AWARD NUMBER: W81XWH-18-1-0709

TITLE: Inflammation and Metabolic Reprogramming of Lupus Monocytes - Mechanisms of the Pathobiology of Lupus Cardiovascular Disease

PRINCIPAL INVESTIGATOR: Dr. Caroline Jefferies

CONTRACTING ORGANIZATION: Cedars Sinai Medical Center
Los Angeles CA90048

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PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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4. TITLE AND SUBTITLE Inflammation and Metabolic Reprogramming of Lupus Monocytes - Mechanisms of the Pathobiology of Lupus Cardiovascular Disease		**5a. CONTRACT NUMBER** **5b. GRANT NUMBER** W81XWH-18-1-0709 **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Caroline Jefferies, PhD Noel Bairey Merz, M.D., FACC, FAHA Mariko Ishimori, M.D E-Mail: caroline.jefferies@cshs.org		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Cedars Sinai Medical Center 8700 Beverly Boulevard Los Angeles CA90048		**8. PERFORMING ORGANIZATION REPORT NUMBER**
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14. ABSTRACT

Lupus is a systemic disease where organs are attacked due to dysfunction of the immune system. Lupus occurs in approximately 0.1-0.3% in the USA, affecting women in their child bearing years with devastating consequences for both patients and their families. Women, non-Hispanic black, and Hispanic, active duty military personnel, have elevated lupus rates, with resultant time away from active duty/work and adverse health consequences. Despite improvement in lupus treatment over the last half century, cardiovascular disease is the leading killer of these young women with lupus. As a systemic disease, young women with lupus often experience chest pain (angina) and an increased risk of cardiac events such as heart attacks and strokes. While many young women have lupus, the effects of the disease can vary widely, making treatment decisions a significant challenge to patients and physicians. This research will examine why some women with lupus get heart disease and why others do not, leading to a better understanding of how to prevent and treat lupus-related organ damage.

Our pilot lupus ischemia study suggests that 44% of lupus patients have evidence of ischemia consistent with coronary microvascular dysfunction (CMD). Our preliminary analysis of monocytes from lupus patietns shows a dysregulation of key enzymes important in regulating metabolism.

Hypotheses: We hypothesize that a distinct profile of metabolic and gene expression changes will associate with the presence and severity of CMD.

Specific Aims: We will study 40 women with lupus and evidence of ischemia but no obstructive coronary artery disease (CAD) to determine the presence and severity of CMD. Concurrently, we will assess lupus specific mechanistic pathways by determining changes in lipid regulating genes and metabolomic pathways in monocytes and correlate this with CMD, cardiac biomarkers and cytokine expression.

Aim 1: To determine the presence and severity of CMD noninvasively using the innovative noninvasive imaging technologies of cardiac magnetic resonance imaging (CMRI) and computed tomographic angiography (CCTA).
Aim 2: To determine whether distinct alterations in metabolic programming in lupus patient monocytes associate with CMD by assessing changes in cellular metabolism and correlating this data with gene expression profiling.

Impact: The cardiovascular system supplies all tissues and organs of the body, has an established relationship to inflammatory metabolomic status, and thus represents an investigative opportunity for improved understanding of a variety of systemic diseases such as lupus. Cardiovascular investigative technology is mature, has provided pathobiological understanding that has led to substantial reductions in cardiovascular disease. The proposed investigation will provide the necessary building blocks of pathobiological understanding to develop treatment, cure and improved quality of life of lupus for Service Members, Veterans and beneficiaries.

15. SUBJECT TERMS

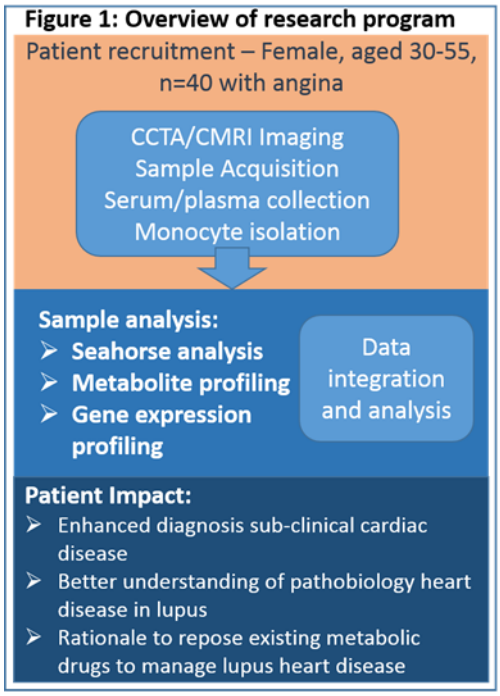
16. SECURITY CLASSIFICATION OF:			**17. LIMITATION**	**18. NUMBER**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

