Abbreviated Title: Tyrosine kinase inhibition in HES

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Title: Efficacy of tyrosine kinase inhibition in reducing eosinophilia in patients with myeloid and/or steroid-refractory hypereosinophilic syndrome

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Investigational Agents:

Drug Name:	imatinib
IND Number:	N/A
Sponsor:	none
Manufacturer:	Varied (generic)

Drug Name:	ruxolitinib
IND Number:	N/A
Sponsor:	none
Manufacturer:	Incyte

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and the following:

- United States 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; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title: Efficacy of tyrosine kinase inhibition in reducing eosinophilia in patients with myeloid and/or steroid-refractory hypereosinophilic syndrome

Study Description: The purpose of this study is to evaluate the efficacy of the tyrosine kinase inhibitors, imatinib mesylate and ruxolitinib in reducing peripheral blood eosinophilia in patients with myeloid and/or steroid-refractory hypereosinophilic syndrome (HES). Patients with HES who meet a set of criteria designed to select patients with the myeloid form of the disease, as well as patients without myeloid disease who are refractory to steroid therapy for HES, will be eligible. Imatinib mesylate will be initiated at a dose of 400 mg daily, the FDA-approved dose for the treatment of chronic myelogenous leukemia (CML), in participants with a known imatinib-sensitive mutation and in participants with myeloid features without a *JAK* mutation. If patients fail imatinib or are steroid-resistant and do not have myeloid features, ruxolitinib therapy will be initiated at a dose of 15 mg twice daily. Patients who demonstrate a clinical and hematologic response at 1 (imatinib arm) or 3 (ruxolitinib arm) months will be eligible to continue therapy. In patients who demonstrate a complete clinical and hematologic response to imatinib therapy (including those who received imatinib prior to enrollment), imatinib may be discontinued. Patients in complete remission for ≥5 years following imatinib are considered cured and will be taken off protocol. Patients receiving ruxolitinib who demonstrate drug-related toxicity and/or a sustained clinical response to ruxolitinib for >2 years may undergo dose descalation.

Objectives: Primary Objective: To assess the efficacy of tyrosine kinase inhibition using imatinib and/or ruxolitinib in reducing peripheral blood eosinophilia in patients with HES.

Secondary Objectives:

1. Assess the durability of hematologic response to imatinib and ruxolitinib.
2. Identify predictors of response to imatinib mesylate and ruxolitinib in *PDGFRA*-negative HES.
3. Determine the cellular and molecular targets of imatinib mesylate and ruxolitinib in *PDGFRA*-negative HES.
4. Evaluate the effects of imatinib mesylate and ruxolitinib on the clinical manifestations of HES.
5. Determine the most appropriate dosing regimen and length of imatinib therapy in *PDGFRA*-positive and *PDGFRA*-negative HES.
6. Determine the most appropriate dose of ruxolitinib for the treatment of HES

Endpoints:	Primary Endpoint: Peripheral blood absolute eosinophil count (AEC) at 1 month (imatinib) or 3 months (ruxolitinib) following initiation of therapy. Secondary Endpoints: 1a. Peripheral blood AEC at 3 months (imatinib), 6, 9, and 12 months (imatinib and ruxolitinib). 1b. Percent of patients who reach an eosinophil count below 1500/mm^3 at 1, 3, 6, 9, and 12 months. 2. Identification of laboratory or clinical findings that predict response to imatinib and/or ruxolitinib therapy. 3. Identification of cellular and molecular targets of imatinib mesylate and ruxolitinib in *PDGFRA*-negative HES. 4. Collection of standardized longitudinal data on disease activity and outcome in patients receiving imatinib or ruxolitinib therapy for HES. 5a. Duration of remission following cessation of imatinib therapy. 5b. Determination of the relationship between the dose and duration of imatinib treatment and the probability of cure (complete remission off therapy for ≥5 years). 6. Determination of the minimum effective dose of ruxolitinib treatment in patients with HES
Study Population:	80 children and adults, aged >2 years (imatinib) and ≥18 years of age (ruxolitinib)
Phase:	2
Description of Sites/Facilities Enrolling Participants:	The NIH Clinical Center will be the only site enrolling participants.
Description of Study Intervention:	Imatinib mesylate 100-400 mg orally (po) daily or ruxolitinib 15 mg po daily.
Study Duration:	30 years
Participant Duration:	Since there are currently no guidelines for length of therapy with imatinib mesylate, drug administration for an individual patient may be continued until the termination of the protocol if there is clinical benefit to the patient and no contraindication to the continuation of therapy. Participants who remain in remission for 5 years following discontinuation of imatinib are considered "cured" and will be taken off protocol. For participants receiving ruxolitinib, drug administration for an individual patient may be continued for up to 7 years or until January 2027 (whichever occurs first) if there is clinical benefit to the patient and no contraindication to the continuation of therapy.

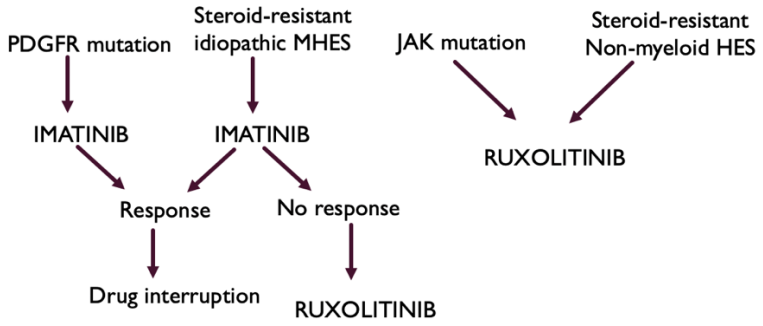
1.2 Schema

Prior to Enrollment

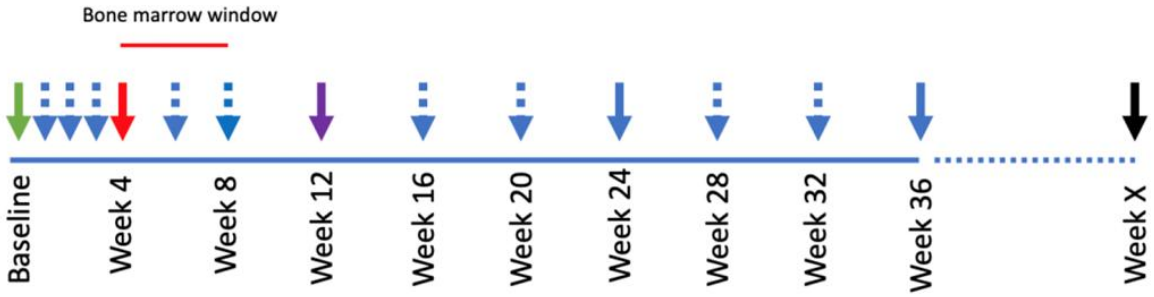
Screen potential participants by inclusion and exclusion criteria; obtain history, document.

Baseline

Obtain informed consent; assign to imatinib or ruxolitinib arm as indicated below; perform baseline studies as listed in Section 1.3 Schedule of Activities.



Study timeline



Green arrow: baseline procedures per section 1.3 SOA and initiation of study drug.
Dashed blue arrows: NIH or local lab testing per section 1.3 SOA.
Red arrow: primary endpoint (imatinib) visit with procedures per section 1.3 SOA.
Purple arrow: primary endpoint visit (ruxolitinib) and assessment of clinical response (both arms) per section 1.3 SOA.
Solid blue arrows: follow-up visits at NIH with procedures, including molecular testing and end organ assessment, per section 1.3 SOA.
Note: the frequencies of testing and visits after week 48 are variable, depending on response and stability of study drug dosing.
Black arrow: end-of-study visit with procedures per section 1.3 SOA.

1.3 Schedule of Activities

	Screening Visit[a]	Baseline Visit	Study Visit 2, Week 4-8[b] ±7 days	Study Visit 3, Week 12 ±7 days	Study Visit 4, Week 24 ±7 days	Study Visit 5, Week 36 ±7 days	Study Visit 6, Week 48 ±7 days	Follow-up Visits, Post-Week 48[c] ±28 days	Final Study Visit ±7 days
Informed consent	X								
Demographics	X								
Medical history	X	X	X	X	X	X	X	X	X
Concomitant medication review	X	X	X	X	X	X	X	X	X
AE review and evaluation	X	X	X	X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X	X	X
Vital signs, including weight	X	X	X	X	X	X	X	X	X
Height	X	X							
Study arm assignment		X							
Administer study intervention		X	X	X	X	X	X	X	
EKG	X	X	X	X	X	X	X	(X)	X
Hematology labs[d]	X	X	X	X	X	X	X	X	X
Serum chemistry labs[e]	X	X	X	X	X	X	X	X	X
PT/PTT	X								
Urinalysis	X	X	X	X	X	X	X	X	X
Tryptase, IgE, B12 labs	X	X	X	X	X	X	X	X	X
Anti-HIV 1/2 serology	X								
Hepatitis B and C screening	X								
Lymphocyte phenotyping, EOS, TBNK	X	X	X	X	X	X	X	X	X

	Screening Visit[a]	Baseline Visit	Study Visit 2, Week 4-8[b] ±7 days	Study Visit 3, Week 12 ±7 days	Study Visit 4, Week 24 ±7 days	Study Visit 5, Week 36 ±7 days	Study Visit 6, Week 48 ±7 days	Follow-up Visits, Post-Week 48[c] ±28 days	Final Study Visit ±7 days
Lipid panel (fasting)[f]	X	X		X			X		
QuantiFERON TB[f]	X								
Pregnancy test[g]	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Other assessments (e.g., immunology assays)	X	X	X	X	X	X	X	X	X
B and T cell clonality testing[h]	X						X	(X)	
Eosinophil phenotyping	X	X	X	X					
EOS purification or PBMC storage[i]	X	X	X	X	X	X	X	X	X
Serum for research and storage	X	X	X	X	X	X	X	X	X
Research urine	X	X	X	X	X	X	X	X	X
Research blood for flow cytometry[j]		X	X	X	X	X	X	X	X
Echocardiogram[k]	X	(X)		(X)	(X)	(X)	X	X	X
PFT[k]	X	(X)		(X)	(X)	(X)	X	X	X
Bone marrow procedure[l]	(X)	(X)	X						
Bone marrow samples[m]	(X)	(X)	X						
Peripheral blood testing for myeloid mutations[n]	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)

