Assessment of Glucocorticoid Responsiveness and Mechanisms of Resistance in Hypereosinophilic Syndromes

NIH Protocol Number:
12-I-0026

Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)

Principal Investigator:
Paneez Khoury MD, LPD, NIAID, NIH
Laboratory of Parasitic Diseases
National Institute of Allergy and Infectious Diseases
Building 10, Rm 12C103
9000 Rockville Pike
Bethesda, MD 20892
Phone: (301) 402-3673
E-mail: paneez.khoury@nih.gov

Version Number:
V13

Day Month Year
September 04, 2020

Investigational Agent:

Drug Name:	Prednisone (under multiple trade names)
IND Number:	N/A (exempt)
Sponsor:	N/A
Drug Supplied By:	NIH Pharmacy

Table of Contents

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

List of Abbreviations

ACR	American College of Rheumatology
AE	Adverse Event/Adverse Experience
AEC	Absolute Eosinophil Count
AF	Activation Function (AF-1, AF-2)
CBC	Complete Blood Count
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CRIMSON	Clinical Research Information Management System of the NIAID
ERR	Eosinophil Response Rate
EGID	Eosinophilic Gastrointestinal Disease
FIP1L1	FIP1-like1
F/P	FIP1L1-PDGFRα
GC	Glucocorticoid
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Element
HES	Hypereosinophilic Syndrome
IL	Interleukin
IRB	Institutional Review Board
LFT	Liver function test
N	Number (typically refers to the number subjects or the sample size)
OHSR	Office of Human Subjects Research
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
PBMC	Peripheral Blood Mononuclear Cells
PDGFR	Platelet-derived growth factor
PHI	Protected Health Information
PI	Principal Investigator
RNA PAX	Ribonuclease PAXgene blood tube
SAE	Serious Adverse Event/Serious Adverse Experience

Protocol Summary

Full Title:	Assessment of Glucocorticoid Responsiveness and Mechanisms of Resistance in Hypereosinophilic Syndromes
Short Title:	Glucocorticoid responsiveness in HES
Principal Investigator:	Paneez Khoury
Sample Size:	N=40
Accrual Ceiling:	40
Study Population:	100 children and adults, ages 7-100
Accrual Period:	6-13 years
Study Design:	Single blinded clinical trial
Study Duration:	14 years
Start Date:	Oct 2011
End Date:	Dec 2026
Primary Objective:	To develop a model to determine whether a single dose glucocorticoid (GC) challenge can be used to predict GC responsiveness in subjects with HES
Secondary Objectives:	To delineate the mechanisms of decreased GC responsiveness in GC-resistant subjects.
Exploratory Objectives:	To assess in-vitro correlates of therapeutic responsiveness in HES.
Endpoints:	**Primary:** GC responsiveness, defined as the lowest dose of prednisone at which eosinophils remain <1000/μL, for at least two consecutive weekly blood draws, and at which symptoms are controlled as determined by the investigator **Secondary:** Identification of GC non-responders and/or suboptimal responders for studies of GC resistance in HES. **Exploratory:** Delineation of the mechanisms of GC resistance and in-vitro correlates of response in subjects with HES.

Précis

This study aims to develop a model to determine whether a single, oral, weight-based dose of glucocorticoid (GC) can predict clinical and biologic response to GC's over the long term in subjects with hypereosinophilic syndrome (HES). Subjects with FIP1L1/PDGFRα-negative HES, who are symptomatic with eosinophil count >1500/μL and receiving ≤10 mg prednisone daily, will be enrolled. A single oral dose of prednisone (1 mg/kg rounded to the nearest 5mg) will be administered. Eosinophil count and various laboratory parameters will be assessed at 2 hours, 4 hours and 24 hours following prednisone administration (investigators will be blinded to the results of the eosinophil counts). The subjects will then begin GC therapy at 30 mg prednisone daily followed by a standardized taper. The lowest dose of GC at which symptoms and eosinophilia are controlled will be compared to the change in eosinophil count at 2, 4 and 24 hours post-challenge. Mechanisms and in vitro correlates of GC resistance will also be explored.

1. Introduction & Background

1.1. Rationale

Although glucocorticoids (GC) are the mainstay of therapy for many autoimmune and inflammatory conditions, not all patients respond equally, and high doses of inhaled or systemic GCs are often used in non-responders. Consequently, GC resistance is an active area of study in asthma, smokers with asthma, chronic obstructive pulmonary disease (COPD), and other inflammatory disorders. GCs are considered standard first-line therapy in patients with hypereosinophilic syndrome (HES) who lack the FIP1L1-PDGFRα (F/P) mutation. In fact, more than 85% of patients with HES studied retrospectively had a complete or partial response to GC at 1 month(1). Although decreased expression of the GC receptor on the surface of eosinophils was implicated in 3 out of 5 patients with GC-resistant HES(2), the mechanisms of decreased or absent GC response in diverse variants of patients with HES are largely unexplored.

A single-dose prednisone challenge has been proposed as a way to assess steroid-responsiveness and measure prognosis in patients with F/P negative HES. Although patients presenting with HES are typically treated with a large dose of GC followed by a steroid taper, the utility of this approach has not been validated. Studying the effects of a steroid challenge would be beneficial for 4 reasons. First, an eosinopenic response after a 1 mg/kg dose could provide information about the efficacy of high dose GC in the event of rapidly progressive disease. Second, if the acute response to a single dose GC challenge were able to predict the maintenance dose after a taper, this might allow patients to spend less time on higher doses. Third, the lack of response to a steroid challenge would identify patients for whom a trial of steroids would be unwarranted allowing faster institution of alternate therapies for HES. Finally, as subjects with GC-resistant HES are systematically identified in a standardized fashion, further mechanistic work into causes of resistance, included elucidation of signaling pathways, glucocorticoid receptor function, and perhaps even genetic reasons for resistance may be explored.

1.2. Background

Glucocorticoids act on a number of glucocorticoid-responsive genes relevant to inflammatory conditions via the glucocorticoid receptor (GR). The GR itself is composed of a DNA ligand and an immunogenic or N-terminal domain. Alternative splicing of the GR gene generates 2 receptor isoforms. The classically active form is GRα, which resides in the cytoplasm and is expressed in cells of most organs and tissues in the human body. GRβ, the second isoform, acts as a dominant negative receptor. Administration of GCs in the setting of inflammatory and allergic conditions results in expression of anti-inflammatory cytokines, as well as diminished expression of inflammatory or adhesion molecules on airway or vascular epithelium. A number of mechanisms have been proposed to explain decreased GC responsiveness. The following section describes some of these mechanisms and their potential relevance in HES (Appendix G, Figure 1) .

1.2.1. Inappropriate Absorption of Glucocorticoids

While there have been no studies of GC absorption in HES, it is possible that patients with eosinophilic involvement of the GI tract might have difficulty with GC absorption (Appendix G, Figure 1). Although GCs are used for the treatment for eosinophilic gastrointestinal disease (EGID), especially in the setting of malabsorption, the mechanism of GI involvement in HES may be different (mucosal edema instead of eosinophilic inflammation, for example). Furthermore, it is not clear that oral dosing in the form of prednisone has similar bioavailability or pharmacokinetics of absorption to the compounded liquid forms of GC used most often in EGID.

1.2.2. Alterations in Glucocorticoid Receptor function

GR function involves multiple steps, including 1) binding of GC to the receptor, 2) translocation of the GC-receptor complex into the nucleus, and 3) direct and indirect effects on GR response elements leading to modulation of the cytokine response (Appendix G, Figure 1). Abnormalities in any of these could potentially lead to decreased GC responsiveness.

Increased expression of GRβ in place of the active GRα has been reported in steroid insensitive nasal polyps(3), fatal asthma(4), steroid resistant asthma(5), and other steroid insensitive inflammatory diseases(6); however, the degree to which differential expression of GR isoforms contributes to steroid resistance remains controversial. To complicate matters further, an additional cohort of receptor proteins produced by alternative translation initiation from a single GR mRNA species was recently described and results in an increased number of GR isoforms(7). GR isoform expression has not been evaluated in HES to date.

