Genomic Effects of Glucocorticoids in Patients with Systemic Lupus Erythematosus

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Table of Contents

List of Abbreviations

AE	Adverse event
BCR	B-cell receptor
BMI	Body mass index
BTRIS	Biomedical Translational Research Information System
CC	Clinical Center
CFR	Code of Federal Regulations
ChIP-seq	Chromatin immunoprecipitation sequencing
CPK	Creatine kinase
CRIMSON	Clinical Research Information Management System of the NIAID
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450 isozyme
DEXA	Dual energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FDR	False-discovery rate
FRAX	Fracture risk assessment tool
GR	Glucocorticoid receptor
GRE	Glucocorticoid response element
GRIS	Genomic Research Integration System
HIV	Human immunodeficiency virus
HPA	Hypothalamic-pituitary-adrenal
HRPP	Human Research Protections Program
hsCRP	High-sensitivity C-reactive protein
IGRA	Interferon gamma release assay
IRB	Institutional review board
IV	Intravenous(ly)
LCTU	Lupus Clinical Trials Unit
NIAID	National Institute of Allergy and Infectious Diseases
NIAMS	National Institute of Arthritis and Musculoskeletal Diseases
NIH	National Institutes of Health
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PT/PTT	Prothrombin/partial thromboplastin time
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
SAE	Serious adverse event
SELENA/SLEDAI	Safety of Estrogens in Lupus Erythematosus National Assessment SLE Disease Activity Index
SLE	Systemic lupus erythematosus
TLR	Toll-like receptor
UP	Unanticipated problem
USP	United States Pharmacopeia

Protocol Summary

Short Title:	Glucocorticoid Genomics in SLE
Sample Size:	N = 40 (20 participants per dose group). The final sample size will be determined by an adaptive study design after the initial recruitment of 20 participants per group.
Accrual Ceiling:	N = 100
Study Population:	Individuals aged 18 to 80 years with systemic lupus erythematous (SLE).
Accrual Period:	48 months
Study Design:	This is a study of the acute effects of glucocorticoids on the immune system of patients with SLE. Participants will undergo baseline blood collection prior to receiving a single intravenous (IV) dose of methylprednisolone sodium succinate. Participants will be randomized into 1 of 2 dose groups: 1 mg/kg or 250 mg. Blood will be collected again at 2 and 4 hours after the methylprednisolone infusion. Individual participation requires 2 visits to the NIH CC and 2 follow-up phone calls. Total length of individual subject participation including screening is 1-12 weeks. Blood samples will be processed for isolation of hematopoietic cell sub-populations (eg, neutrophils, B cells, plasmacytoid dendritic cells, CD4+ T cells, CD8+ T cells, monocytes, and natural killer cells). Laboratory studies will be performed on the purified cells, with the goal of understanding the human response to glucocorticoids in vivo at the level of circulating cell populations (eg, flow cytometry, mass cytometry), RNA (eg, RNA sequencing [RNA-seq], small-RNA-seq, real-time polymerase chain reaction [PCR]), DNA (eg, chromatin immunoprecipitation sequencing [ChIP-seq], methylation analysis, DNA sequencing, genotyping), and protein (eg, flow cytometry, mass spectrometry). At each time point, serum methylprednisolone levels will be measured.
Study Agent/ Intervention Description:	A single IV infusion of methylprednisolone sodium succinate at either 1 mg/kg or 250 mg.
Primary Objective:	To understand the cellular and molecular response to glucocorticoids in individuals with SLE.

Secondary Objectives:

1. To identify candidate targets for therapeutic interventions that could mimic the action of glucocorticoids in patients with SLE, without the significant toxicity caused by the broad range of glucocorticoid actions.

2. To test whether the transcriptional response to glucocorticoids differs between the two doses being studied.

3. To identify similarities and differences, at baseline and in response to glucocorticoids, between the transcriptome of cells from patients and those from the previously studied healthy subjects.

Primary Endpoint:

A list of human protein-coding genes and non-coding RNAs that are differentially expressed in response to glucocorticoids in patients with SLE, for each of the studied cell types and doses.

Secondary Endpoints:

1. A comparison of the transcriptional response to glucocorticoids between the two dose groups.

2. A list of protein-coding and non-coding transcripts, their corresponding proteins, and the molecular pathways representing the best candidates for targeted therapeutic alternatives to glucocorticoids.

3. Validation of the targets identified by functional studies.

4. For each cell type, a list of protein-coding or non-coding transcripts that are shared and a list of transcripts that are different, between patients with SLE and the previously studied healthy controls, at baseline or in response to glucocorticoids.

Précis

Systemic lupus erythematosus (SLE) is a systemic autoimmune syndrome that causes pervasive immune dysregulation in all cells of the innate and adaptive immune system. Most patients with SLE require glucocorticoid treatment at some point of the disease course, but the specific effects of glucocorticoids that lead to therapeutic benefit in this condition are not well understood. In addition, the glucocorticoid dosing regimens that are administered to patients with SLE in clinical practice are arbitrary and not based on detailed knowledge of the effects of glucocorticoids at different doses on cells of the immune system. This study aims to improve our understanding of the effects of glucocorticoids on the immune system of patients with SLE, and to test for possible differences in these effects at a range of clinically relevant doses.

A consistent outcome of glucocorticoid exposure is a significant reprogramming of a cell's transcriptional state. The exact changes, however, vary substantially across cell types. Studies of specific cell populations, therefore, are necessary to gain a realistic view of the transcriptional effects of glucocorticoids. Given that cell composition and molecular behavior are known to differ among pathological states, the study of patients with SLE is necessary to identify the effects of glucocorticoids that are most likely to be responsible for their clinically beneficial effects in this condition.

In this study, participants with SLE, aged 18-80 years, will be randomized to receive a single intravenous infusion of 1 of 2 doses of the glucocorticoid methylprednisolone (1 mg/kg or 250 mg). Blood will be collected within 75 days before infusion, immediately before the infusion, and again at 2 and 4 hours after the start of the infusion. Blood samples will be processed for isolation of hematopoietic cell subsets, including neutrophils, B cells, CD4+ T cells, CD8+ T cells, monocytes, natural killer cells, and plasmacytoid dendritic cells. Laboratory studies will be performed on the purified cells, with the goal of understanding the human response to glucocorticoids in vivo at the level of RNA (eg, RNA sequencing [RNA-seq], small-RNA-seq, real-time polymerase chain reaction), DNA (eg, chromatin immunoprecipitation sequencing, methylation analysis, DNA sequencing, genotyping), and protein (eg, flow cytometry, mass spectrometry). At each time point, serum methylprednisolone levels will be measured and flow cytometry for standard cell-lineage markers will be performed.

1 Background Information and Scientific Rationale

1.1 Scientific Background and Preliminary Data

The goal of this study is to improve our understanding of the effects of glucocorticoids on the immune system of people with SLE.

SLE is a systemic autoimmune syndrome of unclear etiology that primarily affects young women. It is characterized by substantial clinical heterogeneity and unpredictable responses to various forms of immunosuppressive treatment. Immune dysregulation is pervasive in SLE and affects all cells of the innate and adaptive immune system. Most patients with SLE require glucocorticoid use at some point of the disease course, but the specific effects of glucocorticoids that lead to therapeutic benefit in this condition are unclear.

Glucocorticoids were first introduced to clinical practice in the late 1940s.[1] They remain extensively used today due to their rapid and potent immunosuppressive and anti-inflammatory effects. However, even short courses of glucocorticoid therapy are associated with severe, dose-limiting side effects that can affect every organ system. Despite nearly seven decades of intense clinical use, it has been pointed out recently that there are still important gaps in our understanding of glucocorticoid-induced immunoregulation.[2] Two of the most salient knowledge gaps involve disease-specific effects and cell type–dependent effects. This study aims to directly address these two gaps, by analyzing the effects of in vivo glucocorticoid administration on specific cell types in individuals with SLE.

The glucocorticoid dosing regimens that are administered to patients with SLE in current clinical practice are arbitrary and not based on detailed knowledge of the effects of glucocorticoids at different doses on cells of the immune system. This unfortunately means that there may be unnecessary harm in current clinical practice. While repeated exposure to very high doses of glucocorticoids can lead to debilitating side effects, the extent to which such doses have effects on cells of the immune system that differ substantially from those achieved with lower doses remains unclear. Because the level of disease activity in patients with SLE tends to fluctuate over time, and higher doses are generally used to treat patients with higher disease activity, this patient population is particularly susceptible to the potential harm of arbitrary glucocorticoid dosing regimens. Therefore, a secondary aim of this study is to analyze the transcriptional response to in vivo administration of glucocorticoids at a range of clinically used doses.