	Screening Visit[a]	**Baseline Visit**	**Study Visit 2, Week 4-8[b] ±7 days**	**Study Visit 3, Week 12 ±7 days**	**Study Visit 4, Week 24 ±7 days**	**Study Visit 5, Week 36 ±7 days**	**Study Visit 6, Week 48 ±7 days**	**Follow-up Visits, Post-Week 48[c] ±28 days**	**Final Study Visit ±7 days**
Dermatology consult[o]	X						X		
Patient photography[p]		X	X	X	X	X	X	(X)	X

Abbreviations: AE, adverse event; EKG, electrocardiogram; EOS, eosinophil; HIV, human immunodeficiency virus; IgE, immunoglobulin E; PBMC, peripheral blood mononuclear cell; PFT, pulmonary function testing; PT/PTT, prothrombin time/partial thromboplastin time; TB, tuberculosis; TBNK, T, B, and natural killer cell; X, to be performed; (X), to be performed if needed based on criteria below.

a Screening procedures will be done on protocol 94-I-0079 within −28 days of patient enrollment on protocol.
b Participants must come to NIH for required visit between Week 4 and Week 8.
c Post–Month 12, study participants will come to NIH for study visits every 3-6 months. Visit frequency will depend on the participant's clinical response and any changes to study drug.
d Complete blood count w/differential.
e Acute care panel, mineral panel, hepatic panel, C-reactive protein, troponin.
f Ruxolitinib arm ONLY.
g Human chorionic gonadotropin, serum pregnancy test, women of childbearing potential only.
h B and T cell clonality testing, at screening and annually.
i EOS purification should be done at screening, annually, and at follow-up, depending on participant's most recent absolute eosinophil count.
j Ruxolitinib arm ONLY. One 6-mL purple-top tube to Human Eosinophil Section lab for research.
k Echocardiograms and PFTs will performed at baseline (if not available from screening) and yearly thereafter in all participants. Participants with documented cardiac or pulmonary manifestations will undergo additional echocardiogram or PFTs, respectively, at 3-month intervals during the first 48 weeks of the study.
l Bone marrow aspirate and biopsy at baseline and Week 4-8 visit. If bone marrow biopsy and aspirate was performed within 3 months prior to baseline visit, slides are available for review and are of acceptable quality, and all testing as described in footnote m has been obtained, bone marrow does not need to be repeated at baseline visit.
m Bone marrow samples will be obtained for routine histopathology (including immunohistochemistry for mast cells), cytogenetics, flow cytometry, testing for myeloid mutations associated with HES and storage for additional confirmatory or diagnostic testing as needed.
n Myeloid mutations, including mutations involving *PDGFRA, PDGFRB, JAK, KIT* and *BCR::ABL* will be assessed in peripheral blood at screening or baseline. Participants with documented mutations in *PDGFRA* or *PDGFRB* will have mutational testing every 3 months. Participants with mutations involving JAK2 or other potential drivers of myeloid HES will have testing performed at 12 weeks and yearly thereafter. All testing will be performed per standard of care in the NIH Clinical Center.
o Ruxolitinib arm ONLY. Dermatology consult required at baseline (or within 30 days of baseline visit) and annually. May be performed outside the NIH Clinical Center by a credentialed dermatologist.
p Patient photography will be done on participants that have skin symptoms, to facilitate comparison of skin and other visible manifestations.

2 INTRODUCTION

2.1 Study Rationale

HES is a rare group of disorders characterized by peripheral eosinophilia (>1500 eosinophils/mm^3 on at least two occasions) that results in eosinophil-mediated end organ damage (1). HES occurs sporadically in the general population and is a heterogeneous group of disorders with several distinct clinical subtypes, including myeloid, lymphocytic, and idiopathic variants (2). Regardless of the subtype, the severity of clinical pathology in HES is felt to reflect the extent of eosinophil activation in the tissues. Eosinophil granule proteins have been demonstrated in serum of patients with HES (3) and in biopsies of affected tissues. However, neither the triggers of eosinophil activation nor those of the progression from eosinophil activation to specific tissue damage have been clearly defined to date.

Until recently, first-line therapy for patients with HES irrespective of the clinical subtype was prednisone (4). However, despite the fact that complete or partial remission is common in the short term, side effects and secondary resistance frequently limit the utility of prednisone as a long-term therapy and primary steroid resistance occurs in up to 15% of patients (4), particularly in those with features of a myeloid disorder (5). Historically, the most common second-line agents were hydroxyurea and interferon-alpha, due to case series demonstrating moderate success in decreasing eosinophilia and end organ damage in small case series of patients with HES (6–8). Other agents used in the treatment of steroid-refractory HES with varying success include cyclosporin A (9,10), methotrexate, alemtuzumab, 2-chlorodeoxyadenosine (11), vinca alkaloids (9), and chlorambucil (12). Unfortunately, all of these agents have significant toxicity and are effective in suppressing symptoms and eosinophilia in only a subset of HES patients. Whereas bone marrow or stem cell transplantation has proved curative in a small number of cases (13,14) (15,16), the morbidity and mortality associated with this approach has limited its broader utility in the treatment of patients with HES.

Drugs used to treat CML have historically been of benefit in the treatment of HES. Consequently, despite the lack of an obvious mechanism of action in this disorder, the tyrosine kinase inhibitor, imatinib mesylate, was used successfully in the treatment of small numbers of patients with steroid-resistant, clinically aggressive HES (17–20). This ultimately led to the discovery of the imatinib-sensitive *FIP1L1-PDGFRA* fusion gene present in >80% of patients with the myeloid subtype of HES and, in 2006, to the approval of imatinib for the treatment of HES in adults. Subsequent published studies have demonstrated near 100% efficacy in the induction of remission in patients with HES and mutations involving *PDGFRA* and *PDGFRB*, but highly variable results in patients with other subtypes of HES (4,21,22). Although data generated from this protocol suggest that features consistent with a myeloid neoplasm are a predictor of response in *PDGFRA/B*-negative patients with HES, the cellular and molecular targets of imatinib in these patients are unknown. Additional unmet needs include the optimal dose and duration of treatment, key factors that influence outcome following drug discontinuation, and early biomarkers of disease activity in imatinib-responsive patients without a detectable mutation.

Interleukin-5 (IL-5) is a cytokine involved in the development, survival, and activation of eosinophils. Early failure of mepolizumab and reslizumab, two biologics targeting IL-5, to demonstrate efficacy in clinical asthma trials led to the first placebo-controlled clinical trial in

HES beginning in 2004. Although mepolizumab (750 mg iv monthly) was shown to be effective as a steroid-sparing agent in 85% of patients with *PDGFRA*-negative, steroid-responsive HES and dramatically reduced peripheral blood eosinophil counts (23), it was not FDA-approved for HES until late 2020 after a phase 3 trial demonstrated reduction in disease flares using 300 mg monthly administered subcutaneously in a similar cohort of *PDGFRA*-negative, steroid-responsive HES patients (24). Although the approval of mepolizumab has dramatically changed the landscape of HES therapy for *PDGFRA*-negative, steroid-sensitive patients, studies suggest that it is less effective in the treatment of patients with steroid-refractory HES, especially those with the lymphocytic and myeloid variants (25,26). Consequently, additional agents with favorable side effect profiles are clearly needed for the treatment of patients with HES.

2.2 Background

Imatinib mesylate

Imatinib mesylate is a tyrosine kinase inhibitor that selectively inhibits *bcr-abl*, *c-kit*, and the platelet-derived growth factor receptor tyrosine kinases. Imatinib mesylate (Gleevec™, Novartis) was approved by the FDA on May 10, 2001 for use in patients with advanced phase CML and in patients in chronic phase failing first line interferon therapy, in February 2002 for the treatment of gastrointestinal stromal tumors (GIST), and in 2006 for the treatment of HES and for pediatric patients >2 years of age with Ph+ CML, Ph+ acute lymphoblastic leukemia, and GIST. Imatinib is currently available in the US as Gleevec™ and as a generic medication.

Factors initially thought to be associated with a response to imatinib mesylate in HES were the absence of detectable serum IL-5, male sex, and a hypercellular marrow consistent with myeloproliferative disease (20). In view of the data described above, we used imatinib mesylate to treat a single patient with the myeloid form of HES refractory to all standard therapies. After 1 week of therapy, his eosinophil count decreased from 5993 to 22 eosinophils/mm^3 with a concomitant resolution of his symptoms, including regression of severe mucosal ulcerations. His course was complicated only by transient neutropenia requiring interruption of the drug for 4 weeks and resumption at 300 mg po daily. We subsequently treated 6 additional patients with HES and evidence of myeloid disease with imatinib mesylate at 400 mg daily with resolution of eosinophilia within 1 week of therapy in all 6 (27). All 7 of these patients were subsequently found to have an interstitial deletion in chromosome 4 leading to the expression of an imatinib-sensitive, aberrant tyrosine kinase (*FIP1L1/PDGFRA*) (20,28). Since that time, it has been demonstrated that the *FIP1L1/PDGFRA* mutation is present in approximately 10%-15% of patients with HES (4). The percentage of HES patients who are *FIP1L1/PDGFRA*-negative despite clinical and laboratory evidence of myeloid disease is unknown.

In view of data from published studies, including ours, the FDA approved the use of imatinib mesylate for the treatment of HES/chronic eosinophilic leukemia at doses ranging from 100-400 mg daily. Despite reports of imatinib-responsiveness in up to 30% of *FIP1L1/PDGFRA*-negative patients in some series, underlying mutations have been identified in only a small number of patients. Recently published data from this protocol suggest that patients with HES and myeloid features have a 50% response rate; whereas patients who are steroid-resistant but do not have myeloid features are unlikely to respond (21). Whereas the cellular and molecular factors that predict imatinib response in patients with HES and myeloid

features remain unknown, occult genetic abnormalities in imatinib-sensitive genes likely explain response in some cases.