Standard Form 298 (Rev. 8-98)

TABLE OF CONTENTS

1. Introduction

Cardiovascular disease is now the leading killer of lupus patients, and women, non-Hispanic black, and Hispanic active duty military have elevated lupus rates. While obstructive coronary artery disease (CAD) has traditionally been implicated, our pilot lupus ischemia study suggests that 44% have evidence of ischemia with no obstructive CAD, consistent with coronary microvascular dysfunction (CMD). The heterogeneity of this organ involvement is not understood. Cardiovascular investigative technology is mature, has provided pathobiological understanding that has led to substantial reductions in cardiovascular disease (CVD) using existing and novel medications, and therefore represents an opportunity to gain insight into lupus heterogeneity and pathobiology which could lead to new treatment targets and cure.



The overall goal of this grant is to define the prevalence and pathobiology of coronary microvascular dysfunction (CMD) in patients with systemic lupus erythematosus (lupus) using advanced cardiovascular imaging technology, next generation sequencing and metabolomic technologies in order to aid development of new therapeutic directions for treating CMD in lupus. In parallel the bioenergetics and metabolomics profile of monocytes taken from the lupus patients enrolled in this study will be determined and combined with gene expression profiling to identify differences in metabolome and bioenergetics flux that discriminates patients with CMD from those without. The overview of the program is shown in **Figure 1.**

2. Keywords

Bioenergetics
Coronary artery disease
Coronary microvascular dysfunction
Innate immunity
Lupus
Metabolomics
Monocytes

3. Accomplishments

There are two key elements associated with this study: (1) patient recruitment and imaging; (2) metabolomic and genomic assessment of patient monocytes. Our goal for year 1 of this study was to have all the regulatory elements of the study in place and have finalized all requirements for patient recruitment, imaging visits and sample analysis. This has been achieved. We are currently screening a patient so as to have our first visit completed by the end of October. Delays in recruitment are due to unanticipated length of time to receive HPRO approval for the study. However, we do not anticipate that this will negatively impact our overall goal of recruiting 40 patients to the study within the next 24 months.

<u>Our accomplishments to date on each of our two main aims are the following:</u>

Aim 1: Measure the presence and severity of CMD by cardiac magnetic resonance imaging (CMRI) and computed tomographic angiography (CCTA) in lupus patients with angina (Ishimori; Bairey Merz)

Two key tasks are associated with this aim: (1) patient recruitment; (2) patient imaging

<u>In year one we have achieved the following:</u>

We have finalized all regulatory elements for this proposal (IRB and HRPO requirements). IRB approval for this protocol was finalized 18th January 2019 (Appendix 1). However, HRPO approval took longer than anticipated and notification of final approval was sent 07/09/2019 (Log Number E00565.1a). This has delayed initiating

recruitment as we could not advertise the study until we had this approval notice, but we fully anticipate that we will meet our goal of recruiting 40 patients to this study

We have established the Data Monitoring Committee (Dr. Jefferies, Dr. Bairey Merz, Dr. Wei, Dr. Ishimori, Dr. Mehta, Assistant Professor of Medicine, Division of Cardiology, Emory University School of Medicine – independent monitor). Data safety monitoring plan attached (Appendix 2).

We have finalized all SOPs for patient recruitment - detailing steps involved in recruitment and imaging visits and how these are to be coordinated, in addition to finalizing details around sample acquisition and processing and clinical laboratory assessments required (Appendix 3).

Dr. Ishimori has identified a number of patients that are scheduled for a screening visit in October and our aim is to have our first recruit complete her imaging visits by end of November. To meet our target of 40 patients for this study we are aiming to recruit 1-2 patients per month to the study.