Glucocorticoids act primarily by binding in the cytosol to the glucocorticoid receptor (GR), a nuclear receptor of the steroid/thyroid hormone receptor superfamily.[3] The ligand-bound GR translocates into the nucleus and can directly bind DNA at specific recognition sequences known as glucocorticoid response elements (GREs), increasing or decreasing transcription rates depending on the type of GRE.[4,5] In addition, ligand-bound GR can be recruited to specific genomic sites without directly binding DNA, via protein-protein interactions with other

DNA-bound transcription factors.[6] Genomic sites of direct GR binding represent glucocorticoid-induced enhancers, and genomic sites of indirect (tethered) GR binding appear to cluster around and amplify the activity of direct binding sites.[7] Composite sites of direct and tethered interactions with DNA have also been described.[6] Beyond the direct or tethered recruitment of ligand-bound GR to specific genomic sites, a key component of the mechanism of action of glucocorticoids involves interference with the activity of other transcription factors and signaling molecules, most notably NF-κB. This form of interference can be mediated by direct protein-protein interactions between the ligand-bound GR and other transcription factors,[8] but also by indirect effects via inhibitory long non-coding RNAs,[9] proteins that dissociate from the GR-chaperone complex upon glucocorticoid binding,[10] or competition for nuclear co-activators. Finally, some of the most rapid effects of glucocorticoids may occur independently of the cytosolic GR. These include alterations in ion transport across membranes,[11,12] which have been hypothesized to result from intercalation of glucocorticoid molecules into the membrane.[13] They also include interactions with membrane-bound forms of GR.[14-16]

While the mechanisms are diverse, a consistent outcome of glucocorticoid exposure is a significant reprogramming of a cell's transcriptional state.[17,18] The genomic locations of GR binding have been shown to vary widely across cell types,[19-21] a phenomenon that is explained at least in part by differences in chromatin accessibility and expression differences of GR cofactors.[19,22,23] This, in turn, suggests that the transcriptional response to glucocorticoids could vary significantly across cell types. In this context, studies of specific cell populations in the species of interest are necessary to gain a realistic view of the genomic effects of glucocorticoids in any system. Immortalized and tumor-derived cell lines have been valuable tools for the study of the molecular biology of GR signaling. However, their genomic composition and chromatin landscape are known to differ substantially from those of human primary cells. Similarly, complex cell mixtures, such as whole blood and peripheral blood mononuclear cells (PBMCs), have offered an initial glimpse of the genes and pathways affected by a glucocorticoid stimulus in primary human cells,[17,18] but they are limited in their ability to discern cell-specific effects.

To develop a greater understanding of how pharmacologic doses of glucocorticoids regulate immunity and the extent to which they differentially affect distinct cell subsets, we have studied the genome-wide transcriptional response to glucocorticoids in 9 primary human hematopoietic and non-hematopoietic cell types from healthy human volunteers.[24] For each cell type, we performed total RNA-seq before, 2 hours after, and 6 hours after in vitro stimulation with methylprednisolone or vehicle. We found that the number of genes that responded to the glucocorticoid stimulus differed substantially among cell types. In addition, the specific transcripts that responded to the glucocorticoid stimulus were very different in each cell type. Of the 10,665 glucocorticoid-responsive transcripts identified, 5184 (48.6%) were significantly differentially expressed after glucocorticoid treatment in only 1 of the 9 cell types, and only 23 (0.2%) were significantly differentially expressed after treatment in all cell types. In principle, the observed differences in the transcriptional response to glucocorticoids among cell types could

simply result from differences in the baseline transcriptome of each cell type. However, our results suggest that both the magnitude and the direction of transcriptional regulation by glucocorticoids can differ across cell types, even for genes that are similarly expressed at baseline. This suggests that classifying genes as glucocorticoid-induced or glucocorticoid-repressed, as is common in the literature, should only be done in the context of a specific cell type. This also suggests that baseline expression, chromatin accessibility, and glucocorticoid receptor binding can only partially explain how a gene responds to a glucocorticoid stimulus.

An important advantage of genome-wide approaches is that they allow an unbiased view of biological processes, which can reveal previously unrecognized effects. Integrating the transcriptome datasets from the 9 primary human cell types exposed in vitro to glucocorticoids, we generated a pathway-level map of the effects of glucocorticoids.[24] We observed substantial differences in the way that glucocorticoids affect specific molecular pathways in individual cell types, and in hematopoietic versus non-hematopoietic cells.

This pathway-level map of glucocorticoid actions has served as a tool for generating hypotheses about glucocorticoid actions in individual cell types, which can be tested experimentally. We have demonstrated the utility of this approach in uncovering functional effects of glucocorticoids by focusing on B cells, which play a key role in antibody-mediated autoimmune diseases. Cross-talk between the B-cell receptor (BCR) and toll-like receptor (TLR) signaling pathways plays a central role in the integration of functional B-cell responses[25] and in the development of autoreactive B cells.[26] Our pathway-level map suggested that pharmacologic doses of glucocorticoids had strong transcriptional effects on key genes in both pathways. Based on this, we hypothesized that glucocorticoids could functionally impair BCR and intracellular TLR signaling in human B cells.

To determine whether the in vitro findings on BCR signaling genes were consistent with transcriptional changes in B cells exposed to glucocorticoids in vivo, we measured the expression of key BCR signaling genes over time in a cohort of 20 unrelated healthy volunteers who received a single IV dose of methylprednisolone as part of our clinical protocol, 16-I-0126, “Genomic Responses of Human Immune and Non-Immune Cells to Glucocorticoids” (principal investigator: Franco). Consistent with the in vitro data, we observed a significant drop in the expression of *CD79B*, which encodes Igβ, a protein required for BCR assembly and for signal initiation after antigen stimulation.[27,28] In contrast, expression of *CD79A*, which encodes Igα, was not significantly affected. The gene *CR2*, which encodes a key component of the B-cell co-receptor complex that enhances BCR-mediated signaling,[29] was also significantly reduced in expression in response to glucocorticoid.

We also observed decreased expression of *BTK*, which encodes a key tyrosine kinase immediately downstream of the BCR complex, and of *BLNK*, which encodes a B-cell adaptor

protein central to BCR signaling. Interestingly, the gene that encodes the key upstream kinase LYN was significantly reduced in expression after glucocorticoid administration in vivo, although it had not reached statistical significance in vitro, and the opposite was true of the upstream kinase SYK, which emphasizes the importance of studying these phenomena in vivo and in multiple unrelated individuals.

Consistent with our observation of a glucocorticoid-induced drop in transcriptional output from the immunoglobulin and *CD79B* loci, in vitro exposure of human B cells to glucocorticoid led to a significant drop in surface expression of the BCR when compared to vehicle-treated cells. We found, however, that within 4 hours of glucocorticoid exposure, human B cells still had sufficient levels of BCR on the cell surface to allow stimulation with anti-IgM antibodies, with the goal of functionally assessing signaling downstream of the BCR. We then measured phosphorylation of CD79A (an upstream signaling component) and of PLCγ2 (a downstream signaling component) in response to BCR stimulation with anti-IgM, in the presence of glucocorticoid or vehicle. We measured signaling by phospho-flow cytometry to simultaneously assess the response of total, naïve, and unswitched memory B cells. We found that glucocorticoids impair IgM-BCR signaling. Interestingly, for both readouts, the observed reduction in signaling in total B cells appeared to be primarily driven by a stronger and significant effect on unswitched memory cells.[24]

With respect to TLR signaling, our in vitro work revealed a significant glucocorticoid-induced reduction in expression of the gene encoding the intracellular receptor TLR7 in human B cells. TLR7 is involved in RNA-associated antigen recognition and autoantibody production and is believed to play a role in the pathogenesis of human autoimmune diseases.[26] Our clinical data confirmed a significant drop in *TLR7* expression by human B cells after in vivo glucocorticoid administration. To test whether this transcriptional change had a functional effect, we then purified circulating human B cells, stimulated them with the TLR7-specific ligand imiquimod, and measured phosphorylation of p38 MAP kinase as a functional readout of TLR7 activation. As a negative control, we also stimulated the cells with the TLR8-specific ligand motolimod. TLR8 signals similarly to TLR7, but its expression is not affected by glucocorticoids. At saturating or higher ligand concentrations, the proportion of cells that responded to the TLR7 stimulus, and the intensity of the phospho-p38 MAPK signal, were both significantly lower in glucocorticoid-treated than in vehicle-treated cells. In contrast, glucocorticoids did not significantly alter the response to the TLR8 stimulus. To extend these findings, we measured expression of *CCL3* and *CCL4*, two genes that are responsive to TLR7 and TLR8 stimulation but whose expression is unaffected by glucocorticoid treatment of B cells. Consistent with our prior observations, glucocorticoids significantly decreased the transcriptional response of *CCL3* and *CCL4* after TLR7 stimulation, whereas no significant difference was observed after TLR8 stimulation.