The *FIP1L1-PDGFRA* fusion gene is approximately 50-fold more sensitive to imatinib than *BCR-ABL*, prompting early suggestions that cure might be possible. Although molecular relapse occurred in all 5 *PDGFRA*-positive participants in a prior small dose de-escalation trial conducted as part of this protocol (27), participants had been on imatinib for a median of only 25.5 months (range 19-31 months). More recent attempts by our group and others to discontinue imatinib therapy in *PDGFRA*-positive patients suggest that cure is possible in 50%-80% of patients after prolonged remission on drug (21,29,30). The factors that determine whether a patient will be cured are incompletely understood, but based on the data from CML, likely include duration and depth of remission prior to drug discontinuation.

Ruxolitinib

Ruxolitinib (Incyte) is a tyrosine kinase inhibitor that selectively inhibits Janus associated kinases (JAK) 1 and 2. It was approved by the FDA in 2011 for use in adult patients with myelofibrosis, in 2014 for adult patients with polycythemia vera (PV), and in 2019 and 2021 for adult and pediatric patients ≥12 years of age with acute and chronic graft-versus-host disease (GvHD), respectively. Many of the patients with myelofibrosis or PV carry the *JAK2* V617F mutation or the *PCM1-JAK2* translocation (31), both of which have been described in a small subset of patients with HES (32–34). Although we do not routinely screen for mutations other than *JAK2* V617F (and this only for the past several years), we have previously identified *JAK1* or *JAK2* mutations in 7 participants on protocol 94-I-0079. Two participants had the identical mutation in exon 13 of *JAK2*, two had *JAK2* V617F, one had a *PCM1-JAK2* translocation, and two had identical mutations in JAK1 of uncertain clinical significance. One participant with a *JAK2* V617F mutation who failed multiple therapies is now stable with controlled eosinophilia on ruxolitinib. Recent case reports in the literature also suggest that JAK inhibitors may be effective in *PDGFRA*-negative HES with a *PCM1-JAK2* translocation (33,34) and steroid-resistant lymphocytic variant HES due to the involvement of the *JAK-STAT* pathway in cytokine production (35). Taken together, these findings suggest that ruxolitinib might be effective in a subset of patients with myeloid and/or lymphocytic variant HES.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Blood drawing.

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. For children under 16, a numbing medicine such as EMLA cream will be available to minimize discomfort during blood draws.

Bone marrow biopsy and aspiration.

Bone marrow biopsy and aspiration may be associated with pain and/or bleeding into the skin and soft tissues at the site of the procedure. Rarely, the area at the biopsy site can become infected, requiring antibiotic treatment. Although bone marrow biopsies will generally be done under local anesthesia, conscious sedation will be used in all children and in adult patients who are unable to tolerate an awake procedure based on the assessment of the study physician. Since

the risks of conscious sedation in children may include learning and behavioral problems, bone marrow sampling will only be performed in children when medically necessary to assess the effects of imatinib therapy.

Imatinib mesylate.

There are no absolute contraindications to the use of imatinib, and the safety profile of imatinib in HES does not appear to be different from that in CML (Gleevec Package Insert August 2022). Caution is recommended in the following situations: myelosuppression, viral or bacterial infection, liver or renal impairment, gastrointestinal disorders, predisposition to fluid retention/edema, pregnancy or breastfeeding, and concurrent administration of cytochrome P450 (CYP) 3A4 inducers, inhibitors, or substrates.

Although imatinib mesylate is generally well-tolerated, common side effects (incidence ≥30%) at the doses to be used in this study include nausea, vomiting, edema and fluid retention, muscle cramps, arthralgias, diarrhea, skin rashes, fatigue, and abdominal pain (Gleevec Package Insert August 2022). Administration as directed with a meal and a large glass of water reduces the incidence and severity of gastrointestinal side effects. In phase 2 studies of patients with chronic phase CML, grade 3 (absolute neutrophil count, ANC<1000/mm^3) or grade 4 (ANC<500/mm^3) neutropenia resulting in temporary discontinuation of therapy occurred in 25% and 8% of patients, respectively. Transaminase elevations occur less frequently, but led to the discontinuation of therapy in up to 2% of patients. Severe hepatotoxicity, including fatalities, have been described. Cardiogenic shock has been associated with initiation of imatinib in patients with high eosinophil counts and elevated serum troponin or known cardiac involvement (36,37). Prophylactic use of steroids (1-2 mg/kg) is recommended for 1-2 weeks in high-risk patients. Congestive heart failure has also been reported with chronic imatinib use in patients with other cardiac risk factors, such as advanced age or a prior history of cardiac disease. In one international phase 3 study, 0.7% of 1100 patients with chronic CML treated with imatinib (compared to 0.9% of those taking IFN and ara-C) developed congestive heart failure (Gleevec Package Insert August 2022). Other rare side effects include bullous dermatologic reactions, hypothyroidism in thyroidectomy patients on levothyroxine replacement, tumor lysis syndrome, and renal toxicity. Adequate hydration and treatment of hyperuricemia are recommended in patients with high tumor burden. An increased incidence of gastrointestinal bleeding and perforations has been described in patients with GIST. Finally, patients are recommended to exercise caution when driving or operating machinery, as some patients experience dizziness, blurred vision, or somnolence during therapy. Side effects in children receiving imatinib are similar to those in adults except that musculoskeletal pain is less frequent, edema has not been reported, and growth retardation may occur.

Imatinib has been shown to be teratogenic in rats, causing defects such as exencephaly, encephaloceles, and deformities of the skull bones. At doses comparable to a human dose of 400 mg daily, increases in fetal resorption, stillbirths, and early pup mortality were also seen. In a published retrospective analysis of pregnancy outcome in 180 women exposed to imatinib during pregnancy, abnormalities were identified in 12 infants, 3 of whom had similar complex malformations, including 3 with exomphalos (expected incidence in the general population 1 in 3000-4000 births) (38). These data suggest that although most pregnancies in women taking imatinib proceed normally, there is an increased risk of fetal malformations. Imatinib is excreted

in breast milk and breastfeeding is not recommended during imatinib therapy and for 1 month after imatinib discontinuation.

Ruxolitinib.

There are no absolute contraindications to the use of ruxolitinib. Caution is recommended in the following situations: thrombocytopenia, anemia, neutropenia, serious infections, liver or renal impairment, hyperlipidemia, pregnancy or breastfeeding, predisposition to non-melanoma skin cancer, and concurrent administration of strong CYP3A4 inducers, inhibitors, or substrates, or fluconazole. Symptom exacerbation has been reported following interruption or discontinuation of ruxolitinib, and tapering of the drug is recommended in the setting of adverse events (AEs) or disease progression.

Although ruxolitinib is generally well-tolerated, common side effects at the doses to be used in this study include thrombocytopenia and anemia (incidence >20% in patients with myelofibrosis or PV, >50% in acute GvHD, and >35% in chronic GvHD), bruising, dizziness, and headache, and diarrhea in patients with myelofibrosis or PV (>10%), infections and edema in patients with GvHD (≥20%) and weight gain in patients with myelofibrosis or PV (6-7%) (Jakafi Package Insert January 2023). Serious bacterial, mycobacterial, fungal, and viral infections have been reported in patients receiving ruxolitinib. Consequently, in patients with a positive test for latent tuberculosis, the risk/benefit ratio of ruxolitinib therapy will be carefully assessed. Other rare but reported complications include progressive multifocal leukoencephalopathy (PML), herpes zoster, hepatitis B reactivation, non-melanoma skin cancers, and aggressive B-cell lymphomas. Participants receiving ruxolitinib will be monitored closely for these complications. Patients will undergo an initial evaluation and yearly screening for skin cancer by a credentialed dermatologist while on ruxolitinib and yearly screening for B-cell clonality by polymerase chain reaction (PCR). Dermatology evaluation may be performed outside the NIH Clinical Center. Other JAK inhibitors have been associated with major adverse cardiovascular events, thrombosis (especially in current or past smokers), and secondary malignancies other than B-cell lymphoma and skin cancers in patients with rheumatoid arthritis. Although these complications have not been reported in patients receiving ruxolitinib to date, two patients have had thrombotic events while receiving ruxolitinib on this protocol. Patients will be counseled accordingly regarding these potential risks.

Ruxolitinib is not teratogenic or mutagenic in rats. At doses comparable to 34% of the clinical exposure at the maximum recommended dose of 25 mg twice daily, there was no effect on fertility, but increased fetal loss was observed in female rats. There are no published data on ruxolitinib use in pregnant women to date. No data are available regarding the presence of ruxolitinib in breast milk. Nevertheless, use during breastfeeding is not recommended. There does not appear to be any difference in toxicity with pediatric use of ruxolitinib in children ≥12 years of age with GvHD. Although there are case reports of treatment of younger children with comparable side effect profile, ruxolitinib is not FDA-approved for children <12 years of age.

Photography

In patients with cutaneous manifestations of HES and/or visible manifestations of drug toxicity, such as edema or skin rash, photographs may be taken to document drug efficacy or AEs. Taking pictures of the face and body may be embarrassing to some people. We will attempt to preserve

the anonymity of the participants as much as possible while providing the information needed to support the research being published. Participants may decline photographs or place any restrictions on their use. Participants will be given the opportunity to discuss this with the principal or associate investigators.

Alternatives

The alternatives to study participation are treatment by a local provider with 1) imatinib or mepolizumab, both of which are FDA-approved for the treatment of HES, or 2) any of the many non-FDA-approved medications that have been used to treat HES with varying success.

2.3.2 Known Potential Benefits

Participants will benefit from a more thorough medical evaluation and more sensitive mutational testing than is generally available at centers with less expertise in the treatment of eosinophilic disorders. Since the study is designed to evaluate the efficacy of the study drugs in the treatment of hypereosinophilic syndrome, participants may or may not benefit from the experimental therapy, although, based on the package insert and published studies, the prospect of short- and long-term benefit in patients with known imatinib-sensitive mutations is high and may include cure.

