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START DATE: June 18th, 2015 END DATE: June 18th, 2020

NUMBER AND TYPE OF PATIENTS: 100 Lupus Patients
Accrual Ceiling: 100

	Number	Sex	Age Range
Lupus Patients:	100	Females & Males	≥18 ages

PROJECT USES IONIZING RADIATION:
X Research indicated
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Abbreviation	Definition
AE	Adverse Event
BILAG	British Isles Lupus Assessment Group
CAVI	Cardio-Ankle Vascular Index
CRP	C-reactive Protein
CV	Cardiovascular
CVD	Cardiovascular Disease
DAS-28	Disease Activity Score in 28 Joints
DM	Diabetes Mellitus
15-dpGJ2	15-Deoxy-Delta-12,14-prostaglandin J2
EPCs	Endothelial Progenitor Cells
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
HDL	High Density Lipoprotein
HRQOL	Health Related Quality of Life
IC	Immune Complex
ICAM-1	Intercellular Adhesion Molecule-1
IFNs	Interferons
iNOS	Inducible Nitric Oxide Synthase
LFTs	Liver Function Tests
MCP-1	Monocyte Chemoattractant Protein-1
MMF	Mycophenolate Mofetil
MMP	Matrix Metalloproteinase
NETs	Neutrophil extracellular traps
NYHA	New York Heart Association
NO	Nitric Oxide
PAI-1	Plasminogen Activator Inhibitor 1
PAT	Pulse Applanation Tonometry
PGA	Physician Global Assessment
piHDL	Proinflammatory HDL
PPAR	Peroxisome Proliferator Associated Receptor
PWV	Pulse Wave Velocity
RA	Rheumatoid Arthritis
RHI	Reactive Hyperemia Index
RH-PAT	Reactive Hyperemia-Pulse Applanation Tonometry
RXR	Retinoid X Receptor
SAEs	Serious Adverse Events
SF-36	Short Form (36) Health Survey
SLE	Systemic Lupus Erythematosus
TZD	Thiazolidinedione
VCAM-1	Vascular Cellular Adhesion Molecule-1

5 PRÉCIS

Systemic lupus erythematosus (SLE) is an autoimmune disease of unclear cause that affects primarily women of childbearing age. Patients with lupus have a significantly increased risk of developing complications of their blood vessels due to accelerated hardening of the arteries (atherosclerosis). These complications include heart attacks and stroke. No drug to date has proven to prevent this type of complication in lupus and premature vascular disease significantly impacts the quality of life of these patients and enhances their risk of death.

The thiazolidinediones (TZD) are a class of drugs approved for the treatment of patients with type 2 diabetes mellitus (DM); they belong to the family of drugs that activate the peroxisome proliferator-activated receptor-γ (PPAR-γ). They have been proposed to have strong anti-atherogenic and anti-inflammatory effects[1] even in patients without diabetes. Recent work from our group and others indicates that TZDs significantly improve vascular damage, dysfunction of blood vessels and disease activity in mouse models of lupus and abrogate atherosclerosis[2-4]. We recently identified the TZD pioglitazone as an effective drug in modulation of vascular function and disease activity in patients with rheumatoid arthritis[5]. In addition, we have found in mouse models of lupus and in in vitro experiments with human lupus cells, that pioglitazone has important roles in modulating immune function and vascular manifestations[3, 6]. Furthermore, this drug is not immunosuppressive, adding an additional advantage when compared to other medications used in this disease.

We propose that TZDs could significantly improve blood vessel function and play a role in atherosclerosis prevention in human SLE, in addition to modifying lupus disease activity. The major goal of the proposed research is to assess the effects of the PPAR-γ agonist pioglitazone in SLE on vascular function and inflammation and on SLE disease activity. The results of the study may lead to the characterization of a new therapeutic target with dual effects on lupus and its associated blood vessel damage

6 BACKGROUND INFORMATION, SCIENTIFIC RATIONALE AND SIGNIFICANCE

6.1 Pathogenesis of SLE and unmet needs in the treatment of this disease

SLE is a systemic autoimmune syndrome with pleiotropic clinical manifestations that primarily affects women of childbearing age. SLE is highly heterogeneous in its clinical presentation, which renders therapeutic interventions particularly challenging. The current management of patients with SLE is usually stratified by the degree of internal organ involvement; however, most treatment strategies include a variety of immunosuppressive medications that are limited both in their efficacy and by their potential toxicities. FDA-approved treatments for SLE include only hydroxychloroquine, corticosteroids, aspirin, and most recently, belimumab. Potentially devastating side effects of corticosteroids are well known and include infection, avascular necrosis, weight gain, osteoporosis, cataracts and development of diabetes. Although often

beneficial for treatment of active disease, other immunosuppressive medications commonly used in SLE (azathioprine, mycophenolic acid (MMF), cyclophosphamide, etc.) are associated with multiple toxicities. Furthermore, despite the addition of these potentially toxic agents, SLE patients usually require continued treatment with corticosteroids. Thus, lupus patients are typically dependent indefinitely on corticosteroids and/or immunosuppressive agents for disease control even while developing cumulative toxicities from exposure to these drugs. Clearly there is an unmet need for improved treatment of inflammation in this patient population[7].

Both innate and adaptive aberrant immune responses appear to play key roles in the loss of tolerance and the development of subclinical and clinical manifestations[8]. In SLE, genetic, environmental, hormonal, and various epigenetic and immunoregulatory factors act either sequentially or simultaneously on the immune system. This results in the generation of autoantibodies, immune complexes, autoreactive and inflammatory T cells, and inflammatory cytokines that initiate and amplify inflammation and damage to various organs[9]. Various cytokines have been proposed to play important pathogenic roles in SLE, including type I Interferons (IFNs), IL-6, IL-17, BLyS, IL-10 and TNF[10]. Various abnormalities in innate immune responses have been the focus of intense research in the last few years, including the role of aberrant cell death, neutrophil extracellular trap (NET) formation, and the interplay between lupus neutrophils and type I IFNs[11, 12]. Abnormalities in various subsets of T cells have been reported in SLE (including Teffector and Treg subsets), although the mechanism and functional consequences of such changes remain unclear. Th17 cells have been reported at increased frequency and detected at the site of end-organ damage in SLE, while serum levels of IL-17 are increased in SLE and correlate with disease activity[10, 13]. Treg cells are also altered in patients with SLE. Both human and murine studies have reported a deficiency in number and/or function of these cells, which act to suppress the activation of both T-helper cells and B cells. Treg cells isolated from patients with active SLE appear less effective at suppressing T cell proliferation and IFN-γ production in comparison with cells from healthy controls or patients with inactive lupus[14].

6.2 Vascular damage and premature atherosclerosis in SLE

While the life expectancy in lupus has significantly improved[1, 2,] the disease is associated with significant morbidity and increased mortality in significant part due to accelerated atherosclerotic vascular disease[16, 17, 23].The risk of premature cardiovascular disease (CVD), especially in young women with SLE, is striking, and may be as high as 50-fold when compared to matched controls, depending on the study and outcome measure[18]. Atherosclerotic CVD develops or progresses in ~10% of SLE patients/year during short-term follow-up[19] and is one of the most common causes of death[16, 20]. As we now recognize SLE as an independent risk factor for premature CVD, the progressive nature of lupus vascular injury makes this population ideal for the study of mechanisms involved in endothelial dysfunction, a state wherein the vasodilatory, anticoagulant or anti-inflammatory properties of the endothelium are impaired and which predisposes to atherosclerosis development[21]. SLE patients without overt CVD display subclinical vascular

disease (endothelial dysfunction[22, 23], arterial stiffness[24] and coronary perfusion disturbances[25]) and increased subclinical atherosclerosis (carotid plaque and coronary calcification)[26, 27]. Many of these findings are not explained by traditional risk factors[28]. Further, while all-cause mortality in SLE has improved significantly with improved monitoring and immunosuppressive treatments, CVD remains a leading cause of death[29]. Indeed, advances in immunosuppression have decreased organ damage in lupus, but no drug to this date has proven to abrogate atherosclerosis development in SLE, and high uncertainty on how to reduce the enhanced CV risk remains. For example, steroids can induce hypertension, insulin resistance, and dyslipidemias[30], but are anti-inflammatory and therefore may decrease CV complications[26, 31]. Antimalarials and mycophenolate mofetil (MMF) may have vasculoprotective effects[32, 33], but their role in atherosclerosis prevention is unclear. These observations indicate that novel adjuvant therapies that target inflammation, immune dysregulation and metabolic abnormalities without significant side effects are a significant priority in SLE.

6.3 Mechanisms of premature CVD in SLE

Although T and B cells are indispensable for lupus pathogenesis, it is still unclear if they play a major role in lupus CVD. In contrast, links between innate immunity, SLE, and CVD are myriad[12], with proposed significant roles for type I IFNs, platelets and neutrophil NETs among others. We have proposed that type I IFNs promote an imbalance of vascular damage and repair, triggered in part by impairment in the phenotype and function of cells crucial for vasculogenesis including endothelial progenitor cells (EPCs). Furthermore, type I IFNs promote atherogenesis, recruit macrophages to plaque and induce platelet activation[34, 35]. In addition, there is a high prevalence of metabolic syndrome, insulin resistance and dyslipidemia in human and murine SLE[36] and in murine lupus these metabolic abnormalities may precede the development of overt autoimmunity[37].

High-density lipoprotein (HDL) function is not always accurately predicted by HDL cholesterol levels. The functions of HDL include reverse cholesterol transport and modulation of inflammation. In healthy individuals, in the absence of systemic oxidative stress and inflammation, HDL is anti-inflammatory. However, in those with chronic illnesses such as SLE characterized by systemic oxidative stress and inflammation, HDL may actually promote the inflammatory response[38]. Indeed, work from various groups including ours has shown that human and murine lupus are characterized by enhanced HDL oxidation and perturbed function and this has been associated with enhanced atherogenesis[34].

6.4 Current treatments and CVD in SLE

Corticosteroids, widely used as adjuvant treatment in SLE, likely have mixed effects on CVD. While there has long been evidence that duration of prednisone use can independently predict risk of CVD events, there is also evidence that, over time, more aggressive treatment with drugs like cyclophosphamide and corticosteroids, will correlate with reduced CVD burden[19]. In terms of drugs with a specific role in the treatment of the disease manifestations of SLE, two—the

antimalarials and MMF—have received the most attention for their potential cardioprotective benefits. It is still unclear the role that novel and potential biologics may have in modulating CVD in SLE.

In recent years, there has also been increasing interest in whether drugs with defined roles in diseases like hypercholesterolemia and diabetes might have particular benefit in patients with SLE.

6.5 PPAR-γ agonists as potential therapeutic targets in SLE

PPAR-γ agonists are members of the nuclear receptor superfamily of ligand-dependent transcription factors, expressed in various cells including adipocytes, vascular cells, antigen presenting cells and lymphocytes. PPAR-γ activation by agonists determines modulatory effects on different crucial cellular events such as growth factor release, cytokine production, cell proliferation and migration, extracellular matrix remodeling and cell cycle progression/ differentiation. In addition, PPAR-γ agonists have potent antioxidant effects[39]. PPAR-γ regulates gene expression by binding as a heterodimer to the retinoid X receptor (RXR). The PPAR-γ/RXR heterodimer binds to sequence-specific PPAR response elements in the promoter region of target genes, acting as a transcriptional regulator[40]. PPAR-γ also functions in a DNA-binding-independent manner to trans-repress various target genes. PPAR-γ activation by agonists determines modulatory effects on crucial cellular events: growth factor and cytokine production, cell proliferation, migration and differentiation, extracellular matrix remodeling and cell cycle progression. In addition, PPAR-γ/RXR complexes may cause functional inhibition by directly binding to transcription factors, preventing them from inducing gene transcription and may also inhibit phosphorylation and activation of several members of the MAPK family[1].

There are 2 isoforms of PPAR-γ: PPAR-γ1 and PPAR-γ2. Differential promoter usage and alternate splicing of the gene generates 3 mRNA isoforms. PPAR-γ1 and PPAR-γ3 mRNA both encode the PPAR-γ1 protein, which is expressed in most tissues, whereas PPAR-γ2 mRNA encodes PPAR-γ2 protein, which contains an additional 28 amino acids at the amino terminus and is specific to adipocytes[40.] Once tissue injury and inflammatory responses ensue, there is upregulation of PPAR-γ. Activation of PPAR-γ (by endogenous or exogenous ligands) inhibits the activation of the transcription factors NF-κB, AP-1, NFAT, and STAT[41]. This attenuates the formation of cytokines, chemokines, and adhesion molecules, reducing excessive inflammation and tissue injury. Ligands of PPAR-γ inhibit the expression of the pro-inflammatory cytokines IL-1β, IL-6, TNF-α and interferon (IFN)-γ; the chemokine monocyte chemoattractant protein-1 (MCP-1); and the adhesion molecules intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). In addition, other important mediators of inflammatory responses and/or pro-thrombotic cascades such as inducible nitric oxide (NO) synthase (iNOS), COX-2, CD40 ligand, plasminogen activator-I (PAI-I), and MMPs 9 and 1 are inhibited by these compounds[1, 42-46]. Furthermore, within a week of treatment with exogenous PPAR-γ agonists, CRP levels decrease by 30% in patient groups, whereas the reduction produced by statins is only

14%[47, 48]. There are a number of endogenous ligands of PPAR-γ, including eicoisanoids (such as the PGD2 metabolite 15-deoxy-delta prostaglandin J2 (15d-PGJ2)) and long-chain polyunsaturated fatty acids. In addition, various synthetic PPAR-γ agonists have been developed or are under development. The most widely used belong to the TZD or glitazone class of anti-diabetic drugs. The 2 currently available TZDs, pioglitazone and rosiglitazone, are used alone or in combination with other oral anti-diabetic agents for type 2 DM1. While PPAR-γ agonists have potent insulin-sensitizing effects, numerous reports indicate that the therapeutic benefits these drugs may go far beyond their use in DM.

Emerging evidence indicates that the PPAR signaling pathways play critical roles in the regulation of a variety of biological processes within the CV system[49] and that PPAR-γ agonists inhibit the expression of genes that contribute to atherogenesis. PPAR-γ isoforms have been characterized in multiple vascular cell types and prevent in vitro vascular smooth muscle cell growth[50-52] and inflammatory responses, suggesting a role in vascular remodeling and atherosclerosis. Furthermore, TZDs can improve many atherosclerosis risk factors by lowering triglycerides, elevating HDL cholesterol and decreasing levels of biomarkers that are crucial in atherosclerosis development[53]. The positive lipid effects are significantly more prominent for pioglitazone than for rosiglitazone and rosiglitazone can have deleterious lipid effects; therefore, pioglitazone might be a better drug for atherosclerosis prevention[54].

With regards to vasculogenesis, TZDs upregulate both number and functional capacity of EPCs and prevent apoptosis of these cells in mice and humans[55]. Reduction of EPC apoptosis by TZD may be a potentially beneficial mechanism for patients with vascular diseases. TZDs can restore vasodilator responses, improve endothelial function and suppress atherosclerosis progression in various animal models of vascular injury[56,57]. These compounds also have potent antioxidant activity and an ability to inhibit LDL oxidation, further decreasing atherosclerotic risk[58]. In clinical studies, TZDs can inhibit CIMT[59] ameliorate endothelial dysfunction, and inhibit restenosis after cardiac stent[60] in patients with DM independent of glucose control[61]. Pioglitazone decreases visceral and abdominal fat content and blood pressure, and reduces systemic vascular resistance in diabetics[62]. TZDs also ameliorate markers of endothelial activation and improve endothelial function in non-diabetic patients with coronary artery disease[63, 64]; in non-diabetics with the metabolic syndrome[64] in patients with HIV[65], RA[5], and hypertension[66]; and in renal transplant recipients[67]. In nondiabetic patients with CV risk factors, pioglitazone treatment enhances insulin sensitivity, decreases C-reactive protein (CRP), and improves endothelial vasodilator function[68]. Therefore, TZDs might also be effective anti-atherogenic strategies in non-diabetics with other increased risk factors for atherosclerosis.