1.2 Scientific Rationale

In summary, our in vitro and in vivo work to date suggests that:

a. A genomics approach can serve as an entry point for the unbiased study of glucocorticoid responses in primary human cells and it can uncover specific mechanisms of immune regulation that can be further characterized by functional studies.
b. The response to glucocorticoids is highly cell type–dependent. Therefore, information at the level of individual cell types and the study of patients with specific diseases will be necessary to identify the effects of glucocorticoids that are most likely to be responsible for their clinically beneficial effects.

These observations are the basis for the present study. We will analyze the genomic response to in vivo glucocorticoid administration in patients with SLE by serial sampling of individual immune cell populations before and after glucocorticoid administration (Figure 1). This will allow us to study, in a clinically realistic setting, the effects of glucocorticoids at different doses on the immune system of patients with SLE; the level of interindividual variation in glucocorticoid responses at the molecular level; and the differences at baseline and in response to glucocorticoids between cells from patients and those from the previously studied healthy subjects.

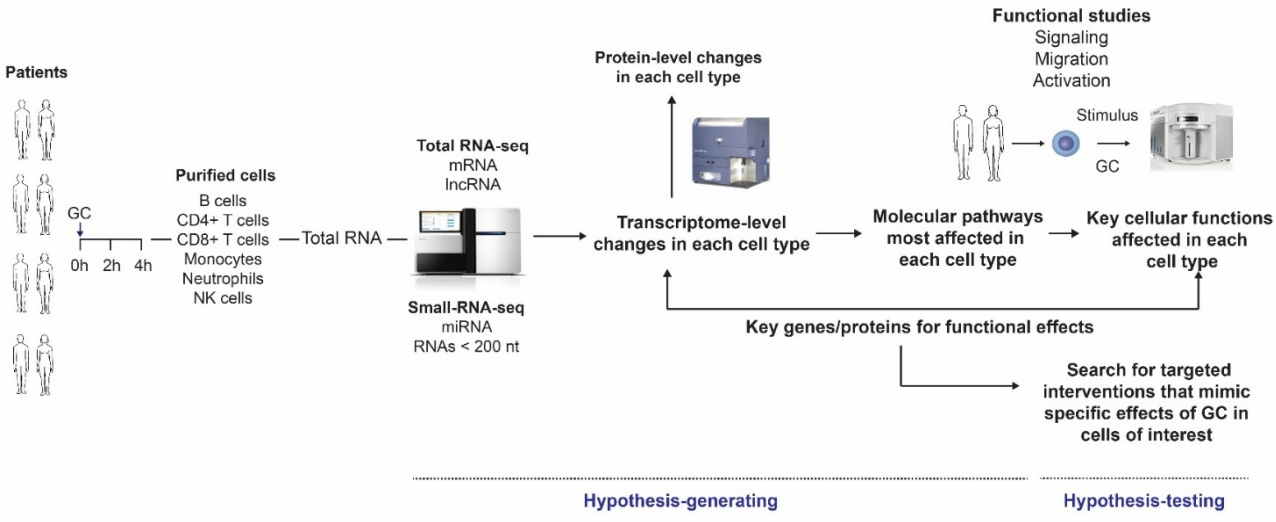

Figure 1. Study design. Patients with SLE will be enrolled. Each participant will be randomized to one of 2 dose groups and will receive a single IV dose of the glucocorticoid (GC) methylprednisolone. Blood will be sampled serially for purification of hematopoietic cell subsets. Total RNA will be purified from each cell subset and total RNA-seq and small RNA-seq will be performed. The transcriptome-level changes in response to GC for each cell type will serve as the basis for the identification of the key molecular pathways and cellular functions that are affected by GC in each cell type. Important observations will be followed up by functional analyses.

Glucocorticoids remain a mainstay of therapy in a broad range of conditions, despite their well-recognized adverse effects and a large and rapidly growing number of other drugs with

immunotherapeutic potential. Identifying the drug targets that, alone or in combination, could best mimic the immunosuppressive effects of glucocorticoids on relevant cell types while limiting off-target actions will be facilitated by greater insight into the mechanisms underlying desirable versus untoward actions of glucocorticoids in distinct human cell types and in relevant disease states. We believe this work will be an important step towards achieving that goal.

1.3 Choice of a Glucocorticoid, Route of Administration, and Dose

Chemical modifications to the 21-carbon corticosteroid backbone (eg, introduction of a carbon 1-2 double bond, methylation at carbon 6, and fluorination at carbon 9) have led to the diverse set of glucocorticoids that is currently available for clinical use. These modifications have mostly altered the pharmacokinetic properties of the drugs, and the relative proportion of mineralocorticoid and glucocorticoid activity, but they do not appear to have significantly altered the mechanism of action or the side effect profile of glucocorticoids. Therefore, the choice of a specific glucocorticoid for our study was based on the frequency of its use in clinical practice and on the availability of an IV formulation (to reduce potential interindividual variation in rates of absorption).

Methylprednisolone is widely used in clinical practice, with indications in the management of diverse conditions including lupus nephritis,[30] dermatomyositis,[31] acute asthma exacerbations,[32] and relapses of multiple sclerosis.[33] IV methylprednisolone has also been used in multiple studies of healthy volunteers since the early 1970s, including our own,[24,34-36] without reports of serious adverse events (SAEs) in those studies. Sodium succinate and phosphate esters are available for IV use. The pharmacokinetics of IV methylprednisolone are also dose-linear,[35,36] which facilitates correlation between the concentrations studied in vitro and the equivalent doses in vivo. Therefore, we have identified methylprednisolone sodium succinate as the glucocorticoid of choice for our study.

An important clinical question with respect to glucocorticoid use is that of the minimum dose that can effectively suppress the immune system. The dosing regimens for glucocorticoids that are commonly used in clinical medicine are mostly arbitrary and based on historical precedent rather than on an objective assessment of their effects on the immune system.[3,13] While the glucocorticoid receptor is nearly saturated at prednisone-equivalent doses in the high-dose range (30-100 mg/day),[3] much higher doses are often used in the clinic, despite limited objective evidence that they are clinically better than a lower dose. To determine the extent to which the transcriptional response to a single dose of glucocorticoid varies by dose, we will randomize study participants into 1 of 2 dose groups: 1 mg/kg or 250 mg.[3] The 1-mg/kg dose was selected because it is commonly used as a starting dose in multiple clinical settings, including in patients with moderate SLE disease activity. The 250-mg dose was selected because that was the dose used in our clinical study of healthy volunteers; administering the same dose to patients with

SLE will facilitate the use of data from the healthy volunteer study in the planned comparison of the genomic response to glucocorticoids between patients with SLE and unaffected controls.

2 Study Objectives

2.1 Primary Objective

To understand the cellular and molecular response to glucocorticoids in individuals with SLE.

2.2 Secondary Objectives

1. To identify candidate targets for therapeutic interventions that could mimic the action of glucocorticoids in patients with SLE, without the significant toxicity caused by the broad range of glucocorticoid actions.
2. To test whether the transcriptional response to glucocorticoids differs between the two doses being studied.
3. To identify similarities and differences, at baseline and in response to glucocorticoids, between the transcriptome of cells from patients and those from the previously studied healthy subjects.

3 Study Design

3.1 Description of the Study Design

Potential participants will present to the NIH CC for a screening visit. Objective documentation of the diagnosis of SLE, the specific clinical manifestations, and the level of disease activity will be established. A baseline whole blood sample will be obtained at screening. Each enrolled participant will be randomly assigned to 1 of 2 methylprednisolone dose groups: 1 mg/kg or 250 mg.

All enrolled participants will return for a day-long study visit ("infusion visit") within 75 days of the screening visit. Following collection of a pre-infusion whole blood sample, participants will receive a single IV dose of methylprednisolone (based on the dose group assignment), infused over 30 minutes. Whole blood samples will then be obtained serially, 2 and 4 hours after the start of infusion.

Participants will be discharged after collection of the 4-hour blood sample, measurement of vital signs, and documentation of any adverse events (AEs). Two follow-up phone calls will be made to document any AEs related to the methylprednisolone. If any AEs that may require treatment are documented on the follow-up phone calls, then participants will be asked to return to the NIH CC's outpatient clinic for evaluation by the principal investigator.

We propose an adaptive study design, in which we will recruit an initial cohort of 20 participants into each of the 2 dosing groups. After processing the samples from these participants, we will perform differential expression analysis to determine if recruitment of additional participants (up to the accrual ceiling of 100) would affect the biological interpretation of the data. If additional data would be unlikely to change the study outcome, then no further recruitment will be pursued.

3.2 Study Endpoints

3.2.1 Primary Endpoint

A list of human protein-coding genes and non-coding RNAs that are differentially expressed in response to glucocorticoids in patients with SLE, for each of the studied cell types and doses.

3.2.2 Secondary Endpoints

1. A comparison of the transcriptional response to glucocorticoids between the 2 dose groups.
2. A list of protein-coding or non-coding transcripts, their corresponding proteins, and the molecular pathways representing the best candidates for targeted therapeutic alternatives to glucocorticoids.
3. Validation of the targets identified by functional studies.
4. For each cell type, a list of protein-coding or non-coding transcripts that are shared and a list of transcripts that are different, between patients with SLE and the previously studied healthy controls, at baseline or in response to glucocorticoids.