2.3.3 Assessment of Potential Risks and Benefits

Given the efficacy and toxicity of current agents used to treat steroid-resistant HES, the potential risks of participation in this protocol are minimally increased over the risks that would be incurred with standard of care. The prospect of direct benefit from the study treatment, access to clinical expertise, and the potential impact of the knowledge gained from this protocol on HES morbidity and mortality both at the individual and population levels, clearly outweighs the small potential risk of participation.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To assess the efficacy of tyrosine kinase inhibition using imatinib and/or ruxolitinib in reducing peripheral blood eosinophilia in patients with HES.	Peripheral blood absolute eosinophil count at 1 month (imatinib) or 3 months (ruxolitinib) following initiation of therapy.	The AEC generally reflects disease activity in HES and is commonly used as an endpoint in clinical trials. The different timing for the two arms was chosen based on our prior clinical experience demonstrating that imatinib causes a rapid reduction in AEC through a direct effect on eosinophils, whereas ruxolitinib can have a slower effect through modulation of cytokines.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Secondary		
1. Assess the durability of hematologic response to imatinib and ruxolitinib.	1a. Peripheral blood AEC at 3 months (imatinib), 6, 9, and 12 months (imatinib and ruxolitinib). 1b. Percent of patients who reach an eosinophil count below 1500/mm^3 at 1, 3, 6, 9, and 12 months.	HES is a chronic disorder. Consequently, efficacy for <1 year would be considered inadequate. 1500/mm^3 was chosen as the cutoff value since this is the level that defines HES.
2. Identify predictors of response to imatinib mesylate and ruxolitinib in *PDGFRA*-negative HES.	2. Identification of laboratory or clinical findings that predict response to imatinib and/or ruxolitinib therapy.	Imatinib is not without toxicity. Therefore, identifying predictors of response would minimize unnecessary treatment.
3. Determine the cellular and molecular targets of imatinib mesylate and ruxolitinib in *PDGFRA*-negative HES.	3. Identification of cellular and molecular targets of imatinib mesylate and ruxolitinib in *PDGFRA*-negative HES.	Delineation of therapeutic targets would assist in the identification of biomarkers and improve management in the clinic and in the context of clinical trials.
4. Evaluate the effects of imatinib mesylate and ruxolitinib on the clinical manifestations of HES.	4. Collection of standardized longitudinal data on disease activity and outcome in patients receiving imatinib or ruxolitinib therapy for HES.	Identification of long-term complications of eosinophilia and/or side effects of therapy through standardized longitudinal follow-up of patients is essential for the development of strategies to further improve prognosis of patients with HES.
5. Determine the most appropriate dosing regimen and length of imatinib therapy in *PDGFRA*-positive and *PDGFRA*-negative HES.	5a. Duration of remission following cessation of imatinib therapy. 5b. Determination of the relationship between the dose and duration of imatinib treatment and the probability of cure (complete remission off therapy for ≥5 years).	An understanding of the impact of dose and duration of therapy on the prospect of cure would potentially reduce the cost and toxicity of therapy by minimizing drug exposure.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
6. Determine the most appropriate dose of ruxolitinib for the treatment of HES	6. Determination of the minimum effective dose of ruxolitinib treatment in patients with HES	The dose of ruxolitinib chosen for this study was based on the dosing for myelofibrosis. Demonstration that a lower dose is effective in HES would potentially reduce the cost and toxicity of therapy by minimizing drug exposure..

4 STUDY DESIGN

4.1 Overall Design

This single-site, open-label, phase 2 clinical trial will be conducted at the NIH Warren G. Magnuson Clinical Center and is designed to assess the efficacy of tyrosine kinase inhibition with either imatinib or ruxolitinib for the treatment of defined cohorts of patients with steroid-refractory HES.

Imatinib arm: Patients with mutations in *PDGFRA/B* (known imatinib-sensitive mutations) or clinical features consistent with a myeloid disorder (and no evidence of a mutation in the *JAK-STAT* pathway) will be treated with imatinib mesylate 400 mg daily. The initial dose may be adjusted, if necessary, per guidelines in the package insert as described in section 6.1.2. If remission is attained, stepwise dose reduction to 100 mg daily will be initiated. Drug may be discontinued in patients in stable remission for ≥5 years on imatinib therapy. Patients receiving imatinib therapy initiated by an outside physician may be eligible for participation in the dose de-escalation portion of the study if documentation of a *PDGFRA/B* mutation is available prior to enrollment. Patients who remain in remission for 5 years after therapy is discontinued (presumed cure) will be taken off protocol.

Ruxolitinib arm: Patients with mutations in the *JAK-STAT* pathway, patients who are steroid-refractory or intolerant and without evidence of a myeloid disorder, and *PDGFRA/B*-negative patients who have evidence of a myeloid disorder but have failed imatinib therapy, will be treated with ruxolitinib 15 mg twice daily. The initial dose may be adjusted, if necessary, per guidelines in the package insert as described in section 6.1.2. Response will be assessed at 3 months, after which drug may be continued in responders for up to 7 years or until Janaury 2027, which ever comes first. Drug dose may be reduced in patients in stable remission for ≥2 years on ruxolitinib therapy.

4.2 Scientific Rationale for Study Design

This study was designed as a pilot proof-of-efficacy trial with a historical control. This study design was chosen because of the lack of sufficient patients to support a single-center randomized trial (as evidenced by the fact that imatinib was FDA-approved for the treatment of HES based on a few small open-label case series) and the fact that statistical assessment

suggested that the study design and number of participants planned would be sufficient to address the study endpoints.

4.3 Justification for Dose

Imatinib: The dose of imatinib was chosen based on the FDA-recommended dosing for CML. Although the current package insert suggests that lower dosing (100 mg daily) is sufficient for the treatment of patients with *PDGFRA/B* mutation, data from CML have demonstrated that cure rates depend on the depth of molecular remission. Preliminary analyses from this protocol have demonstrated higher cure rates than reported in the literature, suggesting that higher initial doses of imatinib and more sensitive monitoring (PCR vs. fluorescence in situ hybridization) may be beneficial.

Ruxolitinib: The dosing regimen was chosen based on the FDA-recommended dosing for the treatment of myelofibrosis with platelet counts of 100-200,000/mm^3 and discussion with Incyte (the supplier of study drug). Preliminary analyses from this protocol suggest that lower doses may be equally effective in some patients.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Male or female, at least 2 years of age for imatinib therapy and ≥18 years of age for ruxolitinib therapy.
2. Documented diagnosis of HES: eosinophilia >1500/mm^3 on two occasions, no secondary etiology for the eosinophilia despite careful clinical evaluation, and evidence of end organ damage (histologic evidence of tissue infiltration by eosinophils and/or objective evidence of clinical pathology in any organ system that is temporally associated with eosinophilia and not clearly attributable to another cause) (2).
3. All participants must fit one of the following four categories:
   a) Myeloid neoplasm associated with a *PDGFRA* or *PDGFRB* rearrangement.
   b) Myeloid neoplasm associated with rearrangement or mutation involving the *JAK-STAT* pathway.
   c) Presence of ≥4 of the following laboratory criteria suggestive of a myeloid disorder:
      i. Dysplastic eosinophils on peripheral smear
      ii. Serum B12 level ≥1000 pg/mL.
      iii. Serum tryptase level ≥12.
      iv. Anemia and/or thrombocytopenia.
      v. Bone marrow cellularity >80% with left shift in maturation.
      vi. Dysplastic (spindle-shaped) mast cells on bone marrow biopsy.
      vii. Evidence of fibrosis on bone marrow biopsy.
      viii. Dysplastic megakaryocytes on bone marrow biopsy.
   d) Refractory to or intolerant of steroids without evidence of a myeloid disorder.
4. Negative serum beta–human chorionic gonadotropin 24 hours prior to drug administration for women of childbearing potential to exclude early pregnancy.
5. Agrees to practice abstinence or effective contraception during administration of imatinib mesylate or ruxolitinib and for 6 months after discontinuation of the drug. Women of

childbearing potential who are using hormonal contraceptives and taking ruxolitinib will also be required to use a barrier method.**
6. Participation in protocol 94-I-0079 (Activation and function of eosinophils in conditions with blood or tissue eosinophilia).

NOTE: Participants who meet inclusion criteria but are already receiving imatinib may be enrolled in the dose de-escalation portion of the study at the investigator's discretion. Patients who meet inclusion criteria but are already receiving ruxolitinib may be enrolled at the investigator's discretion if baseline data are available and they have received ruxolitinib at the dose specified in the protocol for less than 2 months (primary endpoint).

** Effective contraception includes the use of hormonal (birth control pills, for example) and/or barrier (condoms and diaphragms, for example) methods by participants and/or their partners to prevent pregnancy in women of childbearing potential. For women of childbearing potential who use hormonal methods as their primary means of contraception and will be receiving treatment with ruxolitinib, barrier methods will also be required due to possible interference of ruxolitinib with hormonal contraceptives.

Although a private physician is not required for inclusion in the study, it is strongly recommended that all participants have a physician outside the NIH for routine medical care and emergencies.

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Pregnant or nursing women.*
2. *D816V KIT*-positive systemic mastocytosis.
3. Uncontrolled HIV infection (absolute lymphocyte count <200/mm^3 and/or HIV RNA level >500 copies/ml)
4. ANC<1000/mm^3 or platelet count <10,000/mm^3 or <50,000/m^3 with clinical evidence of bleeding.
5. Elevated transaminases (>5 times the upper limit of normal) or elevated bilirubin (>3 times the upper limit of normal).
6. Any condition that, in the investigator's opinion, places the patient at undue risk by participating in the study.

An individual who meets any of the following criteria will be excluded from participation in the ruxolitinib treatment arm of this study:

1. Evidence of B-cell clonality by PCR or flow cytometry.
2. Active tuberculosis, or acute or chronic active infection with hepatitis B or C*.
3. Treatment with fluconazole >200 mg daily.

* Participants with active tuberculosis will be excluded. The most current Infectious Diseases Society of America guidelines will be followed regarding isoniazid therapy for latent tuberculosis. Participants who refuse recommended prophylactic therapy for tuberculosis will be counseled regarding the risks of reactivation of tuberculosis during ruxolitinib therapy but will not be systematically excluded. Molecular and serologic tests for hepatitis B and serology for

hepatitis C will be performed regardless of vaccination history. Participants with evidence of active or chronic infection with hepatitis B or positive hepatitis C serology will be excluded from participation in the ruxolitinib arm of the protocol. Specifically, a positive hepatitis B serology indicative of previous immunization (i.e., hepatitis B anti–surface antibody–positive and hepatitis B anti–core antibody–negative) or a fully resolved acute hepatitis B infection is not an exclusion criterion. Patients with an indolent chronic hepatitis B infection (normal alanine aminotransferase [ALT], aspartate aminotransferase [AST], and albumin, and no radiographic or biopsy evidence of cirrhosis) will be evaluated by an NIH hepatologist and may be eligible. Patients who choose to remain on study with evidence of prior hepatitis B infection will be counseled regarding the risks of reactivation prior to initiation of ruxolitinib therapy.

