformatics and software engineering, who work together with clinical experts in autoimmunity, cancer, allergy and infectious diseases to understand and treat these diseases. The Institute has also established strong local, national and international collaborations. Dr. Pascual's team has a long history of productive research in the fields of human autoinflammatory and autoimmune diseases. Dr. Lyden's group has pioneered the study of exosomes and exomeres, and how these particles horizontally transfer their cargo to recipient cells, thereby acting as vehicles of intercellular communication in both physiological and pathological conditions. The proposed Center is a natural result of the very complementary expertise of these groups and is well-poised to work collaboratively with other Centers to advance clinical and basic discoveries in the field of human autoimmunity

Clinical Research Program

The clinical research component of the ACE program consists of five Centers that provide care and perform clinical investigation across a broad spectrum of autoimmune diseases. These Centers propose innovative, collaborative ACE clinical trials that span multiple sclerosis, IgG4 mediated disease, systemic lupus erythematosus and juvenile inflammatory arthritis. Alternate trials from each Center are also proposed. In addition, legacy trials continue from the prior ACE cycle, including two which are still enrolling and two which have completed enrollment and analysis is underway. As summarized briefly below, one program will explore targeting of a novel fibroinflammatory process to selectively eliminate pathogenic cells (Mass General), one will assess a unique cholinergic anti-inflammatory pathway through a homeostatic, non-immunosuppressive mechanism (Feinstein), one program will evaluate which patients can safely stop selective immunosuppressive therapy (Penn), and one will address disease heterogeneity to enrich for patients with specific molecular profiles for treatment with one or combined immunosuppressive therapy (OMRF). The current status of the ongoing legacy trials is also briefly summarized.

Feinstein Institute

This Center is leading a project assessing the cholinergic anti-inflammatory pathway to treat Juvenile Idiopathic Arthritis (JIA). The cholinergic anti-inflammatory pathway is a homeostatic, non-immunosuppressive mechanism that diminishes inflammation. The research question is whether engaging this pathway by transcutaneous stimulation of the auricular branch of the vagus nerve (taVNS) will ameliorate inflammatory symptoms in children with JIA. This Center is additionally interested in the mechanisms associated with a potential clinical effect and are seeking to find potential biomarkers of response.

The trial is a 12-week study that will be conducted at pediatric rheumatology centers in a randomized double blind, placebo-controlled design of taVNS vs sham stimulation (SS) with a single cross over design. Subjects

will be stratified by JIA disease subtype and medication (i.e. biologic use). At baseline, 68 subjects will be randomized 1:1 to receive taVNS or (SS). At 6 weeks, subjects receiving SS will crossover to taVNS and all subjects will receive taVNS through week 12. The primary endpoint is the 6 week JIA ACR 50 response (a validated response measuring improvement in JIA) in subjects randomized to taVNS compared to subjects randomized to sham stimulation (SS). Safety is monitored throughout the study. Multiple secondary endpoints will be assessed using validated instruments, including pain, fatigue, quality of life, and a functional health assessment. JIA ACR 30 and 70 responses and the individual components of the JIA ACR response will be also be assessed. JADAS, a measure of disease activity is an additional secondary endpoint.

Mechanistic endpoints will include serum levels of CRP, HMGB1, soluble mediators, substance P, lipid mediators of inflammation and of the resolution of inflammation. Blood transcriptome and TLR4 stimulated levels of soluble mediators are additional mechanistic endpoints. Analyses will be performed to compare response rates at 6 weeks between taVNS and SS. Secondary analyses will include comparisons of JIA ACR 30 and 70 and change in disease activity (JADAS) at week six and change in JDAS and of JIA ACR 30, 50 and 70 response rates between the groups at week 12. The trajectory of response rates and of disease activity in each of the two groups will also be analyzed and compared over the 12-week study. Predefined subgroup analyses include explorations of primary and secondary endpoints in individual subtypes of JIA patients and of children entering the study with 3 or 4 active joints. Biomarkers correlating with response and biomarkers predicting a response will be sought from the wide scope of mechanistic markers. An additional exploratory secondary analysis will be correlations of stimulation voltage (measured at study visits) and change in disease activity.

Massachusetts General Hospital

IgG4-RD is a recently described disease for which no approved therapy exists. IgG4-RD causes important end-organ damage, including failure of both the exocrine and endocrine pancreas (autoimmune pancreatitis); hepatic failure (cholangitis); kidney failure from intrinsic renal disease (tubulointerstitial nephritis); obstructive nephropathy and chronic pain (retroperitoneal fibrosis); dysfunction of other organs (orbits, lung); and death from a variety of causes (complications of aortitis, pulmonary disease, meningeal involvement, other). Prednisone is an effective therapy in many patients but does not cure the disease. Moreover, the use of glucocorticoids in a disease that affects older populations and has a predilection for causing exocrine insufficiency of the pancreas is highly problematic.