Current evidence suggests that atherosclerosis prevention induced by these compounds is not only due to the amelioration of systemic metabolic risk factors for atherogenesis but also to downmodulation of inflammatory events that occur within the artery wall[69, 70]. Indeed, in a recent study, pioglitazone was found to counteract the deleterious effects of TNF-α on the

vascular endothelium, further supporting its putative role in inflammatory conditions associated with increased CV risk[71]. Studies suggest that short-term (3 weeks) treatment with TZDs can promote significant improvement in endothelial function in healthy subjects[72]. Regarding lipid control, data on file from Takeda Pharmaceuticals shows that a 30 mg dose of pioglitazone for 26 weeks results in triglyceride lowering of 9.6%, HDL increase of 12%, and a neutral effect on LDL and total cholesterol (www.takeda.com). Low HDL cholesterol is increasingly recognized as a CV risk factor at any given level of total cholesterol or LDL[73]. Thus the HDL elevating properties of TZD therapy may further add to their putative CV benefits. Importantly, a report of 5238 diabetic patients with macrovascular disease has shown that pioglitazone significantly reduces CV morbidity and mortality[74]. Also, treatment of type 2 DM with 45 mg daily pioglitazone exerts powerful effects on endothelial function beyond metabolic control[75]. *This was the dose we used in our RA cohort, where we found significant improvements in arterial stiffness, metabolic parameters and disease activity*[5].

6.6 Safety of PPAR agonists

Regarding safety, recent meta-analyses and observational studies have indicated that rosiglitazone may increase the risk of ischemic CV events and heart failure in DM and the elderly, whereas the association of pioglitazone with these complications is less clear[76]. Ongoing studies are assessing rosiglitazone's effect in CV event prevention in patients with CVD history[77]. There is significant debate regarding the role of rosiglitazone or other TZDs in CV complications, particularly in insulin-dependent DM, heart failure and morbid obesity[78]. Overall, MI and stroke in DM have been significantly reduced by pioglitazone. However, TZDs are contraindicated in New York Heart Association Class III or IV heart failure.

Pioglitazone can cause decreases in hemoglobin and hematocrit of 2-4%. These reductions may be due to an increase in plasma volume and usually pose only minor risk. While pioglitazone is typically not associated with hepatic toxicity or liver function test (LFT) elevations, the pioglitazone package insert states there are post marketing reports of hepatic failure and that causality cannot be excluded; Medwatch report states there is an association with hepatitis, hepatic enzyme elevations to 3 or more times the upper limit of normal. Pioglitazone is structurally related to troglitazone (no longer marketed), which has been associated with idiosyncratic hepatotoxicity and rare cases of liver failure, liver transplants, and death. In a recent study of non-diabetics treated with pioglitazone for similar periods of time to what is proposed for our study, no changes in LFTs were observed[68].

Similarly in diabetics with a mean duration of diabetes of 9.5 years, an increased incidence of bone fracture was noted in female patients taking TZDs. There is significant debate regarding which patients on PPAR-gamma agonists are at increased risk for fracture. For example, a recent study reported that over 1 year, treatment with pioglitazone 30 mg/day did not produce consistent effects on either bone mineral density or bone turnover in people with type 2 DM (T2DM) or glucose intolerance[79] while another study showed decreases in bone density at around 33 months of similar treatment[80] . The majority of fractures observed in female patients

have been nonvertebral fractures including lower limb and distal upper limb. In diabetics, postmenopausal women taking TZDs and the subset of men taking both loop diuretics and TZDs were at increased risk for fractures. In postmenopausal women, risk was associated with higher TZD dose. No difference between rosiglitazone and pioglitazone was apparent[81]. The effects of TZDs on bone appear related, in part, to induction of osteocyte apoptosis[82].

Preclinical and clinical trial data, and results from observational studies suggest an increased risk of bladder cancer in pioglitazone users. The observational data further suggest that the risk increases with duration of use[83]. It has been suggested that patients with bladder cancer (current or previous) should not use pioglitazone. No studies have been published regarding cancer risk with pioglitazone in nondiabetics. A recent meta-analysis of 215,142 of patients with T2DM on pioglitazone revealed a hazard ratio of 1.03(95% CI 0.84-1.26) for patients treated with pioglitazone for less than 12 months. The hazard ratio increased to 1.44(95% CI 1.19-1.74) for patients using pioglitazone for more than 24 months. The p-value for interaction was 0.04, suggesting a significant interaction between duration of pioglitazone use and incidence of bladder cancer. However there was no interaction between cumulative dosage of pioglitazone and incidence of bladder cancer (p-value for interaction=0.19). The number needed to harm (NNH) was 20903 suggesting that more than 20,000 patients needed to be treated for one case of bladder cancer to develop. Use of pioglitazone in patients with SLE and those with history of treatment with cyclophosphamide has not been studied extensively. In their report of 30 SLE patients treated with pioglitazone for 3 months Posadas-Romero et. al. have not reported any increased incidence of bladder cancer[98]. Of note, diabetes may increase the risk of bladder cancer, with varying risk ratios across different duration of diabetes, and glycemic control may also have an impact on the development of cancer through mechanisms related to metabolic changes, accumulation of advanced glycation end-products and increased oxidative stress. As PPAR agonists are usually used as second- or third-line drugs for glycemic control, their use may indicate a poor glycemic condition for a long duration before their use. Many of the previous observational studies could not adequately address the potential confounding effects of diabetes duration and glycemic control in data collection and analyses. As such, randomized clinical trials are needed to assess if there is truly an increased risk in diabetics exposed to long-term pioglitazone and whether this applies to other patient populations.

Over 8500 patients with DM have been treated with pioglitazone in controlled clinical trials. Over 6000 patients have been treated for 6 months or longer and over 3000 patients have received the drug for at least 2 years. The overall incidence and adverse events reported in placebo controlled clinical trials are shown in the table below. The incidence of withdrawals was similar for patients treated with placebo (2.8%) or pioglitazone (3.3%).

Table 1: Three Pooled 16-to 26-Week Placebo-Controlled Clinical Trials of Pioglitazone Monotherapy: Adverse Events Reported at an Incidence > 5% and More Commonly in Patients Treated with Pioglitazone than in Patients Treated with Placebo

	% OF PATIENTS	
	PLACEBO N=259	ACTOS N=606
Upper Respiratory Tract Infection	8.5	13.2
Headache	6.9	9.1
Sinusitis	4.6	6.3
Myalgia	2.7	5.4
Pharyngitis	0.8	5.1

*(Source http://www.rxlist.com/actos-drug/side-effects-interactions.htm)

As mentioned above, our group recently completed a randomized, placebo-controlled, crossover trial on the effect of pioglitazone on endothelial function and disease activity in RA, another inflammatory disease associated to enhanced CV risk. One hundred forty-three non-diabetic adult RA patients (76.2% female, age 55.2 ± 12.1 [mean ± SD]) on stable RA standard of care treatment were enrolled in a randomized, double-blind placebo controlled crossover trial of 45 mg daily pioglitazone versus placebo, with a 3-month duration/arm and a 2-month washout period. Pulse wave velocity of the aorta (PWV), brachial artery flow mediated dilatation, nitroglycerin mediated dilatation, microvascular endothelial function (reactive hyperemia index [RHI]), and circulating biomarkers of inflammation, insulin resistance, and atherosclerosis risk all were quantified. RA disease activity was assessed with the 28-Joint Count Disease Activity Score (DAS-28), CRP and the Short Form (36) Health Survey quality of life questionnaire. When added to standard of care RA treatment, pioglitazone significantly decreased aortic stiffness, while conduit and small vessel function remained unchanged when compared to placebo. Further, pioglitazone significantly reduced RA disease activity and CRP levels while improving lipid profiles. The drug was well-tolerated[5]. In this cohort of patients with overall low Framingham risk factors and preserved heart function, markers of target organ function, including LFTs and CBC, were not different between treatment versus placebo groups at the end of the study. There were more adverse events while on the active treatment group, and these consisted primarily of expected side effects related to this drug class such as weight gain, lower extremity edema, and dyspnea (see table below). Of the 16 serious adverse events (SAEs) in the trial, only one was probably related to the study drug. This was a case of lower extremity edema and chest pain that resolved with discontinuation of the study drug. There were 5 other SAEs considered to be not related to the study drug (dyspnea and chest pain, tachycardia and elevated blood pressure, low potassium and sodium, hip fracture, and intracranial bleed; n=1 each). The study drug was discontinued only for the case of dyspnea with chest pain, and all resolved. The remainder of the SAEs were considered “definitely not” related to the study drug.

Table 2: Adverse Event (AE) by Body System and Treatment in Pioglitazone Trial in RA patients N=143

Body System	Placebo (n=127)	Treatment (n=129)	*P* Value
Body as a whole	3 (2.36%)	5 (3.87%)	0.72
Cardiovascular	4 (3.15%)	8 (6.25%)	0.25
Edema	0 (0.00%)	6 (4.65%)	NA
Hemic and lymphatic	0 (0.00%)	1 (3.87%)	NA
Infection	5 (3.94%)	5 (3.87%)	1.00
Metabolic and nutritional	3 (2.36%)	4 (3.10%)	1.00
Musculoskeletal	5 (3.94%)	8 (6.25%)	0.57
Nervous system	2 (1.57%)	4 (3.1%)	0.68
Respiratory	1 (0.79%)	6 (4.65%)	0.12
Skin and appendages	4 (3.15%)	3 (2.33%)	0.72
Total number of AEs	27 (21.26%)	50 (38.75%)	**0.003**
Total number of subjects with at least one AE	23 (18.11%)	39 (30.23%)	**0.028**

6.7 PPAR agonists in SLE

As mentioned above, both murine and human studies in SLE support the notion that PPAR-γ agonists may be beneficial in this disease and represent an important potential molecular target for vascular dysfunction improvement in SLE (Figure 1). Rosiglitazone and pioglitazone improve disease activity in the MRL/lpr land gld/ApoE-/-lupus mouse model[2] with decreases in autoAbs and renal inflammation and spleen size[2]. Rosiglitazone also decreases hypertension and renal injury in lupus-prone NZB/W F1 mice[4]. A beneficial effect of TZDs in renal injury in other conditions has been well-documented [84, 85]. We have reported that pioglitazone decreases renal inflammation in NZB/W F1 mice[86]. Kidneys from pioglitazone-treated mice showed significant decreases in immune complex (IC) deposition and in mesangial expansion, endocapillary hypercellularity, renal T cell infiltration and renal expression of proinflammatory molecules including TNF and IL-17. While renal function did not differ between treatment groups in the NZB/W mice, our results indicate that further exploring pioglitazone as adjuvant therapy when combined with other treatments targeting SLE activity is warranted, given the potential positive effects on organ damage prevention. These results indicate that TZDs significantly modulate inflammatory responses in SLE and may decrease tissue damage. There is also recent evidence that pioglitazone can downmodulate the proinflammatory effects of type I IFNs on myeloid cells and this may be particularly relevant in SLE pathogenesis, given evidence from our group and others that aberrant neutrophils may play key roles in lupus pathogenesis and its associated vascular disease as mentioned above[89]

Similar to what we reported in murine EPCs[3], when human EPCs are treated with pioglitazone, there is a significant improvement in their capacity to differentiate into mature ECs after 2 weeks

in culture. In contrast, rosiglitazone-treated lupus EPCs do not show improvement in their differentiation capacity (unpublished observations). This further supports testing pioglitazone as the TZD of choice in SLE due to its beneficial effects on vasculogenesis. IFN-α is known to be antiangiogenic and we recently begun to characterize the pathways by which it suppresses EPC differentiation and function[90]. We have performed gene array expression studies on control and lupus circulating EPCs exposed to proangiogenic stimuli for 72 hours, then left untreated or treated for 6 hours with recombinant IFN-α[90]. We identified that IFN-α induces a strong antiangiogenic signature in control and lupus EPCs. One of the most significant changes included repression of various genes involved in PPAR-γ signaling in both SLE and control EPCs. This indicates a potential mechanism by which type I IFNs may interfere with vascular repair, through downregulation of pathways crucial in endothelial health, and further supports testing PPAR agonists in CV prevention in SLE. Interestingly, the level of PPAR-γ repression by IFN-α was significantly more pronounced in SLE EPCs than in control cells, suggesting that the former may be more sensitive to the effects of type I IFNs[91].

In a recent small clinical trial in 30 young female lupus patients, pioglitazone administration over three months improved HDL levels, insulin resistance, and HDL size, while decreasing markers of inflammation such as CRP and serum amyloid A; specific markers of endothelial function were not considered, neither was the effect on SLE disease activity[92].

We therefore propose that these agonists may particularly benefit SLE patients by suppressing inflammatory and immunologic pathways that promote premature atherosclerosis and internal organ damage. Identifying pioglitazone's role in SLE may support an eventual paradigm shift in standard of care for these patients to include therapy with PPAR-γ agonists for treatment and prevention of endothelial injury, atherosclerosis and CV events in this disease and, potentially, in individuals with other systemic autoimmune diseases associated with premature CVD.

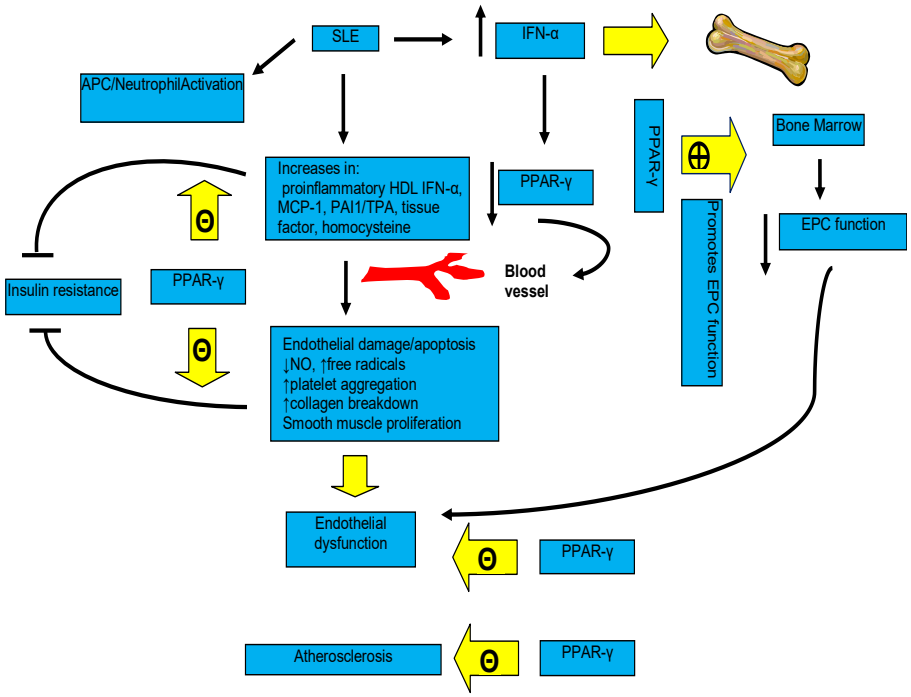

Figure 1: Mechanisms of vascular damage/ atherosclerosis in SLE and potential role of PPAR-γ agonists in atherosclerosis prevention

Predisposition to vascular damage in SLE is likely mediated by multiple pathways including abnormal vascular repair; enhanced endothelial apoptosis through a variety of mechanisms (cytokines such as type I IFNs, autoAbs, cell-mediated cytotoxicity, NETs, traditional risk factors); prothrombotic and metabolic abnormalities including increased piHDL. This leads to blood vessel damage, ↓ NO, increased platelet aggregation and smooth muscle proliferation, all of which can lead to endothelial dysfunction and premature atherosclerosis. Further, inflammation triggers insulin resistance that also contributes to endothelial dysfunction. PPAR-γ agonists may prevent this complication by blocking the majority of these harmful pathways.

7 OBJECTIVES

The major goal of the proposed research is to assess the effect of a targeted pharmacotherapeutic intervention – the administration of the PPAR-γ agonist pioglitazone – in SLE on:

a) vascular endothelial function (primary endpoint), a predictor of CV risk

b) SLE disease activity (secondary endpoint).