5.3 Inclusion of Vulnerable Participants

5.3.1 Participation of Children

Children will only be enrolled on the imatinib arm of the trial. Myeloid variants of HES, including *PDGFRA/B*-positive disease, affect all ages and, similar to the case in adults, present as aggressive HES and have a poor prognosis. The risks of imatinib therapy in children are similar (or decreased) compared to those in adults except for the potential for growth retardation. Imatinib is approved for the treatment of children with CML, ALL, or GIST, and case reports in the literature and data from this protocol suggest that imatinib therapy is equally effective in children with *PDGFRA/B*-positive disease as in adults (39–41). Thus, the potential for direct benefit outweighs the risks of participation in children with this rare life-threatening disorder.

5.3.2 Participation of Adults Who Lack Capacity to Consent to Research Participation

Adults who lack capacity to consent to research participation will not be excluded from enrolling in this protocol. HES is a rare disease with the potential for life-threatening complications, including eosinophilic cardiac involvement, thromboembolism, and encephalopathy, especially in patients with myeloid forms of the disease and/or who are refractory to glucocorticoid therapy. It would be unethical to exclude such patients from access to the therapies provided in this protocol because they lack the capacity to consent. In the case of imatinib, which is FDA-approved for the treatment of HES, this protocol provides drug and access to enhanced monitoring that has been successful in guiding drug discontinuation. In the case of ruxolitinib, this protocol provides access to off-label use of an FDA-approved drug that has efficacy against *JAK2*, the second most common mutation driving myeloid HES and an important molecule in lymphocyte production of IL-5 (a major driver of eosinophilia in lymphocytic variant and idiopathic HES). Risks will be minimized for this population as procedures for the purposes of research, other than blood draws and administration of the study medication, will not be performed in adults who are unable to consent.

5.3.3 Participation of NIH Staff or Family Members of Study Team Members

NIH staff and family members of study team members may be enrolled in this study as these populations meet the study entry criteria. Neither participation nor refusal to participate in the research will have an effect, either beneficial or adverse, on the participant’s or their family member’s employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the *NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* and the *Leave Policy for NIH Employees Participating in NIH Medical Research Studies* (NIH Policy Manual 2300-630-3). Please see section 10.1.3 for consent of NIH staff and family of study team members.

5.4 Inclusion of Pregnant Women, Fetuses, or Neonates

Not applicable.

5.5 Lifestyle Considerations

During this study, participants receiving imatinib are asked to:

- Refrain from consumption of grapefruit or grapefruit juice while taking imatinib therapy.
- Limit alcohol and nicotine use due to the potential for accelerated metabolism of the drug and/or increased side effects.

5.6 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of transiently abnormal laboratory results, pregnancy or breastfeeding on the day of enrollment may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.7 Strategies for Recruitment and Retention

HES is a rare and heterogeneous group of disorders that primarily affects adults, although pediatric cases have been reported, and include cases of *FIP1L1/PDGFRA*-positive and *JAK* mutation-positive disease (2). Whereas *PDGFR* mutations show a strong male predominance, other forms of HES appear to be equally distributed between the sexes. Adult and pediatric patients meeting eligibility criteria will be enrolled in the study without regard to race, sex, or religion. Patients residing outside of the US may be enrolled if able to meet the visit requirements.

This is a single-site study. Patients will be recruited from among patients referred from outside physicians for evaluation of hypereosinophilia and already evaluated on protocol 94-I-0079 (“Eosinophil activation and function in parasitic infections and other conditions with increased tissue or blood eosinophilia in humans”) in reducing eosinophilia in patients with HES or eosinophilic gastroenteritis refractory to or intolerant of conventional therapy). In addition, a small number of physicians who study this condition will be contacted directly and informed of

the study. The anticipated accrual rate for this study is limited by the rarity of HES and is anticipated to be 5-10 patients per year. Visit reminders have been sufficient to ensure long-term participation.

5.7.1 Costs

Participants may be asked to have complete blood counts with differential or other standard-of-care testing performed locally after enrollment on the study. For participants with insurance, fees associated with these tests should be submitted to their insurance carrier. For participants who do not have insurance or insured participants who may incur expenses for copays or unpaid balances, arrangements will be made in advance with NIAID to have expenses, up to $5000 per year, paid to the performing laboratory facility. Unexpected protocol-related complications that require transfer to other hospitals will be addressed per NIAID policy.

5.7.2 Compensation

Patients will not be compensated for participation in this study. Reimbursement for travel, lodging and meals will be provided per the NIAID travel policy.

6 STUDY INTERVENTION

6.1 Study Interventions(s) Administration

6.1.1 Study Intervention Description

Imatinib. Generic imatinib is available from multiple manufacturers, including Novartis (Gleevec™) as 100-mg and 400-mg tablets. Imatinib is on formulary in the Clinical Center Pharmacy and commercial product will be dispensed in appropriately labeled bottles per standard pharmacy protocol.

Ruxolitinib. Ruxolitinib for use in this study is provided to the Clinical Center Pharmacy by Incyte as 5-mg tablets that are indistinguishable from their commercial product (Jakafi™). The research drug will be dispensed in appropriately labeled bottles per standard pharmacy protocol.

There will be no IND obtained for the use of any of the commercial agents used in this study.

This study meets the criteria for exemption for an IND as this investigation is not intended to support a new indication for use or any other significant change to the labeling; the drugs are already approved and marketed, and the investigation is not intended to support a significant change in advertising; and the investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. Imatinib is FDA-approved for HES at the dose being used in this study. Ruxolitinib is being used at the dose commonly used for myelofibrosis, a rare myeloid disorder that can be associated with similar mutations to those in myeloid HES. Based on the mechanism of action, there is no reason to suspect eosinophil-related complications of therapy and, although limited, the data to date do not indicate a different or more severe side effect profile in HES as compared to the FDA-approved indications for ruxolitinib.

6.1.2 Dosing and Administration

Imatinib

Participants who are found to have imatinib-sensitive mutations, including *FIP1L1/PDGFRA* and translocations involving *PDGFRB*, and those with myeloid features and no detectable mutation will be treated with imatinib. Treatment duration will range from one month (in the event of non-response) to 30 years (the duration of this protocol), although it is anticipated that most responding patients will receive drug for 5-10 years. In rare cases, treatment may be discontinued prior to 1 month for toxicity.

The dosing regimen to be used initially (400 mg po once daily in adults and 260 mg/m^2/day in children with food and a glass of water) is identical to that recommended by the FDA for the treatment of the chronic phase of CML (Gleevec Package Insert August 2022). In patients with ANC<1500/mm^3, platelet counts <75,000/mm^3 or abnormal liver function tests (ALT or AST >2.5 or bilirubin >3 times the upper limit of normal), the starting dose will be reduced to 300 mg po once daily. This regimen was selected based on the safety-versus-efficacy margin in terms of achieving therapeutic drug levels (trough plasma levels above the concentrations required for in vitro cell growth inhibition) and data from phase 2 studies, which showed a decreased rate of cytogenetic remission at doses less than 300 mg/day despite decreased blasts in the peripheral circulation. Although there have been many reports of imatinib mesylate treatment of HES using lower doses than those recommended for CML (17–19), bone marrow studies were not reported post-treatment and follow-up has not been sufficient to assess the development of resistance to low dose or intermittent therapy.

The recommended guidelines for dose adjustment and discontinuation of therapy in CML will be followed. Specifically,

> Drug administration will be <u>discontinued</u> if there is no clinical response (eosinophil count has not fallen by ≥30%) at 1 month, at the request of the patient, or for any condition that, in the investigator's opinion, places the patient at undue risk for continued participation in the study.
>
> Drug administration will be <u>interrupted</u> for:
>
> 1) ANC<1000/mm^3.
> 2) Platelet count <10,000/mm^3 or 50,000/mm^3 with clinical evidence of bleeding.
> 3) ALT or AST >5 times the upper limit of normal.
> 4) Bilirubin >3 times the upper limit of normal.
> 5) Other grade 3 or 4 AEs that the investigator feels are related to study drug or negatively impacted by continuation of study drug.
>
> In the event of neutropenia or thrombocytopenia, drug will be restarted once the ANC≥1500/mm^3 and the platelet count is ≥75,000/mm^3. If the time to the recovery of counts is >2 weeks or this is the second episode of cytopenia, the dose will be decreased to 300 mg daily. For abnormalities in liver transaminases or bilirubin, drug will be restarted at 300 mg daily (or 195 mg/mm^2/day for children) once levels have returned to 2.5 or 1.5 times the upper limit of normal, respectively. Following interruptions due to other adverse reactions, drug will be restarted at a reduced dose (300 mg daily) once the event has resolved.
>
> Initiation of imatinib therapy will be <u>delayed</u> in the event of intercurrent viral or bacterial infection.

In patients who demonstrate a complete clinical and hematologic response, including resolution of bone marrow abnormalities, molecular remission (in patients with the *FIP1L1/PDGFRA* mutation), and lack of progression of end organ involvement, the dose of imatinib will be decreased to 200 mg daily (or 130 mg/mm^2/day for children). This dose will be continued for a minimum of 3 months. The dose will be further decreased to 100 mg (or 75 mg/mm^2/day for children) daily in patients who remain clinically and hematologically stable at this time. Discontinuation of imatinib may be considered at the discretion of the investigator in patients who have remained stable on 100 mg daily for an extended period of time (>5 years). Patients on imatinib therapy at the time of enrollment will follow the same de-escalation protocol once complete clinical and hematologic remission is documented. If at any time during the study, the eosinophil count begins to rise, the patient becomes symptomatic, or there is other evidence of disease progression (e.g., increased levels of *FIP1L1/PDGFRA*), the dose of imatinib will be doubled every week (for eosinophilia or symptoms) or every month (for molecular relapse) until disease progression is arrested (up to a total dose of 600 mg daily [or 390 mg/mm^2/day for children]). If the patient does not respond to 600 mg daily (or 390 mg/mm^2/day for children), their participation in the protocol will be terminated and they will be referred for alternative therapy.