Our original patient recruitment chart:

	Year 1				Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3** 5	**Q4** 5	**Q1** 5	**Q2** 5	**Q3** 5	**Q4** 5	**Q1** 5	**Q2** 5	**Q3**	**Q4**
Target Enrollment (cumulative)			**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**		

Our revised patient recruitment chart is below:

	Year 1				Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1** 6	**Q2** 7	**Q3** 7	**Q4** 7	**Q1** 7	**Q2** 6	**Q3**	**Q4**
Target Enrollment (cumulative)					**6**	**13**	**20**	**27**	**34**	**40**		

Aim 2: Perform metabolomics and genomic analyses on lupus patient monocytes (Jefferies)
In anticipation of the requirements for this study we have established all SOPs with respect to handling of patient samples on day of collection and lab measurements to be made (Appendix 4).

Initial data: We have established all the conditions for performing our metabolomic and genomic analyses for this study. Briefly we have achieved the following: We have optimized cell numbers and conditions for conducting extracellular flux analysis to measure basal oxygen consumption rates and extracellular acidification rates on CD14+ monocytes. Specifically, we have completed analyses of inhibitors to be used in this study. The inhibitor of fatty acid transportation Etoxomir decreased both OCR and ECAR in lupus patient monocytes to that of controls, without any apparent toxicity (Figure 1a). As expected 2-deoxyglucose and metformin completely abolished responses in monocytes (Figure 1a). Analyses of effects on interferon-induced gene expression indicated that etoxomir and 2-DG could dose dependently reduced IFN-induced changes in gene expression in monocytes (Figure 1b). We have also established conditions for assessing phospho-STAT1 activation in CD14+ monocytes by flow cytometry (Figure 1c). All conditions and doses of inhibitors to be used in this study are now established.