4 Study Population

4.1 Recruitment Plan

Participants will primarily be recruited from NIH study 94-AR-0066, “Studies of the Pathogenesis and Natural History of Systemic Lupus Erythematosus” (principal investigator: Hasni). Because there are NIH employees enrolled in 94-AR-0066, employees may also enroll in this study.

Participants may also self-refer, be referred by outside physicians, or be referred by other investigators at the NIH CC. Any individuals who self-refer must first be enrolled onto study 94-AR-0066 to be eligible for this study.

4.2 Inclusion Criteria

Individuals must meet all of the following criteria to be eligible for study participation:

1. Aged 18-80 years.

2. Has a diagnosis of SLE based on the 1997 Update of the 1982 American College of Rheumatology Revised Criteria for Classification of SLE.
3. Currently enrolled in study 94-AR-0066.
4. Able to provide informed consent.
5. Willing to allow genetic testing.
6. Has a primary care physician or other healthcare provider who will manage all health conditions related or unrelated to the study objectives.
7. If receiving immunosuppressive therapies other than glucocorticoids for SLE, then on a stable dose (defined as no increases in dose for the 60 days prior to screening).
8.

    A. If receiving glucocorticoid therapy and not experiencing a lupus flare at the time of the screening visit, then potential participants must be on a daily prednisone or prednisone-equivalent dose ≤ 10 mg/day and agree to undergo a glucocorticoid taper (sections 6.1 and 6.2).

    OR

    B. If receiving glucocorticoid therapy experiencing a lupus flare at the time of the screening visit, then potential participants must be on a daily prednisone or prednisone-equivalent dose ≤ 15mg/day. A glucocorticoid taper will not be performed.

4.3 Exclusion Criteria

Individuals meeting any of the following criteria will be excluded from study participation:

1. Body mass index (BMI) < 18 or > 40.
2. History of a severe allergic reaction to glucocorticoids.
3. History of a severe adverse cardiovascular or psychiatric event related to glucocorticoid administration.
4. Use of a glucocorticoid at a prednisone-equivalent dose 10 mg/day in the 15 days prior to screening (> 15 mg/day if experiencing a flare on the day of the screening visit).
5. A previously established diagnosis of an active solid or hematologic malignancy.
6. A previously established diagnosis of untreated osteoporosis. For the purpose of this study, osteoporosis is defined as a dual-energy X-ray absorptiometry (DEXA) scan obtained within 2 years of screening demonstrating a hip or spine bone mineral density T score ≤ −2.5 in men ≥ 50 years of age or in postmenopausal women.
7. A previously established diagnosis of untreated osteopenia with a high fracture risk. For the purpose of this study, osteopenia is defined as a DEXA scan obtained within 2 years of screening demonstrating a hip or spine bone mineral density T score < −1 and > −2.5 in men ≥ 50 years of age or in postmenopausal women. For the purpose of this study, a

high fracture risk is defined as a fracture risk assessment tool (FRAX) 10-year risk of major osteoporotic fracture > 20%, or a FRAX 10-year risk of hip fracture > 3%.

8. A previously established diagnosis of diabetes mellitus or a fasting blood glucose level ≥ 125 mg/dL at the time of screening. For patients without a previously established diagnosis of diabetes mellitus and a fasting blood glucose level < 125 mg/dL at the time of screening, no additional screening tests (e.g., HbA1c or oral glucose tolerance test) will be performed. A history of glucocorticoid-induced hyperglycemia that is not present at the time of screening in the absence of treatment will not be considered an exclusion criterion.
9. Cancer chemotherapy within the 5 years prior to screening.
10. Surgery requiring general anesthesia in the 8 weeks prior to screening.
11. History of an infection requiring IV antibiotics in the 30 days prior to screening.
12. A positive test for HIV or hepatitis A, B, or C virus infection.
13. A positive or indeterminate test for active or latent tuberculosis (interferon gamma release assay [IGRA]) without evidence of prior treatment.
14. History of chronic or possible latent untreated parasitic, amebic, fungal, or mycobacterial infections.
15. Use of desmopressin in the 30 days prior to screening.
16. Use of one of the following cytochrome P450 isozyme (CYP) 3A4 inducers in the 30 days prior to screening: nafcillin, rifabutin, rifampin, St. John's wort, or troglitazone.
17. Use of one of the following CYP3A4 inhibitors in the 30 days prior to screening: clarithromycin, itraconazole, or ketoconazole.
18. Use of belimumab or rituximab within the past 180 days.
19. Vaccination within the past 30 days.
20. Pregnancy, current or in the 90 days prior to screening.
21. Currently breastfeeding.
22. Any electrocardiogram (ECG) abnormality that is clinically significant.
23. Any condition that, in the opinion of the investigator, contraindicates participation in this study.

Laboratory evaluations that will be used to establish eligibility are listed in sections 6 and 7, below. For exclusion criteria that involve previously established diagnoses, such as active malignancy, osteoporosis, or diabetes mellitus, any previously obtained diagnostic studies will be considered, but diagnostic testing for the sole purpose of establishing eligibility will not be performed.

Co-enrollment guidelines: Co-enrollment in other studies is permitted. Study staff should be notified of co-enrollment on any other protocol to ensure the schedules are compatible and that the volume of blood drawn for research is within NIH CC limits.

4.4 Justification for Exclusion of Special Populations

Pregnancy is an exclusion criterion because there are no adequate and well-controlled studies of glucocorticoids in pregnant people. Glucocorticoids should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Additionally, pregnancy itself may alter the immune system, and thus may affect study results. Breastfeeding is an exclusion criterion because of the potential for serious adverse reactions in nursing infants from glucocorticoids.

People younger than 18 years are excluded from this study because there is the potential for greater than minimal risk and no prospect for direct benefit.

5 Study Agent

5.1 Disposition, Dispensation, and Accountability

The study agent, methylprednisolone sodium succinate, will be distributed and accounted for by the NIH pharmacy according to standard pharmacy procedures.

5.2 Formulation, Packaging, and Labeling

Methylprednisolone sodium succinate for injection, United States Pharmacopeia grade (USP), sold as SOLU-MEDROL sterile powder by Pfizer, Inc, is an anti-inflammatory glucocorticoid that occurs as a white, or nearly white, odorless hygroscopic, amorphous solid.[37] It is very soluble in water and in alcohol but is insoluble in chloroform and is very slightly soluble in acetone.

Methylprednisolone sodium succinate has the same metabolic and anti-inflammatory actions as methylprednisolone. When given parenterally and in equimolar quantities, the two compounds are equivalent in biologic activity.

The study agent is packaged in the preservative-free Act-O-Vial System, which is a single-use vial.

Each vial will be individually labeled with the patient identification number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, and a statement that the agent should be kept out of reach of children.

5.3 Study Agent Storage and Stability

The study agent must be protected from light. Unreconstituted product and solution are stored at a controlled room temperature of 20°C to 25°C (68°F to 77°F). Solution must be used within 48 hours after mixing.

5.4 Randomization

Study participants will be randomized evenly to 2 dose groups. The purpose of randomization in this setting is to produce balanced dose groups where participants are comparable between groups in terms of possible confounding factors such as age, sex, or concomitant medication use. To further ensure that participants are similar in terms of SLE severity between the groups, we will adopt stratified randomization according to their disease activity using the Safety of Estrogens in Lupus Erythematosus National Assessment SLE Disease Activity Index (SELENA/SLEDAI) score on the day of enrollment. Participants will be classified into 1 of 3 disease-severity categories: low (SELENA/SLEDAI score = 0-4), intermediate (SELENA/SLEDAI score = 5-8), or high (SELENA/SLEDAI score > 9). Randomization will be carried out within each category. This stratified randomization method ensures the proportion of participants with high disease activity is the same between the dose groups.

The study statistician will design the randomization scheme. The randomization code list will be maintained by the study statistician and the study coordinator.

5.5 Preparation, Administration, and Dosage of Study Agent

Methylprednisolone will be prepared according to the package insert and depending on the dose group to which each participant has been assigned.[37] The study agent solution will be administered as an IV infusion over 30 minutes, for a total single dose of 1 mg/kg or 250 mg of methylprednisolone. Pulse oximetry will be followed during the infusion for safety monitoring.

5.6 Concomitant Medications and Procedures

All concomitant prescription and nonprescription (including over-the-counter) medications taken during study participation will be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

5.7 Prohibited Medications and Procedures

Treatment with the following drugs/medications will not be permitted during participation in this study:

- Desmopressin.
- CYP3A4 inducers nafcillin, rifabutin, rifampin, St. John’s wort, or troglitazone.
- CYP3A4 inhibitors clarithromycin, itraconazole, or ketoconazole.
- Belimumab or rituximab.
- Receipt of any vaccine.