Work from this group at the Massachusetts General Hospital (MGH) and the Ragon Institute (of MGH, MIT, and Harvard) has implicated CD4+ cytotoxic T lymphocytes (CD4+CTLs) as the linchpin of IgG4-related disease (IgG4-RD). The CD4+CTLs identified by our group express a protein called SLAMF7. Our publication marked the first report of SLAMF7 on a CD4+ T cell. We have demonstrated a predominance of these cells within the infiltrates of affected tissues of IgG4-RD subjects and shown that circulating counterparts of these cells are also present in the blood. We hypothesize that CD4+CTLs play a crucial role in the pathophysiology of IgG4-RD. Further, we believe they are an important driver of disease-associated fibrosis, and the implications of our work may extend to other diseases characterized by tissue scarring.

Elotuzumab, the first drug ever awarded a breakthrough designation for any indication by the U.S. Food & Drug Administration, is a monoclonal antibody directed against SLAMF7. Our fundamental hypothesis is that elotuzumab will eliminate the CD4+CTLs that are crucial to the fibroinflammatory process of IgG4-RD. We hypothesize, further – because plasmablasts and certain other activated B cells also express SLAMF7 and participate intimately in the pathophysiology of IgG4-RD – that this intervention will have similar effects on cells of the B lymphocyte lineage, contributing more broadly to an excellent therapeutic effect of interventions designed to target SLAMF7. The trial therefore represents a proof-of-concept study capitalizing upon work done within the MGH/Ragon collaboration to define the current model of disease pathophysiology.

The clinical aims of this project are to: 1) confirm that targeting SLAMF7 is a safe therapeutic strategy in IgG4-RD, 2) define the optimal dosing regimen of elotuzumab in the treatment of IgG4-RD, 3) assess the efficacy of elotuzumab in IgG4-RD and 4) evaluate the impact of elotuzumab on serological markers of fibrosis.

The trial is divided into two parts, Part 1 and Part 2. Part 1, designed to identify the optimal regimen with which to proceed in Part 2 (the randomized, double-blind portion of the trial), is divided into Part 1A and Part 1B, during which patients will receive either four or eight doses of elotuzumab combined with prednisone. Part 1A of the trial began enrollment in November, 2021. In Part 2, we will proceed with a randomized trial comparing a regimen of elotuxumab plus prednisone to prednisone alone.

The clinical aims will be accomplished in concert with a carefully designed series of mechanistic studies conducted at both the MGH and Emory University. All sites participating in the trial will collect mechanistic study samples. The participating sites in this trial for Part 1 include the MGH, Emory, and the Mayo Clinic. Part 2 of the trial will involve an expansion of the trial to include 5-7 additional sites.

The second project of the MGH Clinical ACE is a tocilizumab withdrawal trial in giant cell arteritis. This trial will begin later in the current ACE cycle.

University of Michigan

The current work of the University of Michigan Clinical ACE is built on the hypothesis that organ-targeted autoimmune diseases depend on unique pathogenic interactions between cells of the immune system and parenchymal or stromal cells of the target organ, both in disease initiation and target organ destruction.

Based on advances over the past five years, we propose here a new but related central hypothesis, that novel, safer and more effective precision-targeted and personalized therapies for autoimmune diseases can be developed based on insights into two critical and interacting components of autoimmune diseases: 1) the molecular mechanisms by which target organ stromal and parenchymal cells initiate, orchestrate and control the evolution and consequences of autoimmune diseases and 2) the critical roles of localization of unusual lymphocyte populations to target organs in subsets of patients with autoimmune diseases, and the opportunity for elimination of these cells without substantial impairment of normal host defenses.