One of the ways that glucocorticoids exert their anti-inflammatory effects is through inhibition of transcription factors, which in turn alter cytokine expression profiles. Differential expression of the NFκB DNA binding unit (NFKB1), STAT-4 and IL-4R in stimulated PBMCs predicted the GC resistant phenotype in asthmatics(8) Furthermore, cytokine footprints of decreased GC responsiveness have been described in patients with eosinophilic esophagitis and asthma. In one study of patients with eosinophilic esophagitis, non-responders to budesonide therapy had elevated numbers of TGFβ1 positive cells compared with responders(9). Studies in asthmatic patients have demonstrated increased number of cells expressing Th2 cytokines, including IL-4(5, 10), IL-5(5), and IL-13(10), as well as increased IFNγ(5) mRNA in bronchoalveolar lavage (11) from GC resistant subjects. The cytokine phenotype of GC resistance in HES has not been studied.

Despite the complexity of GC signaling (Appendix G, Figure 2)(12), some progress has been made in evaluating kinetic phosphorylation events occurring at the N-terminus phosphorylation sites of the GR after binding of corticosteroids. Phosphorylation events affect subcellular transport of the GR and therefore have effects on GR function in the setting of inflammatory conditions. Examples of assessment of phosphorylation events in asthma include: demonstration of elevated p38 MAPK and pERK1/2 in asthma

patients compared to controls(11), and elevated p38 MAPK reduction of nuclear GR-ligand binding affinity in steroid-dependent asthmatics compared with controls(13). It has also been proposed that phosphorylation of the GR might affect ligand affinity suggesting a role for tissue or cell specific responses.

1.2.3. Glucocorticoid effects on trafficking and eosinophil transcriptomics

At the time of initiation of this study, early (<4h) kinetics of glucocorticoid action on eosinophils were not well described. Samples from this and other studies were subjected to transcriptomic evaluation to determine early kinetics of eosinophil decline. More recent knowledge suggests that there is a substantial decline of eosinophils in the peripheral circulation (at least in normally responsive eosinophils) by 3 hours. More striking, the major genes of interest in apoptosis and trafficking were noted to be up or down-regulated by 2 hours after GC administration. Of the 414 genes differentially expressed in eosinophils in the first 2 hours after prednisone administration, 12 are known to be involved in leukocyte migration: *CXCR4*, *RIPOR2*, *PREX1*, *CCR1*, *PTGER4*, *SWAP70*, *IL16*, *PIK3CG*, *NLRP12*, *CD244*, *S1PR1*, and *CSF1*. Four of these genes were significantly differentially expressed prior to the initial decline in circulating eosinophils, which occurred in the interval between 60 and 120 minutes. In vitro and flow cytometric studies demonstrated that CXCR4 is upregulated in response to steroids and we hypothesize this might be important in trafficking of eosinophils from the peripheral blood into tissues (14). For this reason, we are splitting amount of research blood drawn in the 4 hour timepoint into a 2 and 4 hour timepoint in order to be able to study the exploratory endpoints without losing valuable data at the 4 hour timepoint. The primary clinical endpoint at 4 hours remains unchanged as it is not appropriate or possible to change the primary endpoint at this juncture.

Ultimately, understanding the various processes that predict decreased GC responsiveness would assist in numerous ways in the care of patients with HES. First, it would allow tailoring of treatment regimens for patients with HES. Second, development of assays or biomarkers that can predict decreased responsiveness could reduce GC use in such patients by preventing unnecessary treatment trials in patients unlikely to respond and accelerating the time to maintenance dosing. Thirdly, understanding the underlying mechanisms of GC resistance might pave the way for more effective anti-inflammatory treatments or adjuncts to GC, as has been demonstrated in COPD, where theophylline and selective PI3Kδ inhibitors have a synergistic effect with GC administration (15). Better understanding of transcriptional changes in eosinophils might provide additional insight into receptor ligands on eosinophils, their role in trafficking, and might pave the way for further research into therapeutic implications of these findings. Finally, insights into the causes of decreased responsiveness of eosinophils to GC and in HES may have implications for other inflammatory and allergic disorders.

2. Study Objectives

2.1. Primary Objective

To develop a model to determine whether a single dose steroid challenge can be used to predict GC responsiveness in subjects with HES

2.2. Secondary Objectives

To define a group of GC non-responders and/or suboptimal responders for further study of the mechanisms and biologic correlates of GC resistance in HES

2.3. Exploratory Objectives

To delineate some of the mechanisms of decreased GC responsiveness in GC-resistant subjects and to assess in-vitro correlates of therapeutic responsiveness in HES in a subset of subjects.

3. Study Design and Methods

3.1. Description of the Study Design

The study is designed as a single center, open label, single-blinded observational study to develop a model to assess whether a single dose steroid challenge can predict GC responsiveness in subjects with HES. GC responsiveness is defined as the minimum dose of GC required to control eosinophilia and symptoms.

3.2. Screening

Subjects with HES enrolled on Protocol #94-I-0079 (Eosinophil Characterization, Activation, and Function in Parasitic Infections and other Conditions with Increased Tissue or Peripheral Blood Eosinophilia in Humans) will be recruited for enrollment in this protocol. Since eligibility for this protocol will be determined on the basis of the standard clinical and laboratory evaluations performed as part of Protocol #94-I-0079, a separate screening visit will not be necessary for the GC challenge. Since the HES workup may need to take place over several visits, to determine eligibility, a CBC with differential will be performed at the NIH or at the patient's local site within 14 days prior to enrolling on this protocol.

Informed consent will be obtained and subjects will be enrolled during a scheduled visit for protocol 94-I-0079 or via telephone prior to undergoing any procedures specific to this protocol. Telephone consents will be obtained according to current NIH policy (section 7.5.3).

3.3. Baseline Visit

Baseline assessments and procedures will be performed up to 90 days prior to dosing with prednisone and will include (see Appendix B for complete list):

- A complete history and physical examination, with emphasis on signs and symptoms associated with eosinophilia (i.e. fatigue, pruritus, skin involvement, neuropathy, arthralgias, myalgias etc.)
- Routine laboratory studies, including complete blood count with differential, routine chemistries including electrolytes, liver and kidney panels.
- Additional diagnostic testing, as clinically indicated, to characterize baseline clinical manifestations of eosinophilia.
- Blood β-HCG at baseline visit. Urine β-HCG will be performed in the morning prior to prednisone administration in women of childbearing potential.
- Assessment of eosinophil activation, additional research studies and storage of samples as outlined in Appendix C**.**

3.4. Procedures and Laboratory Testing

Subjects will receive a single dose of prednisone prescribed by the PI or designee to be taken by mouth at a dose of 1 mg/kg (rounded to the nearest 5 mg) between the hours of 7:30 and 9am on the day of study. Laboratory testing, including a baseline cortisol level, CBC with differential, and research specimens (see Appendix B**)** will be drawn prior to prednisone administration and at 2, 4 and 24 hours after prednisone administration. Investigators will be blinded to the 2-hour, 4-hour and 24-hour eosinophil counts (the results will not appear in the NIH Clinical Research Information System (CRIS), but will be available from the Clinical Pathology internal system for analysis once the trial is completed). All blood draws will be timed and will occur in outpatient phlebotomy, outpatient clinic or inpatient unit that is able to draw at specific times to prevent variability in draw times across the subject cohorts.

In normal subjects, prednisone is rapidly absorbed and metabolized with peak levels at 2-4 hours and a biologic half-life of 12-36 hours. Serum will be stored from blood drawn at the 4-hour time point for assessment of synthetic glucocorticoid levels, including prednisone and its metabolite prednisolone. Subjects may be asked to undergo further workup for causes of poor absorption by their home physician or at NIH if indicated.