5.8 Investigational New Drug Exemption

Use of methylprednisolone sodium succinate in this study is exempt from an investigational new drug application to the Food and Drug Administration (FDA) because its use meets the following criteria, per Title 21 of the United States Code of Federal Regulations (CFR) Part 312.2(b)[38]:

1. The drug product is lawfully marketed in the United States.
2. The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug.
3. In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug.
4. The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product.
5. The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR 56) and with the requirements for informed consent (21 CFR 50).
6. The investigation is conducted in compliance with the requirements of 21 CFR 312.7 (ie, the investigation is not intended to promote or commercialize the drug product).

6 Study Schedule

This study will involve 2 study visits at the NIH CC (screening and infusion visits) and 2 follow-up phone calls. Only those participants found to be eligible after the screening procedures will return to continue the study. Participants will be referred to their primary care physician or other healthcare provider if any test results need further follow-up.

6.1 Screening (day −75 to −1)

The following tests will be conducted after informed consent is obtained:

- Physical exam (including BMI assessment) with vital signs.
- Medical history and medication review.
- ECG.
- SELENA/SLEDAI score.
- Urine collection for the following:
  - Urinalysis with microscopic examination.
  - Spot urine protein, creatinine, and protein-to-creatinine ratio.
- Blood draw for the following:
  - Complete blood count with differential.
  - Acute care panel.
  - Calcium and magnesium levels.

- Random morning serum cortisol level.
- Liver function tests (hepatic panel).
- Complement levels: C3 and C4.
- Anti–double-stranded DNA (dsDNA) antibodies.
- Erythrocyte sedimentation rate (ESR).
- High-sensitivity C-reactive protein (hsCRP).
- Lipid panel.
- Serum creatine kinase (CPK).
- Serum pregnancy test (for participants capable of becoming pregnant).
- Prothrombin/partial thromboplastin time (PT/PTT).
- HIV and hepatitis A, B and C (viral markers screen test, which includes HBsAg, anti-HCV IgG, anti-HAV IgM). Test results obtained within the past 30 days will be accepted.
- Interferon gamma release assay.
- Baseline purification of hematopoietic cell sub-types.
- Baseline flow cytometry.
- Banking of baseline plasma, peripheral blood mononuclear cells, and DNA.

With the exception of the serum pregnancy test, test results obtained within 30 days of the screening visit under protocol 94-AR-0066 will be accepted for screening.

After eligibility is confirmed, participants who are receiving glucocorticoid therapy may be directed to undergo a taper before the infusion visit, as described in section 6.2, below.

6.1.1 Management of Patients who are Experiencing a Lupus Flare at the Time of the Screening Visit

Patients who are eligible for enrollment based on the study's inclusion and exclusion criteria, but who are experiencing a lupus flare at the time of their screening visit, will be considered for enrollment only when the following two criteria are met:

a. The infusion visit can be scheduled for a date that is within 7 days of enrollment, and
b. The severity of the flare does not require administration of glucocorticoids prior to the anticipated date of the infusion visit.

The above criteria will apply irrespective of whether the patients are on glucocorticoid therapy at the time of their screening visit, but patients who are experiencing a lupus flare while on glucocorticoid therapy will not be deemed eligible for a glucocorticoid taper. For patients on glucocorticoid therapy who are experiencing a lupus flare at the time of their screening visit, the maximum prednisone or prednisone-equivalent dose will be ≤ 15 mg/day. Patients on higher doses of chronic glucocorticoid therapy who are experiencing a flare are deemed likely to require an infusion earlier than 7 days after evaluation and will thus be excluded.

If a patient who is experiencing a lupus flare on the day of the screening visit is enrolled, but the infusion visit is later delayed beyond 7 days after enrollment or the severity of the flare increases to the point where glucocorticoids must be administered before the infusion visit can be scheduled, glucocorticoid treatment will be administered as clinically indicated and the patient will be withdrawn from the study.

6.2 Glucocorticoid Taper

Given the known toxicity of glucocorticoid therapy, it is standard clinical practice to attempt to reduce the lifetime exposure of patients with SLE to glucocorticoids, by periodically tapering their glucocorticoid to the minimum effective dose. An ideal tapering regimen, or a reliable way to identify patients on chronic glucocorticoid therapy in whom the dose can be tapered, have not been defined. Similarly, the biological changes that take place upon glucocorticoid tapering, or the glucocorticoid-responsive genes that help maintain patients in clinical remission, are very poorly understood. In this protocol, we will enroll patients who are in clinical remission and receiving chronic glucocorticoid treatment. In those patients, a glucocorticoid taper will be performed. These are patients who would be good clinical candidates for tapering their glucocorticoid dose under standard clinical practice. In the setting of this study, the rationale for tapering is two-fold:

i. Patients in whom the dose of glucocorticoid can be tapered to zero can be studied in the same context as those who were not receiving glucocorticoids prior to the study.
ii. Patients in whom the dose of glucocorticoid is tapered, but their disease activity increases during the taper, can provide very valuable information. Identifying the genes and molecular pathways that differ between a chronic stable dose, a disease flare at a lower dose, and the subsequent response to a glucocorticoid infusion, can help identify the genes that glucocorticoids regulate chronically to sustain clinical remission.

To maximize the safety of glucocorticoid tapering in this study, enrolled participants will be considered eligible for a glucocorticoid taper if they are on a stable prednisone or prednisone-equivalent dose ≤ 10 mg/day and have not had escalation of other immunosuppressive therapies for SLE, as indicated in inclusion criteria 7 and 8, and in exclusion criterion 4.

The taper will be managed by the clinical staff of the NIAMS Lupus Clinical Trials Unit (LCTU). The LCTU staff are actively involved in the care of patients in the study of the natural history of SLE (94-AR-0066), in which all participants in this study will also be enrolled. The tapering protocol is described in **Error! Not a valid bookmark self-reference.**.

Table 1. Glucocorticoid Tapering Protocol

Stable glucocorticoid dose (prednisone-equivalent)	Tapering regimen
7.5-10 mg/day	Decrease dose by 2.5 mg/week until a dose of 5 mg/day is reached. Then, decrease dose by 1 mg/week until the taper is complete.
≤ 5 mg/day	Decrease dose by 1 mg/week until the taper is complete

The taper will be considered complete when a participant reaches one of two endpoints:

1. The participant is no longer receiving a glucocorticoid, or
2. Upon clinical evidence of a disease flare.

For participants whose taper ends because they are no longer receiving a glucocorticoid, the infusion visit will be performed approximately 10 calendar days after the last dose of glucocorticoid. After the infusion visit, participants will be followed up by the LCTU team and/or their outside rheumatologist for further management of the disease and decision to restart glucocorticoid medication.

For participants whose taper ends due to clinical evidence of a disease flare, or if a disease flare occurs between the end of the taper and the infusion visit, then the infusion visit will be scheduled within 7 days after the documentation of the flare. After the infusion visit, participants will be followed by the LCTU team and/or their outside rheumatologist for further management of the disease and decision to restart glucocorticoid medication. If the severity of the flare requires intervention prior to a scheduled infusion visit, then the participant will be withdrawn from the study and will continue to be managed by the LCTU team and/or their outside rheumatologist, as appropriate.

Of note, exogenous glucocorticoid administration suppresses the hypothalamic-pituitary-adrenal (HPA) axis, and the time following a glucocorticoid taper that it takes for the HPA axis to return to normal function can vary among individuals. Formal documentation of the function of the HPA axis by a cosyntropin stimulation test is not part of routine clinical practice after glucocorticoids are tapered, unless a person is experiencing signs or symptoms of adrenal insufficiency. Therefore, cosyntropin stimulation testing will not be part of this study. It is unclear whether the baseline function of the HPA axis influences the transcriptional response by cells of the immune system to high doses of glucocorticoids. Although this study is not designed to address that question, the glucocorticoid panel that will be obtained before and after medication administration on the day of infusion (Table 2) includes measurement of plasma cortisol. Morning cortisol levels > 15 µg/dL are predictive of a normal result on a short cosyntropin stimulation test in nearly all patients, and values < 5 µg/dL predict an abnormal result in nearly all patients.[39,40] Therefore, depending on the distribution of values in this group

of participants, these data could eventually be used to stratify the participants by baseline level of function of the HPA axis, to assess whether this leads to measurable differences in the response to exogenous glucocorticoids.

6.3 Infusion Visit (day 0)

- Targeted physical exam with vital signs (on initial encounter and prior to discharge).
- Acute care panel (results will be obtained before infusion).
- IV catheter insertion.
- Serum pregnancy test (for those capable of becoming pregnant; results will be obtained before infusion). For participants tested within the past 24 hours, the test will not be repeated.
- Interim medical history and medication review.
- Pre-infusion blood draw.
- IV infusion of methylprednisolone sodium succinate (30 minutes).
- Blood pressure monitoring every 30 minutes from the time of infusion until the time of collection of the last blood sample.
- Pulse oximetry monitoring during the infusion.
- Nursing staff will monitor participants for the presence of behavioral abnormalities (altered mood, hyperactivity, disorientation, psychosis).
- Blood draws at 2 and 4 hours (± 10 minutes) after the start of infusion.