The primary clinical project, CD319 as a novel target for treatment of systemic sclerosis: Treatment with Elotuzumab, led by Dinesh Khanna, MD, MSc is built on evidence for a pathogenic role of CD4+CD319+ lymphocytes in autoimmune diseases that leads to fibrosis of target organs. The alternate clinical project: Tofacitinib for treatment of photosensitivity and cutaneous inflammation in systemic lupus, led by J. Michelle Kahlenberg, MD, PhD, is based on our observation that keratinocyte-derived interferon-kappa drives photosensitivity and cutaneous inflammation in lupus skin. Treatment of these aspects of lupus with a Janus kinase inhibitor will be a step towards more precise targeting of interferon-kappa in lupus. Our collaborative project: Validation of novel molecular targets for more precise treatment of autoimmune diseases, led by David A. Fox, MD, will assess expression and function of selected molecules produced by stromal cells in target organs of a broad range of autoimmune diseases -- AIRE (the autoimmune regulator protein), CD318 (a novel ligand of CD6) and CD13, which may have major pathogenic roles in human autoimmune conditions. Blocking the CD6/CD318 interaction also shows promise as a new target for cancer immunotherapy that would avoid autoimmune toxicities that occur frequently with currently employed checkpoint inhibitors. These projects will be supported by an Administrative Core and a Funds Management Core, and by numerous patient cohorts, disease-focused clinical programs, core facilities and biorepositories at the University. The Principal Investigators, Drs. Dinesh Khanna and David A. Fox, work in close collaboration on our existing Clinical ACE and have substantial experience and productivity in clinical and translational research in systemic sclerosis, rheumatoid arthritis and other human rheumatic/autoimmune diseases. Together with a team of colleagues at the University of Michigan and future collaborators from other ACE institutions, this group of investigators is poised to make valuable contributions to our understanding and treatment of autoimmune diseases.

University of Pennsylvania

The University of Pennsylvania (Penn) ACE focuses on B cells as drivers of autoimmunity for three chronic and potentially highly debilitating autoimmune diseases - multiple sclerosis (MS), pemphigus vulgaris, and type 1 diabetes. Prospective randomized clinical trials have shown that B cells are established drivers of autoimmunity in these conditions, but the diseases differ in the ways in which B cells contribute to disease. The Penn ACE aims to better understand causes and mechanisms of autoimmunity, accelerate clinical research, and increase interdisciplinary collaborative research across these and other B cell-mediated autoimmune diseases.

The Penn ACE primary clinical project is a randomized-discontinuation study of anti-CD20 B-cell depleting therapy in patients with MS. While the recently approved anti-CD20 antibody ocrelizumab has emerged as highly effective at limiting new relapsing disease activity in MS patients, the approved regimen calls for ongoing B-cell depletion using repeated courses of treatment and there are growing concerns about the long-term safety of chronic B cell depletion. Prior work including by members of our ACE team has indicated that the therapeutic mechanism of action associated with the benefit of anti-CD20 therapy in MS relates to non-antibody dependent functions of B cells, including their capacity to induce abnormal pro-inflammatory responses of T cells and myeloid cells that mediate disease attacks. Indeed, B cell depletion in patients results in reduced pro-inflammatory T cell and myeloid cell responses. We have also demonstrated that the B cells that reconstitute after transient anti-CD20 treatment are less pro-inflammatory and, in some patients, may be actively downregulatory such that their T-cell and myeloid-cell responses remain acquiesced. Based on these observations, our randomized-discontinuation trial of anti-CD20 in MS will test the intriguing hypothesis that, in a subset of MS patients, transient rather than continuous B cell depletion may result in durable disease quiescence - effectively representing a form of restored tolerance.

Patients in our trial will all initiate treatment with anti-CD20 and at 12 months will be randomized to either placebo; an additional 12 months of ocrelizumab followed by placebo; or continuous ocrelizumab treatment. Following randomization, we will use frequent exams and magnetic resonance imaging (MRI) of the brain to monitor for any evidence of both clinical and subclinical disease activity, as well as carry out comprehensive molecular profiling of B cells and non-B cells (T cells and myeloid cells). Our primary outcome will be to assess the frequency of patients who achieve durable treatment benefit defined as entire absence of disease activity for at least 3 years following anti-CD20 discontinuation. As mechanistic outcomes, we will assess the cellular immune profiles associated with durable vs. non-durable disease control, as well as possible predictive markers using pre-treatment samples. Results of this clinical trial will be potentially paradigm-shifting for the treatment of multiple sclerosis in addition to providing novel insights into fundamental mechanisms underlying autoimmunity and immune tolerance in humans.

Oklahoma Medical Research Foundation

The Oklahoma ACE strives to understand the biology of autoimmune diseases through interdisciplinary, collaborative research that integrates clinical and basic questions. Although significant progress in unveiling mechanisms of autoimmune disease pathogenesis has been made, application of emerging pathophysiologic insights to the development of targeted therapies is critically lacking. For autoimmune disease therapeutic development to succeed and patient outcomes to improve, deepened understanding of molecular disease heterogeneity, therapeutic pharmaco-biology and improved trial designs are needed. The Oklahoma ACE will pursue a novel, comprehensive theme of accelerating discovery and translation by deconstructing molecular heterogeneity to enrich for patients with common molecular pathways, partnered with repurposed therapies from other fields and novel trial designs that eliminate confounding background polypharmacy, to address these unmet needs.