After the 24-hour challenge, subjects will be started on a GC taper to determine the lowest dose of GC that is able to suppress signs and symptoms of HES and maintain the peripheral eosinophil count below 1000/µl. GC will be initiated at 30 mg in all subjects, and the taper will proceed according to an algorithm (Appendix E). GC will be tapered by 5 mg weekly until a dose of 15 mg is reached. The dose will then be tapered by 2.5 mg weekly until a dose of 5 mg is reached or the eosinophil count rises.

In some cases, an initial starting dose of 30 mg of prednisone will be insufficient to control eosinophilia. If AEC is >1000/µL 1 week after the initiation of GC, the GC dose will be increased to 60 mg and a taper initiated from this higher dose. A CBC with

differential will be drawn after 2 days on this higher dose and if the AEC remains >1000/μL, the subject will be withdrawn and total GC dose used for the final analysis will be the weight-based challenge dose for that individual. If the subject is responsive at the higher dose, the subject will be kept at 60 mg for one week after which the GC dose will be tapered by 10 mg every week until a dose of 30 mg of prednisone is reached. If AEC is still suppressed and they are able to continue the taper, they will then taper according to the standard taper schedule outlined above and shown in Appendix E.

Weekly eosinophil counts will be obtained with the taper until the AEC rises above 1000/μL or the subject experiences HES symptoms. HES symptoms will be defined by the investigator and will be based on data collected at the time of the baseline visit, as well as clinical experience in the treatment of HES. If either eosinophils rise or HES symptoms are present, prednisone will be increased to the previous dose, and a taper will be re-attempted. If the AEC increases after an attempted decrease in dose, the rate of the tapering schedule will be decreased to 2.5 mg/week from 5 mg/week. The taper will continue according to this schedule until 2 attempts to taper from a given dose fail or a dose of 5 mg of prednisone is reached. If a subject's AEC continues to climb despite a dose increase, at the discretion of the investigator the dose may be increased by a larger dose than outlined in Appendix E. Attempts to capture the true effective dose of glucocorticoid that controls counts and symptoms will be attempted; however if not practical due to time or side effect profile of prednisone, subjects will return for an end of study visit and be removed from the study. If a subject is unable to be tapered, their responsiveness, or lack thereof, will be captured at that dose. Depending on the dose and the clinical presentation they may be continued on prednisone however they will be withdrawn from the protocol at the dose at which they were no longer able to taper.

The GC taper will be individualized in subjects in whom a prolonged course of a higher dose is clinically indicated (e.g., Churg Strauss syndrome/EGPA) or a slower taper is needed to prevent steroid withdrawal. Investigators will not be blinded to the eosinophil counts obtained during the taper, as the primary endpoint is dependent on the initial results of the steroid challenge to which the investigators are blinded.

3.5. Study Endpoints

3.5.1. Primary Endpoint

The primary response variable is GC responsiveness, defined as the lowest dose of prednisone at which eosinophils remain <1000/μl, for at least two consecutive weekly blood draws, and at which symptoms are controlled as determined by the investigator; OR, dose of prednisone at which subject is unable to taper prednisone further without rebound of eosinophilia and/or symptoms after three attempts; OR, the challenge dose, if there is no drop in eosinophil counts within the first week of challenge or taper.

GC responsiveness in each individual will be assessed using the percent change in eosinophil count (eosinophil response rate [ERR]) from baseline to 4h (or 24h) post-steroid challenge.

3.5.2. Secondary Endpoint

Subjects will be classified into 3 categories on the basis of GC responsiveness: unresponsive, suboptimally responsive, and steroid-responsive. These categories are defined in Section 6.1 “Description of the analyses.”

Clinical factors associated with GC responsiveness (including HES variant, pattern of organ involvement and laboratory parameters) will be explored.

3.5.3. Exploratory Endpoints

The mechanisms of GC resistance will be explored (Appendix F) by dividing subjects into response categories for the following assessment in all subjects:

a) GI absorption of prednisone by evaluation of plasma levels of prednisone/prednisolone

b) intracellular cytokine profiles in lymphocytes using multiparameter flow cytometry

c) RNA expression profiles by microarray profiling of whole blood

d) GR isoform assessments: GR isoforms will be assessed on all subjects using RT-PCR to understand relationship between responsiveness and predominant isoforms

e) Proteomics: A collaboration with Dr. Konrad Pazdrak (Assistant Professor, 2.230 Basic Science Building, 301 University Boulevard, Galveston, TX 77555-0635) has been initiated using samples from the screening protocol for eosinophilia (94-I-0079). If preliminary findings are promising, coded samples, without personal identifying information, from 12-I-0026 will be sent to him for further proteomic evaluation of steroid responsiveness.

Not all studies will be performed for each subject due to time or sample constraints, although efforts will be made to proceed with exploratory investigations in a uniform manner. Additionally, investigations not outlined above may be performed based on preliminary findings and collaborations with other investigators in the field of GC responsiveness.

4. Inclusion and Exclusion Criteria

4.1. Subject Inclusion Criteria

Subjects on Protocol #94-I-0079 will be eligible for participation in the study only if all of the following criteria apply:

1. Subjects must be 7 years of age or older to enroll
2. Subject meets diagnostic criteria for HES (AEC >1500/μL, absence of a secondary cause and signs and/or symptoms attributable to the eosinophilia)
3. AEC >1500/μL obtained within 14 days prior to enrollment

4. Willingness to perform the timed steroid challenge
5. Appropriate candidate for GC treatment after challenge
6. Willingness to have samples stored for future research

4.2. Subject Exclusion Criteria

A subject will not be eligible to participate in the study if any of the following apply:

1. Receiving >10 mg prednisone or equivalent at the time of enrollment.
2. Receiving ≤10 mg of prednisone or equivalent but have not been on a fixed dose for at least 3 weeks (subjects on a current corticosteroid taper will be excluded)
3. AEC ≤1500/µl on the day of the steroid challenge
4. Use of immunomodulatory medications, (other than ≤10 mg/day prednisone) including but not limited to biologics, within the past 6 months
5. Pregnant at the time of screening.
6. Have a known mutation in the FIP1L1-PDGFR gene
7. Any condition that, in the opinion of the investigator, places the subject at undue risk by participating in the protocol
8. Weight less than 48 kg (106 lbs) in subjects less than 18 years of age

5. Monitoring and Withdrawal

5.1. Clinical Evaluations

Standard safety assessments for this study will include history and physical examinations, and vital signs at baseline and at 24 hours. Clinical assessments of SAEs and AEs will be performed at all study visits, as well as during phone encounters.

5.2. Laboratory Evaluations

Standard laboratory evaluations will include clinical chemistry panel including renal, hepatic panels and electrolytes, CBC with differential, cortisol levels and stored samples as described in Appendix B and Appendix C.

5.3. Follow-up

Follow-up will consist of phone-calls within a window of 7 ± 3 days to coordinate the tapering schedule of prednisone. All changes in prednisone doses and tapers will be appropriately documented with concurrent eosinophil counts as applicable. Blood for RNA analysis will be obtained at 1 week to assess medium term effects of GC administration. The blood draw will be performed at the subject's home institution (with sample sent to NIH in accordance with appropriate procedures), or may be performed at NIH if the subject lives locally.

Subjects will be asked to return 2-4 weeks after the completion of their GC taper for their final visit on this protocol.

5.4. Early Termination Visit

Subjects who withdraw from the study prematurely (see below) will continue to be followed on Protocol #94-I-0079.

5.5. Re-contact of Subjects after Study Termination

After study termination, subjects will be contacted yearly as part of the required study visits for Protocol #94-I-0079. In settings where subjects request to withdraw consent for Protocol #94-I-0079, they will be asked whether they would be willing to be re-contacted in the future.