In participants whose glucocorticoid taper ends due to clinical evidence of a disease flare, the following additional procedures will be performed on the day of the infusion visit:

- SELENA-SLEDAI score.
- Urine collection for the following:
    - Urinalysis with microscopic examination.
    - Spot urine protein, creatinine, and protein-to-creatinine ratio.
- Blood draw for the following:
    - Complete blood count with differential.
    - Complement levels: C3 and C4.
    - Anti–double-stranded DNA (dsDNA) antibodies.

6.4 Follow-up Phone Calls

A follow-up phone call will be made between days 1 and 3 after the infusion visit to document the presence or absence of side effects related to the study drug. A second follow-up phone call will be made 5 days (± 2 days) after the infusion visit. If any AEs that may require treatment are documented on the follow-up phone calls, then the participant will be asked to come to the NIH CC's outpatient clinic for evaluation by the principal investigator. Participation in this study will be complete after the second phone call or, if applicable, after any follow-up visits.

7 Study Procedures/Evaluations

Methylprednisolone sodium succinate infusion: Participants will receive a single IV dose of methylprednisolone sodium succinate (based on the dose group to which they are assigned) administered through an IV catheter over a period of 30 minutes.

Blood draw: Blood will be drawn at screening, immediately prior to the study agent infusion, and 2 hours (± 10 minutes) and 4 hours (± 10 minutes) after the start of the infusion. Blood will be used for laboratory and research evaluations and storage as indicated in Table 2. The amount of blood drawn for research purposes will be within the limits allowed for adult research participants by the NIH CC (Medical Administrative Series Policy M95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf).

ECG: 12-lead ECG will be performed at screening to rule out electrical cardiac abnormalities and ensure eligibility to receive the study agent.

SELENA/SLEDAI: The SELENA/SLEDAI index is a tool for measuring disease activity in patients with SLE. The score is determined by points assigned to symptoms and laboratory values the patient had within 28 days of evaluation.[41,42] A higher score represents a more significant degree of disease activity.[43] The score will be calculated by a qualified practitioner.

7.1 Return of Research Results

Any clinically relevant test results will be shared with the participant throughout the study. Unexpected or incidental medical findings unrelated to the study may occur and may require the participant to follow up with an appropriate physician/healthcare provider for standard medical care or follow-up.

The genetic research in this study is not designed or intended to produce data regarding health risks, incurable conditions, or information contradictory to stated biological relationships between individuals. Therefore, it is not expected that the results from these studies will be returned to volunteers. In the rare event of detecting an incidental finding related to a participant's health, we will share the information with the participant. Genetic counseling and advice is available from the NIH to help participants understand the implications of incidental findings, if necessary.

Table 2. Blood Volumes and Research Uses

Visit	Blood sample	Initial processing	Downstream use
Screening visit	Baseline whole blood sample (116 mL)[a]	60 mL:[b] Isolation of hematopoietic cell sub-types (eg, neutrophils, B cells, CD4+ T cells, CD8+ T cells, monocytes, NK cells)	RNA-level, DNA-level, protein-level, and/or functional assays
		10 mL: PBMC isolation	PBMC banking
		8 mL: Plasma purification	Plasma banking
		8 mL: Hepatitis A, B and C	N/A
		6 mL: HIV	N/A
		5 mL: DNA purification	Genome-wide genotyping and/or DNA sequencing
		4 mL: Acute care panel, C3 and C4 complement levels, calcium, CPK, hsCRP, LFTs, lipid panel, magnesium, random morning serum cortisol, serum pregnancy test	N/A
		4 mL: Anti-dsDNA	N/A
		4 mL: IGRA	N/A
		3 mL: PT/PTT	NA
		3 mL: CBC/diff	N/A
		1 mL: ESR	N/A
Infusion visit	Pre-infusion whole blood sample (90 mL)	60 mL: Isolation of hematopoietic cell sub-types (eg, neutrophils, B cells, CD4+ T cells, CD8+ T cells, monocytes, NK cells)	RNA-level, DNA-level, protein-level, and/or functional assays
		10 mL: PBMC isolation	PBMC banking
		8 mL: Plasma purification	Plasma banking
		5 mL: Serum	Glucocorticoid panel
		4 mL: Acute care panel, serum pregnancy test, C3 and C4 complement levels[c]	N/A
		4 mL: Anti-dsDNA[c]	N/A
		3 mL: CBC/diff	N/A
	Serial post-infusion whole blood samples (90 mL × 2)	60 mL: Isolation of hematopoietic cell sub-types (eg, neutrophils, B cells, CD4+ T cells, CD8+ T cells, monocytes, NK cells)	RNA-level, DNA-level, protein-level, and/or functional assays
		10 mL: PBMC isolation	PBMC banking
		8 mL: Plasma purification	Plasma banking
		5 mL: Serum	Glucocorticoid panel
		4 mL: Acute care panel	N/A
		3 mL: CBC/diff	N/A

CBC/diff = complete blood count with differential; CPK = creatine kinase; dsDNA = double-stranded DNA; ESR = erythrocyte sedimentation rate; hsCRP = high sensitivity C-reactive protein; IGRA = interferon gamma release assay; LFT = liver function test; N/A = not applicable; NK = natural killer; PBMC = peripheral blood mononuclear cell; PT/PTT = prothrombin/partial thromboplastin time.

a Baseline blood will be collected at screening from all potential participants and will be processed as described even if the participant is determined to not be eligible for the study.

b The purpose of this initial sample will be to assess the level of variation at baseline.

C C3 and C4 complement levels and anti-ds DNA antibodies will only be ordered on the day of infusion in patients whose glucocorticoid taper ended due to clinical evidence of a disease flare.

8 Potential Risks and Benefits

8.1 Potential Risks

Methylprednisolone sodium succinate infusion: Side effects from a one-time IV dose of methylprednisolone may include metallic or bitter taste in the mouth during and possibly after infusion, upset stomach, sleep disturbance (insomnia, sleepiness/fatigue), increased appetite, water retention, weight gain, temporary increase in blood glucose, temporary increase in blood pressure, changes in white blood cell counts (eg, leukocytosis , neutrophilia, monocytopenia, or eosinopenia), mood disturbance or unusual behavior, electrolyte imbalance, muscle cramps, back pain, headaches, flushing, and frequent urination lasting 1-2 weeks after infusion.[37]

Individuals who received methylprednisolone to treat illness have had skin problems such as rash or dry skin, easy bruising, growth of body hair, irregular menstrual cycles, abnormal results of liver enzyme tests, enlargement of the liver, inflammation of the pancreas, numbness in the feet and hands, eye problems, and bone problems such as osteoporosis and damage to the hip bone. Many of these effects were dependent on the duration and frequency of treatment and we do not expect that they will occur with a single dose.

Glucocorticoids may decrease resistance to infection and the ability to localize infection. Infections with any pathogen in any location of the body may be associated with the use of glucocorticoids alone or in combination with other immunosuppressive agents. Glucocorticoids may reactivate or exacerbate latent fungal, bacterial, or viral infections. Individuals with known or suspected active or latent infections will be excluded from study participation. In addition, participants will be advised to limit travel for 4 weeks following the methylprednisolone infusion.

Allergic reactions including anaphylactoid reaction, anaphylaxis, and angioedema have been reported in patients receiving methylprednisolone.

Serious cardiac, musculoskeletal, neurologic/psychiatric, and other side effects of IV methylprednisolone have been reported in patients with underlying disease.

There are reports of cardiac arrhythmias and/or cardiac arrest following the rapid administration of large IV doses of methylprednisolone (greater than 0.5 g administered over a period of less than 10 minutes). Bradycardia has been reported during or after the administration of large doses of methylprednisolone sodium succinate and may be unrelated to the speed or duration of infusion.

Glucocorticoid taper: For individuals who are eligible for the study and undergo a glucocorticoid taper, the progressive reduction in the dose of glucocorticoid may result in destabilization of SLE, potentially leading to a disease flare.

Blood draw and IV placement: The risks of drawing blood include pain, bruising, bleeding, fainting, and, rarely, infection.

ECG: ECGs are of minimal risk to the participants and cause no significant side effects. The electrodes may feel cold when applied; in rare cases, some people will develop a rash or skin irritation where the patches are placed. This type of irritation usually resolves by itself or occasionally with topical medication.