The Oklahoma ACE primary clinical project is a phase-2, double-blind, placebo-controlled trial of mycophenolate mofetil alone or in combination with voclosporin for systemic lupus: Examining distinct immunophenotypes to validate and enhance rational treatment. This project seeks to discover homogenous patient subsets for whom mycophenolate mofetil (MMF) is an immunologically relevant and therefore effective treatment and to determine changes in biomarkers associated with clinical response.

Since past studies in nephritis suggest that the combination of MMF and a calcineurin inhibitor may increase clinical response proportions, patients with treatment failure on MMF will be offered another course of brief steroids and re-randomized to continue MMF for longer or to receive MMF plus voclosporin, a calcineurin inhibitor with a relatively safe track record that has recently been approved for use in SLE. Monitoring of the immunophenotypic impact during both stages of treatment may help determine immunological differences in response vs non-response to MMF and correlates of subsequent response to combination treatment for those who do not obtain optimal clinical efficacy from MMF alone.

To safely study patients with a serious chronic disease while providing scientific sample collection by limiting background medications, this trial utilizes an innovative SLE trial design developed at OMRF. In this approach, depomedrol injections are given during the screening period to suppress disease, while background immunosuppressive drugs are stopped. Partnered mechanistic studies will test our soluble mediator flare index and other select activated immune cell subsets for the ability to predict upcoming flares, as well as to test specific hypotheses of MMF response/resistance and of SLE disease flare mechanisms.

New Clinical Projects/Trials

AIG01: Elotuzumab in Immunoglobulin G4-Related Disease (IgG4-RD)
Immunoglobulin G4-Related Disease (IgG4-RD) is a chronic fibro-inflammatory condition that can affect virtually every organ system, including the pancreas, biliary tract, salivary and lacrimal glands, orbits, lungs, kidneys, meninges, pituitary gland, prostate and thyroid. It may also involve the retroperitoneum. This multi-organ immune-mediated condition, once regarded as a group of isolated, single-organ diseases, is now recognized to be an overarching, single-disease entity linked by common histopathological and immunohistochemical features. IgG4-RD tends to afflict middle-aged to elderly individuals. Although IgG4-RD can affect a single organ at presentation, it is not uncommon for participants to present with or develop multi-organ disease. As the disease progresses, additional organs develop lesions and the cellular inflammation characterizing early disease moves toward a more fibrotic stage, causing major tissue damage, dysfunction and ultimately organ failure. It is unclear whether IgG4 itself is involved in the pathogenesis of the disease. The goals of IgG4-RD treatment are to reduce inflammation and organ swelling and to prevent or reverse tissue fibrosis. No approved therapy exists for IgG4-RD. [Text from ClinicalTrials.gov: NCT04918147]

ALE11: Tofacitinib for lupus photosensitivity
The “Evaluation of tofacitinib in prevention of photosensitivity in lupus” is a new phase I proof-of-concept study to investigate the impact of JAK inhibitors on parameters of ultraviolet (UV) light sensitivity in patients with systemic lupus erythematosus. The trial will begin enrolling patients in early 2022 and will test apoptotic and inflammatory changes in the skin following UV light exposure before and after a 25 day course of tofacitinib 11 mg daily. Skin biopsies and blood will be saved for additional explorations of NETosis and spatial sequencing. A total of 10 patients will be enrolled. The Michigan ACE leads this project. ClinicalTrials.gov: NCT05048238.

AMS05: Ocrelizumab withdrawal in early relapsing multiple sclerosis
AMS05 is a Randomized Blinded Discontinuation Trial of the anti-CD20 B-cell depleting treatment ocrelizumab (OCR) in patients with early relapsing multiple sclerosis (RMS). The pivotal clinical trials of OCR leading to its approval for patients with RMS demonstrated that it is highly effective at limiting new disease relapses when used as a continuous regimen of 6 monthly infusions. The overarching goal of AMS05 is to test the hypothesis that a subset of patients would experience durable remission of relapsing biology (a state akin to restored immune tolerance) following transient treatment with OCR. The rationale is based on prior work indicating that the therapeutic mechanism of action of anti-CD20 therapy in MS relates to depletion of pro-inflammatory B cells that drive pro-inflammatory T cells and myeloid cells to mediate relapses. B cells that reconstitute after transient anti-CD20 treatment are less pro-inflammatory and, in some patients, may be actively downregulatory such that the T-cell and myeloid-cell responses in these patients are actively acquiesced. We hypothesize that such patients will benefit from durable disease remission defined as absence of clinical or brain MRI evidence of new disease activity, without ongoing therapy. The study’s randomized and blinded design with frequent monitoring and frequent biological sampling will enable it to meet its primary objective of determining the rate