5.6. Premature Withdrawal of a Subject

Subjects may be withdrawn for the following reasons:

1. Significant adverse events from GC therapy requiring withdrawal of subject and/or switching to alternate therapies
2. Determination of positive F/P gene status during steroid therapy that was previously unknown.
   NOTE: Most subjects referred to NIH with HES have known F/P gene status and are already on tyrosine kinase inhibitors if indicated. In extremely rare cases where gene status is unknown, GC therapy might be initiated while F/P gene status is being investigated as part of Protocol #94-I-0079. If a positive result were obtained, the subject would be withdrawn from the study and imatinib therapy would be recommended. Such subjects would be excluded from the analysis.
3. Complete non-response to GC's evidenced by lack of reduction of peripheral eosinophil count from baseline at the initial 30 mg dose or the increased 60 mg dose. Subjects will be switched to an alternative therapy either by their primary care physician or at the follow-up visit. Although subjects will be withdrawn from further steroid tapering, evidence of non-response will be included in the analysis.
4. AEC ≤1500/μL on the day of steroid challenge.
5. Subject becomes pregnant (subjects who become pregnant after the first GC dose will be followed for safety on 94-I-0079).

6. Analysis Plan

6.1. Description of the Analyses

The primary objective of this study is to develop a model to predict GC responsiveness (see section 2) using the percent change in the eosinophil count at 4 or 24 hours following steroid challenge (ie, ERR). GC responsiveness is defined as the dose required to control disease manifestations and eosinophilia. Since this response is necessarily subject to many factors including physician expert opinion, it is important that the PI and Accountable Physician be blinded to the predictor variables. In order not

to compromise patient care, the 24 hour post-challenge CBC with differential will be assessed by an independent physician (Dr. Irina Maric or her designee) who will decide whether un-blinding is necessary for patient safety.

To allow for flexibility in building the predictive model, we will first try the class of linear models on the GC responsiveness and pick the best model in terms of a small sample adjusted Akaike Information Criterion(16). If the fit of that model is not good (for example, the errors look far from normal), then transformations on the GC responsiveness may be tried. If none of those models have good fit, then we will consider a cumulative logit model where we partition the GC responsiveness into 3 ordered categories: steroid responsive (<15 mg of prednisone or equivalent alternate corticosteroid daily), moderately responsive (requiring between 16-40 mg of prednisone or equivalent), or resistant (>41 mg of prednisone required daily with or without a response).

The primary objective of this study is exploratory, because we are looking for the best model for prediction and do not want to limit ourselves to 1 class of models. In order to protect ourselves from creating a model that appears to predict the response when in fact there is no real relationship between the predictors and the response, we will first test for a significant Spearman correlation between GC responsiveness and ERR to make sure that there is a significant effect. If there is a significant correlation, then we have some comfort that our final model is not just modeling noise. For our sample size justification (section 6.2), we assume that if we have a large enough sample size to show a significant correlation between GC responsiveness and 1 of the continuous predictive variables, then we would have a sample size large enough to explore these models. Because our objective is to find a reasonable model, and sample sizes are limited by availability of subjects, the sample size calculation is mostly to ensure that with such small sample sizes we would be able to detect moderate (i.e., correlations of about 0.5) effects.

6.1.1. Appropriate Methods and Timing for Analyzing Outcome Measures

Outcome measures will be measured using weekly or bi-weekly eosinophil count assessments at the subject's home physician office. Outcome measures will be assessed once 30% of subjects have been enrolled and challenged, and again at completion of the final subject enrollment.

6.1.2. Addressing Study Objectives

Although the primary objective is an exploratory model building, we do consider the simple null hypothesis that there is no correlation between % change in eosinophil count at 4 and 24 hours after challenge and GC response. Regardless of whether that test is significant or not, we will attempt to predict the GC response through the model building strategy described in the Section 6.1 on Description of the Analyses.

6.2. Sample Size Justification

The sample size of this study is practically limited by the number of subjects available for study. Currently, about 30-35 new hypereosinophilic subjects per year are seen at the NIH and enrolled on protocol 94-I-0079, of which approximately 10% would meet inclusion criteria for this protocol. Consequently, we expect that we can enroll 30-40 subjects in this study over a 13-year period.

To show that this number of subjects is not unreasonably small, we calculate the sample size for a Spearman's correlation coefficient. If true correlation is 0.5 between a predictor variable (ERR at 4 or 24 hours) and the GC responsiveness (see Description of the Study, Section 3.1), the total number of subjects needed to show that the correlation is different from zero at the two-sided 0.05 level with 80% power is about 32 subjects. We calculated this by simulating 10,000 data sets of 32 bivariate random normal variables with correlation 0.5 and seeing that the Spearman coefficient is significantly different from zero 79.5% of the time. This is similar to the usual sample size calculation for Pearson's correlation(17) or (18) that gives a sample size of 29 for the same situation.

Because the predictor and outcome variables are both continuous, if the true change in the dependent variables is 0.5 standard deviations per 1 standard deviation change in the independent variable, a clinically important measurement of association between response to a steroid challenge and final required dose of GC will be possible.

6.3. Final Analysis Plan

1. Study Endpoints:

- Primary: GC responsiveness.
  - Definition: See primary endpoint for definition of GC responsiveness.
  - Validity and Reliability: This is a rare disease, and standard care for this disease is still being developed. There is no standard endpoint, and this endpoint has not been used before. One of the purposes of this study is to see if this endpoint can be reliably predicted. The endpoint has face validity because the dose that controls eosinophil concentration is useful for determining the extent of a subject's disease and their responsiveness to steroid therapy. We will first try and predict GC responsiveness as described in section "Description of the Analyses" (Section 6.1), but may also try to predict the categorization of the GC responsiveness into 3 ordered categories described in section "Description of the Analyses".
  - Predictor Variables: All predictor variables will be measured in the first 24 hours after initial dose, and will be blinded to the PI and Accountable Physician. These variables will be used to predict the GC responsiveness:

percent change in eosinophil count from baseline to 4 and 24 hours and steroid absorption.

- Secondary:
    - Classification of subjects by GC response for assessment of clinical factors associated with GC responsiveness (including HES variant, pattern of organ involvement and laboratory parameters)
- Exploratory
    - Exploratory analyses of potential mechanisms and in vitro correlates of GC resistance, including impaired steroid absorption, cell surface markers on lymphocytes, intracellular and serum cytokine profiles.

2. Description of the Statistical Methods to Be Employed:
    - Spearman correlation of GC responsiveness and percent change in eosinophil from baseline to 4 and 24 hours, with confidence intervals and testing whether it is different from zero.
    - Model building as described in section “Description of the Analyses” (Section 6.1).

3. Level of Significance to be Used*:* Two-sided 0.05

4. Exploratory Analyses: See section 6.1 for primary objective. Similar model building strategies will be used for secondary endpoints and exploratory endpoints.

5. Handling of Missing and Spurious Data: We have defined the primary endpoint so that there should be little missing data. For example, subjects that are unable to control their eosinophil counts with 30-60 mg prednisone will still have a defined endpoint and their results will be included in the analysis. Based on prior experience with this subject cohort, we anticipate an extremely low dropout rate (<5%/year).

6.4. Interim Analysis Plan

Due to the rare nature of the disease, various investigators have asked whether an interim analysis would be appropriate in this rare population. Since approximately two thirds of the projected subjects have completed the study, we are able to perform an interim analysis using ERR to predict GC responsiveness. Although this is an additional analysis, since the original final analysis plan allowed for an unspecified number of different models, this does not change the overall analysis plan in any substantial way. Nevertheless, trying many models may result in an overfit final result, therefore, for the interim analysis we will try only one flexible model.

We use a linear model to predict the GC responsiveness using either a first, second or third order polynomial of log(ERR) at 4 hours in the model and the final end-prednisone dose of each subject as the GC response as defined in section 3.5.1. The choice between the three models will be made by the one with minimum AIC (Akiake

information criteria). To evaluate the prediction, we will use a leave-one-out cross validated estimate of R squared, where for each fit with a point left out we use the minimum AIC model of the three. Because the entire model finding procedure is cross validated, the estimate of R squared will not be biased upward by using the lowest AIC from the three models. As in the final analysis plan, to test for significance between log(ERR) and GC responsiveness, we test whether the Spearman (i.e., rank) correlation is significantly different from zero, and calculate estimates and associated confidence intervals for Spearman's correlation.