Since imatinib mesylate is an inhibitor and substrate of CYP3A4, caution is recommended when administering imatinib mesylate with 1) inhibitors of CYP3A4, such as clarithromycin, erythromycin, ketoconazole, and itraconazole, as plasma levels of imatinib may be increased, 2) inducers of CYP3A4, such as phenytoin, rifampin, and St. John's Wort, as plasma concentrations of imatinib may be decreased and 3) CYP3A4 substrates, such as cyclosporine, calcium channel blockers, and pimozide, whose concentrations may be altered by the administration of imatinib mesylate. Although every effort will be made to avoid the coadministration of drugs that are known to interact with imatinib mesylate, in the event that this is not possible, dosage adjustments will be made based on the recommendations of the Clinical Center pharmacist.

Ruxolitinib

Adult participants with mutations in *JAK1* or *JAK2*, those with myeloid features who have no detectable mutation and have failed imatinib, and steroid-resistant participants without myeloid features will be treated with ruxolitinib. Treatment duration will range from 3 months (in non-responders) to 7 years (based on the existing agreement with Incyte). In rare cases, treatment may be discontinued prior to 1 month for toxicity.

The dosing regimen to be used initially (15 mg po twice daily) is identical to that recommended by the FDA for the treatment of myelofibrosis with platelet counts of 100-200,000/mm^3 (Jakafi Package Insert January 2023). In patients with platelet counts <100,000/mm^3, moderate renal impairment (creatinine clearance <60 mL/min) or abnormal liver function tests (ALT or AST >2.5 or bilirubin >3 times the upper limit of normal), the starting dose will be reduced to 10 mg twice daily. The recommended guidelines for dose adjustment during therapy and discontinuation of therapy in myelofibrosis will be followed. Specifically,

> Drug administration will be <u>discontinued</u> if there is no evidence of clinical response at 3 months, at the request of the patient, or for any condition that, in the investigator's opinion, places the patient at undue risk for continued participation in the study. Clinical

response will be defined as one of the following: 1) reduction in AEC by ≥30% at 3 months, 2) objective improvement in clinical symptoms with stable or decreased eosinophil count at 3 months, or 3) ability to taper background therapy with stable or decreased eosinophil count at 3 months.

Drug administration will be interrupted for:

1) ANC<500/mm^3.
2) Platelet count <50,000/mm^3 or bleeding at any platelet count.
3) Other grade 3 or 4 AEs that the investigator feels are related to study drug or negatively impacted by continuation of study drug.

In the event of neutropenia or thrombocytopenia, drug will be restarted once the ANC≥750/mm^3 and the platelet count is ≥75,000/mm^3 at the higher of 5 mg once daily or 5 mg twice daily below the largest dose in the week prior to interruption. Following interruptions due to other adverse reactions, drug will be restarted at a reduced dose (as above) once the event has resolved.

Initiation of ruxolitinib therapy will be delayed in the event of intercurrent active viral, bacterial, mycobacterial, or fungal infection.

Drug administration may be continued in patients experiencing clinical benefit who develop anemia or gastrointestinal symptoms. However, the dose of ruxolitinib may be decreased at the discretion of the PI.

In patients who demonstrate a clinical response (as defined above) for ≥2 years, the dose of ruxolitinib may be decreased to 10 mg orally twice daily. This dose will be continued for a minimum of 3 months. The dose may be further decreased to 5 mg orally twice daily in patients who remain clinically and hematologically stable at this time.

Since ruxolitinib is an inhibitor and substrate of CYP3A4, caution is recommended when administering ruxolitinib with 1) inhibitors of CYP3A4, such as clarithromycin, erythromycin, ketoconazole, and itraconazole, as plasma levels of ruxolitinib may be increased, 2) inducers of CYP3A4, such as phenytoin, rifampin, and St. John's Wort, as plasma concentrations of ruxolitinib may be decreased, and 3) CYP3A4 substrates, such as cyclosporine, calcium channel blockers and pimozide, whose concentrations may be altered by the administration of ruxolitinib. Although every effort will be made to avoid the coadministration of drugs that are known to interact with ruxolitinib, in the event that this is not possible, dosage adjustments will be made based on the recommendations of the Clinical Center pharmacist.

Due to the potential interaction between ruxolitinib and hormonal contraceptives, women of childbearing potential who are using hormonal contraceptives will also be required to use a barrier method.

6.2 Preparation/Handling/Storage/Accountability

Both imatinib and ruxolitinib will be used as specified in the package inserts.

6.2.1 Acquisition and Accountability

In most cases, imatinib will be provided by the Clinical Center Pharmacy and prescribed through the Clinical Research Information system (CRIS). For some patients enrolled after initiation of

imatinib therapy by an outside provider, imatinib will continue to be provided by the outside provider. Ruxolitinib will be provided directly to the pharmacy by Incyte and prescribed through CRIS. Standard pharmacy procedures will be followed. At the end of the trial, any unused drug will be destroyed.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Not applicable.

6.2.3 Product Storage and Stability

Both imatinib and ruxolitinib can be stored at room temperature per standard Clinical Center Pharmacy procedures.

6.2.4 Preparation

The tablets will be provided to the patient in appropriately labeled bottles per standard Clinical Center Pharmacy procedures.

6.3 Measures to Minimize Bias: Randomization and Blinding

Not applicable.

6.4 Study Intervention Compliance

Study intervention compliance will be assessed by patient report at each visit and missed doses (if any) recorded in the Clinical Research Information Management System of the NIAID (CRIMSON).

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in CRIMSON and/or CRIS are concomitant prescription medications, over-the-counter medications, and supplements.

Concomitant medications will be recorded in CRIMSON at enrollment and updated at every visit. Patients will also by counseled to notify the study team of any changes in concomitant medications between visits.

Imatinib. Although there are no absolute contraindications to concomitant therapy for participants in the imatinib arm of this study, imatinib has significant interactions with agents that induce or inhibit CYP3A or are metabolized by CYP3A4 or CYP2D6. These include many commonly prescribed drugs, including benzodiazepines, calcium channel blockers, warfarin, clarithromycin, erythromycin, ketoconazole, itraconazole, phenytoin, rifampin, and cyclosporine. Wherever possible, these agents will be discontinued or replaced by an equally effective agent that does not interact with imatinib. When this is not possible, dose modification may be implemented at the recommendation of a Clinical Center pharmacist.

Ruxolitinib. The use of fluconazole at doses greater than 200 mg daily is a contraindication to ruxolitinib therapy and dosage adjustment is indicated in patients receiving fluconazole at doses lower than 200 mg daily due to the fact that fluconazole has been shown to increase drug levels of ruxolitinib. Ruxolitinib has significant interactions with agents that induce or inhibit CYP3A4. These include many commonly prescribed drugs, including ketoconazole, erythromycin, and rifampin. Wherever possible, these agents will be discontinued or replaced by an equally

effective agent that does not interact with imatinib. When this is not possible, dose modification may be implemented at the recommendation of a Clinical Center pharmacist.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation of imatinib or ruxolitinib does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified after enrollment (including but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

The data to be collected at the time of study intervention discontinuation will include the following:

- Reason for study intervention discontinuation.
- Disease status, including AEC and molecular status (if applicable).

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue study intervention for the following reasons:

- Completion of study intervention.
- Disease progression which requires discontinuation of the study intervention.
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Investigator discretion.
- Participant unable to receive study drug for 4 weeks.

7.2 Participant Discontinuation/Withdrawal from the Study

Prior to removal from study, effort must be made to have all participants complete a safety visit approximately 4 weeks following the last dose of study therapy.

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- Positive pregnancy test.
- Participant has completed the study follow-up period.
- Death.
- Screen Failure.

In view of a report demonstrating a small but significant increased risk of aggressive B-cell lymphomas in people with a myelofibrosis and a pre-existing B-cell clone receiving *JAK1/2* inhibitors, B-cell clonality testing will be performed at screening and yearly during ruxolitinib

therapy. Participants with a detectable B-cell clone at screening will be excluded from the study. However, if a B-cell clone develops during ruxolitinib treatment, the risks and benefits of continuing ruxolitinib therapy will be discussed with the patient. Patients who have demonstrated a clear response to ruxolitinib and choose to remain on study will be followed closely for signs and symptoms of developing lymphoma, including B symptoms (fever, night sweats, unintentional weight loss), splenomegaly, and lymphadenopathy.

The reason for participant discontinuation or withdrawal from the study will be recorded in CRIMSON. Participants who sign the informed consent form but do not receive the study intervention may be replaced. Participants who sign the informed consent form and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if they fail to return for 1 scheduled visit or miss 3 consecutive laboratory assessments and are unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 1 month and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

8.1.1 Screening Activities Performed Prior to Obtaining Informed Consent

Minimal risk activities that may be performed before the participant has signed a consent include the following:

- Email, written, in person or telephone communications with prospective participants.
- Review of existing medical records to include history and physical exam, laboratory studies, etc.
- Review of existing magnetic resonance imaging, X-ray, or computed tomography images.
- Review of existing photographs or videos.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

Potential participants will undergo screening assessments as part of NIH protocol 94-I-0079. At a minimum, the following procedures will be performed within 28 days prior to enrollment on this protocol, unless an alternative time period is specified. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

All study participants:

- Complete medical history and physical examination by a credentialed NIH clinician.
- Complete blood count (CBC) with differential.
- Routine chemistries (acute care, hepatic and mineral panels, lactate dehydrogenase [LDH]).
- Prothrombin/partial thromboplastin time.
- Serum or urine pregnancy test (for patients of childbearing potential only).
- Serum quantitative immunoglobulin levels including IgE.
- Serum B12 and tryptase levels.
- Serum troponin level.
- Anti-HIV1/2 serology.
- Hepatitis B and C screen.
- Urinalysis.
- Bone marrow biopsy and aspirate with routine histopathology (including immunohistochemistry for mast cells), cytogenetics, flow cytometry, testing for myeloid mutations associated with HES (including *KIT* D816V, *FIP1L1-PDGFRA*, fusions involving *PDGFRB*, *PCM1-JAK2*, *JAK2* V617F), and storage for additional confirmatory or diagnostic testing as needed*.
- Peripheral blood testing for *FIP1L1-PDGFRA, BCR-ABL, JAK2* V617F, and for other myeloid mutations (if clinically indicated).
- Testing for T- and B-cell clonality by PCR.
- Peripheral blood flow cytometry (T/B/natural killer [NK] and eosinophil panels).
- Peripheral blood for peripheral blood mononuclear cell (PBMC) and eosinophil purification and storage for additional testing as needed.
- Echocardiogram.
- Electrocardiogram (EKG).
- Pulmonary function testing.
- Other diagnostic testing if clinically indicated.