This randomized clinical trial, with a modest sample size, can both serve as a "proof of concept" study for the potential utility of TZDs in SLE, and inform us on which surrogate outcomes or biomarkers may be useful in future lupus studies aimed at CV/immunomodulatory outcomes.

Aim 1: To conduct a randomized, double-blind, placebo controlled, crossover, proof-of-concept clinical trial in adult SLE patients comparing the effects of pioglitazone vs. placebo on vascular endothelial function and blood vessel inflammation. Primary outcome measures will be large

vessel arterial stiffness, measured either by cardio-ankle vascular index (CAVI) or pulse wave velocity (PWV, assessed by Sphygmocor) (both tests to assess large vessel arterial stiffness), and assessment of microvascular peripheral arterial tone (PAT). Secondary outcome measures of atherosclerosis risk and metabolic disturbances will include proinflammatory HDL (piHDL) levels and HDL cargo, lipoprotein function, lipid profile and insulin resistance and EPC phenotype and function. In addition, modulation of arterial inflammation will be assessed in a subset of patients by neck to groin fluorodeoxyglucose positron emission tomography CT (FDG PET/CT).

Hypothesis 1: Endothelial function and arterial compliance will significantly improve in SLE patients treated with pioglitazone when compared to placebo, while arterial inflammation will decrease.

Hypothesis 2: Pioglitazone will alter HDL cargo function, decrease proinflammatory HDL levels and insulin resistance, and improve markers of vascular repair in SLE.

Aim 2: To assess the ability of pioglitazone to reduce SLE disease activity, when added to standard lupus care, as assessed by the SLEDAI-2K disease activity index (SLEDAI-2K), lack of A or B flares on the British Isles Lupus Activity Group (BILAG 2004) score, and physician's global assessment (PGA) (See Appendix sections 2,3,7). Serological markers of SLE activity and various cytokines considered important in lupus pathogenesis will also be quantified.

Hypothesis 3: SLE Disease activity will decrease in SLE patients when treated with pioglitazone vs. placebo.

Aim 3: To investigate the role of in vivo administration of PPAR-γ agonists in modulating aberrant immune responses in SLE. These will include lymphocyte phenotype and function, autoantibody and cytokine production and myeloid cell phenotype and functional abnormalities.

Hypothesis 4: Pioglitazone will normalize innate and adaptive immune responses in SLE.

8 STUDY DESIGN AND METHODS

This will be a single-center, randomized, double-blind, placebo-controlled, crossover, proof-of-concept treatment trial enrolling 100 SLE patients including replacements due to screen failures and withdrawals. If a patient drops out of the study before completion both arms, another patient may be enrolled so that up to 75 patients complete the study. They will be treated for 3 months with 45 mg daily pioglitazone or placebo, undergo an 8 week washout period, then undergo crossover to the other arm for 3 additional months. Throughout the study, functional, anatomical and blood biomarkers of vascular damage, atherosclerosis risk and SLE activity will be collected. Pioglitazone has been chosen over rosiglitazone because it appears to have a significantly better safety profile regarding CV effects and lipid profile alterations[93], based on our experience in the rheumatoid arthritis (RA) trial[5] and the small pilot study published by

another group that showed improvement in some metabolic parameters in SLE[92], as well as our preliminary data which indicate improvement of vasculogenesis by pioglitazone but not rosiglitazone[94] in human SLE. We also have significant experience using this drug due to the recently completed clinical trial in RA[3]. The dose and study time period have been selected based on pioglitazone's macrovascular protective effects[74] and to allow detection of meaningful changes in endothelial function and disease activity, respectively. This is also based on the dose and study time period used in the RA trial, which showed significant effects on arterial stiffness, metabolic parameters, inflammatory markers and RA disease activity[5].

TZD studies indicate improvements on endothelial function from within a few weeks to a couple of months. Previous crossover studies assessing pioglitazone's role in endothelial function, insulin resistance and inflammation have used treatments from 6-12 weeks with washout periods of 4-6 weeks[68, 72, 95]. In our studies in RA, 3 months was sufficient to observe significant improvements in arterial compliance and disease activity[5]. These washout periods appeared sufficient to avoid carryover effects and, indeed, in the RA trial we found no evidence of carryover effect after the same washout period[5]. Carryover issues will also be addressed by randomizing the sequence of placebo and pioglitazone treatment. Previous studies did not reveal that the sequence (i.e. placebo followed by pioglitazone vs. pioglitazone followed by placebo) had any effect on the observations[68]. The study will take approximately 5 years to complete. Figure 2 represents the study sequence.

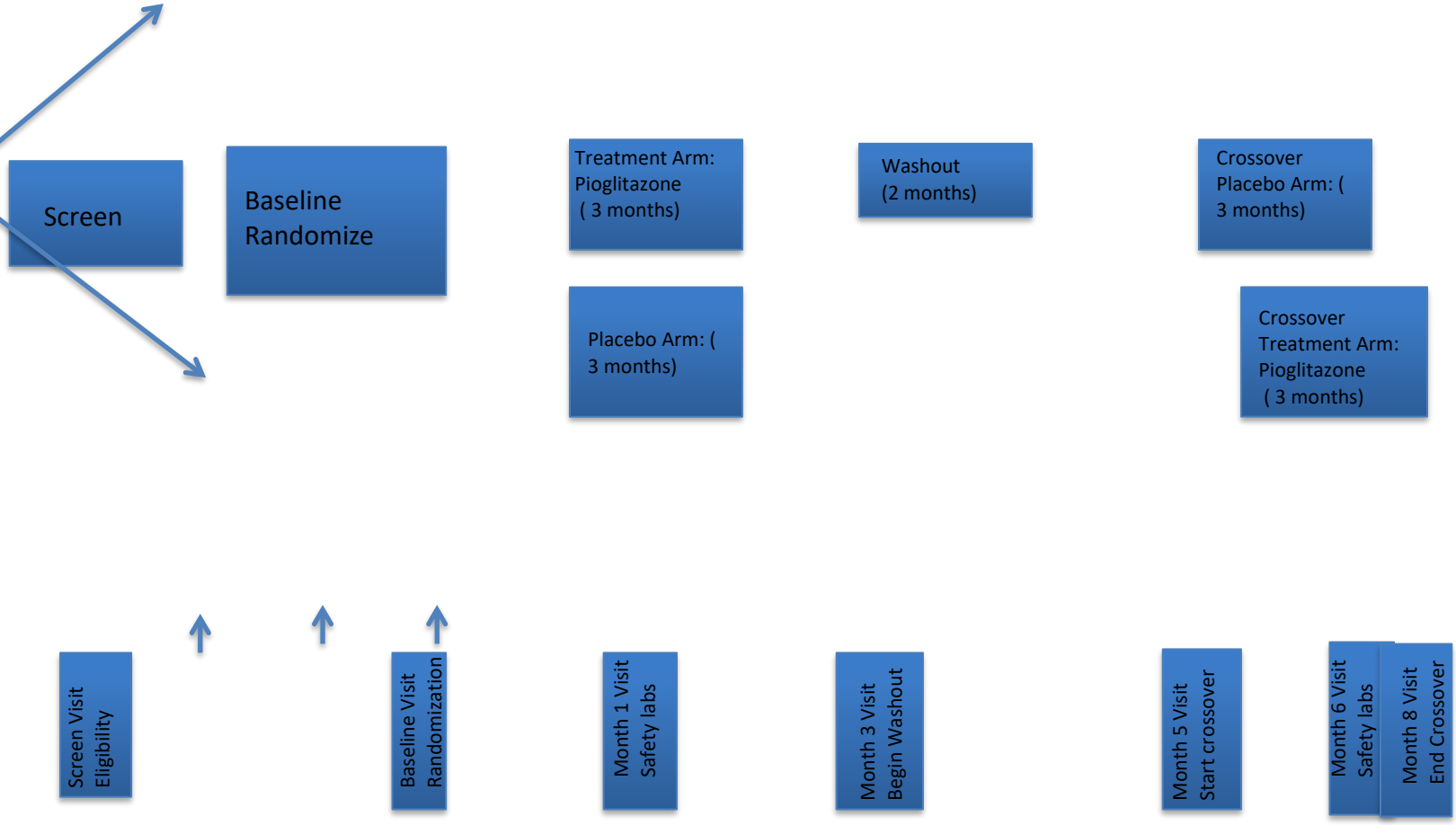

Figure 2: Overall Study Schematic

8.1 Proposed Surrogate Outcomes and Biomarkers

8.1.1 ***Specific Aim 1: Effect of Pioglitazone on vascular function and cardiometabolic risk.***

a) Vascular function and vascular inflammation.

SLE patients demonstrate endothelial dysfunction independent of Framingham risk factors and in the setting of subclinical CVD [22, 23]. Endothelial dysfunction is an early event in atheroma development and a trigger of CV events[96]. PPAR-γ agonists reverse endothelial dysfunction in other diseases[61, 64, 98], and pioglitazone improves endothelial function in murine lupus[99].

Arterial compliance/CAVI and PWV: Arterial stiffening is caused by structural and/or functional changes within the major conduit artery walls, resulting in increases in pulse wave velocity, with important hemodynamic consequences[100]. SLE patients without overt atherosclerosis have increased arterial stiffness[24, 101-103], indicating it may be a sensitive marker of early CV dysfunction and increased risk and a target of treatment efficacy. In

RA, arterial compliance can improve after 12 weeks of etanercept[104], suggesting that short-term treatment of inflammatory processes may improve vascular compliance. In animal models and humans with other disease states, PPAR-γ agonists significantly improve arterial compliance during short duration trials [105-108]. In our pioglitazone trial in RA, arterial stiffness as assessed by PWV was significantly improved in patients on the pioglitazone arm when compared to placebo arm[5]. CAVI is an index reflecting the stiffness of the artery from the heart to ankles; it increases with atherosclerosis progression[108]. An advantage of CAVI over other tests that assess vascular stiffness is that it is not significantly affected by changes in blood pressure. CAVI has been widely applied clinically to assess arterial stiffness in subjects with known CVD, as well as those at risk (hypertensive patients, diabetics, the elderly, and the obese). CAVI has also been utilized in studying normal individuals to assess their potential risks of developing CVD[109]. Changes in CAVI have been observed when measures to decrease CVD risk factors have been implemented[110-112]. Specifically for SLE, a small study recently showed that CAVI is increased in SLE patients when compared to age- and sex-matched controls[113]. Similarly, PWV assessment using a Sphygmocor device is a non-invasive, reliable measurement to assess vascular compliance and was used in our RA trial[5]. Either of these two tests will be used in this trial, depending on which machine will be me more readily available once these studies are initiated.

Pulse wave analysis (SphygmoCor): SphygmoCor is a set of non-invasive tools used to determine central blood pressures and arterial stiffness. It (1) derives the pressure wave from the ascending aorta to the carotid artery and (2) gives an accurate measurement of pressure at the heart, brain, and kidneys. However, it cannot be used on patients who may suffer from heart arrhythmias or arterial stenosis.

The SphygmoCor consists of the following:

SphygmoCor Px Pulse Wave Analysis System – a diagnostic tool to measure central blood pressure

SphygmoCor Pulse Wave Analysis System – an algorithm used to determine central aortic pressure and visualize ventricular- vascular interactions

SphygmoCor Pulse Wave Velocity System- a tool that derives a pressure pulse waveform using the pressure tonometer and an ECG signal simultaneously. Arterial tonometry uses a pressure sensor to detect the speed of a pulse wave and may indicate a problem in the arteries.

SphygmoCor Pulse Wave Monitoring System– a tool that provides an estimated pressure waveform from the ascending aorta.

PAT: The Endopat2000 device measures finger microvascular endothelial function in a fully-automated fashion before and after reactive hyperemia (RH-PAT). RH-PAT response is independently predicted by traditional and novel CV risk factors[114]. PAT test is operator-independent, with higher inter-day reproducibility than US conduit studies[115]. PAT dysfunction occurs in SLE in association with IFN signatures[116]. While pioglitazone did not modulate PAT in RA patients[5], it will be important to establish the role of this drug in microvascular function in SLE. Finger PAT signal is recorded by non-invasive, modified plethysmography-based probes, then to digital media. PAT software calculates various outcomes, the main one being the RH-PAT index=ratio of average RH-PAT PWA in a finger of the occluded arm for a 60 second period beginning 1 minute after blood pressure cuff-release, compared to average PWA during the 210 second-long baseline pre-occluded period. The ratio is normalized to the PAT index in the control arm.

To simplify, we will refer to the CAVI, PWV and EndoPAT tests as "vascular function studies".

Assessment of vascular inflammation using [18F]-2-fluoro-2-deoxy-D-glucose (FDG) imaging using positron-emission tomography (PET)/computed tomography (CT)): FDG-PET/CT enables highly precise, novel measurements of inflammatory activity including vascular, visceral, and whole body inflammation in vivo. The range of applications for FDG-PET imaging has been expanded to include the evaluation of activity of atherosclerotic plaque. Importantly, in diabetics or patients with glucose intolerance, pioglitazone attenuated left main trunk inflammation in a glucose-lowering independent manner when examined after 4 months of treatments_9_References[4]. This will be performed in a subset of 30 patients given the logistics of scheduling imaging studies at the Clinical Center.

b) Metabolic parameters and vasculogenesis profile

Insulin resistance: This is an important CV risk factor[117-119] and a significant proportion of SLE patients exhibit this abnormality[120, 121]. Homeostatic measurements like the HOMA calculation allow for an easy assessment of insulin resistance[122, 123]. In lupus-prone mice, pioglitazone induced significant changes in insulin resistance. Furthermore, in the RA trial, 3 months of pioglitazone changes led to substantial improvements in this parameter, as assessed by HOMA2 calculations[5].

piHDL and lipids: Patients with active SLE exhibit a proatherogenic lipid profile with low HDL, high triglycerides and dysfunctional HDL[124]. Pioglitazone improves lipid profiles l[125, 126] in DM, in non-diabetics and in murine SLE[86]. We will monitor lipid profile, oxidized HDL and cholesterol efflux activity of HDL from all subjects ex vivo before and after therapy. HDL oxidation and proteome profiles will be determined by MS in a subset of patients in the trial, NMR to assess lipoprotein particles will also be performed. These

studies will identify whether pioglitazone treatment improves HDL oxidation, remodels HDL proteome and renders the lipoprotein more functional.

EPC numbers and function: As SLE EPCs are decreased in number and display decreased function, and pioglitazone has been shown to have positive effects on vasculogenesis, we will quantify EPC numbers and function as previously described by our group, at various time-points[35].

8.1.2 ***Specific Aim 2: Effect of pioglitazone on SLE disease activity***

In clinical trials, the main methods to assess SLE activity are the SLEDAI-2K and the BILAG 2004. The investigators on this study have significant expertise in the use of these tools and have participated in clinical trials that used both methods to score disease activity.

a) –SLEDAI 2K: This validated scoring system is a modification of the original SLEDAI-2K system to reflect persistent, active disease in those descriptors that had previously only considered new or recurrent occurrences[5], The score can be between 0-10[5]. It is based on the presence of 24 descriptors in nine organ systems over the preceding 30 days. Descriptors are documented as present or absent. Each descriptor has a weighted score. Higher scores represent higher disease activity.(see Appendix).

b) Lack of A or B flares on BILAG 2004: The BILAG 2004 assesses SLE activity based upon the premise of "intention to treat"[129, 130]. It has been widely used and is well validated (sensitivity 87%, specificity 99%, and positive predictive value of at least 80%). Within each organ/system, a combination of answers provides an activity score from A (very active) to E (not or never active) (see Appendix 3). Recent trials have modified BILAG 2004's use to only the presence or absence of an A or B flare during the observation period as an outcome measure, thus incorporating BILAG's sensitivity to flares in individual organ systems with the SLEDAI-2K. For the proposed study, the clinical disease activity endpoints will be patients achieving SLEDAI-2K improvement + absence of a BILAG 2004 A or B flare +improvement in PGA (see Appendix 3 and 7). There are eight systems: general, mucocutaneous, neurological, musculoskeletal, cardiorespiratory, vasculitis, renal and hematological. A score is calculated for each system depending on the clinical features present and whether they are new, worse, the same or improving in the last 4 weeks compared with previously. The most severe features in each system, which are deemed to require high dose steroids (prednisolone >20 mg daily or equivalent) and/or cytotoxic agents, characterize an A score. One or more features may have to be present to score an A, depending on the system. More moderate disease

items that would be considered appropriate to treat with lower dose steroids, antimalarials or non-steroidal anti-inflammatory drugs (NSAIDs) contribute to a B score. Mild symptomatic features that require just symptomatic therapy, for example with analgesics and NSAIDs, can only contribute to a C score. If there are no current symptoms but the system has previously been involved then a D is recorded. If the system has never been involved, it is scored E

c) Health Related Quality of Life Questionnaire (HRQOL) (SF-36) and physician global assessment (PGA): This is a widely used tool to assess HRQOL with prior successful application in SLE[131, 132]. SF-36 assesses physical wellness, social and emotional well being and overall sense of mental health[131]. Completion takes 5-15 minutes (see Appendix 5).

d) Other: At each visit, C3, C4, anti-dsDNA, CRP and sedimentation rate will be quantified.