If the interim model appears to explain the variation of the GC responsiveness (i.e., has a large cross validated R squared), then we can use the interim model to predict the GC responsiveness of the subsequent subjects that enter the study as a validation cohort. Because we expect few additional subjects (<15), our validation would only be able to detect extreme departures from the model. We test for extreme departures from the model by creating a 95% prediction interval and testing whether we can show that significantly less than 95% of the subsequent subjects fall within that prediction interval. For all sample sizes 5 or greater, we have at least 80% power to show that the model fails (the 95% prediction interval covers less than 95% of subsequent responses) if the true proportion that fall within the 95% prediction interval is 50% or less. It is possible that the prediction intervals from the model are so wide that they will not be clinically useful, perhaps as a result of insufficient subjects enrolled, in which case the study will continue since the secondary and exploratory outcomes of the study will continue to be useful for studies of GC effects on eosinophils.

7. Human Subjects Protection

7.1. Study Population

7.1.1. Rationale for Subject Selection

New and returning subjects enrolled on Protocol #94-I-0079 who have documented HES, AEC >1500/μL on ≤ 10 mg prednisone equivalent daily and are candidates for GC therapy will be recruited for this protocol. Subjects receiving higher prednisone doses at baseline will be excluded to maximize the likelihood that the steroid challenge will have observable effects. Subjects with chronic eosinophilic leukemia, including those with PDGFR-associated disease, will also be excluded since imatinib is first line therapy for this condition.

7.1.2. Recruitment Plan

Individuals contacting the investigators for evaluation of HES as well as subjects already enrolled in Protocol #94-I-0079 (Eosinophil Activation and Function in Parasitic Infections and other Conditions with Increased Tissue or Blood Eosinophilia in Humans) will be considered for participation in this protocol.

7.2. Justification for Eligibility of Special Populations

7.2.1. Exclusion of Pregnant Women

Subjects who are pregnant, including those for whom GC are clinically indicated, will be excluded from participation in this study because of the increased risk of gestational diabetes in the setting of prolonged GC exposure in pregnancy. These subjects will be referred to their referring/treating physician for management.

7.2.2. Exclusion of Children Weighing <48 kg

Children younger than 18 years of age weighing <48 kg will be excluded from the study due to limitations of maximal blood draw over time for research purposes.

7.2.3. Inclusion of Children Weighing ≥48 kg

Children younger than 18 years of age weighing ≥48 kg will be eligible to enroll in this study because HES is a genetic disease that can affect children. Therefore, there is important information about this disease and the use of GCs as treatment to be learned from pediatric participants. Additionally, children stand to benefit from this study the same as adults.

7.3. Potential Risks

Venipuncture

The drawing of blood may be associated with discomfort, occasional bruising at the site of puncture, and rarely, fainting. All blood will be drawn from arm veins.

IV line insertion

The risks of having an IV inserted including pain, bruising, swelling, and in rare instances, fainting, thrombosis, or infection.

Prednisone

Prednisone (or its equivalent) is commonly used to treat a wide variety of disorders, including HES. The common side effects of prednisone are well-described and include increased appetite, insomnia, fluid retention, indigestion, headache, facial swelling, menstrual irregularities, and visual changes. When used over extended periods of time, GCs (such as prednisone) may cause weight gain, cataracts, myopathy, osteoporosis, glucose intolerance, increased risk of infections such as *P. jiroveci*, and can affect mood. Abrupt cessation of prednisone may cause headaches, vomiting, hypotension, fatigue, and in severe cases, may result in loss of consciousness and even death. If participants become severely ill or need to undergo surgery during the prednisone taper, their doses may need to be increased termporarily. There is evidence that participants who take high doses of prednisolone may secrete the steroid in breast milk. Waiting to breastfeed for 3-4 hours after receipt of prednisolone reduces this risk. In any

case, there are no known effects of corticosteroids in breastfed infants. Participants on long-term dosing will be monitored closely for these side effects. In addition, for subjects that will be on greater than 30-mg prednisone for 3 weeks or longer (ie those on the increased 60-mg dose), standard *P. jiroveci* prophylaxis will be instituted. Similarly, evidence-based approaches for GC bone loss prevention or treatment will be followed according to the latest ACR 2010 recommendations for prevention of GC induced osteoporosis(19, 20).

7.4. Potential Benefits

Since the study is designed to evaluate the degree of response to GC's in the treatment of hypereosinophilic syndrome, subjects may or may not benefit from therapy. The information learned from this study may improve understanding of how GCs can be used to treat HES, which may lead to improvements in clinical care of these patients.

7.5. Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers that begins before consent is given and continues until the end of the subject'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. Subjects will be given the opportunity to ask questions and have them answered.

Subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject's medical record. The informed consent process will emphasize that the quality of subjects medical care will not be adversely affected if they decline to participate in this study.

7.5.1. Assent of Children

Where deemed appropriate by the clinician and the child's parent(s) or guardian, the child will also be included in all discussions about the trial and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts and benefits of participation. Verbal assent will be obtained as appropriate for children ages 8 years and older. Children under the age of 18, but who are age 8 or older will be asked to sign an age appropriate assent form. Children under the age of 8 will not be required to provide assent as they typically do not have the ability to fully understand the nature of research. The consent/assent process will be documented in the child's medical record, including the assessment of the child's ability to provide assent (verbal versus written) as applicable. All children will be contacted

after they have reached the age of 18 to determine whether they wish to continue on the trial and informed consent will be obtained from them at that time.

7.5.2. Consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

If reconsent is not feasible, we request waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.

Requirements for Waiver of Consent consistent with pre-2018 45 CFR 46.116 (d):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
(3) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

7.5.3. Telephone Consent Process

Subjects with HES will be screened for this study on Protocol 94-I-0079. If for any reason a subject chooses not to or is unable to sign the Informed Consent during a scheduled visit on Protocol 94-I-0079 but he/she chooses to enroll on this protocol, permission to consent subjects over the telephone may be permitted. In these instances, permission to email, mail or fax the consent document will be obtained from the subject. Once consent is received, the subject is instructed to call the investigator for discussion of the study and consent document. The study will be thoroughly explained with ample time for questions or concerns related to participation. Informed consent will be obtained by the principal investigator or a designated associate

investigator on this protocol. The subject will identify him/herself by name and date of birth, and state that he/she gives consent to participate in this study. The staff member will verify the subject's name and the verbal consent to participate. Upon completion of the telephone call, the manner of obtaining consent and the names of the person administering and providing consent will be documented in the medical record, signed and dated. The subject must sign and date the consent in his/her possession. This signed and dated consent must be sent to the investigator. No testing will occur without this consent. Once received, the consent will be checked for accuracy and signed by the NIH investigator/designee. The signed consent will then be filed in the medical record.

7.6. Subject Confidentiality

All records will be maintained in a locked file in the Clinical Center and/or in the Medical Records Department of the NIH where they are accessible only to authorized personnel.

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 OHRP.

8. Adverse Event Reporting Plan

8.1. Specification of Safety Parameters

HES is a complex multisystem disorder. Exacerbations of symptoms or signs related to eosinophilia are expected to occur during this protocol and will be recorded, but not reported as AEs. Similarly, side effects of GC are well-described (see section 7.3) and are expected to occur during this protocol. Although GC administration at the doses to be used in this study is considered standard of care for HES, we will record but not report disease-related AEs, and record and report all research-related AEs (including expected events from the GCs) per NIH Human Research Protections Program (HRPP) Policy 801 described in section 8.3.