of durable remission at the 48-month end-of-study, as well as achieve its mechanistic objectives of defining the cellular immune response profiles associated with durable remission (versus lack thereof); and identifying potential biomarkers able to predict which patients will (or will not) experience such durable remission. All eligible patients will be treated with OCR at least through the month-12 infusion. We anticipate then randomizing 150 patients in a 1:1:1 parallel-arm design to either switch to placebo infusions; continue OCR for another 12 months and then switch to placebo; or remain on OCR throughout the 48-month end-of-study. Results of this clinical trial will be potentially paradigm-shifting for the treatment of multiple sclerosis in addition to providing novel insights into fundamental mechanisms underlying autoimmunity and immune tolerance in humans.

ALE10: A Phase 2, Double-Blind, Placebo-Controlled Trial of Mycophenolate Mofetil (MMF) alone or with Voclosporin for Systemic Lupus: Examining Distinct Immunophenotypes to Validate and Enhance Rational Treatment (DIVERT)

This will be a multicenter, double blind, placebo-controlled Phase 2 trial of 120 participants with SLE. This protocol design is based on the Biomarkers of Lupus Disease (BOLD) study. The trial is limited to participants without organ-threatening disease and mandates withdrawal of all lupus medications except for low dose steroids, antimalarials and/or non-steroidal anti-inflammatory drugs. After withdrawal of background medications (Stage 1), participants enter into Stage 2 designed to test the clinical and immunologic effects of MMF, compared to placebo in phenotypic clusters of participants with SLE. This first part of the trial (Stage 2) is expected to identify molecular clusters associated with clinical success or failure of MMF treatment. Stage 3 is designed to test the clinical and immunologic effects of adding voclosporin (or not) for those participants who do not respond to MMF alone and to compare MMF vs MMF + voclosporin in a subset of non-responders who received placebo in the first part (Stage 2) of the trial. If one or more clusters of participants who have not responded to MMF alone can be identified by immunophenotypes, either at baseline or after receiving MMF, the question of whether all of these or only certain subsets are candidates for voclosporin can be addressed in an exploratory way.

ACV01: Booster Effects with Autoimmune Treatments in Patients with Poor Response to Initial Covid-19 Vaccine

This is a Phase 2, randomized, multi-site, adaptive, open-label clinical trial entitled “Booster Effects with Autoimmune Treatments in Patients with Poor Response to Initial Covid-19 Vaccine,” comparing the immune response to different COVID-19 vaccine booster doses in participants with autoimmune disease requiring immunosuppressive medications. All study participants will have negative serologic or sub-optimal responses (defined as a Roche Elecsys® Anti-SARS-CoV-2 S (RBD) result ≤ 50 U/mL) to initial COVID-19 vaccine regimen with Moderna COVID-19 vaccine, Pfizer-BioNTech COVID-19 vaccine, or Janssen COVID-19 vaccine. The primary objective is to determine the proportion of participants who have a serologic response, defined as at least a four-fold increase in anti-COVID-19 antibody response (Spike 1), after receiving either the vector based or mRNA based vaccine booster and either do or do not hold immunosuppressive medications around the time of vaccine booster. We will initially focus on 5 autoimmune diseases: Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Multiple Sclerosis (MS), Systemic Sclerosis (SSc), and Pemphigus. Clinicaltrials.gov: NCT05000216

ACV02: Observational study of SARS-CoV-2 vaccine responses in patients with autoimmune diseases

This is a five ACE site, observational study entitled, “Observational study of SARS-CoV-2 vaccine responses in patients with autoimmune diseases.” The primary objective is to determine whether vaccine responses reach a predetermined titer in patients with autoimmune diseases with or without immunosuppression. This is a prospective observational registry. Adult patients with Rheumatoid Arthritis, Psoriatic Arthritis, SLE, Sjogren’s Disease, Multiple Sclerosis, Scleroderma, Vasculitis or Blistering Diseases will undergo a blood draw and collection of clinical information prior to receipt of COVID-19 vaccination. Subjects will undergo a repeat blood draw and collection of clinical information at 4-8 weeks, 6 months and 12 months following their second vaccination (or 8-12 weeks after single vaccination for vector based vaccine).

Legacy Clinical Projects/Trials

The ongoing legacy trials of the ACE Program include a project to evaluate the safety of an endocannabinoid in inflammatory musculoskeletal pain (ALE09), to prevent the development of rheumatoid arthritis in at-risk individuals (ARA08), to test the safety and predictors of systemic lupus patients who can stop mycophenolate mofetil (ALE06), and to determine the safety and efficacy of anti-CD20 therapy in pulmonary hypertension associated with systemic sclerosis (ASC02).