8.1.3 *Specific Aim 3: Assess the immunoregulatory role of pioglitazone in lymphoid and myeloid cell subsets in SLE*

We propose to investigate the effects of pioglitazone on intracellular signaling molecules, serum cytokines and peripheral blood gene expression as a measure of biological effects that can potentially be used as outcome measures to power for larger trials. At each visit, serum and plasma will be saved for quantification of putative biomarkers of disease activity and vascular damage in all patients. We will assess the effects of oral pioglitazone on immune cell phenotype and function. Some of the proposed studies include:

- Alteration in the "interferon signature", "T cell signature" and the "granulocyte signature" in PBMCs using RNA seq or Affymetrix (to be performed by Center for Human Immunology);
- Alteration in peripheral blood immune cell populations with special attention to CD4+, CD25+, Foxp3+ regulatory subsets and Th17 cells, monocyte subsets and a subset of aberrant neutrophils present in lupus patients (low-density granulocytes).
- Measures of serum cytokines and chemokines: this will include Th1, Th2 and Th17 markers, type I IFNs and myeloid cell-relevant cytokines such as IL-6, Il-8, MCP-1, IL-1beta.
- Levels of autoantibodies (anti-dsDNA, ENA-Abs, APL Abs) and total IgG

Some of the studies will be performed by the Center for Human Immunology (NIH) and others will be performed by Dr. Kaplan's laboratory.

9 INCLUSION AND EXCLUSION CRITERIA

9.1 Study Population and Recruitment

Patients 18 years old or older who fulfill revised SLE American College of Rheumatology (ACR) and 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria (1 and 4) will be recruited from the Outpatient Rheumatology Clinic of the Clinical Center at the NIH or the NIAMS Community Health Center, from referrals for treatment and/or second opinion, by advertising the study to rheumatologists within the larger DC area, and by direct advertising through publications of patient advocate organizations, such as the Arthritis Foundation, the Lupus Foundation, Lupus Research Institute, Alliance for Lupus Research, the American College of Rheumatology meeting, and in the news outlets. These documents will be sent to IRB for approval before being used.

Patients will be considered enrolled once they have signed consent.

9.2 Inclusion Criteria

1. For females and males 18 years old or older~~:~~ females should be on adequate contraception if they are of child-bearing potential, which should be documented by a clinician, unless patients or their spouse/partner(s) have previously undergone a sterilization procedure. Adequate contraception will be considered:
    - Intrauterine device (IUD),
    - Hormone implants,
    - Injectable contraceptives,
    - Oral contraceptives plus a barrier method (male condom, female condom or diaphragm),
    - Abstinence, or
    - A vasectomized partner.
2. Meet revised ACR criteria and 2012 SLICC criteria for SLE and have: a) a baseline SLEDAI-2K ≥ 4 and <20 or a clinical SLEDAI-2K ≥ 2 (not considering anti-dsDNA or complement levels) and b) lack of BILAG A flare at baseline (See Appendix 3).
3. Stable doses of immunosuppressants and/or antimalarials for at least 3 months, and/or corticosteroids for at least 2 weeks. The prednisone dose may be increased after screening visit as long as the total dose is less than 20 mg of prednisone or equivalent per day and the subject is on stable dose for at least 2 weeks prior to their Day 1 visit. If on statins, should have been on stable doses for at least 6 months.
4. Allowed concomitant lupus-related medications

    Antimalarials,

    Prednisone $\leq$ 20mg daily or equivalent doses of other corticosteroids;

    Immunosuppressants:

Mycophenolate mofetil, up to 3000 mg/day,

Methotrexate, up to 30 mg/week,

Azathioprine, up to 3 mg/kg/day,

Leflunomide, up to 20 mg/day,

Cyclosporine, up to 5 mg/kg/day

Tacrolimus, up to 0.1 mg/kg/day

NSAIDS and aspirin

Note: While it would be highly desirable to maintain corticosteroid dosage at constant level for trial duration, it is impractical to anticipate that all patients with active SLE can be maintained without therapy modification for an 8-month period. Because corticosteroids are acceptable agents for treatment of the vast majority of minor lupus flares, and patients with major flares (BILAG 2004 A or recent change in medications) will be excluded, this will provide standardized treatment across study population that can be easily analyzed. An increase in prednisone dose of less than or equal to 10 mg/day from their prednisone dose at study entry will be permitted during the trial for increased disease activity with a standardized taper allowable in small monthly decrements to the patient's baseline dose or 5 mg/day, whichever is less.

9.3 Exclusion Criteria

1. Pregnant or lactating women
2. Expected need for major surgery during trial.
3. Current or previous diagnosis of malignant disease, except for basal cell or squamous cell carcinoma of the skin with complete excision and clear borders or adequately treated in situ carcinoma of the cervix.
4. Acute infections identified during screening that require antibiotics. These subjects would be eligible to participate following resolution of infection before Day 1 visit within the allowed 46 days screening period. The subject will re-screen if it extends beyond the allowed 46 days screening period. See more details of information about the 46 days screening window period in section 9.4 Screening and Randomization, line 9-10.
5. Chronic infections such as hepatitis B, hepatitis C, HIV or Tuberculosis.
6. Current use of cyclophosphamide or having received cyclophosphamide within the last year.
7. Prior history of hemorrhagic cystitis or hematuria while receiving cyclophosphamide that could not be explained by other causes.
8. Current use (within 3 months) of tocilizumab, rituximab, belimumab, intravenous gammaglobulin or other biologic.
9. History of poor compliance with medical care, study visits and/or medication use.

10. Receipt of any investigational new drug or device within 30 days prior to screening or 5 half-lives of the agent (whichever is longer), or any investigational new drug with known long-term effects.
11. Pioglitazone is not recommended in patients with symptomatic heart failure. Patients with current heart failure (NYHA class II, III or IV) and/or a left ventricular ejection fraction of <45% by echocardiogram at screening will be excluded.
12. Significant impairment of major organ function (lung, heart, liver, kidney) or any condition that, in the opinion of the Investigator, would jeopardize the subject's safety following exposure to the study drug.
13. Known hypersensitivity to TZDs
14. Serum hepatic transaminase levels > 2 times upper normal limit, or clinical evidence of active liver disease at screening. The only exception is patients with confirmed non-alcoholic fatty liver disease (NAFLD) where pioglitazone has been reported to have a therapeutic role.
15. Diagnosis of DM or meeting DM criteria at screening visit, as established by new classification criteria[96]: Patients with diabetes are excluded because diabetes by itself will induce profound changes in endothelial function and we want to assess the effects of PPAR agonists in vascular risk beyond changes in insulin resistance.
16. Known latex allergy for EndoPAT test
17. Patients with severe Raynaud's phenomenon, history of finger ulcers or finger gangrene will not undergo Endopat testing.
18. Patients with severe SLE at baseline, as quantified as SLEDAI-2K >20.
19. Patients with active lupus nephritis or active CNS lupus at baseline even if SLEDAI-2K <20. Active disease will be considered as CNS or renal disease that require aggressive immunosuppression. Active CNS disease will be diagnosed based on clinical presentation and physical exam, exclusion of other conditions that could explain symptomatology and, when warranted, ancillary tests (imaging) that support the diagnosis.

Patients that are not on induction therapy for lupus nephritis and have chronic (more than 6 months), stable proteinuria <750 mg/gram in protein: creatinine ratio but otherwise considered to have no evidence of active lupus nephritis (e.g. no cellular casts and stable serum creatinine < 2 mg/dL) over the last 6 months, will be included in the study.

In selected patients with potentially confounding clinical factors, consults will be requested to help clarify the nature of any underlying renal disease that may affect inclusion.

20. Postmenopausal women who have not undergone a DEXA scan over the last year will undergo a DEXA scan at screening. Patients with a better than -2.5 will be included. Postmenopausal women who have undergone a DEXA scan during the last year and have a T score better than -2.5 will be included without repeating the DEXA scan prior to enrollment. If the T score is worse than -2.5, they will be excluded from participating unless the subject is willing to begin appropriate treatment for osteoporosis by Visit Day 1. Postmenopausal

women who have undergone a DEXA scan during the last year, have a T score worse than -2.5 and are not on bisphosphonates or other appropriate therapy will be excluded.

Study Visits and Procedures

Table 3: Study Visits and Procedures

Procedure	Screen	Baseline	Month 1	Month 3	Month 5	Month 6	Month 8	Unscheduled visit
Consent and Randomization. Eligibility Questionnaire	X							
Medical History, Physical Exam including vital signs, SLEDAI-2K	X							
Presenting Symptoms-Abbreviated Medical History		X	X	X	X	X	X	X
Physical Exam including vital signs		X	X	X	X	X	X	X
BILAG 2004, SLEDAI-2K ,PGA, SF36		X		X	X		X	
Blood Draw: insulin, glucose, lipids, EPCs, C3, C4, autoAbs, piHDL, cytokines, CRP, ESR , Aim 3 labs, Acute care panel, Lymphocyte Phenotyping TBNK, lipoprotein profile, Urinalysis (includes microscopic), p/c ratio.		X		X	X		X	X
Blood Draw: safety labs (CBC, LFTS, pregnancy tests in women of childbearing potential)		X	X	X	X	X	X	X
Screening labs and imaging. Blood draw: (pregnancy test, lipids, glucose, LFTs, CBC, Anti-ds-DNA, C3, C4, Urinalysis (includes microscopic), p/c ratio, Infectious disease	X							

testing), Echocardiogram (if it has not been obtained within the last 6 months) and DEXA scan (postmenopausal women without DEXA scan in the last year)								
Vascular function Studies		X		X	X		X	
Dispense Drug		X			X			
AE Review			X	X	X	X	X	X
Drug Accountability				X			X	
Health lifestyle Counseling (PRN)		X		X	X		X	
Urine analysis	X						X	X
PET-CT (subset of patients)		X		X				

9.4 Screening and Randomization

Subjects will review and sign informed consent document, and medical history, physical exam, disease activity (SLEDAI-2K), screening labs and echocardiogram will be performed. Echocardiogram will not be performed if patient is rescreened within 6 months and previous result is normal or if patient had normal Echocardiogram within 6 months prior to screening. Routine laboratory data obtained within 30 days of the screening visit under the natural history protocol 94-AR-0066 can be used for screening. Screening labs will include infectious disease testing for HIV, Hepatitis B, Hepatitis C and Tuberculosis if they have not been obtained within the last 6 months. As mentioned above, postmenopausal women without a DEXA scan in the last year will undergo a DEXA scan to assess eligibility. The window between screening and baseline must be less than 46 days, otherwise subjects will need to be rescreened regarding labs. Inclusion/Exclusion Criteria will be signed off based on screening labs and assessment. Patients who meet these criteria will be randomized to a sequence of active drug followed by placebo, or placebo followed by active drug in a 1:1 allocation ratio. Randomization and drug/placebo allocation will be done by the NIH clinical center research pharmacy after screening visit.

We will test Urinalysis (includes microscopic) on Screening and Month 8 visits on all patients. If patients have evidence of microscopic hematuria > 20 RBCs/uL(excluding normal menstruation cycle) it will be repeated twice at one month (+/- 7 days) intervals and if RBCs

are consistently more than 20 RBCs/uL then Urology consult will be placed. The following algorithm will be put in place, per recommendations by the Urology service at NCI:

1. Question patient about macroscopic hematuria > 20 RBCs/uL times 3 results.

    a. If YES → Urology consult → Cytology

        i. If abnormal cytology → Cystoscopy → FISH @ Mayo Labs for genetic changes for bladder cancer detection

        ii. If normal cytology → Nothing

9.5 Study Visits

9.5.1 *Baseline visit and 5-month +/- 7 days (post-washout) visit*

a. Baseline visit will include medical history. The baseline and 5-month (post-washout) visits will consist of a physical exam, and blood draw for biomarkers (Table 3). SF-36, PGA, disease activity (BILAG 2004, SLEDAI-2K) and vascular measurements will be determined and subjects will be issued a 3-month supply of blinded study drug or placebo with instructions. The first week of each treatment arm, patients will take two 15 mg capsules of pioglitazone daily (30 mg total daily dose) and, if no significant side effects develop (primarily fluid retention), will increase to three 15 mg capsules (45 mg total daily dose). Subjects will be instructed to follow the same titration for the placebo arm. Patients will be called prior to increasing the dose to 45 mg to question patients about rapid weight gain (>5 pounds in a week), significant edema or other side effects. Patients will be called 1-2 weeks after increasing dose to 45 mg daily to assess tolerability. If 45 mg dose is not well tolerated but the 30 mg dose is well tolerated, dose will be decreased back to 30 mg daily for duration of trial. PET/CT exams will be performed in 15 patients initially randomized to placebo and 15 patients initially randomized to pioglitazone and the investigators will be blinded to this. PET/CT studies will only be performed at baseline and month 3. Subjects can opt out of the PET/CT scan and still participate in the study. Patients will be asked to participate at baseline until we reach 15 patients/group. If a patient is found to have an acute infection at the baseline visit, they will be given antibiotics and their next visit will be scheduled for no more than 4 weeks later, at which time they will start the study medication. We will repeat only baseline clinical labs at this extra visit; we will not repeat PET/CT, vascular function studies or research sample collection. If a patient has an abnormal lab result at the baseline visit that requires a repeat, the patients next visit will be scheduled no more than 4 weeks later, at which time if the abnormal lab has improved or normalized they will

start the study medication. We will not repeat PET/CT, vascular function studies or research sample collection. If any subjects are enrolled in our Natural History of SLE Protocol (94-AR-0066) and have undergone a research-indicated PET/CT scan as part of that study within the 6 months prior to their baseline visit, we will use that data for their baseline values and will only perform the PET/CT scan at the month 3 visit.

9.5.2 *Visits at months 1 (+/- 7 days) and 6 (+/- 7 days)*

Visits at months 1 and 6 are safety visits after study drug initiation. Only liver function tests (LFTS), complete blood count (CBC) and pregnancy test (in women of child-bearing potential) will be obtained. Adverse events will be assessed/reviewed. Abbreviated medical history and a physical exam will be performed to assess any changes in medical conditions or medications. If feasible, blood may be obtained at a local lab and results faxed to NIH and a phone call from the research coordinator or investigators will assess adverse events.

9.5.3 *Visits at 3 (+/- 7 days) and 8 months (+/- 7 days)*

Subjects will undergo a physical exam and blood will be drawn for biomarkers and safety labs. SF-36, PGA, disease activity and vascular measurements will be obtained. Abbreviated medical history will be performed to assess any changes in medical conditions or medications. Any leftover study drug will be collected and drug accountability performed. No additional study drug will be dispensed at these visits. PET exams will be performed in 15 patients initially randomized to placebo and 15 patients initially randomized to pioglitazone and the investigators will be blinded to this. PET studies will only be performed at baseline and month 3. A urine analysis will be obtained at the end of the study to assess for hematuria.