8.2. Reporting Procedures

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

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

8.3. Type and Duration of the Follow-up of Subjects after Adverse Events

Subjects who have AEs or SAEs will be followed per standard clinical practice until their condition stabilizes or the AE resolves. AEs and other reported events are defined in HRPP Policy 801.

Subjects who withdraw from the protocol as a result of an AE or SAE will be followed on Protocol #94-I-0079.

8.4. Assessing Adverse Events

All laboratory and clinical AE's that occur in a subject will be assessed for severity and classified into one of the categories below:

- **Graded 1 (Mild):** Event requires minimal or no treatment and does not interfere with the patient's daily activities.
- **Grade 2 (Moderate):** Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Grade 3 (Severe):** Event interrupts a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating
- **Grade 4 (Life Threatening):** Any adverse drug experience that places the patient or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
- **Grade 5: (Death)**

8.5. Relationship Assessment

For all collected AE's the clinician who examines and evaluates the subject will determine the adverse event's causality based on the temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below.

Unrelated: Clearly not related to research

Unlikely: Doubtfully related to research

Possible: May be related to the research

Probable: Likely related to the research

Definite: Clearly related to the research.

9. Data and Safety Monitoring and Management Plan

Study data will be collected at the study site(s) and maintained on an electronic data system (CRIMSON). These forms or systems are to be completed on an ongoing basis during the study. Data entered into electronic data systems shall be performed by authorized individuals. Corrections to electronic data systems shall be tracked electronically (password protected or through an audit trail) with time, date, individual making the correction, and what was changed.

The Investigator is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected and must be signed and dated by the person recording and/or reviewing the data. Source documents include all recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the subject's medical records, laboratory reports, ECG tracings, x-rays, radiologist's reports, subject's diaries, biopsy reports, ultrasound photographs, progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the study. Data from CRIMSON Data System will be collected directly from subjects during study visits and telephone calls or will be abstracted from subjects' diaries/memory cards and medical records. The subject's medical record must record his/her participation in the clinical trial and, study treatment/vaccination (with doses and frequency) or other medical interventions or treatments administered, as well as any adverse reactions experienced during the trial.

Clinical data will be collected during the initial evaluation and at subsequent follow-up visits and will be stored using the CRIMSON database using a pre-specified Case Report Form (CRF).

9.1. Study Records Retention

The investigator is responsible for retaining all essential documents listed in the International Council for Harmonisation Good Clinical Practice Guideline. All essential documentation for all study subjects are to be maintained by the investigators in a secure storage facility for a minimum of three years per NIAID policies. These records are also to be maintained in compliance with IRB, state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator must provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID must be notified in writing and written NIAID/OCRPRO permission must be received by the site prior to destruction or relocation of research records.

10. Protocol Monitoring Plan

Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor several aspects of the study in accordance with the appropriate regulations and the approved protocol. Only pediatric subjects will be monitored and the objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the IC process for each monitored pediatric subject; 2) to verify AEs and SAEs, including the prompt reporting of all SAEs; 3) to compare applicable CRIMSON data abstracts 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 investigator (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts and pertinent hospital or clinical records) readily available for inspection by the local IRB, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

The study team is responsible for monitoring data collected from our adult population.

11. Plan for Research Use and Storage of Human Samples

11.1. Intended Use

Samples and data collected under this protocol may be used to study mechanisms of responses to GCs.

11.2. Storage and Tracking

Serum and plasma will be stored in a –80^{o}C freezer, cells in liquid nitrogen, and tissue samples at room temperature or frozen as appropriate. Access will be limited using locked rooms or freezers depending on the type of sample. Stored research samples will be labeled with a code that only the study team can link to the participant. The coded samples may be sent for research purposes to other investigators. Data will be kept in password-protected computers. Only investigators or their designee(s) will have access to the samples and data.

Samples will be tracked using BSI-II (Biological Specimen Inventory) v.7.2. Samples sent to the NCI at Frederick Central Repository for processing after blood draw will be stored in the same freezers after receipt and assignment of a unique code.

11.3. Disposition at the Completion of the Protocol

In the future, other investigators (both at NIH and 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 and linked samples or data and getting results that they can link back to their subjects.

11.4. Reporting Loss or Destruction of Samples/Specimens/Data to the IRB

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, and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIH IRB.

12. Remuneration Plan for Subjects

No remuneration will be provided for participating in the study.

Appendix A: Definition of Hypereosinophilic Syndrome

Historically, HES has been defined according to the original Chusid's criteria (21): sustained peripheral blood eosinophilia of unknown origin, exceeding 1500/µl for more than 6 consecutive months, and responsible for the development of organ dysfunction and/or damage.

More recently the definition of HES has been refined as follows, and will be used for the purposes of this protocol (22).

1. Blood eosinophilia of greater than 1500/µL on at least 2 occasions or evidence of prominent tissue eosinophilia associated with symptoms and marked blood eosinophilia

2. Exclusion of secondary causes of eosinophilia, such as parasitic or viral infections, allergic diseases, drug-induced or chemical-induced eosinophilia, hypoadrenalism, and neoplasms

Appendix B: Schedule of Procedures/Evaluations

Evaluation	Baseline/ H&P	GC Challenge				GC Taper Weekly	End of Study Visit
		0 hour	2 hour	4 hour	24 hour		
Informed Consent	X						
Demographics (update CRIS and CRIMSON)	X						
H&P and Medication History	X						X
Clinical Assessment (Vital signs and weight, Symptom evaluation (HES and steroid related)	*X	*X			X	#X	X
Acute care, mineral and hepatic panels	X	X			X		X
CBC with differential	X	X	X	X	X	X	X
PT/PTT, INR	X						
Flow cytometry	X						X
Urinalysis	X						
Pregnancy Testing – Serum Beta Hcg	X						
Pregnancy Testing – Urine Hcg		X					
Cortisol		X					
Research blood	X	X	X	X	X		X

* Symptom evaluation to be performed at baseline/H&P or prior to dosing of steroids

Weekly taper for assessments not completed at NIH will not include vital signs and weight.

Appendix C: Adult Blood Volumes for Specimen Collection

Evaluations	**Study Schedule**									
[1] Visit	**Baseline H&P**	**GC Challenge**				**Weekly draws**		**Weekly draws**	**Approx. max # of weeks**	**End of Study Follow-up [2]**
		0 hr	**2 hr**	**4 hr**	**24 h**					
Week of Study		1	W1	W 1	W 1	W 1	W 2-8	W 9-16	W 16-24	W 16-24
Day of Study	X	D 1	D1	D 1	D 2					
Clinical										
CBC, differential, platelets	3 mL	3 mL	3mL	3 mL	3 mL	5 mL	5 mL	5 mL	5 mL	3 mL
PT, aPTT, INR	4.5 mL									
β-HCG	4 mL	4 mL								
Acute care, mineral and hepatic	4 mL	4 mL			4 mL					4 mL
Flow cytometry	6 mL									6 mL
Cortisol		4 mL								
Research										
PBMC Eosinophil & Plasma Storage	60 mL	50 mL	30 mL	30 mL	60 mL					60 mL
Serum Storage	8 mL	8 mL		8 mL	8 mL					8 mL
RNA PAX		3 mL		3 mL	3 mL	3 mL				3 mL
Volume (mL)**	**89.5 mL**	**76 mL**	**33 mL**	**44 mL**	**78 mL (177.5)****	**43 (5 mL x 8 wks)+3**		**40 (5 mL x 8 wks)**	**45 (5 mL x 9 wks)**	**120 (45 mL + follow up)**
[3] Cumulative 8 week Volumes (mL)						**363.5**		**40**	**45**	**165**

[1] Visit Windows: Screening may be completed over 2 visits. Other visits and permitted windows are as follows: [2] Baseline within 90 days of GC challenge. Follow up 2-4 weeks after completion of taper (window +/- 1 week). Weekly calls/draws within 3 days of timepoint [3]Per NIH MEC Policy M95-9, maximum blood volumes drawn for *research purposes* for an *adult* subject (aged 18 years or older) may not exceed: 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. Exceptions to this policy shall be approved by the IRB. ** Denotes 24 hour volume

[4] Day X evaluations are the baseline for subsequent safety assessments. Day X evaluations must take place within 90 days prior to GC challenge. A CBC with differential must be available within 14 days of the enrollment.