APG01: This phase 1, open-Label, multicenter clinical trial tests the safety of ex vivo expanded autologous polyclonal regulatory T cells (PolyTregs) in subjects with active pemphigus (vulgaris and foliaceus). Associated mechanistic studies will evaluate significant events that occur when using PolyTregs and establish the dose recommended for further clinical investigation in subjects with pemphigus, in addition to characterizing immune system responses. It is led by Dr. Haley Naik at the University of California, San Francisco. Four sites participate (UCSF, Iowa, UTSW, and Duke) in screening, enrolling, and infusing 4 subjects before the study was closed to enrollment in 2019. Clinical database lock and results are expected in 2022. Clinical Trials.gov: NCT03239470

ALE09: A Phase 2, Double-blind, Randomized, Placebo-controlled Multicenter Study to Evaluate Efficacy, Safety, and Tolerability of JBT-101 in Systemic Lupus Erythematosus is a randomized double-blind placebo-controlled study assessing the effects of JBT-101 on inflammatory musculoskeletal pain in patients with SLE. JBT-101 is a synthetic endocannabinoid receptor type 2 (CB2) agonist and an activator of the body's normal processes to resolve innate immune responses without immunosuppression. JBT-101 induces a class switch in production of lipid mediators to increase production of "Specialized Pro-resolving lipid Mediators" (SPMs) while reducing production of pro-inflammatory mediators. In turn, SPMs trigger a physiologic network to resolve inflammation and fibrosis, and, when present, increase clearance of pathogens. With return to homeostasis, clinical benefit is expected to occur in people with SLE, for example, in those who have inflammation of the musculoskeletal system and associated pain. The primary objective of this study is to evaluate the efficacy on inflammatory pain related to active musculoskeletal disease in SLE, as well as safety, tolerability, and biologic effects of JBT-101. This ACE study is also accompanied by evaluation of multiple mechanistic endpoints. This project has completed enrollment and results are expected in 2022. The Feinstein Clinical ACE leads this project. Clinical Trials.gov: NCT03093402

ARA08: The "Strategy to prevent the onset of clinically-apparent rheumatoid arthritis" or Stop RA trial is the first prevention research study for rheumatoid arthritis conducted within the United States. This project identifies at-risk individuals who have anti-cyclic citrullinated peptide antibody levels which are concerning for the development of RA within the next two years and randomizes them to receive modest immunosuppression with hydroxychloroquine or placebo with careful monitoring for the development of new symptoms or serological biomarkers for development of clinical rheumatoid arthritis or new measures of epitope spreading. Biospecimens are also being collected for mechanistic studies for disease progression and for non-progression or resolution. This project completed enrollment in 2021 and results are expected in 2024. Investigators at the University of Colorado lead this project. Clinical Trials.gov: NCT02603146

ALE06: The "An Investigator-Initiated, Phase II, Randomized, Withdrawal Study of Mycophenolate Mofetil (MMF) in Patients with Stable, Quiescent Systemic Lupus Erythematosus (SLE)" is another legacy trial of the ACE Consortium which has completed enrollment, locked the clinical database, and conducted statistical analysis of the primary and secondary clinical outcomes. Mechanistic studies of gene expression profiles, soluble mediators and autoantibodies of samples from this study have been done to determine serologic markers of impending SLE disease flare and to help identify at the molecular level patients who may need to remain on MMF therapy for ongoing disease suppression. Additional samples are available for other immunologic evaluation of mechanisms of flare and disease quiescence. The Oklahoma ACE leads this project. ClinicalTrials.gov: NCT01946880.

ASC01 is a randomized, double-blind, placebo-controlled, phase II multicenter trial of anti-CD20 (rituximab) for the treatment of systemic sclerosis-associated pulmonary arterial hypertension. Systemic sclerosis-associated

pulmonary arterial hypertension (SSc-PAH) is a serious, life-threatening manifestation of systemic sclerosis (SSc), an autoimmune disease of the connective tissue characterized by scarring (fibrosis) and atrophy of the skin, joints and tendons, skeletal muscles, and internal organs, and immunological disturbances. Compelling pre-clinical data and anecdotal clinical reports that suggested that modulation of the immune system may be an effective strategy for treating SSc-PAH. This trial tests whether rituximab has a beneficial effect on clinical disease progression in patients with SSc-PAH when compared to placebo. This trial is completed; mechanistic studies and data analysis are ongoing. Stanford investigators lead this project. ClinicalTrials.gov: NCT01086540