The minimum time between baseline and visit 3-month and between visit 5 month and visit 8 month will be 75 days and the maximum 105 days. Overall, total study duration will not exceed 9 months (+/- 1 month).

9.5.4 *Unscheduled Visits*

Subjects will undergo a physical exam and abbreviated medical history will be performed to assess any changes in medical conditions or medications. Reason for the visit will be assessed related to AE, flare or study unrelated procedure/visit. Blood may be drawn for clinical and safety labs when deemed appropriate by the study investigator. We may also perform a urine test called urine analysis. Research blood may also be drawn for biomarkers as needed.

10 MONITORING SUBJECTS AND CRITERIA FOR WITHDRAWAL

10.1 Patient Withdrawal and Termination Criteria.

Subjects will be informed that they may withdraw or be excluded from the study at any time. Our intention is to maintain stable doses of immunosuppression and antimalarials for the

duration of the trial. On occasion, doses are adjusted to treat SLE activity. While not ideal, we anticipate that over the duration of 8 months, outside providers may want to adjust or add new immunosuppressive therapy. In the event of these changes, each of these instances will be reviewed by the Principal Investigator to determine continuation or withdrawal from the trial to maintain scientific integrity.

The following conditions will require the discontinuation of study agent:

1. Subjects may voluntarily withdraw at any point
2. Flares not responding to the treatment above or if, in the opinion of the responsible investigator, the subject needs immediate immunosuppressive therapy that is not allowed in the protocol
3. Any Grade 4 adverse event that is unexpected and at least possibly, probably or definitely related to study drug
4. More than 1 "no-show" for a visit
5. Becoming pregnant after baseline visit. The subject will be followed for the outcome of the pregnancy.
6. Any other reason that, in the opinion of the responsible investigator, poses unacceptable risk to the subject.
7. Nevertheless, LFTs will be monitored on all patients at each study visit and those patients with LFTs >2x upper limit at Screening visit will be excluded. If during the trial, LFTs increase to > than 3x fold the upper limit of normal (ULN), levels will be rechecked within 36 hours and if elevation >3 ULN persists and there are not alternative etiologies, therapy will be discontinued. If LFTs are elevated >2 but < 3x ULN (after confirming within 48 hours), we will investigate etiology of liver enzyme elevation and cautiously reinitiate therapy if alternative etiology is found. For patients with NAFLD that are enrolled, based on recent clinical trials that have been published (Cusi K et al.Annals of Internal Medicine 2016) assessing the role of long-term pioglitazone treatment for nonalcoholic steatohepatitis, we will include only patients that have LFTs < 3 times ULN. If there is an increase to >3 times ULN once study drug starts during monitoring (confirmed within 24 hours), drug will be stopped.

10.2 Study Completion Criteria

A subject is considered to have completed the study when he/she has completed 8 months in the trial and received both treatment arms of the study. Enrolled subjects who withdraw from the trial prior to receiving study agent or who only complete one arm will be replaced; however, patients who complete only one arm will still be included in analysis.

10.3 Results Given to Participants and Physicians

Clinically relevant results will be transmitted to patients as part of this protocol. Participants are encouraged to remain in contact with the responsible investigators regarding advances in the field, and will be invited to enroll in pertinent future studies. Participants may request research publications resulting from this protocol. Medical care after study completion will occur with

the primary treating physician and rheumatologist. Results of tests will be shared with the medical provider and patient if consider medically/clinically relevant.

10.4 Blinding, Packaging, Labeling and Storage

Study agent (15 mg pioglitazone or placebo) will be packaged in identical vials each containing 300 capsules. Both study agents will be blinded using identical-looking capsules prepared by a vetted vendor selected by the NIH Pharmaceutical Development Service (PDS). Each vial will be labeled with a treatment number specific to the subject. A 300 capsule (one vial) supply of study agent will be given to each subject at enrollment at the baseline visit and at month 5. The amount of study agent dispensed will be sufficient to last from baseline to month 3, and from month 5 to month 8. The randomization code will be provided by the Clinical Center Research Pharmacy, as mentioned above. Emergency unblinding will occur in the case of a serious adverse event (SAE) that is considered potentially secondary to the drug. Unblinding will be performed by PDS. Only the pharmacists will be unblinded throughout the trial. The subjects, the investigators, and the study site staff will remain blinded to study medication assignment during the entire study period. Compliance with study treatment and pill counts will be performed by the research nurse/study coordinator and this information will be provided to PDS as well.

11 DATA ANALYSIS

The study measures the primary and secondary outcome variables at 4 time periods in each subject. We will begin with a univariate investigation of the outcomes to check for outliers and distributional properties followed by an assessment for carryover effects. The analysis of the study will be conducted using a 2-period cross-over model by comparing matched pair differences for the subjects in the first sequence with those in the second sequence. We expect this analysis will indicate no difference between the groups. If we find no carry over effect the modeling will continue without controlling for sequence. If we do find a sequence effect we will account for it in our models. The analysis will be based on a repeated measures analysis accounting for treatment and period effects and incorporating the two baseline measures. We will use a generalized linear model and a Generalized Estimating Equations (GEE) framework with two dummy variables, one indicating treatment condition and the other indicating the baseline condition (the omitted or the reference condition is Placebo). We will use an unstructured covariance matrix and robust standard errors. The primary parameter of interest is the regression coefficient for the treatment condition (a contrast between the placebo and pioglitazone). This approach is attractive because it can handle non-normal and categorical outcomes. The analysis is robust to model mis-specification[154].

The approach used in testing the hypothesis under the three specific aims will be similar. For Specific Aim 1, we will perform a separate analysis for each outcome variable: CAVI/Sphygmocor, PAT and FDG/CT. We will also perform secondary analysis using serum and cellular CV biomarkers. Our first analysis will be a randomization based analysis with no

covariates other than treatment and period effects. There are several confounding variables, especially medications that could contribute towards the variation in the outcome. We will expand the regression models by including them as predictors. If the number of confounding variables is large, we will use investigate a propensity score matching analysis. For Specific Aim 2, we will define a dichotomous clinical endpoint measuring lupus disease activity. Patients achieving an improvement of the SLEDAI-2K by 20%, a significant improvement in PGA and the absence of a BILAG 2004 A or B flare will be considered to have improved. Others will be defined as not having improved. The analysis for this score will be similar to the one proposed for Specific Aim 1. A GEE model will be used to assess treatment differences. We will also investigate the separate effects of each of these measures. The same approach used in Aim 1 will be taken for confounding variables using regression or propensity score adjustment. We will begin the investigation of Aim 3, assessment of innate and adaptive immune abnormalities in SLE, with a graphical analysis of the variables. We use multivariate methods to determine good summary measures of the various factors and compare the two treatments using the same models as defined for Aims 1 and 2.

Our primary analysis will be performed as an intent-to-treat analysis. If the subjects providing complete data are systematically different from those who dropped out, then the estimates based only on completers will be biased. We will investigate adjusting for differences in the populations who are completers and noncompleters and will consider multiple imputation to impute the missing values. Before the database is locked and final analysis performed, violations will be assessed on a case-by-case basis by the PI to determine if the medication change was such that it would adversely affect the study results. If changes may adversely affect data interpretation, subjects will be dropped from final analysis. A post hoc analysis of disease activity, measured by SLEDAI-2K, will be adjusted for burst steroids in excess of that permitted by the protocol. Analysis of prednisone doses, daily and cumulative will also be included.

11.1 Power and Sample Size

We arrived at sample size based on previous publications for arterial stiffness and vascular dysfunction observed in SLE[92, 101-103] as well as on our recently completed RA trial. To achieve this sample size, we will be recruiting 100 patients, assuming a 20% or more dropout rate (see below). The actual analysis will be more powerful and will use data on all subjects including those who provide partial information. Based on our previous experience with the CV lupus cohort and on published literature on cross-over designs using pioglitazone to measure CV markers in other populations, we estimate our dropout rate to be between 3-20%. We have then projected a 20% drop-out rate that includes both drop-outs, as well as the few people who are deemed protocol violators because of significant changes in immunosuppressive or vascular medications. This sample will give us power to detect moderate standardized differences for the other variables measured in this study. As this is a “proof of concept” study, we estimate it is

important to detect at least moderate differences in outcome for them to be clinically meaningful.

11.2 Data Management

The primary source data for the study will be maintained by NIH Clinical Research Information System (CRIS) and the NIH Clinical Trials Database (CTDB), with subject surveys and interval questionnaires administered by the secure Clinical Trials Survey System (CTSS). Study data and outcomes will be monitored by a Data Safety Monitoring Committee (DSMC) consisting of NIH staff not affiliated with the study who will meet every 6 months and review un-blinded data. Possible adverse outcomes of treatment will be as per NIH and GCP monitoring guidelines. An IND waiver request was granted by FDA for this study.

11.3 Study Timeline

Table 4: Study Timeline

Time Period	**Year 1**		**Year 2**		**Year 3**		**Year 4**		**Year 5**	
	Q 1/2	Q 3/4	Q 1/2	Q 3/4	Q 1/2	Q 3/4	Q 1/2	Q 3/4	Q 1/2	Q 3/4
Finalize Protocol CRFs	**X**									
Submit IRB	**X**									
Finalize SOPs	**X**									
Build Database	**X**	**X**								
Pilot Test CRFs and Database	**X**	**X**								
Recruit Participants		**X**	**X**	**X**	**X**	**X**	**X**	**X**		
Conduct Study Visits				**X**	**X**	**X**	**X**	**X**		
Interim Analysis					**X**		**X**			
DSMC Reviews		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Clean Data and Lock Database									**X**	
Analysis and Publication										**X**

12 HUMAN SUBJECT PROTECTION ISSUES

12.1 Selection

12.1.1 ***Human Participants Involvement and Characteristics***

For the purposes of this study, human participants will undergo physical examination and questionnaire completion, CAVI, Endopat, FDG PET CT and DXA scan (a subset of subjects), blood draws, Echocardiogram, and exposure to study drug (pioglitazone and placebo). There will be no involvement of fetuses, neonates, children, pregnant women, prisoners, or institutionalized individuals. Women of childbearing potential will be included, but risk reduction measures (including required contraception and pregnancy testing) are in place to protect them.

12.1.2 ***Inclusion of Women, Minorities, and Children:***

12.1.2.1 Women

Women and men with SLE will be recruited into this study. Research consistently shows that women are 7-10 times more likely to develop SLE than men. For this reason, we expect approximately 80-90% of participants to be female. Based on the past record of the investigative team and the SLE natural history protocol, we believe specialized outreach for the recruitment of women will not be necessary.

12.1.2.2 Inclusion of Minorities

No ethnic groups will be excluded from this protocol. All participants must have a diagnosis of SLE. Research reviews indicate an increased risk for SLE development in African-Americans and Hispanics. Targeted enrollment rates will be based on data from the natural history protocol of SLE at NIH. Based on the past record of the investigative team, we expect that minorities will be recruited in adequate numbers. However, the PI will monitor recruitment progress on an annual basis, and will implement outreach strategies if enrollment falls short of targets. Outreach strategies include recruiting participants from physician offices in the area that service a predominance of minority patients.

12.1.2.3 Inclusion of Children

Children will be excluded from this study as pioglitazone is not approved in children and the proposed vascular tests have not been validated in children.

12.1.2.3 Inclusion of NIH employees

NIH employees are allowed to participate if they fulfill criteria. NIH employees who become subjects will be protected, as specified in SOP14F

12.2 Benefits and Risks/Discomforts

12.2.1 ***Benefits***

The knowledge to be gained may result in better, more focused treatment approaches, implemented earlier in the course of disease, to reduce cardiovascular morbidity and mortality associated with SLE and other inflammatory autoimmune diseases. This would directly benefit future patients and greatly improve our understanding of these debilitating comorbidities.

This study will provide important preliminary information about the effect of PPAR pathways in SLE patients and may contribute to a better understanding of the pathophysiology of SLE. Patients will benefit from comprehensive clinical exams and laboratory tests during the trial, as well as assessment of CV risk and thorough evaluation by experts in SLE. Otherwise, there may be no direct benefits to participants. The study agent is not expected to be associated with the most common toxicities of therapies commonly used in the treatment of SLE, such as severe immunosuppression, myelosuppression, or amenorrhea. This study involves more than minimal risk.

12.2.2 ***Potential Risks***

12.2.2.1 Blood Draws

The risks of venous puncture include the occurrence of discomfort and/or bruising at the site of puncture. Less commonly, there may be formation of a small blood clot or swelling of the surrounding area as well as bleeding from the puncture site. Syncope and local infection may occur. These are minor risks.

12.2.2.2 Physical Exam and Questionnaire-based Data Collection

Some participants may experience discomfort or frustration these procedures. However, the risks for the participants are felt to be similar to those encountered in a standard physical examination. Breach of confidentiality and the possible discomfort associated with being asked personal questions about health history and the completion of questionnaires pose minimal risks. Risks due to physical exam and questionnaire-based data collection are considered negligible.

12.2.2.3 Vascular Function Studies

No complications are expected from these procedures. Minor discomfort from the use of blood pressure cuffs may be experienced and is similar to having one's blood pressure checked.

12.2.2.4 FDG/PET/CT

The NIH Clinical Center PET department uses only commercially available FDG.

The effective radiation dose from 10 mCi FDG is approximately 0.62 rem. Thus the total radiation from one FDG scan and a transmission CT scan is 1.32 rem. In addition to any radiation concern, there could be psychological distress caused by an incidental finding of asymmetric FDG uptake that would likely necessitate additional investigation to exclude cancer or other lesions (see below).

Additional minimal risks include bleeding or bruising at the venous site of FDG administration. We have provided radiation dosimetry at the end of this protocol. This procedure will not be performed more often than twice per patient during the 8 month duration of this trial and will be performed in 30 out of the 70 patients. Overall, minimum exposure will be 0 rem/year (only

vascular function tests but no imaging) and maximum exposure will be 2.64 rem/year, which is within the annual exposure limits.

Participants may experience mild claustrophobia from the PET CT. The participant will be able to notify the technician throughout the scan if he/she needs to come out of the scanner.

12.2.2.5 Incidental Findings

Incidental findings from imaging studies of coronary disease, obstructive or non-obstructive, will be reported to the subject's referring physician. This protocol will follow the NIH Clinical Center Guidelines for Management of Small Pulmonary Nodules Detected on CT Scans. The literature has noted incidental findings on PET/CT scans to include neoplastic and non-neoplastic lesions. We have broadened our findings to include non-neoplastic and also inflammatory lesions such as lymph nodes as well as solitary pulmonary nodules. Neoplastic findings include cancer of the colon, lung and soft tissue (sarcoma); however, except for lung cancer, these other 2 cancer types have not been highly detected by PET/CT. Information regarding incidental findings is explained in the informed consent to help the subjects understand that these findings will be included on their report, sent to their physician, and may require clinical testing as a follow up to define the abnormality. Prior to sending report, the investigators will provide dedicated follow up time with subjects to inform them of findings and as needed, will provide consult with appropriate medical personnel.