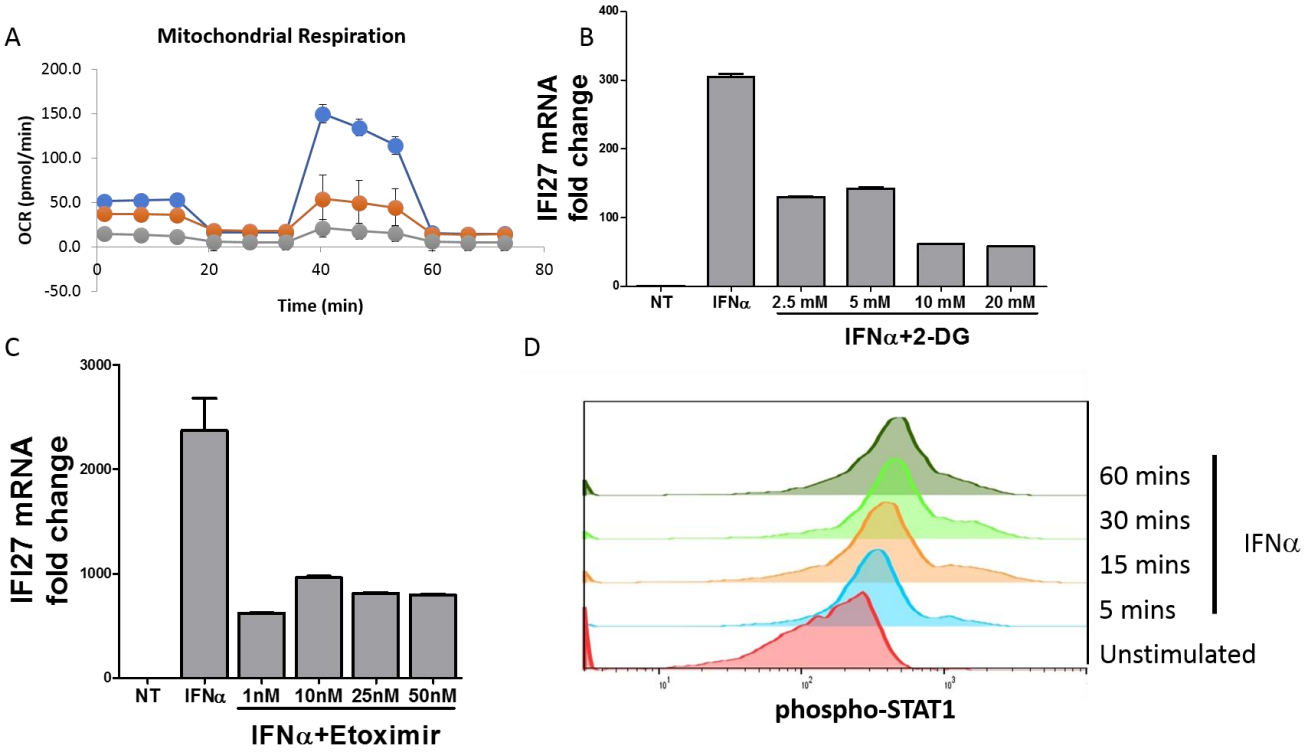


Figure 1: (A) Pretreatment of CD14⁺ monocytes with 2-deoxyglucose (2-DG) and etoxomir reduces oxidative phosphorylation; (B&C) pretreatment of CD14⁺ monocytes with 2-DG (B) and Etoxomir (C) results in corresponding inhibition of IFNα-driven gene expression; (D) IFNα treatment of CD14⁺ monocytes drives STAT1 phosphorylation as measured by intracellular FACs analysis.

4. Impact

The cardiovascular system supplies all tissues and organs of the body and thus represents a window of opportunity for improved understanding of lupus variability and why some women get heart disease and others do not. Further work beyond the current application, will include using the identified biomarker panels for identifying specific lupus subtypes of heart disease. We will also test specific treatments aimed at the biomarkers such as medications currently used for heart disease (metformin, statins) and new agents (monoclonal antibody therapies such as canakinumab) shown to be effective for heart disease in clinical trials.

5. Changes/Problems

The only changes noted is a change to recruitment which was detailed above:

Our original patient recruitment chart:

	Year 1				Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3** 5	**Q4** 5	**Q1** 5	**Q2** 5	**Q3** 5	**Q4** 5	**Q1** 5	**Q2** 5	**Q3**	**Q4**
Target Enrollment (cumulative)			**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**		

Our revised patient recruitment chart is below:

	Year 1				Year 2				Year 3			
Target Enrollment (per quarter)	**Q1**	**Q2**	**Q3**	**Q4**	**Q1** **6**	**Q2** **7**	**Q3** **7**	**Q4** **7**	**Q1** **7**	**Q2** **6**	**Q3**	**Q4**
Target Enrollment (cumulative)					**6**	**13**	**20**	**27**	**34**	**40**		

6. Products – Not applicable

7. Participants & Other Collaborating Organizations

Key Study Personnel (Include Degrees and Credentials)	Study Roles and Responsibilities	Primary Point of Contact (Select one)
Name: Caroline Jefferies, PhD Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): PI Responsibilities: Sample and data analysis	x☐
Name: Mariko Ishimori, M.D. Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): Co-Investigator Responsibilities: recruitment, data/specimen collection, analysis	X☐
Name: Bairey Merz, C. Noel, M.D., FACC, FAHA Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): Co-Investigator Responsibilities: recruitment, data/specimen collection, analysis	X☐
Name: Janet Wei, M.D. Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): Investigator Responsibilities: recruitment, data/specimen collection, analysis	☐
Name: Daniel Wallace, M.D. Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): Co-investigator Responsibilities: Recruitment	☐
Name: Dan Berman, M.D. Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): Co-Investigator Responsibilities: Data/specimen collection, analysis	☐
Name: Louise Thomson, MBChB Affiliated Institution: Cedar Sinai Medical Center	Study Role(s): Co-Investigator Responsibilities: Data/specimen collection, analysis	☐

8. Special Reporting Requirements - none

Appendices
Appendix 1:

Webridge Compliance Extranet

Office of Research Compliance and Quality Improvement, 6500 Wilshire Blvd., Suite 1800, Los Angeles, CA 90048

1/18/2019

To: CAROLINE JEFFERIES
CC: BONNIE PAUL
From: Stephen Lim, M.D. Executive Chairperson
On Behalf of the CSMC Institutional Review Boards
Subject: IRB Approval for Pro00054998

Please note that the Cedars-Sinai Institutional Review Board (CSMC IRB) has approved you to conduct research involving human subjects . Please review the following information summarizing the approval granted:

IRB No.:	Pro00054998
Study Title:	Inflammation and metabolic reprogramming of Lupus monocytes – mechanisms of the pathobiology of Lupus cardiovascular disease
Approval Period:	**1/18/2019 through 12/31/2019**
	Approved via Full IRB Review.