*For patients receiving imatinib therapy and enrolling for dose de-escalation only, bone marrow examination will not be performed unless clinically indicated. For all other patients, a bone marrow will be performed unless they have had a recent bone marrow examination (within the prior 3 months) and there have been no major changes in HES medication, or clinical or laboratory findings.

Ruxolitinib arm only:

- Fasting lipid profile (cholesterol, low-density lipoprotein, very low–density lipoprotein, high-density lipoprotein, and triglyceride levels).
- QuantiFERON tuberculosis test.

- Dermatology consult (cancer screening). Dermatology evaluation may be performed outside the NIH Clinical Center by a credentialed dermatologist.

8.1.2 Screening activities Performed After Consent Has Been Signed

Pregnancy testing will be performed in patients of childbearing potential after consent has been signed and prior to the first dose of study medication. Other screening procedures may be repeated prior to initiation of study drug treatment at the request of the PI (i.e., if there has been an interval change in clinical status or medications).

8.2 Study Evaluations & Procedures

Baseline study visits will be conducted in person at the NIH Clinical Center, after which study drug will be prescribed through the NIH Pharmacy.

The following procedures will be performed at the baseline visit (and before the first dose of study drug):

- Complete medical history and physical examination by a credentialed NIH clinician.
- CBC with differential.
- Routine chemistries (acute care, hepatic and mineral panels, LDH).
- Serum or urine pregnancy test (for patients of childbearing potential only).
- Serum quantitative immunoglobulin levels including IgE.
- Serum B12 and tryptase levels.
- Serum troponin level.
- Urinalysis.
- Peripheral blood testing for previously identified genetic abnormalities (i.e, *FIP1L1-PDGFRA, BCR-ABL, JAK2* V617F).
- Peripheral blood flow cytometry (T/B/NK and eosinophil panels).
- Peripheral blood for PBMC and eosinophil purification and storage for additional testing as needed.
- EKG.
- Other diagnostic testing if clinically indicated.

Note: bone marrow examination, echocardiogram, and pulmonary function tests may be repeated at baseline at the discretion of the PI if there has been a significant change in therapy or clinical status.

Following the first dose of study drug, all patients will undergo weekly laboratory safety/efficacy assessments for 4 weeks, biweekly for 6 weeks, and monthly thereafter. These laboratory visits may be conducted at the NIH or at a local laboratory with test results sent by fax or other secure means to the study team. The dose of study drug will be modification if needed as per section 6.1.2. The primary efficacy visit will be conducted at the NIH at 1 month (for imatinib) or 3 months (for ruxolitinib). Follow-up visits will be conducted every 3 months after initiation of study drug for at least 1 year to assess end organ manifestations and response to therapy.

Patients who are clinically asymptomatic and without a history of cardiac or other life-threatening involvement and have been on a stable dose of imatinib for 6 months following dose de-escalation may have their visit frequency decreased to every 6-12 months at the discretion of the investigator, and their routine laboratory study (CBC and serum transaminases)

frequency reduced to every 3 months. Participants who did not qualify for dose de-escalation but have been on a stable dose of imatinib for ≥2 years may also have their visit frequency decreased to every 6-12 months at the discretion of the investigator. Routine laboratory studies will continue to be performed at least every 3 months (CBC and serum transaminases) by the participant's local physician or a commercial laboratory. Testing for abnormalities in *PDGFRA/B* or other imatinib-sensitive genes present at baseline will be performed every 3-6 months (at the investigator's discretion) at the NIH or using a blood specimen mailed to the NIH.

Participants who are clinically asymptomatic and without a history of cardiac or other life-threatening involvement and have been on a stable dose of ruxolitinib for 12 months may have their visit frequency decreased to every 6-12 months at the discretion of the investigator, and their routine laboratory study (CBC and serum transaminases) frequency reduced to every 3 months. Testing for abnormalities in *JAK2* or other ruxolitinib-sensitive genes present at baseline will be performed at the investigator's discretion at the NIH or using a blood specimen mailed to the NIH.

In cases where in person visits are not possible for logistical reasons (e.g., COVID-19 restrictions), telehealth visits are acceptable except for the baseline, bone marrow, and primary endpoint visits, at the discretion of the PI. Telehealth or telephone visits will be conducted by credentialed NIH health care providers in a manner compliant with MAS policy M20-1 Utilization of Telehealth/Telemedicine by NIH Healthcare Providers for NIH Clinical Center Patients utilizing Information Technology platforms that have been determined by appropriately qualified NIH information technology personnel to be compliant with all necessary information security standards.

A description of the study assessments performed to address the primary and secondary endpoints follows:

Medical history.

All patients will undergo a detailed medical history by a credentialed NIH clinician at the baseline visit, including history of present illness, past medical history, review of systems, family history, social history, allergies, and medications. Medical histories at subsequent visits will be performed by a credentialed NIH clinician and include interim clinical history, review of systems and updated allergy and medication information.

Physical examination.

All patients will undergo a complete physical examination, including vital signs and weight, by a credentialed NIH clinician at every visit. Height will be documented at the baseline visit only.

Absolute eosinophil count.

CBC with differential will be performed by the NIH Clinical Laboratory to assess AEC at baseline and the primary endpoint visit. Subsequent assessments may be performed at the NIH or a local laboratory.

Molecular testing.

In patients in whom a fusion gene or other abnormality associated with eosinophilic myeloid neoplasms is detected prior to treatment, standard Clinical Center tests will be used to follow levels of the abnormal gene. For patients with mutations involving PDGFRA or PDGFRB

(imatinib arm), testing will be performed on blood and bone marrow at 4-8 weeks and on blood drawn at the NIH Clinical Center at 3months following initiation of therapy. Subsequent testing will be performed on blood drawn at the NIH Clinical Center or mailed in every 3-6 months thereafter while therapy is ongoing. If imatinib therapy is discontinued, monthly assessments will be performed for a minimum of 3 months. Additional evaluations will be performed in the setting of other evidence of disease progression. For patients with other mutations (imatinib or ruxolitinib arm), molecular testing will be performed on blood and bone marrow at 3 months following initiation of therapy and at least yearly thereafter while therapy is ongoing.

Bone marrow examination.

The screening bone marrow aspirate and biopsy will serve as the baseline examination unless there has been a significant change in HES therapy since that time, and the PI determines that repeat baseline examination is clinically important to ensure that there has not been interval disease progression or development of bone marrow pathology that would alter the risk/benefit of study participation. Bone marrow aspirate and biopsy will be performed at 4-8 weeks following initiation of study drug to assess response to treatment and exclude pathology unmasked by a reduction in bone marrow eosinophilia (42). Bone marrow testing will include routine histopathology (with immunostaining for mast cells) and flow cytometry to assess eosinophil and mast cell numbers and activation. Genetic testing at the primary endpoint will be restricted to assessment of mutations found to be present at baseline testing. Bone marrow aspirate (5 cc) will be collected, and bone marrow mononuclear cells purified and stored, in the event that additional testing is clinically indicated (i.e., testing for mutations associated with resistance to imatinib or ruxolitinib, assessment for novel mutations not recognized at the time of the initial bone marrow).

Assessment of end organ manifestations.

At 3-month intervals, patients will undergo standard-of-care clinical evaluation at the NIH to assess progression of end organ involvement by HES during the first year of therapy. This evaluation will include history and complete physical examination, laboratory testing, and EKG. Pulmonary function tests and echocardiogram will be performed at baseline in all patients and every 3 months for the first year in patients with evidence of clinical involvement of the lungs or heart, respectively. Patient photography will be performed in patients with visible clinical manifestations) every 3 months for the first year or until the visible clinical manifestations have resolved. Additional testing will be performed if clinically indicated. After the first year of therapy, history and complete physical examination, EKG and laboratory testing will be performed at each study visit. Echocardiography will be performed yearly or more frequently if clinically indicated. Other end organ assessments, including PFTs and radiologic imaging, will be performed as clinically indicated.

8.2.1 Biospecimen Evaluations

All clinical and safety laboratory evaluations will be performed by the NIH Clinical Center Laboratory or an accredited outside laboratory compliant with the Clinical Laboratory Improvement Amendments (CLIA) of 1988. Shipping of laboratory samples for testing at the NIH will follow all applicable regulations for the shipping of hazardous materials.

The following special studies will be performed solely for research purposes:

a. Drawing of additional blood for immunologic and molecular studies of the various components of the cellular and humoral immune systems. In most instances, 68 mL of research blood will be drawn at the initial visit and at scheduled follow-up visits for purification and storage of eosinophils, PBMCs, serum, and plasma. Additional blood samples may be drawn for whole blood flow cytometry or other specialized assays. The frequency of the blood draws will depend on the frequency of visits as described in sections 1.3 and 8.2. The amount of blood to be drawn from adult patients 18 years of age or older for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period. For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. Although most research blood samples will be drawn at the NIH Clinical Center during an in-person visit, research blood may be drawn remotely and shipped to NIH for analysis in some cases.

b. Collection of an additional 5-mL bone marrow aspirate to evaluate the effects of imatinib mesylate or ruxolitinib on myeloid precursors prior to (if the bone marrow collection is performed at the NIH Clinical Center) and at 4-8 weeks following the start of drug administration in consenting adult patients only. Unused samples may be recovered for research purposes from clinically indicated procedures in pediatric or adult patients, including those performed by local physicians outside the NIH.

8.2.2 Correlative Studies for Research/Pharmacokinetic Studies

The laboratory research evaluation of all patients will focus on the identification of features predictive of a response to imatinib mesylate or ruxolitinib and on the identification of the cellular and molecular targets of these therapies in HES. For these experiments, eosinophils, lymphocytes, and/or CD34+ cells will be purified from peripheral blood and bone marrow using standard techniques.

1) Predictors of response to tyrosine kinase inhibitors

In addition to the collection of demographic data (sex, age, race), laboratory testing, including serum tryptase, IgE, and B12 levels, will be performed to attempt to identify the subgroup of myeloid patients most likely to respond to imatinib mesylate. The utility of bone marrow features (such as cellularity and extent of fibrosis) in predicting response will also be examined.