12.2.2.6 Study Drug

Participants will be randomized to pioglitazone or placebo and all patients will at some point receive pioglitazone given the crossover nature of trial. Risks of pioglitazone use in this population (i.e. non-diabetic) are as follows:

- Decreases in hemoglobin and hematocrit of 2-4%. These reductions may be due to an increase in plasma volume and usually pose only minor risk. CBC will be monitored on all patients at each study visit.
- Mild to moderate edema. Pioglitazone is contraindicated in patients with NYHA Class III or IV cardiac failure. In diabetics, concern over the use of rosiglitazone and worsening CV outcomes is still a matter of debate but a black box has been included in the medication guide describing a risk for congestive heart failure exacerbation in individuals taking TZDs. For our study, all patients will undergo a baseline echocardiogram and will be excluded for an ejection fraction <45% and/or evidence of NYHA class II-IV.
- Hepatic effects. Pioglitazone can cause decreases in hemoglobin and hematocrit of 2-4%. These reductions may be due to an increase in plasma volume and usually pose only minor risk. While pioglitazone is typically not associated with hepatic toxicity or liver function test (LFT) elevations, the pioglitazone package insert states there are post marketing reports of hepatic failure and that causality cannot be excluded; Medwatch report states there is an association with hepatitis, hepatic enzyme elevations to 3 or more times the upper limit of normal. Pioglitazone is structurally related to troglitazone

(no longer marketed), which has been associated with idiosyncratic hepatotoxicity and rare cases of liver failure, liver transplants, and death. In a recent study of non-diabetics treated with pioglitazone for similar periods of time to what is proposed for our study, no changes in LFTs were observed[68]. Similarly, in our RA study, we did not detect any significant LFT abnormalities, even in those patients concomitantly taking methotrexate. Nevertheless, LFTs will be monitored on all patients at each study visit and those patients with LFTs >2x upper limit at Screening visit will be excluded. If during the trial, LFTs increase to > than 3x fold the upper limit of normal (ULN), levels will be rechecked within 36 hours and if elevation >3 ULN persists and there are not alternative etiologies, therapy will be discontinued. If LFTs are elevated >2 but ≤ 3x ULN (after confirming within 48 hours), we will investigate etiology of liver enzyme elevation and cautiously reinitiate therapy if alternative etiology is found. For patients with NAFLD that are enrolled, based on recent clinical trials that have been published (Cusi K et al.Annals of Internal Medicine 2016) assessing the role of long-term pioglitazone treatment for nonalcoholic steatohepatitis, we will include only patients that have LFTs < 3 times ULN. If there is an increase to ≥3 times ULN once study drug starts during monitoring (confirmed within 24 hours), drug will be stopped.

- Interaction with hormonal contraceptives may reduce the effectiveness of hormonal contraception. Patients will be required to also use a barrier method during the trial if they are taking oral contraceptives.
- The most common side effects reported were upper respiratory tract infection, sinusitis, pharyngitis, headache, myalgia, and tooth disorder.
- Very rarely, pioglitazone may be associated with sporadic, transient elevations in CPK levels, which have been self-resolving.
- Small weight gains (<5 kg) are associated with pioglitazone use, usually with more prolonged use than the treatment course we are proposing. Weight and edema will be monitored closely and patients will be instructed to notify investigators if weight is gained or edema or dyspnea develop.
- An increased incidence of bone fracture was noted in female patients taking TZDs. During a mean follow-up of 34.5 months, the incidence of bone fracture in females was 5.1% (44/870) for pioglitazone versus 2.5% (23/905) for placebo. This difference was noted after the first year of treatment and remained during the course of the study. No increase in fracture rates was observed in men treated with pioglitazone 1.7% (30/1735) versus placebo 2.1% (37/1728). Given the short duration of the trial, we will not assess the effect of this drug on bone metabolism. Given that the mechanism of bone fractures with TZDs is considered different than the one associated with corticosteroid use, we will not change inclusion/exclusion criteria based on medication use. In female patients, attention will be given to assessing and maintaining bone health according to current standards of care and in communication with treating rheumatologist. As mentioned in the exclusion criteria, we will exclude postmenopausal women who have a DXA scan showing a T score worse than -2.5, unless they are willing to begin a bisphosphonate by visit day 1.
- As mentioned above, there have been data suggesting an increase in bladder cancer in diabetics that have been on TZDs for > 2 years. Given the short duration of this trial, no significant concerns are present. Nevertheless, we will exclude patients who have received cyclophosphamide over the last year, whoever developed hemorrhagic cystitis,

or those with a previous history of bladder cancer, or with a history of hematuria not explained by another cause.

Overall, given the short duration of exposure to pioglitazone (3 months), we do not expect repercussions on bone health, bladder or adverse CV outcomes.

12.2.2.7 Echocardiogram:
No side effects are associated with echocardiogram.

12.2.2.8 DXA scan:
The exposure to radiation is minimal from this procedure. No side effects are otherwise expected and this will only be performed in postmenopausal subjects that have not undergone a DXA scan over the last year.

12.3 ADVERSE EVENT AND UNANTICIPATED PROBLEM REPORTING:

The Principal Investigator will be responsible for detecting, documenting, and reporting AEs and SAEs in accordance with the protocol, IRB requirements, and federal regulations.

12.3.1 Definitions:

12.3.2 Adverse Event:

An adverse event (AE) is any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome, or disease that either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. All AEs will be graded for intensity (severity) and relationship to study drug.

12.3.3 Unanticipated Problem:

The Office for Human Research Protections considers unanticipated problems to be any incident, experience, or outcome that meets all of the following criteria:

- Is unexpected in terms of nature, severity, or frequency given a) the research procedures that are described in the IRB-approved research protocol and informed consent, and b) the characteristics of the subject population being studied;
- Is related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Places subjects or others at a greater risk for physical, psychological, economic, or social harm than was previously known or recognized.

An incident, experience, or outcome that meets the 3 criteria above will generally warrant consideration of substantive changes in order to protect the safety, welfare, or rights of subjects or others. Examples of corrective actions or substantive changes that might need to be considered in response to an unanticipated problem include the following:

- Changes to the research protocol initiated by the investigator prior to obtaining IRB approval to eliminate apparent immediate hazards to subjects.
- Modification of inclusion or exclusion criteria to mitigate the newly identified risks.
- Implementation of additional procedures for monitoring subjects.
- Suspension of enrollment of new subjects.
- Suspension of research procedures in currently enrolled subjects.
- Modification of informed consent documents to include a description of newly recognized risks.
- Provision of additional information about newly recognized risks to previously enrolled subjects.

Per the definition, only a subset of AEs would be further characterized as unanticipated problems. Additionally, there are other sorts of events that, while not AEs, would also be characterized as unanticipated problems (e.g., contaminated study drug).

12.3.4 Serious Adverse Event:

A serious adverse event (SAE) is defined as any untoward medical occurrence that:

- Results in death,
- Is life-threatening (defined as a subject at immediate risk of death at the time of the event; it does not apply to an AE which hypothetically might have caused the death if it were more severe),
- Requires or prolongs hospitalization (i.e. the AE required at least a 24-hour inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion),
- Results in a congenital anomaly or birth defect (i.e., an adverse outcome in a child or fetus of a patient exposed to the trial drug prior to conception or during pregnancy),
- Causes a persistent or significant disability/incapacity (i.e. the AE resulted in a substantial disruption of the patient's ability to carry out normal life functions), or

- Is any other condition that, in the judgment of the investigator, represents a significant hazard or it does not meet any of the above serious criteria but may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

12.3.5 Medical Events Not Qualifying as Adverse Events or Serious Adverse Events:

Signs and symptoms of pre-existing medical conditions will not be recorded or reported as AEs or SAEs, unless they represent a clinically significant change from the baseline disease status documented at the Pre-screening Visit. In addition, hospitalization for elective procedures or surgeries will not be considered SAEs, nor will inpatient hospitalizations for convenience.

12.3.6 Clinical Laboratory Test Results Not Qualifying as Adverse Events or Serious Adverse Events:

A clinically significant laboratory result that is present at baseline and does not change significantly during the study will not be reported as an AE or SAE. The clinical significance of a change in a laboratory result will be determined by the investigator.

The Principal Investigator or designated AI will evaluate all clinical laboratory and imaging results for clinically significant abnormalities and document the evaluation in the medical record and case report form. A laboratory abnormality will be documented as an adverse event using the following criteria:

- The abnormality is not already encompassed by a reported adverse event (e.g., elevated AST need not be reported as an AE if Liver Failure has already been reported as an AE).
- The abnormality is considered clinically significant by the Investigator. Clinically significant lab abnormality is defined as meeting the following:
    - Necessitates study drug dosing modification (i.e., dose reduction, interruption or discontinuation); and/or
    - Requires a therapeutic intervention (e.g., concomitant medication, blood transfusion or dialysis); and
    - Is unexplainable by the patient's current and past medical conditions

The Principal Investigator will follow significant abnormalities until they return to baseline or stabilize.

12.3.7 Lupus Flare:

A lupus flare is any significant worsening of the signs, symptoms and laboratory test abnormalities associated with lupus. Any increase in the SLEDAI 2K index of 3 or more will be considered as a SLE flare.

12.3.8 Reporting of Adverse Events, Unanticipated Problems and Protocol Deviations:

12.3.8.1 Intensity of Adverse Event:

The intensity (severity) of AEs and SAEs will be graded according to a descriptive scale based on the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. If AEs and SAEs is not available at CTCAE, it will be graded according to below chart.

Severity Definitions

Grade	Definition
1. Mild:	Causing no limitation of usual activity
2. Moderate:	Causing some limitations of usual activities
3. Severe:	Causing inability to carry out usual activities
4. Life Threatening:	Patient was at immediate risk of death from the event

For lupus flares, we will use the following criteria based on validated activity scores: (changes are points increased compared to baseline visit)

Increase in SLEDAI 2K: 2-4: Mild
Increase in SLEDAI2K: 5-8: Moderate
Increase in SLEDAI 2K: > 9: Severe

12.3.8.2 Relationship to Study Drug and Procedures:

For all AEs and SAEs, the investigator will provide his/her best estimate of the causal relationship between the event and study drug, and the causal relationship between the event and study procedures. The degree of certainty about causality will be graded according to the criteria in Table. 5.

Table 5. Relatedness of Adverse Event to Intervention

Causality	Description
Not Related Category	
Unknown	Not enough information exists for the assessment of causality at the time of occurrence.
Unrelated	Adverse event is clearly due to extraneous causes (e.g., underlying disease, environment)
Related Category	
Unlikely (must have at least 2)	1) does not have temporal relationship to intervention 2) could readily have been produced by the subject's clinical state 3) could have been due to environmental or other interventions 4) does not follow a known pattern of response to intervention 5) does not reappear or worsen with reintroduction of intervention
Possible (must have at least 2)	1) has a reasonable temporal relationship to intervention 2) could not readily have been produced by the subject's clinical state 3) could not readily have been due to environmental or other interventions 4) follows a known pattern of response to intervention
Probable (must have at least 3)	1) has a reasonable temporal relationship to intervention 2) could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions 3) follows a known pattern of response to intervention 4) disappears or decreases with reduction in dose or cessation of intervention
Definite (must have all 4)	1) has a reasonable temporal relationship to intervention 2) could not readily have been produced by the subject's clinical state or have been due to environmental or other interventions 3) follows a known pattern of response to intervention 4) disappears or decreases with cessation of intervention and recurs with re-exposure

12.3.8.3 Expectedness of Adverse Events:

For purposes of regulatory reporting, the medically responsible investigator will determine whether an AE or SAE is expected or unexpected. Expected adverse events are those adverse events that are listed or characterized in the Package Insert or in the Physicians' Desk Reference.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or Physicians' Desk Reference (PDR), published medical literature, protocol, and informed consent document or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I., PDR, published medical literature, protocol and informed consent. For example, under this definition, hepatic necrosis would be unexpected if the P.I. only referred to elevated hepatic enzymes or hepatitis.

For consistency of labeling and categorizing adverse events, the NCI CTCAE, Version 4.0 will be used in this study.

12.3.9 Procedures for Reporting

Serious adverse events, unexpected AEs, and unanticipated problems will be reported to the IRB, NIAMS Clinical Director, and DSMC according to the NIH-OHSRP SOP 16, "Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations." All patients who receive at least one dose or part of a dose of the trial medication (pioglitazone), whether withdrawn prematurely or not, will be included in the safety analyses. All data relating to safety will be listed and summarized separately for the treatment period and for the entire study. All safety reports will be reviewed by the Principal Investigator.

12.3.10 Reporting Timeline:

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations, will be reported to the IRB, DSMC and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB, DSMC and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol

deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director, DSMC and IRB within 7 days after the PI first learns of the event.

12.3.11 Reporting of Non-Serious Protocol Deviations:

Non-serious protocol deviations will only be reported to the IRB (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

12.3.12 Reporting of Adverse Events:

The PI is responsible for summarizing all serious adverse events and adverse events at least possibly related to the research procedure and interventions at the time of Continuing Review.

12.3.13 Reporting of Deaths:

All deaths that have occurred among study participants since the previous review will be summarized at the time of continuing review.

12.3.14 Reporting Waivers:

Waiver of Reporting to the IRB of anticipated minor protocol deviations, adverse events and deaths due to underlying disease or population under study unless determined to be an Unanticipated Problem.

- Non-unanticipated problems (UP)/ adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in patients with SLE. If events are occurring substantially more frequent that would be anticipated in typically treated patients with SLE, they will also be reported to IRB. The following anticipated adverse events will not be reported to the IRB unless they occur at a severity greater than that known to occur in patients taking pioglitazone: lower extremity edema, decrease in Hb which is less than 1.5 g/dl, weight gain <5 pounds/week. Examples of expected adverse events include those events detailed in the Investigator's protocol for pioglitazone and

in the pioglitazone drug insert. No more than a total gain of 15% weight gain if associated with signs and symptoms of volume overload such as shortness of breath on exertion, paroxysmal nocturnal dyspnea or elevated jugular venous pressure will be allowed for the total duration of the study. If patient gained weight during study due to other reasons such as lifestyle changes or increase in Prednisone dose or other specific reasons and the patient is not at risk of health compromising condition, these reasons will be documented in CRIS and will not be reported as Unanticipated Problems. Patient will be advised to lose weight through NIH nutrition consults or exercising. If the rate of these events exceeds the rate specified in the protocol or investigator's brochure the events will be classified and reported as though they are Unanticipated Problems.

12.3.15 Adverse Event, Protocol deviation and Unanticipated Problem Assessment and Follow-up:

In the event of an adverse event, protocol deviation and unanticipated problem the first concern will be for the safety of the patients. Investigators are required to collect and document all adverse events (AEs), protocol deviations and unanticipated problems. At each study visit, the Principal Investigator will inquire about the occurrence of AE/SAEs since the last visit, and review any protocol deviations and unanticipated problems. Adverse events (including SAEs), protocol deviations, and unanticipated problems may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject in an objective manner.
- Receiving an unsolicited complaint from the subject.
- Review of all source documentation related to study procedures; abnormal values or results from clinical or laboratory evaluations (including, but not limited to, radiographs, ultrasounds, or electrocardiograms) can also indicate adverse events.

Events will be followed for outcome information until they return to baseline or stabilize. Study-related AEs will be followed and/or treated at the NIH until resolution or stabilization of the AE, after which the subject will be referred to a physician(s) outside of the NIH for care and follow-up.

12.3.16 Adverse Event Recording:

Adverse events will be monitored throughout this study, and these events will be recorded on the appropriate AE eCRF at each visit after starting study medication. The record for each event will include the following information:

- Description of the event.
- Onset and stop dates of the event.
- Seriousness of event.
- Intensity (or severity) of the event.
- Action taken because of the event.
- Relationship of the event to study drug and/or study procedure.
- Outcome of the event.
- Expectedness of the event.

12.3.17 Pregnancy Reporting and Follow-up:

This study includes pregnancy information as safety data and pregnancies will be recorded if they begin any time after enrollment. Information about any pregnancy should be reported promptly to the NIH NIAMS/NIDDK IRB, NIAMS Clinical Director, and DSMC on the same timeline as a SAE. All pregnancies identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy in a study subject or a partner of a study subject. A pregnant subject should be instructed to stop taking study medication. The investigator will refer patient to High-risk Obstetrics for counseling and follow-up. Pioglitazone has a pregnancy risk factor Category C; there are no adequate and well controlled studies in pregnant women. Animal studies show increased rates of post-implantation loss, delayed development, reduced fetal weights, and delayed parturition at doses 10 to 40 times the maximum recommended human dose (Label, 2013). Monitoring of the pregnant subject should continue until the conclusion of the pregnancy, and a follow-up Pregnancy Monitoring form detailing the outcome of the pregnancy should be submitted to the IRB. When possible, similar information should be obtained for a pregnancy occurring in a partner of a study subject. Information requested about the delivery will include:

- Subject's enrollment ID
- Gestational age at delivery

- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

  Should the pregnancy result in a congenital abnormality or birth defect, an SAE also must be submitted to the NIH NIAMS/NIDDK IRB using the SAE reporting procedures described above.