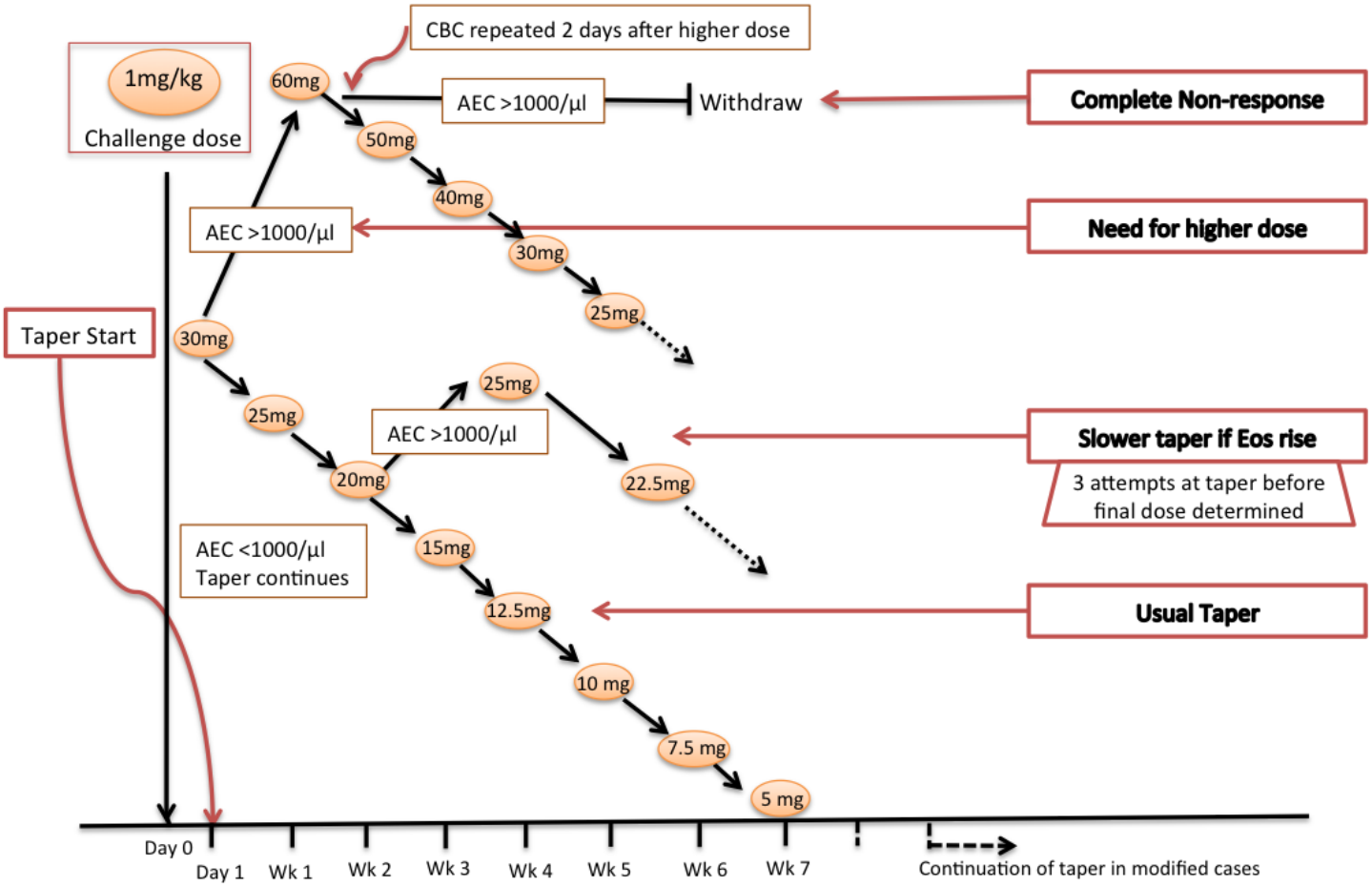
Appendix D: Pediatric Blood Volumes for Specimen Collection

Evaluations	**Study Schedule**								
(1) Visit	**Baseline H&P**	**GC Challenge**			**Weekly draws**		**Weekly draws**	**Approx. max # of weeks**	**Follow-up (2)**
		0 hr	**4 hr**	**24 hr**					
Week of Study	-1	1	W 1	W 1	W 1	W 2-8	W 9-16	W 16-24	W 16-24
Day of Study	X	D 1	D 1	D 2					
Clinical									
CBC, differential, platelets	3 mL	3 mL	3 mL	3 mL	5 mL	5 mL	5 mL	5 mL	3 mL
PT, aPTT, INR	4.5 mL								
β-HCG	4 mL	4 mL							
Acute care, mineral and hepatic	4 mL	4 mL		4 mL					4 mL
Flow cytometry	6 mL								6 mL
Cortisol		4 mL							
Research									
PBMC Eosinophil & Plasma Storage	30 mL	20 mL	30 mL	30 mL					30 mL
Serum Storage	4 mL	4 mL	4 mL	4 mL					4 mL
RNA PAX		3 mL	3 mL	3 mL	3mL				3 mL
Volume (mL)**	**55.5**	**42**	**40 mL**	**44 mL (163)****	**43 (5 mL x 8 wks)+3**		**40 (5 mL x 8 wks)**	**45 (5 mL x 9 wks)**	**95 (45 mL + follow up)**
(3) Cumulative 8 week Volume (mL)					**260.5**		**40**	**45**	**105**

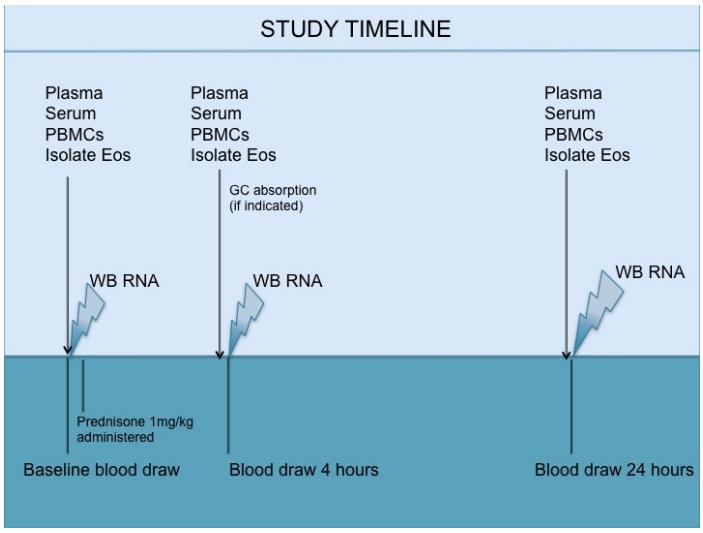
[1] Visit Windows: Screening may be completed over 2 visits. Other visits and permitted windows are as follows: (2) Baseline within 90 days of GC challenge. Follow up 2-4 weeks after completion of taper (window +/- 1 week). Weekly calls/draws within 3 days of timepoint [3]Per NIH MEC Policy M95-9, maximum blood volumes drawn for *research purposes* for *pediatric* subjects: no more than 5 mL/kg in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. Exceptions to this policy shall be approved by the IRB. ** Denotes 24 hour volume

[4] Day X evaluations are the baseline for subsequent safety assessments. Day X evaluations must take place within 90 days prior to GC challenge

Appendix E: Steroid Taper Schedule



Appendix F: Exploratory Endpoints Timeline



1) All subjects will be evaluated for GC absorption 4 hours after GC dosing by evaluation of blood prednisone and prednisolone levels and assessment of pharmacokinetics.