RNA-seq, DNA sequencing, and genome-wide genotyping: Studies of RNA molecules will be performed to identify changes in gene expression in response to the study agent. Studies of DNA molecules (genome-wide genotyping) will be performed to identify variants in the genome that

may influence gene expression. These studies will involve assays of specific regions of the genome. Depending on the findings, sequencing of DNA (either specific regions or the entire DNA molecule) may be performed in the future. Incidental findings from genetic studies may have psychological implications for participants and family members. Genetic counseling and advice is available from the NIH to help participants understand the implications of incidental findings, if necessary. Following genetic testing, data will be shared in a controlled-access public database in accordance with NIH guidelines, for other investigators to benefit from the information (eg, dbGaP, the Database of Genotypes and Phenotypes). However, no personal, identifiable information will be shared in this process, as the results will only be shared with a code.

8.2 Potential Benefits

This research is not designed to benefit study participants. This research is designed to provide generalizable knowledge about SLE pathogenesis and the mechanisms of action of glucocorticoids in SLE. The study may also inform the development of future anti-inflammatory and immunosuppressive medications.

9 Research Use of Stored Human Samples, Specimens, and Data

Intended Use: Samples, specimens, and data collected under this protocol may be used to study the body's response to glucocorticoids. Genetic testing will be performed.

Storage: Samples are stored in a secure facility with limited access. Samples and data will be de-identified prior to storage using a code assigned by the investigators. All data, including the database linking the code with a specific individual, will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples and data acquired under this protocol will be tracked using the Clinical Research Information Management System of the NIAID (CRIMSON). After de-identification, laboratory specimens will be tracked using the Biological Specimen Inventory system, under a NIAID-wide contract for laboratory specimen banking and tracking.

Disposition:

- In the future, other investigators (at NIH or outside) may wish to study these samples and/or data. If the planned research falls within the category of "human subjects research" on the part of the NIH researchers, IRB review and approval will be obtained. This includes the NIH researchers sending out coded or linked samples or data, or getting results that they can link back to their participants.
- At the time of protocol completion, samples will be either destroyed or, after IRB approval, transferred to another existing protocol. Data will be archived by the study

team in compliance with requirements for retention of research records; alternatively, after IRB approval, the data may be transferred to another repository.

Loss or Destruction:

- Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a protocol deviation, unanticipated problem (UP), and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIH IRB.
- Additionally, participants may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the participant and to the IRB. Participants who revoke permission to have their samples stored will be withdrawn from the protocol. However, this decision will not affect the individual's participation in any other protocols at NIH.

10 Data Sharing Plan

Human data generated in this study will be shared for future research as follows:

- De-identified data in an NIH-funded or approved public repository. Gene expression data will be made available through the Gene Expression Omnibus and Sequence Read Archive repositories.
- De-identified data in public repositories.
- Identifiable data in the Biomedical Translational Research Information System (BTRIS, automatic for activities in the CC) or the Genomic Research Integration System (GRIS).
- De-identified or identifiable data with approved outside collaborators under appropriate agreements.
- In publications and/or public presentations.

Data may be shared before publication, at the time of publication, or shortly thereafter.

Study participants will be offered co-enrollment in the NIAID Centralized Sequencing Program (protocol 17-I-0122). For participants who choose to enroll in that protocol, genome sequencing and microarray analysis will be performed as part of that protocol. Data generated as part of the Centralized Sequencing Program is shared with the referring investigators via the NIH Genomic Research Integration System (GRIS).

11 Remuneration Plan for Participants

Participants will be compensated for their time and inconvenience according to the following schedule:

- Screening visit: $60
- Infusion visit: $180
- Total = $240

In the event that additional study-related visits need to be scheduled after the infusion visit, as detailed in section 12.5 below, each additional visit will be compensated at $60. Travel costs may be covered as per the current NIH/NIAMS policy.

12 Assessment of Safety

AEs and other reportable events are defined in NIH Human Research Protections Program (HRPP) Policy 801.

12.1 Toxicity Scale

The Investigator will grade the severity of each AE according to the "Common Terminology Criteria for Adverse Events (CTCAE)" (v 5.0) which can be found at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

12.2 Causality

Causality (likelihood that the event is caused by the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship
  OR
- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship
  OR
- definitely due to an alternative etiology

Note: Other factors should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

12.3 Recording/Documentation

At each contact with the participant, information regarding AEs will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents may include, but not limited to, progress notes, laboratory reports, consult notes, and phone call summaries. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and the principal investigator's judgment of the AE's relationship and expectedness to the study agent will also be recorded in CRIMSON.

12.4 Expected Events

Some signs or symptoms are induced by or associated with methylprednisolone sodium succinate, and any combination of these is expected to occur. These events will not be reported unless they reach a grade of 3 or higher (as defined in the CTCAE, v 5.0), are deemed a risk to the participant's rights or wellbeing, or are deemed appropriate to report under specific circumstances by the principal investigator. Examples include:

- Hyperglycemia
- Leukocytosis
- Neutrophilia
- Monocytopenia
- Eosinopenia
- Flushing

- Change in appetite
- Bitter taste in mouth
- Metallic taste in mouth
- Anxiety
- Changes in mood
- Insomnia
- Lymphopenia (only reported if grade 4 or above)

12.5 Type and Duration of the Follow-up of Participants after Adverse Events

AEs related to research procedures will be followed through resolution or until the investigator determines the event has stabilized and no additional follow-up is required. AEs related to the methylprednisolone infusion that have not resolved by the end of study participation will be followed until they have returned to baseline values. If it is not possible to obtain a final outcome for an AE (eg, the participant is lost to follow-up), then the reason a final outcome could not be obtained will be recorded by the investigator in CRIMSON.

12.6 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all participants and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The halting rules are:

- 2 or more participants experience the same or similar SAEs that are possibly, probably, or definitely related to the study agent;
  OR
- any safety issue that the principal investigator determines should halt the study.

The principal investigator will determine if the study should be halted. The data and safety monitoring board (DSMB) may also recommend a halt.

12.6.1 Reporting a Study Halt

If a halting rule is met, then a description of the AE(s) or safety issue must be reported by the principal investigator within 1 business day to the IRB. The principal investigator will also notify the DSMB.

12.6.2 Resumption of a Halted Study

The principal investigator, in collaboration with the DSMB, will determine if it is safe to resume the study. The principal investigator will notify the IRB of the decision on resumption of the study.

12.7 Study Discontinuation

The IRB, the NIAID, and other oversight bodies as applicable, as part of their duties to ensure that research participants are protected, may discontinue the study at any time. Subsequent review of serious, unexpected, and related AEs by the IRB may also result in suspension of enrollment and further administration of study agent.

12.8 Premature Withdrawal of a Participant

An individual participant will be withdrawn for any of the following:

- An individual participant's decision. (The investigator should attempt to determine the reason for the participant's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the participant or to the integrity of the study data.
- A participant becomes pregnant between screening and the infusion visit.
- A participant experiences a disease flare during tapering of current glucocorticoid therapy that requires intervention before the infusion visit.
- A participant experiences an anaphylactoid reaction, anaphylaxis, or angioedema requiring medical therapy during the glucocorticoid infusion.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.
- A participant who has a flare at screening visit and not scheduled for infusion within 7 days or needs to receive glucocorticoids before the scheduled infusion visit.

12.9 Replacement of Withdrawn Participants or Participants Who Discontinue Study Treatment

Participants who withdraw or are withdrawn from the study prior to the infusion visit will be replaced.

13 Reporting Procedures

13.1 Reporting to the NIH IRB

UPs, non-compliance, and other reportable events will be reported to the NIH IRB according to HRPP Policy 801.

13.2 Reporting to the NIAMS Clinical Director

The principal investigator will report UPs, major protocol deviations, and deaths to the NIAMS clinical director according to institutional timelines.

14 Clinical Monitoring Structure

To help ensure that NIH Office of Research Support and Compliance procedures and Good Clinical Practices are being carried out, a Clinical Trials Management designee within the Office of Clinical Research Policy and Regulatory Operations, Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH Office of Human Subjects Research Protections reporting requirements for reportable events. In addition, the quality management and data management plan for the study will be reviewed.

14.1 Quality Management Plan

During the study, the principal investigator and study team will be responsible for implementing a quality management plan. Additionally, the study team will be responsible for completing and submitting a summary report on the quality plan to the NIAID clinical director or designee at least annually as detailed in the quality management plan. A courtesy copy will also be sent to Clinical Trials Management.

14.2 Safety Monitoring Plan

The NIAID intramural DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interest as defined by NIAID policy. The DSMB will review the study protocol and informed consent document prior to initiation and twice a year thereafter, or as may be determined by the DSMB.

The DSMB may convene additional reviews as necessary. The DSMB will review the study data as needed to evaluate the safety, efficacy, study progress, and conduct of the study.

All deaths, SAEs, and UPs will be reported to the DSMB at the same time they are submitted to the IRB unless otherwise specified herein.

The principal investigator will notify the DSMB at the time halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The principal investigator will submit the written DSMB summary reports with recommendations to the IRB.

15 Statistical Considerations

15.1 Definition of the Study Endpoints

The primary endpoint of this study is a list of human protein-coding genes and non-coding RNAs (small RNAs and long non-coding RNAs) that are differentially expressed in response to glucocorticoids in patients with SLE, for each of the studied cell types and doses.