12.3.18 Lost to follow up patient reporting:

After three attempts to contact the patient via phone, a certified letter will be sent to notify him or her that they have been withdrawn from the study.

12.3.19 Stopping Rules:

If any of the involved entities believes there is any evidence of a pattern of unanticipated AEs (regardless of causality) or SAEs an unscheduled independent third party review by the DSMC will be requested. Based on the conclusions of the review, the PI will either terminate the study or modify the protocol. Additional stopping rules may be set at any time by the IRB or DSMC. Due to the interventional nature of the protocol, we expect to work closely with an assigned DSMC.

12.3 Compliance with Good Clinical Practices

This trial will be conducted in compliance with the protocol, current GCPs recommended by the International Conference on Harmonization (ICH) and the applicable regulatory requirements for participating institutions. These include the tenets of the Declaration of Helsinki and review and approval by the appropriate ethics review committee or IRBs of participating organizations.

12.4 Data Safety and Monitoring

The study will be conducted according to Good Clinical Practice (GCP) guidelines, the Manual of Procedures (MOP), U.S. 21 CFR Part 50 – Protection of Human Subjects, 21CFR312 subpart D and Part 56 – Institutional Review Boards.

12.5 Data Safety Monitoring Committee

The NIAMS DSMC will have safety oversight responsibilities for the study. DSMC will be comprised of 3 members after discussion with NIAMS Clinical Director. The DSMC Chair will not be affiliated to NIAMS.

Approximately twice a year (as mentioned in section 11.2), the DSMC will review data related to enrollment progress, study implementation, subject safety, and protocol violations. The CTDB will generate reports that compile all newly submitted and accumulated AEs, SAEs, toxicities, pregnancies, and concomitant medications. Subsequent review of periodic reports will be performed by the Principal Investigator.

The DSMC will also consider current information from other sources on the biology of the disease and the subject population under study. Based on these reviews, the DSMC will make recommendations to the Principal Investigator and the NIAMS Clinical Director concerning the continuation, modification, or termination of the study. The DSMC will also meet ad hoc if relevant issues arise that require committee review.

The Principal Investigator will be responsible for reporting incidents of non-compliance to the NIH IRB (in compliance with regulations on the protection of human subjects and institutional policy and procedures) and responsible for securing compliance.

Clinical monitoring for this study will be based on a clinical monitoring plan developed by Leidos Biomedical Research, Inc., Clinical Monitoring Research Program, Clinical Trials Management team in collaboration with the principal investigator. The purposes of the clinical monitoring activities are:

1) To verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject;

2) To verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;

3) To compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and

4) To help ensure investigators are in compliance with the protocol.

The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP) and applicable guidelines (ICH-GCP) are being followed.

During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The Clinical Monitoring Plan will specify the frequency, procedures, and levels of monitoring activities. Some monitoring activities will be performed remotely (e.g., review of regulatory documents), while others will take place on site (e.g., verification of study databases against source documentation).

The staff from Leidos Biomedical Research Inc. CMRP/CTM will conduct the monitoring activities and provide the follow-up letters describing the findings. The frequency of reporting for monitoring activities will be specified in the monitoring plan. The Principal Investigator will receive copies of the final follow-up letters.

12.6 Research Use, Storage, and Disposition of Human Samples If, Specimens, or Data

12.6.1 *Data Collection*

Study staff will complete electronic case report forms (eCRFs) that will be compiled and stored in a computerized central database CTDB. Security of the database system is maintained through an application firewall, military grade encryption and SSL certificates, removal of personal identifiers consistent with HIPAA requirements (45 C.F.R. 164.514(a),(b)&(c)).

Research samples collected from subjects consenting to this protocol will be stored in locked secure freezers belonging to NIAMS. The freezers are located in Building 10 at the NIH. Samples will be kept indefinitely unless there is a significant justification for destroying them. The Principal Investigator will report to the Institutional Review Board (IRB) the loss or destruction of samples collected under this protocol.

All samples will be coded and will not have personal identifiers. The codes for identifiers will be contained in a secure electronic database (CTDB) and a subject code log that is maintained in secure research files. An electronic record log with identifiers of all collected research specimens will be kept. These will be stored in either research charts or on secure NIH computers.

If coded samples are shared with collaborators within or outside the NIH in the future, tech transfer agreements, MTAS or CRADAS will be obtained and an amendment will be submitted to IRB to describe the nature of this collaboration. Currently, we do not have any planned collaborations. Samples will be stored in the NIAMS and may be used by the investigators in this proposal for studies in the pathogenesis of SLE. Approval from the IRB will be obtained prior to any research use of stored samples beyond the scope of this study.

All patient samples will be coded and used for research purposes without sharing identifying information and all collaborators will follow federal rules for clinical research.

The investigators will retain all study-related records for at least 3 years after discontinuation of the study. Some of the research data might be maintained indefinitely for research purposes. Research records and all source documents will be kept in locked cabinets or rooms, and computer research databases will be stored in a secure, password-protected environment, per standard NIH policies. Only study investigators and participating research personnel will have access to the data.

The site investigators are required to keep accurate records to ensure the conduct of the study is fully documented. The period of record retention should be consistent with the record retention

policies of the sponsoring agency or applicable regulatory agencies. Medical and research records will be maintained in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, Principal investigator must permit authorized representatives of the IND sponsor (if applicable), Leidos, CTDB at the NIH and health authorities to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other subject data may be copied (obscuring any personally identifying information). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. Participating sites will normally be notified in advance of auditing visits.

Study staff will complete electronic case report forms (eCRFs) via a web-based electronic data capture (EDC) system (Clinical Trials Database, CTDB) that is compliant with Part 11 Title 21 of the Code of Federal Regulations. Subject's electronic medical records in the Clinical Research Information System (CRIS) will be used as source documents for these eCRFs.

CRFs and subject questionnaire data will be kept in the CTDB database.

The data will be further validated via a series of manual edit checks, and all relevant data queries will be raised and resolved on an ongoing basis. Complete, clean data will be frozen to prevent further inadvertent modifications. All discrepancies will be reviewed and any resulting queries will be resolved with the investigators and amended in the database. All elements of data entry (i.e., time, date, verbatim text, and the person performing the data entry) will be recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

12.7 Remuneration

Patients will be offered remuneration as follows:
$60/visit for screening, baseline, month 3, month 5 and month 8 visits.
$30/visit for safety visits at months 1 and 6.
$150/ FDG/PET CT scan.

Travel costs may be covered as per the current NIH/NIAMS policy.

12.8 Protocol Consent Processes and Documents

The principles of informed consent in the current edition of the Declaration of Helsinki, as well as compliance with all IRB requirements, will be implemented in the study, before any protocol-specified procedures are carried out. A standard consent form for subject participation will be provided with the protocol to the IRB and Office of Protocol Services (OPS) of the NIH. Any modifications to the standard information in the template will require review and approval by the IRB. All subjects will receive a consent form that will include the purposes, procedures, benefits, and potential hazards of the study. This information will be reviewed with the subject

by either the principal or a qualified associate investigator. All prospective subjects will be given ample time to read the consent form, and ask questions, before signing.
The consent documents will be translated into Spanish. For Spanish speaking subjects the consent will be explained by the PI/AI through an interpreter. Subjects who are unable to read and/or understand English or Spanish will be given a short consent form in their native language, if available. The short consent form will be explained to the subject through an interpreter. The NIH IRBs will review and approve the short consent form process for non-English-speaking subjects. If these approaches are not satisfactory, the subject will be excluded from the study because of an inability to understand the consent. All subjects will be informed of their right to withdraw from the study. Translated documents must be certified to contain the complete descriptions provided in the English version of the document.

Short Form Consent Process:

We anticipate the enrollment of Spanish speaking subjects into this study for which English consents have been fully translated. If there is unexpected enrollment of a research participant for which there is no translated extant IRB-approved consent document, the Principal Investigator and/or those authorized to obtain informed consent will use the short form consent process as described in MAS Policy M77-2, NIH SOP 12, and 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB-approved consent document. We request prospective IRB approval of the use of the short form consent process for up to a maximum of 5 requests (either for individual participants or families of participants) in a given language, and will notify the IRB at the time of continuing review of the frequency of the use of the short form. Should we reach the threshold of 5 subjects and/or families speaking a single language, we will request an additional use of the short form from the IRB and will notify the Board that we plan to have any consent documents frequently used with that population translated into the language(s) they speak.

For inclusion in the study, each subject will be required to sign the consent form. The original forms will become part of the permanent medical record and kept on file in the subject's study chart, available for inspection by regulatory authorities, both federal and institutional, as well as the monitoring entity, Leidos Biomedical Research, Inc. Copies will be provided to the subjects. The fact that informed consent was obtained prior to the initiation of study procedures will be documented in the subject's medical or research record.

13. Participation of NIH employees:

We anticipate eligible NIH employees may participate in this study. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH information sheet on Staff Research Participation" as per NIH SOP 14F, "Research Involving NIH Staff as Subjects." If the employee is within the same branch, section, or unit such that the individual obtaining consent is a supervisor then independent monitoring of the consent process through Clinical Center Department of Bioethics Consultation Service will be requested. Study staff will be trained that communication of any personal or medical information about an NIH employee), including the fact that they are participating in this study, should be restricted to those investigators who need to know this information, and such information will not be discussed with anyone outside of the study without permission from the subject.

We will discuss the following applicable safeguards to the eligible NIH employee :

- Unbiased participation for protocol integrity and participant risk assessment.
- Ensure there is no perceived workplace pressure or expectation on either participation or deciding not to participate on the protocol in regards to a benefit or adverse effect on their NIH employment or staff position.
- Protection of privacy and confidentiality will be maintained, but also with acknowledgement of the limits due to sensitive information that may be in their NIH file.
- Discussion of time commitments of the study and compensation in accordance with NIH policy 2300-630-3, *Leave Policy for NIH Employees Participating in NIH Medical Research Studies*.

14. ASSOCIATE INVESTIGATORS

1. Dr. Thomas: Associate Investigator.

Role: Dr. Thomas will be referring subjects to the NIH clinical center for possible participation in the protocol. He will not be involved in consenting subjects or any other research procedures or analyses. He will not be receiving any identifiable research samples from the study on the subjects he refers to the NIH. However, as he is the primary physician listed in CRIS he would get progress notes and clinical labs from subject's visit to the NIH clinical center.

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16. APPENDICES

1. **American College of Rheumatology Revised Classification Criteria for Systemic Lupus Erythematosus (1A) and SLE-disease activity index.**

1A. ACR Revised Classification Criteria for SLE

Criteria	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
: pDiscoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring occurs in older lesions
Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
Serositis	**a.** Pleuritis—convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion *or* **b.** Pericarditis—documented by ECG or rub or evidence of pericardial effusion
Renal disorder	**a.** Persistent proteinuria >0.5 g/day >3+ if quantitation is not performed *or* **b.** Cellular casts—may be red blood cell, hemoglobin, granular tubular, or mixed
Neurologic disorder	**a.** Seizures—in the absence of offending drugs or known metabolic derangements (e.g., uremia, acidosis, or electrolyte imbalance) *or*

Criteria	Definition
	b. Psychosis—in the absence of offending drugs or known metabolic derangements (e.g., uremia, acidosis, or electrolyte imbalance)
Hematologic disorder	**a.** Hemolytic anemia with reticulocytosis, *or* **b.** Leukopenia—<4000/mm^3, *or* **c.** Lymphopenia—<1500/mm^3, *or* **d.** Thrombocytopenia—<100,000/mm^3 in the absence of offending drugs
Immunologic disorder	**a.** Anti-DNA—antibody to native DNA in abnormal titer, *or* **b.** Anti-Sm—presence of antibody to Sm nuclear antigen, *or* **c.** Positive finding of antiphospholipid antibodies based on (1) abnormal serum concentration of IgG or IgM anticardiolipin antibodies, (2) positive test result for lupus anticoagulant using a standard method, or (3) false-positive serologic test for syphilis known to be positive for at least 6 mo and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
ANA	Abnormal titer of ANA by immunofluorescence or equivalent assay at any point in time and in the absence of drugs known to be associated with drug-induced lupus syndrome

2A. **SLEDAI-2K disease activity questionnaire:**

For an item to be scored the indicated weight, the manifestation must have been present in the past 10 days.

SLEDAI 2K		Descriptor	Definition
Weight	SCORE		
8	_____	Seizure	Recent onset, exclude metabolic, infectious or drug causes.
8	_____	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes
8	_____	Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features, inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes.
8	_____	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	_____	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	_____	Lupus headache	Severe, persistent headache; may be migrainous, but must be nonresponsive to narcotic analgesia.
8	_____	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	_____	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4	_____	Arthritis	$\geq$ 2 joints with pain and signs of inflammation (i.e., tenderness, swelling or effusion).
4	_____	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4	_____	Urinary casts	Heme-granular or red blood cell casts.
4	_____	Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	_____	Proteinuria	>0.5 gram/24 hours

4	_____	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	_____	Rash	Inflammatory type rash.
2	_____	Alopecia	Abnormal, patchy or diffuse loss of hair.
2	_____	Mucosal ulcers	Oral or nasal ulcerations.
2	_____	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	_____ P	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2	_____	Low complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory
2	_____	Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1	_____	Fever	>38◦ C. Exclude infectious cause.
1	_____	Thrombocytopenia	<100,000 platelets / $x10^9$/L, exclude drug causes.
1	_____	Leukopenia	< 3,000 white blood cells / $x10^9$/L, exclude drug causes.

TOTAL SCORE: _____

3. BILAG SCORE.

BILAG 2004: The scoring system for the BILAG index of disease activity is based upon the principle of the physician's intention to treat. There are eight systems: general, mucocutaneous, neurological, musculoskeletal, cardiorespiratory, vasculitis, renal and haematological. A score is calculated for each system depending on the clinical features present and whether they are new, worse, the same or improving in the last 4 weeks compared with previously. The most severe features in each system, which are deemed to require high dose steroids (prednisolone >20 mg daily or equivalent) and/or cytotoxic agents, characterize an A score. One or more features may have to be present to score an A, depending on the system More moderate disease items that would be considered appropriate to treat with lower dose steroids, antimalarials or non-steroidal anti-inflammatory drugs (NSAIDs) contribute to a B score. Mild symptomatic features that require just symptomatic therapy, for example with analgesics and NSAIDs, can only contribute to a C score. If there are no current symptoms but the system has previously been involved then a D is recorded. If the system has never been involved, it is scored E

BILAG-2004 INDEX Centre: Date: Initials/Hosp No:

- **Only record manifestations/items due to SLE Disease Activity**
- **Assessment refers to manifestations occurring in the last 4 weeks (compared with the previous 4 weeks)**
- **TO BE USED WITH THE GLOSSARY**

Record: **ND** Not Done
0 Not present
1 Improving
2 Same
3 Worse
4 New
Yes/No OR Value (where indicated)
***Y/N** Confirm this is due to SLE activity (Yes/No)

CONSTITUTIONAL
1. Pyrexia - documented > 37.5ºC ( )
2. Weight loss - unintentional > 5% ( )
3. Lymphadenopathy/splenomegaly ( )
4. Anorexia ( )

MUCOCUTANEOUS
5. Skin eruption - severe ( )
6. Skin eruption - mild ( )
7. Angio-oedema - severe ( )
8. Angio-oedema - mild ( )
9. Mucosal ulceration - severe ( )
10. Mucosal ulceration - mild ( )
11. Panniculitis/Bullous lupus - severe ( )
12. Panniculitis/Bullous lupus - mild ( )
13. Major cutaneous vasculitis/thrombosis ( )
14. Digital infarcts or nodular vasculitis ( )
15. Alopecia - severe ( )
16. Alopecia - mild ( )
17. Peri-ungual erythema/chilblains ( )
18. Splinter haemorrhages ( )