Collaborative Projects

The NIAID Autoimmune Centers of Excellence (ACE) in its current configuration includes five Basic ACEs and five Clinical ACEs, thus bringing together ten centers from across the country. While Clinical ACEs are centered around clinical trials and Basic ACEs each have a Principal project, each ACE also includes a Collaborative Project designed to serve as the glue binding different ACE projects and centers together. A number of the ACE centers also have exploratory Pilot projects that also contribute newer approaches to the overall effort. The broader purpose and long-term goal of the ACE is to recognize that a deeper understanding of as well as newer therapies for autoimmune diseases will more readily emerge from collaborative efforts, such as those summarized in this document as the Collaborative Agenda of the ACE. The ACE has been created to foster collaborations in the realm of autoimmune disease research, recognizing, as the old adage so aptly encapsulates, that the whole is always considerably greater than just the sum of its parts.

This Collaborative Agenda can be broadly visualized as seeking to bring groups together around three broad and inclusive goals. One goal is to better understand *the regulation of B and T cell tolerance* across a range of autoimmune diseases. The second broad goal is to use a range of approaches *to subset patients with systemic lupus erythematosus i*n order to better understand the pathogenesis of this disease and to also more effectively target therapeutics in lupus patients. The final goal is to better *understand autoimmune fibrotic diseases*, by appreciating the contributions of clonally expanded T and B cells as well as stromal cells in tissue sites using novel imaging approaches and cell distance mapping and to use the knowledge gained to leverage newer therapeutic approaches.

Group 1: The regulation of B cell and T cell tolerance across several different autoimmune diseases

Studies linked to this goal will include analysis of disease initiation as modeled by the induction of autoimmunity in patients exposed to TNF inhibitors, epigenetic studies of B cells in SLE, rheumatoid arthritis and Type I diabetes and studies of B cell subsets and clonal networks in the effector organ as exemplified by the pancreas in Type 1 diabetes. Investigators working together will use high throughput technologies including multiparameter flow cytometry to identify B and T cell subsets and immunoglobulin repertoire analysis and ATAC-Seq to analyze B cell abnormalities during induction and progression of autoimmunity. The ACE will use shared definitions of B and T cell subsets for phenotyping and cell sorting, use shared protocols for sample storage, use shared technology for epigenetic analyses and share data analysis pipelines and databases for repertoire analysis. In addition, the ACE will provide samples as needed for collaborative experiments including serum for microparticle analyses and tissue blocks for immunohistochemical analyses.

The overall goals are to understand the characteristics of autoreactive B cells and T cells during different stages of disease from disease initiation to target organ damage with respect to phenotype, repertoire (B cells) and epigenome. Both extrafollicular B cell responses and germinal center B cell responses can contribute to autoreactivity and will likely need to be targeted differently. As disease evolves, B cell epigenetic changes may contribute to driving and maintaining autoimmunity. Published studies from the ACE have suggested that abnormalities in the B cell epigenome are already present in naïve B cells from lupus patients and that the transcriptional pathways turned on in extrafollicular B cells differ from those of total plasma cells. Extension of

these studies to both B and T cells in a well characterized cohort of lupus nephritis patients will help determine whether responders and non-responders differ in their lymphocyte epigenetic profiles.

Application of the same technologies to B cells from patients with evolving SLE-like autoimmunity and B cells in target organs will help to determine whether the transcriptional B cell landscape changes as disease evolves and in different sites (PBMCs vs. target organ, including the role of the microbiome in driving autoimmunity) and whether there are shared drivers, B cell subsets or transcriptional or epigenetic signatures of B cell dysregulation across diseases. Repertoire studies in the same patients will determine the degree of class switching, somatic mutation and selection of B cells and clonal relatedness of the extrafollicular and germinal center derived pathways and may point to the origins of autoantibodies in each of the diseases under study.

Similarly, analysis of newly identified T helper cell subsets will help to define the T cells that are driving autoantibody formation in each disease. Application of single cell technologies in selected patients will allow further dissection of the heterogeneity of B cell responses across diseases and disease stages. Stored samples will be available for further studies as the data become available.

Group 2: Sub-setting of lupus patients to understand pathogenesis and appropriately direct therapies

Systemic lupus erythematosus (SLE) remains an autoimmune disease with a relatively poor prognosis and a paucity of effective therapies. The broad questions driving this project focus on why some patients progress to more severe disease and why some patients respond to certain therapeutic regimes while others do not. It is generally believed that a significant issue in improving the outcomes of clinical trials in SLE and improving outcomes for patients in clinical practice is patient heterogeneity with respect to mechanisms of disease. This heterogeneity is reflected well in animal models which differ substantially with respect to pathogenetic mechanisms and therefore, not surprisingly, with respect to effective therapy. It is also clear that patients cannot be subsetted based on clinical parameters. Biomarkers that reflect mechanism(s) of pathogenesis are needed.