IRB Review Date:	1/18/2019
Principal Investigator:	CAROLINE JEFFERIES
Co-Investigators:	DEBIAO LI MICHAEL NELSON JANET WEI BEHZAD SHARIF BALAJI TAMARAPPOO C. NOEL BAIREY MERZ MARIKO ISHIMORI LOUISE THOMSON

Data and Safety Monitoring Plan Template for Moderate or High Risk Studies

Study Title: Inflammation and metabolic reprogramming of Lupus monocytes – mechanisms of the pathobiology of Lupus cardiovascular disease
Principal Investigator: Caroline Jefferies, PhD
Monitor: _X_Principal Investigator

_X___Independent Monitor (Dr. Mehta, Assistant Professor of Medicine, Division of Cardiology, Emory University School of Medicine)

_____Data and Safety Monitoring Board (Give names, affiliations and areas of expertise for all members)

Introduction

This data and safety monitoring plan is designed to enhance the safety of participants in the study and to improve the quality of the data collected.

The overall goal of this study is to define the mechanistic pathways of cardiovascular disease (CVD) in patients with systemic lupus erythematosus (lupus). The cardiovascular system supplies all tissues and organs of the body, has an established relationship to inflammatory metabolomic status, and thus represents an investigative opportunity for improved understanding of a variety of systemic diseases, specifically lupus.

Safety Considerations

PATIENT RISKS

Study participants who undergo CCTA will be exposed to iodinated contrast, and patients with history of iodine allergies and renal disease will therefore be excluded. CCTA involves radiation risk that will be relayed to all individuals who enroll in the program at a low radiation dose of approximately 2 milliSieverts for the angiography. Low dose coronary calcium scanning will be performed with a radiation dose of approximately 1 milliSievert for an overall total of 3-8 milliSevert for the CT testing. The average annual background radiation of individuals in the United States is estimated to be 3mSv. CCTA uses iodinated contrast which can cause contrast induced nephropathy (CIN). This risk is minimized by excluding patients with renal dysfunction.

Adenosine stress CMR involves the use of gadolinium-based contrast, which recently has been linked to nephrogenic systemic fibrosis (NSF), a severe disease first described in 1997. Nephrogenic systemic fibrosis is characterized by swelling, induration and tightening of the skin and, rarely, fibrosis of the lungs, skeletal muscles, pleura, pericardium, myocardium, kidneys, muscle, bone, and testes. NSF usually occurs in patients with severe renal impairment, denoted by an estimated glomerular filtration rate (eGFR) less than 60 ml/min or need for dialysis. A few case studies have suggested that inflammation in addition to renal insufficiency can increase the risk of developing NSF. To decrease the risk of NSF, participants with an eGFR less than 60 ml/min will be excluded from the study. Additionally, the imaging department at CSMC uses a gadolinium contrast that is believed to be the most stable contrast in its chelated form.

For those patients who have current history of asthma, the possible side effects of regadenoson used in the adenosine stress CMR include headache, dizziness, chest pain, nausea, abdominal discomfort, flushing, bronchoconstriction and heart rhythm abnormalities. Patients who undergo a blood draw may experience some pain and there is a small risk of bleeding, bruising, or infection at the puncture site. There is also a small risk of fainting.

Reporting of Adverse Events

Adverse events will be assessed by Dr. Ishimori and reported by study coordinator as per IRB regulations.

Data Accuracy and Protocol Compliance

Standard security procedures will be employed to assure patient information confidentiality. Subjects will be identified only by a study ID code. The study data and samples will be de-identified using the study ID code. The linkage list will be saved in a password-protected excel spreadsheet. All paper records will be kept under lock and key. Only study staff will have access to the study material.