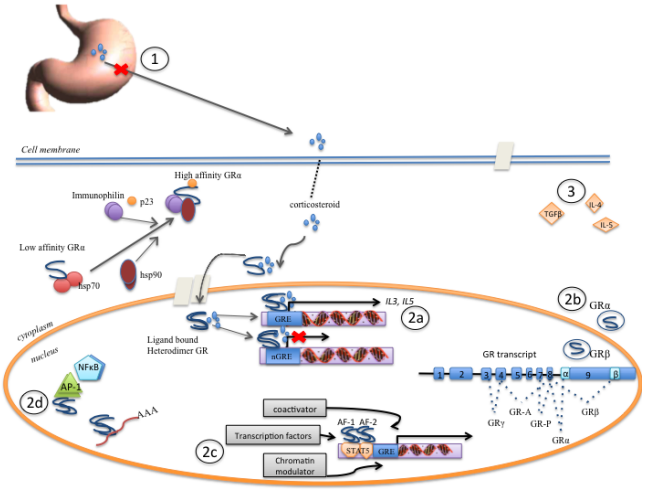
2) All subjects will have RNA extracted from whole blood and frozen for evaluation of changes in expression after dosing with GC. Results will be evaluated using microarray technology once all subjects have been enrolled.

3).GR isoforms will be performed by RT-PCR

4) Additionally, plasma, PBMC's and eosinophil RNA will be stored for further studies not described above.

Appendix G: Figures

Figure 1: Glucocorticoid Action



1 – GC absorption through the gastrointestinal tract

2a –Cytoplasmic GRα becomes ligand bound and enters the nucleus where it undergoes homo- or heterodimerization. The GC/GR complex binds to glucocorticoid response elements (GREs) leading to activation or repression of cytokine transcription

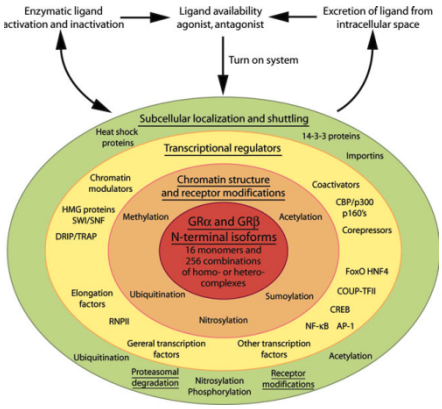
2b – Alternative splicing of the GR transcript into GRα and GRβ as well as alternate transcript forms. GRβ is primary localized in the nucleus, whereas GRα is cytoplasmic before it binds ligand.

2c – The formation of the transcriptional complex: involvement and interaction of transcription factors, co-activators, and chromatin modulators modulate the AF-1 and AF-2 portions of the GR complex.

2d – Post-transcriptional regulation mechanisms including GC/GR complex binding to transcription factors AP-1, NFκB, and cyclic AMP response element binding protein (CREB) and GR binding to mRNA(23)

3 – Cytokine expression profile/footprint in GC resistance

Figure 2: Altered GC receptor signaling



From: Kino, T, Chrousos GP. Intracellular Glucocorticoid Signaling: A Formerly Simple System Turns Stochastic. Science Oct 2005(12).

Scientific References

1. Ogbogu PU, Bochner BS, Butterfield JH, Gleich GJ, Huss-Marp J, Kahn JE, et al. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. J Allergy Clin Immunol. 2009;124(6):1319-25 e3.
2. Prin L, Lefebvre P, Gruart V, Capron M, Storme L, Formstecher P, et al. Heterogeneity of human eosinophil glucocorticoid receptor expression in hypereosinophilic patients: absence of detectable receptor correlates with resistance to corticotherapy. Clin Exp Immunol. 1989;78(3):383-9.
3. Hamilos DL, Leung DY, Muro S, Kahn AM, Hamilos SS, Thawley SE, et al. GRbeta expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone. J Allergy Clin Immunol. 2001;108(1):59-68.
4. Christodoulopoulos P, Leung DY, Elliott MW, Hogg JC, Muro S, Toda M, et al. Increased number of glucocorticoid receptor-beta-expressing cells in the airways in fatal asthma. J Allergy Clin Immunol. 2000;106(3):479-84.
5. Leung DY, Hamid Q, Vottero A, Szefler SJ, Surs W, Minshall E, et al. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. J Exp Med. 1997;186(9):1567-74.
6. Webster JC, Oakley RH, Jewell CM, Cidlowski JA**.** Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci U S A. 2001;98(12):6865-70.
7. Oakley RH, Cidlowski JA**.** Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. J Biol Chem. 2011;286(5):3177-84.
8. Hakonarson H, Bjornsdottir US, Halapi E, Bradfield J, Zink F, Mouy M, et al. Profiling of genes expressed in peripheral blood mononuclear cells predicts glucocorticoid sensitivity in asthma patients. Proc Natl Acad Sci U S A. 2005;102(41):14789-94.
9. Aceves SS, Newbury RO, Chen D, Mueller J, Dohil R, Hoffman H, et al. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. Allergy. 2010;65(1):109-16.
10. Spahn JD, Szefler SJ, Surs W, Doherty DE, Nimmagadda SR, Leung DY**.** A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity. J Immunol. 1996;157(6):2654-9.
11. Liu W, Liang Q, Balzar S, Wenzel S, Gorska M, Alam R**.** Cell-specific activation profile of extracellular signal-regulated kinase 1/2, Jun N-terminal kinase, and p38 mitogen-activated protein kinases in asthmatic airways. J Allergy Clin Immunol. 2008;121(4):893-902 e2.
12. Kino T, Su YA, Chrousos GP**.** Human glucocorticoid receptor isoform beta: recent understanding of its potential implications in physiology and pathophysiology. Cell Mol Life Sci. 2009;66(21):3435-48.

13. Irusen E, Matthews JG, Takahashi A, Barnes PJ, Chung KF, Adcock IM. p38 Mitogen-activated protein kinase-induced glucocorticoid receptor phosphorylation reduces its activity: role in steroid-insensitive asthma. J Allergy Clin Immunol. 2002;109(4):649-57.
14. Khoury P, Stokes K, Gadkari M, Makiya MA, Legrand F, Hu Z, et al. Glucocorticoid-induced eosinopenia in humans can be linked to early transcriptional events. Allergy. 2018;73(10):2076-9.
15. To Y, Ito K, Kizawa Y, Failla M, Ito M, Kusama T, et al. Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2010;182(7):897-904.
16. Burnham KP, and D. R. Anderson. Model Selection and multimodel inference: a practical information-theoretic approach. 2nd ed. New York: Springer-Verlag; 2002.
17. Hulley SB CS, Browner WS, Grady DG and Newman TB. Designing Clinical Research. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
18. Lachin JM. Introduction to sample size determination and power analysis for clinical trials. Control Clin Trials. 1981;2(2):93-113.
19. Chrousos GP, Kino T. Intracellular glucocorticoid signaling: a formerly simple system turns stochastic. Science's STKE : signal transduction knowledge environment. 2005;2005(304):pe48.
20. Grossman JM, Gordon R, Ranganath VK, Deal C, Caplan L, Chen W, et al. American College of Rheumatology 2010 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Care & Research. 2010;62(11):1515-26.
21. Chusid MJ, Dale DC, West BC, Wolff SM. The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. Medicine. 1975;54(1):1-27.
22. Simon HU, Rothenberg ME, Bochner BS, Weller PF, Wardlaw AJ, Wechsler ME, et al. Refining the definition of hypereosinophilic syndrome. The Journal of allergy and clinical immunology. 2010;126(1):45-9.
23. Umland SP, Schleimer RP, Johnston SL. Review of the molecular and cellular mechanisms of action of glucocorticoids for use in asthma. Pulm Pharmacol Ther. 2002;15(1):35-50.