NEUROPSYCHIATRIC
19. Aseptic meningitis ( )
20. Cerebral vasculitis ( )
21. Demyelinating syndrome ( )
22. Myelopathy ( )
23. Acute confusional state ( )
24. Psychosis ( )
25. Acute inflammatory demyelinating polyradiculoneuropathy ( )
26. Mononeuropathy (single/multiplex) ( )
27. Cranial neuropathy ( )
28. Plexopathy ( )
29. Polyneuropathy ( )
30. Seizure disorder ( )
31. Status epilepticus ( )
32. Cerebrovascular disease (not due to vasculitis) ( )
33. Cognitive dysfunction ( )
34. Movement disorder ( )
35. Autonomic disorder ( )
36. Cerebellar ataxia (isolated) ( )
37. Lupus headache - severe unremitting ( )
38. Headache from IC hypertension ( )

MUSCULOSKELETAL
39. Myositis - severe ( )
40. Myositis - mild ( )
41. Arthritis (severe) ( )
42. Arthritis (moderate)/Tendonitis/Tenosynovitis ( )
43. Arthritis (mild)/Arthralgia/Myalgia ( )

Weight (kg): **Serum urea (mmol/l):**
African ancestry: Yes/No **Serum albumin (g/l):**

CARDIORESPIRATORY
44. Myocarditis - mild ( )
45. Myocarditis/Endocarditis + Cardiac failure ( )
46. Arrhythmia ( )
47. New valvular dysfunction ( )
48. Pleurisy/Pericarditis ( )
49. Cardiac tamponade ( )
50. Pleural effusion with dyspnoea ( )
51. Pulmonary haemorrhage/vasculitis ( )
52. Interstitial alveolitis/pneumonitis ( )
53. Shrinking lung syndrome ( )
54. Aortitis ( )
55. Coronary vasculitis ( )

GASTROINTESTINAL
56. Lupus peritonitis ( )
57. Abdominal serositis or ascites ( )
58. Lupus enteritis/colitis ( )
59. Malabsorption ( )
60. Protein losing enteropathy ( )
61. Intestinal pseudo-obstruction ( )
62. Lupus hepatitis ( )
63. Acute lupus cholecystitis ( )
64. Acute lupus pancreatitis ( )

OPHTHALMIC
65. Orbital inflammation/myositis/proptosis ( )
66. Keratitis - severe ( )
67. Keratitis - mild ( )
68. Anterior uveitis ( )
69. Posterior uveitis/retinal vasculitis - severe ( )
70. Posterior uveitis/retinal vasculitis - mild ( )
71. Episcleritis ( )
72. Scleritis - severe ( )
73. Scleritis - mild ( )
74. Retinal/choroidal vaso-occlusive disease ( )
75. Isolated cotton-wool spots (cytoid bodies) ( )
76. Optic neuritis ( )
77. Anterior ischaemic optic neuropathy ( )

RENAL
78. Systolic blood pressure (mm Hg) value ( ) Y/N*
79. Diastolic blood pressure (mm Hg) value ( ) Y/N*
80. Accelerated hypertension Yes/No ( )
81. Urine dipstick protein (+=1, ++=2, +++=3) ( ) Y/N*
82. Urine albumin-creatinine ratio mg/mmol ( ) Y/N*
83. Urine protein-creatinine ratio mg/mmol ( ) Y/N*
84. 24 hour urine protein (g) value ( ) Y/N*
85. Nephrotic syndrome Yes/No ( )
86. Creatinine (plasma/serum) µmol/l ( ) Y/N*
87. GFR (calculated) ml/min/1.73 m^2 ( ) Y/N*
88. Active urinary sediment Yes/No ( )
89. Active nephritis Yes/No ( )

HAEMATOLOGICAL
90. Haemoglobin (g/dl) value ( ) Y/N*
91. Total white cell count (x 10^9/l) value ( ) Y/N*
92. Neutrophils (x 10^9/l) value ( ) Y/N*
93. Lymphocytes (x 10^9/l) value ( ) Y/N*
94. Platelets (x 10^9/l) value ( ) Y/N*
95. TTP ( )
96. Evidence of active haemolysis Yes/No ( )
97. Coombs' test positive (isolated) Yes/No ( )

Revision: 1/Sep/2009

5. SLICC Classification Criteria for SLE

Requirements: 4 or more criteria (at least 1 clinical and 1 laboratory criteria)

OR biopsy-proven lupus nephritis with positive ANA or anti-DNA.

Clinical Criteria	Immunologic Criteria
1. Acute Cutaneous Lupus	1. ANA
2. Chronic Cutaneous Lupus	2. Anti-DNA
3. Oral or nasal ulcers	3. Anti-Sm
4. Non-scarring alopecia	4. Antiphospholipid Ab
5. Arthritis	5. Low complement (C3, C4, CH50)
6. Serositis	6. Direct Coombs
7. Renal	
8. Neurologic	
9. Hemolytic Anemia	
10. Leukopenia	
11. Thrombocytopenia (<100,000)	

6. 36-item Short Form Survey

Medical Outcomes Study: 36-Item Short Form Survey Instrument

1. In general, would you say your health is:	
Excellent	1
Very good	2
Good	3
Fair	4
Poor	5

2. **Compared to one year ago**, how would your rate your health in general **now**?	
Much better now than one year ago	1
Somewhat better now than one year ago	2
About the same	3
Somewhat worse now than one year ago	4
Much worse now than one year ago	5

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

(Circle One Number on Each Line)

	Yes, Limited a Lot	Yes, Limited a Little	No, Not limited at All

3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	[1]	[2]	[3]
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	[1]	[2]	[3]
5. Lifting or carrying groceries	[1]	[2]	[3]
6. Climbing **several** flights of stairs	[1]	[2]	[3]
7. Climbing **one** flight of stairs	[1]	[2]	[3]
8. Bending, kneeling, or stooping	[1]	[2]	[3]
9. Walking **more than a mile**	[1]	[2]	[3]
10. Walking **several blocks**	[1]	[2]	[3]
11. Walking **one block**	[1]	[2]	[3]
12. Bathing or dressing yourself	[1]	[2]	[3]

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

(Circle One Number on Each Line)

	Yes	No
13. Cut down the amount of time you spent on work or other activities	1	2
14. **Accomplished less** than you would like	1	2
15. Were limited in the **kind** of work or other activities	1	2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

During the **past 4 weeks,** have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

(Circle One Number on Each Line)

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	1	2
18. **Accomplished less** than you would like	1	2
19. Didn't do work or other activities as **carefully** as usual	1	2

20. During the **past 4 weeks,** to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

(Circle One Number)

Not at all 1

Slightly 2

Moderately 3

Quite a bit 4

Extremely 5

21. How much **bodily** pain have you had during the **past 4 weeks**?

(Circle One Number)

None 1

Very mild 2

Mild 3

Moderate 4

Severe 5

Very severe 6

22. During the **past 4 weeks,** how much did **pain** interfere with your normal work (including both work outside the home and housework)?

(Circle One Number)

Not at all 1

A little bit 2

Moderately 3

Quite a bit 4

Extremely 5

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks** . . .

(Circle One Number on Each Line)

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	None of the Time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6

28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

32. During the **past 4 weeks,** how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

(Circle One Number)

All of the time 1

Most of the time 2

Some of the time 3

A little of the time 4

None of the time 5

How TRUE or FALSE is <u>each</u> of the following statements for you.

(Circle One Number on Each Line)

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5

7. NIH Clinical Center guidelines for the management of allergic reactions

ANAPHYLAXIS TREATMENT MEDICATION DOSE GUIDELINES – PRIMARY THERAPY			
DRUG	**CONCENTRATION**	**ADULT DOSE** [1]	**PEDIATRIC DOSE** [1,2,3]
First-line Treatment			
EPINEPHRINE AUTO-INJECTOR (EpiPen)	1:1,000 (0.3 MG Fixed Dose Inj)	0.3 mg > 25 Kg [3] **IM** [4] MAY REPEAT q 5 to 15 mins	0.3 mg > 25 Kg [3] **IM** [4] MAY REPEAT q 5 to 15 mins
EPINEPHRINE AUTO-INJECTOR Jr (EpiPen Jr.)	1:2,000 (0.15 MG Fixed Dose Inj)	N/A	0.15 mg 10 to 25 Kg[3] **IM** [3] or SQ MAY REPEAT q 5 to 15 mins
EPINEPHRINE AMPULE	1:1,000 (1 mg/mL)	0.2 to 0.5 mg per dose **IM** [4] or Subcutaneous MAY REPEAT q 5 to 15 mins	0.01 mg/Kg per dose **IM** [4] or Subcutaneous MAY REPEAT q 5 to 15 mins MAX SINGLE DOSE 0.5 mg (0.5 mL)

1. The diagnosis and management of anaphylaxis practice parameter: 2010 Update. J Allergy Clin Immunol 2010;126: 477-80.
2. The Harriet Lane Handbook, 18th Edition
3. This differs from the package insert recommendation as per Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel. J Allergy Clin Immunol 2010;126: S1 – S58.
4. The intramuscular (IM) route is preferred. Epinephrine absorption in adults: Intramuscular versus subcutaneous injection. J Allergy Clin Immunol 2001;108:871-3.

SEE REVERSE SIDE FOR ADJUNCTIVE THERAPY➔

Approved by P&T Committee on February 24, 2011. Revised on XX/XX/2011

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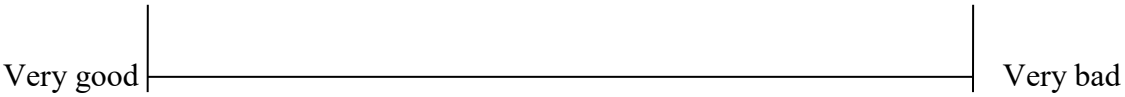
7. Physician Global Assessment (PGA)

Mark an X on the line below to indicate disease activity (independent of patient's self assessment):



8. Systemic Lupus International Collaborating Clincs/American College of Rheumatology (SLICC/ACR) Damage Index

Score Item

Ocular (either eye, by clinical assessment)
0,1 Any cataract ever
0,1 Retinal change or optic atrophy

Neuropsychiatric
0,1 Cognitive impairment (e.g. memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) OR major psychosis
0,1 Seizures requiring therapy for 6 months
0,1,2 Cerebrovascular accident ever (score 2 if >1)
0,1 Cranial or peripheral neuropathy (excluding optic)
0,1 Transverse myelitis

Renal
0,1 Estimated or measured glomerular filtration rate < 50%
0,1 Proteinuria > 3.5g/24h
or 3 OR End-stage renal disease (regardless of dialysis or transplantation)

Pulmonary
0,1 Pulmonary hypertension (right ventricular prominence, or loud P2)
0,1 Pulmonary fibrosis (physical and radiograph)
0,1 Shrinking lung (radiograph)
0,1 Pleural fibrosis (radiograph)
0,1 Pulmonary infarction (radiograph)

Cardiovascular
0,1 Angina OR coronary artery bypass
0,1,2 Myocardial infarction ever (score 2 if > 1)
0,1 Cardiomyopathy (ventricular dysfunction)
0,1 Valvular disease (diastolic murmur or systolic murmur > 3/6)
0,1 Pericarditis for 6 months, OR pericardectomy

Peripheral vascular
0,1 Claudication for 6 months
0,1 Minor tissue loss (pulp space)
0,1,2 Significant tissue loss ever (e.g. loss of digit or limb)(score 2 if > 1 site)
0,1 Venous thrombosis with swelling, ulceration, OR venous stasis

Gastrointestinal
0,1,2 Infarction or resection of bowel below duodenum, spleen, liver or gallbladder ever, for any cause (score 2 if > 1 site)

0,1 Mesenteric insufficiency
0,1 Chronic peritonitis
0,1 Stricture OR upper gastrointestinal tract surgery ever
0,1 Chronic pancreatitis

Musculoskeletal
0,1 Muscle atrophy or weakness
0,1 Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)
0,1 Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)
0,1,2 Avascular necrosis (score 2 if > 1)
0,1 Osteomyelitis
0,1 Tendon rupture

Skin
0,1 Scarring chronic alopecia
0,1 Extensive scarring of panniculum other than scalp and pulp space
0,1 Skin ulceration (excluding thrombosis for > 6 months)
0,1 Premature gonadal failure
0,1 Diabetes (regardless of treatment)
0,1,2 Malignancy (exclude dysplasia) (score 2 if >1 site)

9. Tabulation of Radiation Doses to Subjects

Radioactive Material or Procedure: F18FDG, 2 studies/duration of trial

Administered quantity or view of exposure: 10mCi

Subject Age: Adult

	RADIATION DOSE (rem)	
ORGAN	**Per single Administration**	**Per Year (12 mos.)**
Adrenals	0.48	0.96
Brain	0.70	1.40
Breasts	0.34	0.68
Esophagus 1	0.44	0.88
Gallbladder Wall	0.49	0.98
GI-tract: Lower Large Intestine	0.51	1.01
Small Intestine	0.47	0.94
Stomach	0.47	0.94
Upper Large Intestine	0.46	1.84
Colon 2	0.48	0.96
Heart Wall	2.20	4.40
Kidneys	0.74	1.48
Liver	0.58	1.08
Lungs	0.64	1.28
Muscle	0.39	0.78
Ovaries	0.53	1.03
Pancreas	0.96	1.92
Red Marrow	0.47	0 .94
Bone Surfaces	0.41	0.82
Skin	0.30	0.60
Spleen	1.40	2.80
Testes	0.41	0.82
Thymus	0.44	0.88
Thyroid	0.39	0.78
Urinary Bladder Wall 3	3.20	6.40
Lens of Eye4	0.30	0.60
Uterus	0.62	1.24
EFFECTIVE DOSE	**0.62**	**1.24**

NOTES:

1. Since no dose is explicitly tabulated for esophagus, thymus dose is used (as per ICRP80)
2. Colon Dose estimated by [0.57 ($Dose_{ULI}$) + 0.43 ($Dose_{LLI}$)] (as per ICRP 80).
3. Dynamic urinary bladder model used; void interval of **1.5 hours**
4. Lens of Eye Dose does not contribute to effective dose

DOSIMETRY SOURCE: Coronado, L. (F-18)FDG Internal Radiation Dosimetry for use in Research Protocols, 10/30/91, NIH,RSB; updated to ICRP 103 by Corina Millo, May 2010 (note some remainder organs doses are not defined;10 are included)

Radioactive Material or Procedure: attenuation whole body CT, mCT Siemens Biograph.

Administered quantity or view of exposure: 120 kV, 92mA, pitch=0.8, collimation=19.2, 2 scans/duration of trial (8 month trial). Subject Age: Adults

	RADIATION DOSE (rem)	
ORGAN	**Per single Administration**	**Per Year (12 mos.)**
Adrenals	0.58	1.16
Brain	0.68	1.36
Breasts	0.56	1.12
Esophagus 1	0.75	1.50
Gallbladder Wall	0.62	1.24
GI-tract: Lower Large Intestine	0.66	1.32
Small Intestine	0.58	1.16
Stomach	0.64	1.28
Upper Large Intestine	0.60	1.20
Colon 2	0.63	1.26
Heart Wall	0.67	1.34
Kidneys	0.67	1.34
Liver	0.62	1.24
Lungs	0.69	1.38
Muscle	0.99	1.98
Ovaries	0.59	1.18
Pancreas	0.56	1.12
Red Marrow	0.57	1.14
Bone Surfaces	1.84	3.68
Skin	0.93	1.86
Spleen	0.60	1.20
Testes	1.34	2.68
Thymus	0.75	1.50
Thyroid	1.00	2.00
Urinary Bladder Wall	0.73	1.46
Lens of Eye3	0.81	1.62
Uterus	0.68	1.36
EFFECTIVE DOSE	**0.70**	**1.40**

DOSIMETRY SOURCE: CT dosimetry by Craig Baker, physicist, Aug. 2012, based on ICRP 103.
1. Since no dose is explicitly tabulated for esophagus, thymus dose is used (as per ICRP80)
2. Colon Dose estimated by [0.57 ($Dose_{ULI}$) + 0.43 ($Dose_{LLI}$)] (as per ICRP 80).
3. Lens of Eye Dose does not contribute to effective dose