The secondary endpoints of this study are:

1. A comparison of the transcriptional response to glucocorticoids between the 2 dose groups.
2. A list of protein-coding and non-coding transcripts, their corresponding proteins, and molecular pathways representing the best candidates for targeted therapeutic alternatives to glucocorticoids.
3. Validation of the targets identified by functional studies.
4. For each cell type, a list of protein-coding or non-coding transcripts that are shared and a list of transcripts that are different, between patients with SLE and the previously studied healthy controls, at baseline or in response to glucocorticoids.

15.2 Bioinformatics Pipeline and Data Handling

The output of the RNA and small-RNA sequencing runs (bcl files) will be converted to FASTQ format with the software program bcl2fastq. Quality control of the resulting FASTQ files will be performed with the software program FastQC. RNA-seq data will be aligned to the most recent human genome assembly using the software program STAR.[44] The resulting BAM files and the most recent version of the GENCODE will be used to obtain raw read counts for each transcript, with the featureCounts program of the package Subread.[45]

15.3 Identification of Glucocorticoid-responsive Genes

To identify glucocorticoid-responsive genes, differential expression analysis for the RNA-seq and small-RNA-seq data will be performed in R, with the package DESeq2.[46]

For each cell type, we will assess the level of variation in the baseline measurements by comparing the expression values on the day of the screening visit to the 0-hour (pre-infusion) values on the day of the glucocorticoid infusion. For participants who undergo a glucocorticoid taper, this analysis will contrast the pre- and post-taper patterns of gene expression. We will then perform differential analysis comparing each of the two post-infusion time points (2 hours and 4 hours) independently against the baseline. DESeq2 (section 15.6) adopts the method of Benjamini-Hochberg to adjust for multiplicity. We will draw conclusions based on the Benjamini-Hochberg adjusted p values, which control for the family-wise false discovery rate (FDR) in the presence of multiple comparisons. We will employ a cutoff FDR[47] value of $< 5\%$ to

select the transcripts that will be considered differentially expressed in each cell type at each time point. The resulting gene lists will be contrasted to determine, for example, which genes are uniquely differentially expressed in a specific cell type, or common to a specific hematopoietic lineage. They will also be used for pathway enrichment analysis, to determine the molecular pathways and biological functions that are most responsive to the glucocorticoid stimulus in each cell type.

A separate analysis will compare data obtained at the same time point (2 hours or 4 hours) between the high-dose and low-dose groups, to test whether there are significant differences in the transcriptional effect of glucocorticoids at the two doses. Finally, we will identify differences and similarities between the baseline transcriptome and the transcriptional response to glucocorticoids in patients with SLE recruited in this study and in healthy volunteers recruited in our previous study. Since the healthy and the SLE participants come from two different studies, they are not directly comparable in many aspects. Therefore, these results will be derived from the two studies separately and no formal comparison will be made across the two studies. We will present the baseline distribution of gene expression, the distribution of gene expression changes, and the subset of differentially expressed genes. For each cell type, we will generate a list of protein-coding or non-coding transcripts that are shared and a list of transcripts that are different between SLE patients and healthy controls.

Sequencing library preparation and sequencing batches will be carefully designed such that samples from the same participant and cell type are run in the same batch and samples from different participants are evenly distributed across batches. This will keep batch effects to a minimum in the identification of differentially expressed genes. For analyses across different participants, such as comparisons among different cell types, linear regression will be adopted with batch as a regression variable to adjust for batch difference. While prior experience suggests that library preparation is the greatest source of batch-to-batch variation in RNA-seq experiments, if the source of batch effects is unclear or cannot be adequately modeled, then the R package SVA will be used.

15.4 Analysis of Flow Cytometry and Cytokine Data

Visualization and gating for the flow cytometry data will be performed with the software program FlowJo.

Analysis of flow cytometry and high-throughput cytokine measurement data will be performed in R using custom scripts.

15.5 Summary Statistics for Demographic and Clinical Data

Demographic characteristics and medical history will be summarized, eg, proportion, mean, median, standard deviation, and minimum and maximum values. Means and standard deviations will be reported for all continuous variables; percent will be reported for categorical variables.

15.6 Adaptive Sample Size Determination

Given the high-throughput data generation strategy of next-generation sequencing, the use of different starting materials for sequencing (mRNA, small RNA, DNA), and the use of serial sampling, standard sample size calculation methods are not appropriate in this case and an adaptive study design is a more realistic approach. Sample size consideration will be based primarily on the power to identify differentially expressed genes. The primary endpoint of the study is a list of human protein-coding genes and non-coding RNAs that are differentially expressed in response to glucocorticoids in patients with SLE, for each of the studied cell types and doses. With a sample size of 20 in each dose group, there would be 90% power to detect a change of effect size 0.72 before and after glucocorticoid by the standard paired t-test. That means, within each dose arm and for each cell type, we could detect a differentially expressed gene with 90% power if the gene expression difference before and after glucocorticoid was no less than 0.72 SD.

Due to the specific properties of RNA-seq data, an advanced mixed-effects negative binomial modeling approach will be adopted for evaluating gene expression change and the significance of the change. This will be achieved with the R package DESeq2, a widely adopted tool for differential expression analysis on sequencing data, which targets the issue of large number of genes and small number of samples. In DESeq2, data from all genes are shared and utilized in assessing the differential expression of each gene. This leads to more stable estimation of differential expression and its variability, and consequently to better power for detecting differentially expressed genes.

Based on the data from our previous study of healthy volunteers (protocol 16-I-0126) and a bootstrap procedure, with a sample size of 20 we can detect differentially expressed genes from 0 to 2 hours after glucocorticoid infusion with a median of 0.45 log2 fold change at a significance level of 0.05 after adjusting for multiplicity, and 0.62 log2 fold change from 0 to 4 hours. In SLE patients, there may be higher variability in the data than what we observed in healthy volunteers. Meanwhile, the magnitude of gene expression changes is also expected to be higher, given the state of activation of many immune cells in SLE compared to healthy controls. Therefore, we estimate that the proposed initial sample size in this study will be sufficient to detect the genes associated with the effect of glucocorticoids in SLE patients.

To empirically evaluate whether this estimate is correct, we propose an adaptive study design similar to the one we performed on the study with healthy volunteers. Specifically, we will

recruit an initial cohort of 20 participants in each dose group. Based on the observed data from 20 participants, we will then assess the gain in discovery with up to 30 additional participants per group. We will perform a bootstrap procedure, sampling with replacement up to 50 units from the 20 observed participants in each group to evaluate the improvement in gene detection. For example, if the subset of genes that can be detected as differentially expressed over time and the subset of genes that can be detected as differentially expressed over the 2 doses do not change significantly above a number, then we will stop at that number. If there is change in the gene subsets, but the pathways and the biological interpretation of the data remain unchanged, then additional participants will be unlikely to change the outcome of the study and no further recruitment will be pursued. Due to the adaptive sample size determination, we will again use a nominal significance level $\alpha < 0.05$.

16 Ethics/Protection of Human Subjects

16.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate in research. It is an ongoing conversation between the human research participant and the researchers that begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Participants will be given the opportunity to ask questions and have them answered.

The participants will sign the informed consent document prior to undergoing any research procedures. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's medical record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

We anticipate the enrollment of Spanish-speaking participants in this study. The English language consent document will be translated in full into Spanish by the NIH Library. For Spanish-speaking participants, the Spanish language consent form will be presented by the investigator through an interpreter, unless the investigator is fluent in Spanish.

Two Step Consenting Process

In certain cases, when prospective participants would like to learn additional details about the study before coming to the NIH (e.g., due to geographical distance or other logistical and personal reasons), we would like to do a two-step informed consent process. In the first step, we will either mail or email the informed consent document to the patient; this will be followed by a

telephone call or videoconference, to go over the informed consent form document orally. For patients who agree to participate at that point, the second step will involve scheduling a screening visit at the NIH Clinical Center. As with any other screening visit, a member of the study team will then answer any additional questions and obtain the patient's written signature in the informed consent form. This informed consent process will then be documented in the subject's medical records.

16.2 Participant Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized individuals may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NIAID, and the Office for Human Research Protections.

16.3 Participation of NIH Employees

NIH employees may participate in this protocol. 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 Employee Research Participation."

For NIH employees:

- NIH staff may be a vulnerable class of participants.
- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation.
- The employee participant's privacy and confidentiality will be preserved in accordance with NIH CC policies, which define the scope and limitations of the protections.
- For NIH employee participants, consent will be obtained by an individual independent of the employee's team. Those in a supervisory position to any employee and co-workers of the employee will not obtain consent.
- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

17 Data Handling and Record Keeping

17.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from participants during study visits and telephone calls, or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

17.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the International Council on Harmonisation Good Clinical Practice guidelines. Study records will be maintained by the principal investigator for a minimum of 7 years, and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

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