The PI, Dr. Jefferies, will be monitoring protocol compliance and data accuracy. This will also monitored by the study coordinator and Co-I, Dr. Ishimori.

Pathobiology of Lupus Heart Disease SOP: Grant # 230605, IRB # Pro00054998
PI: Caroline Jefferies, PhD

Print copy of ICF/HIPAA from Webridge. Do not use saved versions as revisions may have occurred. Check language of preference. If not English, make sure short forms are available and Interpreter Services has been called/available x3-5353. An interpreter MUST be present when using short forms. If the language of preference is one other than what is available in the short forms, we may NOT enroll the patient under any circumstances, regardless of whether or not an interpreter is present.

https://irb.cedars-sinai.edu/csirb/sd/Rooms/DisplayPages/LayoutInitial?container=com.webridge.entity.Entity%5BOID%5B460372F66084D04F9B89BD4E4CB51CE6%5D%5D&tab2=F902D7C4E3FAA848A018152D336BFA8F

All other documents will be available in shared drive:

Z:\REGULATORY\Current Studies - IRB Approved\Pathobiology of Lupus Heart Disease – 54998

Patient will be referred to Investigator for eligibility.

- Review chart against protocol. Key items to look for:
  - Age (**30-55 y/o**) at enrollment
  - Preferred Language
  - **Iodine/contrast or gadolinium allergy**
  - Renal function (**GFR ≥45**)
  - History of CAD
    - other **cardiac interventions** (past or future)
      - No CABG, stent, SHD, etc.
    - cardiology consults/diagnosis
  - **ACR Criteria for SLE**
  - **Pregnancy (exclusionary)**
  - Other
    - Inability/willingness to follow up
    - Recent or need for future intervention or surgery
    - Inability to provide ICF (language/dementia etc.)

Consent w/ Investigator (likely Ishimori or Wallace).
Co-Investigators: **Mariko Ishimori, MD** **Daniel Wallace, MD**
Noel Bairey Merz, MD Janet Wei, MD, FACC
Daniel Berman, MD Louise Thomson, MBChB
Balaji Tamarappoo, MD, PhD

Provide signed consent (copy or email) to patient

Baseline:

- Investigator will perform physical exam, cardiac/med history, SELDAI, SLICC, and SLE Damage Index.
- CRC will administer Rose, Diamond Index, SAQ, WISE and PROMIS-29 questionnaires.

- Fasting Labs and ECG *(can used SOC if available <30days)*
    - If in CTRC:
        - Schedule accordingly and Provide staff with order set and tubes.
            - Deliver labs to central pathology (South Tower, 4th FL) and Davis as appropriate
    - Other:
        - Investigator will enter all orders, ECG and lab draw in approved clinic.
            - Provide order set, IRB#, and research tubes/instruction

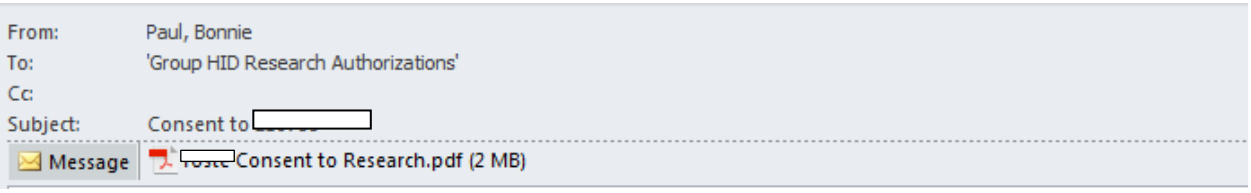
- Labs to be drawn:
    - Research/Biobank, Chemistry, CBC, Compliments, Differential, Anti-centromere, Autoimmune, U/A w/ microscopy
    - Fasting:
        - Lipoprotein, lipids, glucose, insulin

  If unable to acquire labs at baseline, repeat during imaging follow up, or otherwise prior according to patient availability.

Escort the patient to CT unless coming back another day

After Baseline:

- Upload ICF/HIPAA to Group HID Research Authorizations at earliest convenience (must be done same day for billing purposes). Name/MRN will need to be on first page. GroupHIDResearchAuthorizations@cshs.org



Create Subject Binder ASAP

Enter data in RedCap within a week.