In these proposed collaborative studies both prognosis and response to therapy will be assessed in patients based on the following parameters:

1. Profiling of surface markers on B and T cell subsets, monocytes and granulocytes, intracellular and secreted analytes identified in blood by flow cytometry and Fluidigm Cytof
2. Analyses of the transcriptomes and chromatin states of different immune cells in blood
3. Analyses of the protein cargo of exosomes in blood and urine

The approach will include a number of studies of blood and urine including immunophenotyping by flow cytometry of blood, gene expression of blood and urine cells, assays of chromatin accessibility, exosome analysis, and focused blood proteomics.

This broad approach is necessary as little information is available to drive more focused studies. Importantly, the analyses outlined below are expected to provide more than one algorithm for characterizing patient heterogeneity. It will be important to test these in large longitudinal cohorts and in clinical trials to determine which algorithms predict clinical response or long-term outcome.

While these collaborative studies will focus primarily on the molecular sub-setting of lupus patients the studies will be extended across the ACE to other Systemic Autoimmune Disorders or through samples from ACE clinical trials.

Group 3: Obtaining a better understanding of the contributions of adaptive immune cells and stromal cells to autoimmune diseases that lead to target organ remodeling

One of the questions bringing ACE investigators together is the study of autoimmune disorders that lead to target organ remodeling – either fibrosis or tissue destruction – and the analyses of the contribution of adaptive immune cells and stromal cells to these processes and to autoimmune inflammation in general. Fibrosis is a major clinical issue and in many disorders such as systemic sclerosis and systemic lupus erythematosus the inability to keep fibrotic processes in check with existing therapies contributes to the morbidity and mortality of these diseases.

One of the disorders that is being examined is IgG4-related disease, a relatively indolent autoimmune fibrotic disorder that is generally, but not always, responsive to B cell depletion and prednisone. The potential contribution of apoptosis induced by clonally expanded CD4+CTLs and CD8+ cytotoxic cells, as well as the contributions of tissue infiltrating activated B cells and their interactions with specific T cells in tissues are being explored. The other disorders that will be examined by the ACE include but are not limited to systemic sclerosis, lupus nephritis and rheumatoid arthritis. In studies involving three centers of the ACE, striking similarities were identified between the pathogenesis of IgG4-related disease and systemic sclerosis. Both disorders were shown to be linked to an expansion of cytotoxic CD4+ T cells that infiltrate tissues. Other collaborative studies also involving three ACE centers have examined in great detail the gut microbiomes of patients with IgG4-related disease and systemic sclerosis. Strong similarities in the metagenomic microbiome signatures of systemic sclerosis and IgG4-related disease were discovered, supporting the idea that similar mechanisms may operate in these two autoimmune fibrotic diseases. Collaborative studies to discover the T cell antigens that drive these diseases are ongoing Some studies will be undertaken on other diseases including thyroid associated ophthalmopathy, cutaneous lupus, autoimmune retinopathy, and multiple sclerosis.

These collaborative studies will focus in a synergistic manner on developing high resolution imaging of a range of adaptive, innate, and stromal cell types in tissue section using multicolor immunofluorescence combined with multispectral imaging as well as confocal microscopy and cell distance mapping approaches. The abundance of different immune cell subsets in disease tissues, the molecules they express and secrete and the specific interactions that occur between cell types will be comprehensively mapped. Tissues that will be examined include lesions from IgG4-related disease, systemic sclerosis, lupus nephritis and rheumatoid arthritis along with some control skin conditions including bullous pemphigoid. Collaborations between different ACEs on systemic sclerosis and IgG4-RD are already ongoing and these include clinical trials attempting to target CD4+CTLs and activated B cells.

Studies on the specific interactions between CD6 on T cells and its ligands CD318 and CD166 on stromal cells, on shed CD13 from stromal cells and the bradykinin 1 receptor on monocytes and T cells, and also on the regulation in disease stromal cells of AIRE expression by the secreted protein Slit2 will be undertaken on tissues from a number of cohorts.

What are the potential triggers that might induce autoimmune Fibrosis? Collaborations between ACEs have already revealed that remarkable changes occur in the gut microbiome at a species level in both IgG4-RD and systemic sclerosis. Functional studies to look for these potential microbial and metabolic triggers of fibrosis will be undertaken.

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