CT
Coordinate with Taper Imaging:

- Schedule patient visit according to Imaging/patient availabity. Send appointment information and instructions. Source available in shared drive:

- Send MRI MOP to staff as needed. Source available in shared drive:

Day of Visit:

- Meet patient in lobby of main hospital or Taper (unless escorting from CTRC)

- Escort to location of CT
    - Ensure staff is aware of Research visit/MOP before leaving
    - Provide MRI prep before patient leaves

MRI

Coordinate with Bairey Merz Group:

- Schedule patient visit according to Imaging/patient availabity (check iLab). Send appointment information and instructions.

- Contact patients 2 business days prior to appointment to review MRI prep and confirm attendance.

- Fasting labs to occur immediately prior to imaging at time of IV insertion.
    - Research/biobanking draw
    - Lipoprotein, lipids, glucose, insulin
    - All labs not previously drawn at Baseline

- Email Bairey Merz Group.

- Send itinerary and order set to staff as needed. Source available in shared drive.

Day of Visit:

- Meet patient in lobby of main hospital
- Escort to location of MRI (Davis)
    - Ensure staff is aware of Research visit
    - Provide tubes for clinical and research draws
    - Deliver as appropriate

Update patient log. EDC will need to be updated within 5 days of enrollment. Save all documents (orders, consents, etc.) in shared drive.

DoD Lab SOP

Blood to be collected on day of MRI visit:

70ml of heparinized blood.
1 paxgene RNA and 1 Paxgene DNA tube
1 tiger top (serum)
1 small lavender (Plasma)

Preparation

1. Monocytes will be prepared from 70ml Heparanized blood by Ficoll density centrifugation. Resulting PBMCs will be washed and monocytes isolated by CD14+ selection (Stemcell Technologies).
2. 2.5mn Monocytes will be pelleted and snap frozen for analysis of mitochondrial proteins by mass spec (Mitoplex) and metabolites

The following assays will be carried out:

1. **Seahorse** - assessed in realtime following stimulation with IFNα, immune complexes (IC),or following overnight stimulation. Basal and maximal OCR rate, spare respiratory capacity, ATP production and ECAR will all be measured using the XF96 extracelllular flux analyzer (EFA) (Seahorse Biosciences). To assess the relative requirement of cells under basal and stressed conditions to utilize exogenous or endogenous fatty acids, BSA-conjugated palmitate will be used as an exogenous source of energy in monocytes. The carnitine palmitate transferase 1 inhibitor, Etomoxir (Eto) will be used to prevent BSA-palmitate uptake and allow discrimination between endogenous and exogenous FA usage. EFA measurements will be coupled with metabolomics profiling of unstimulated monocytes following methanol extraction of cell lysates and targeted MRM-based mass spectrometry using Agilent MassHunter Metabolomic dMRM database in collaboration with Dr. Van Eyk (Cedars Sinai Medical Center).

   2.5 x 105 cells per well. 10mn cells
   Cells will be plated in 6 well plates, stimulated/treated as indicated and next day counted and seeded on rehydrated XF96 plate for Seahorse analysis

Ctl	IFN (o/n)
2DG	
Met	
BPTES	
Etoxomir	

2. **Functional changes:** To address the effect of inhibiting glycolysis or OXPHOS using 2-DG (which inhibits hexokinase) or metformin (which inhibits the ETC) on monocyte activation, IFN-induced changes in ISG, upregulation of co-stimulatory molecules (CD86, ICOS) will be assessed by qPCR and flow cytometry as appropriate, following pre-incubation of monocytes (isolated from healthy controls or lupus patients (with inhibitors for 30 mins followed by 3 hour stimulation with IFN-α. phosho-sSTAT1/p-STAT2 will also be measured by phosflow to determine the ability of metabolic inhibitors to inhibit signaling through the IFN receptor (IFNAR).

   Gene expression – 10mn cells

Ctl	IFN (3 hrs)
2DG O/N	
Met O/N	
BPTES O/N	
Etoxomir O/N	

   FACs – 1mn cells

Ctl	IFN (15mins)
2DG O/N	
Met O/N	
BPTES O/N	
Etoxomir O/N	