2) Cellular targets of tyrosine kinase therapy in HES

The most common mutation in patients with HES and myeloid features is *FIP1L1/PDGFRA*. This aberrant tyrosine kinase is inhibited in vitro by imatinib at concentrations up to 50-fold lower than those needed to inhibit *bcr-abl*, a finding consistent with the rapid normalization of eosinophil levels in response to low dose imatinib that has been observed in several studies. Other *PDGFRA* fusions, as well as translocations and mutations involving *PDGFRB* are known to be sensitive to imatinib and can present as HES. *JAK2* mutations have also been described in patients with HES, although whether the eosinophilia responds to JAK inhibitors in these patients is unknown.

In the myeloid form of HES, myeloid precursors are increased and often dysplastic despite the lack of an obvious cytogenetic abnormality. Other lineages do not appear to be involved, with the exception of mast cells, which are diffusely increased and may show spindle-shaped morphology. The stage of development at which the proliferation arises is unknown. Bone

marrow biopsies will be examined prior to and at 4-8 weeks after the initiation of imatinib mesylate or ruxolitinib therapy to assess the effects of the drug on the different lineages and cell types. Special staining for tryptase, reticulin, CD34, and endothelial cell markers will be performed. In patients in whom adequate material is available from the bone marrow aspirate, flow cytometry will also be performed at the two time points. Finally, peripheral blood and bone marrow aspirate samples, when available, will be used to isolate PBMCs, eosinophils, and neutrophils for in vitro studies of the effects of tyrosine kinase inhibition in HES.

3) Molecular targets of imatinib mesylate and ruxolitinib in HES

Imatinib mesylate is known to selectively inhibit *bcr-abl*, *c-kit*, and the platelet-derived growth factor receptor tyrosine kinases through competitive inhibition at the adenosine triphosphate–binding site of the enzyme (43). Despite the recent identification of *FIP1L1/PDGFRA*, in 30% of imatinib responsive patients with HES, the molecular target remains unknown.

Ruxolitinib has activity against JAK1 and JAK2, and is FDA-approved for the treatment of PV and myelofibrosis, both of which are associated with mutations in *JAK2* (*JAK2* V617F and *PCM1-JAK2* being the most common). *JAK2* mutations have been identified in patients with *PDGFRA*-negative HES (32), and response to ruxolitinib has been reported in some cases (33,34). Despite this, the frequency of *JAK2* mutations in HES remains largely unknown. Furthermore, routine testing is often limited to *JAK2* V617F and standard cytogenetics, which may miss less-common mutations.

a) Sequence analysis

Twenty-five of the 36 participants with myeloid HES enrolled on the imatinib arm of the protocol have the *FIP1L1/PDGFRA* fusion. One *FIP1L1/PDGFRA*-positive participant also has a transmembrane mutation in *c-kit* that is of unknown significance, and three participants have translocations involving *PDGFRB*. Among the 11 participants without mutations involving *PDGFRA* or *PDGFRB*, three had a durable and complete response to imatinib. Two of the three have subsequently been removed from protocol for presumed cure. The third participant, who had a mutation in *JAK2*, subsequently relapsed after 6 months on imatinib therapy and was taken off protocol. No genetic abnormality potentially underlying the eosinophilia has been identified to date in the other two patients. We will continue to look for *FIP1L1-PDGFRA, BCR-ABL,* and *JAK2* V617F using standard techniques. In patients in whom these abnormalities are not present, additional mutations will be assessed in the clinical hematology laboratory using a variety of techniques, including commercially available customized microarray panels for the identification of mutations associated with myeloid disorders.

b) Analysis of RNA expression

Since CD34+ stem cells are precursors to the eosinophil lineage, it is likely that the tyrosine kinase being affected by imatinib mesylate and/or ruxolitinib in patients with HES is present in these cells. To identify potential molecular targets other than those suggested above, CD34+ cells will be purified from the PBMCs and/or bone marrow mononuclear cells prior to and 1 month after the initiation of treatment with imatinib mesylate. RNA expression in these two populations of cells will be compared using microarray analysis and/or reverse transcriptase (RT)-PCR techniques, with particular attention to the expression of molecules involved in signal transduction pathways.

Preliminary studies have revealed that the percentage of CD34+ cells is not elevated in the peripheral blood of patients with myeloid HES. Nevertheless, if enough cells are available, RNA expression comparisons after in vitro culture of CD34+ cells in the presence and absence of imatinib mesylate and/or ruxolitinib will also be performed.

8.2.3 Samples for Genetic/Genomic Analysis

8.2.3.1 Description of the Scope of Genetic/Genomic Analysis

Genetic analysis performed as part of this study will be limited to targeted DNA or RNA sequencing of genes associated with eosinophilic myeloid neoplasms.

8.2.3.2 Description of How Privacy and Confidentiality of Medical Information/Biological Specimens Will Be Maximized

Since the results of genetic testing performed as part of this protocol have potential clinical implications for the individual study participants, samples/data will be stored with the patient's name and/or a unique identifier (EOS study ID). Bone marrow and blood cells will be stored 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. Samples sent for research purposes to other investigators will be labeled with a code that only the study team can link to the identity of the participant. Data will be kept in password-protected computers.

Some genetic data will be generated in CLIA-certified laboratories (i.e., by the NIH Clinical Center Laboratory or as a send-out from the NIH Clinical Center Laboratory). These results will be documented in the patient's medical record in CRIS. Genetic abnormalities with potential clinical implications that are identified as part of non-validated panels or RNA sequencing will be validated in a CLIA-certified laboratory (i.e., by the NIH Clinical Center Laboratory or as a send-out from the NIH Clinical Center Laboratory) at no cost to the patient.

8.2.3.3 Management of Results

Validated results of genetic testing that have clinical implications will be shared with patients and their physicians. Since the goal of the research is to identify driver mutations, the majority of identified abnormalities will be somatic and have no implication for other family members. Rarely, abnormalities associated with eosinophilic myeloid neoplasms, including *JAK2* V617F, can be germline and occur in multiple family members (44). However, due to the low frequency of this occurrence, screening of family members is not generally recommended. Patients with mutations other than those in *PDGFRA/B*, which have not been identified in multiple family members to date, will be informed of the possibility that mutations are rarely present in multiple family members.

As new mutation driving eosinophilia are identified, sequencing may be repeated at multiple time points. Patients will be informed that this may occur and that, if a genetic abnormality is identified at a later date, they will be contacted and informed of this result.

Incidental findings are likely to be extremely uncommon given the study design, which primarily involves targeted analysis. Nevertheless, participants will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary

analysis. Participants will be contacted at this time with a request to provide a blood sample to be sent to a CLIA-certified laboratory. If the research findings are verified in the CLIA-certified lab, the participant will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the participant does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

8.2.3.4 Genetic counseling

Genetic counseling will be performed by a credentialed NIAID genetics counselor.

8.3 Safety and Other Assessments

Clinical data will be collected during the initial evaluation and at subsequent follow-up visits and will be stored using the CRIMSON database. Monitoring of this data will be by the PI only, since 1) the diagnostic procedures to be used will be restricted to those acceptable for optimal clinical practice, 2) the research-related procedures are of low and well-defined risk (see above), and 3) administration of the study drug is not blinded or randomized.

The following procedures will be performed to assess patient safety during the trial:

- ***Concomitant medication review –*** Medication review will be conducted by a credentialed clinician to identify potential drug interactions requiring study drug dose modification.
- ***Physical examination*** – Complete skin examination for cancer screening will be performed by a credentialed dermatologist in patients receiving ruxolitinib.
- ***Laboratory evaluations*** – CBC with differential, renal function, serum transaminases, and serum troponin will be monitored in all patients to assess drug toxicity and the need for dose adjustment. CBCs will be monitored weekly for the first month and whenever the ANC falls to <1500/mm^3 and/or the platelet count falls to <100,000/mm^3. If blood counts are higher than these levels, monitoring will be reduced to every 2 weeks until 12 weeks of treatment is reached, at which time if the counts are stable, monitoring will be performed monthly. Liver function tests will be monitored weekly for the first month of therapy, every 2 weeks until 12 weeks of treatment, and monthly thereafter, with more frequent monitoring in patients with elevated transaminases or bilirubin levels. Pregnancy testing will be performed at the baseline visit prior to initiation of drug therapy in patients of child-bearing potential and repeated at the discretion of the PI.

  Patients receiving ruxolitinib will also be monitored with fasting lipid profiles. Routine laboratory testing being performed both for safety and to assess the primary endpoint will be performed at the NIH. Other routine testing may be performed either at the NIH or at a local laboratory.
- ***EKG and Echocardiography*** – Routine EKG and echocardiography will be performed to assess risk and provide a baseline prior to initiation of study drug in all participants. Follow-up assessments will be performed to assess drug toxicity.
- ***Flow cytometry*** - A T/B/NK flow cytometry panel will be performed at baseline and at least yearly thereafter in all patients receiving ruxolitinib to monitor B-cell numbers and

every three months in patients with aberrant T-cell populations on either study arm to monitor clonal expansion.

- ***Assessment of AEs*** - AEs related to study procedures or interventions will be assessed at every visit. If the event has not resolved prior to the end of the visit, follow up will be arranged prior to discharge. The timing of the follow up will depend on the nature and severity of the AE/SAE. Clinical exacerbations and laboratory abnormalities related to eosinophilia or therapeutic interventions will not be classified as AEs unless they are possibly, probably, or definitely related to the research or meet criteria for an SAE.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event

AE means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

AEs will be assessed by the study clinician using the Common Terminology Criteria for Adverse Events v4.0 (CTCAE), published May 28, 2009 at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/Archive/CTCAE_4.0_2009-05-29_QuickReference_8.5x11.pdf.

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

8.4.3.2 Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.4.3.3 Expectedness

The PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured in CRIMSON. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI or appropriate designee as defined in the study delegation log will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 Adverse Event Reporting

AEs will be reported to the NIH IRB per NIH Human Research Protection Program (HRPP) Policy 801.

8.4.6 Serious Adverse Event Reporting

SAEs will be reported to the NIH IRB per NIH HRPP Policy 801.

For participants enrolled on the ruxolitinib arm of the study only:

Every SAE, regardless of suspected causality (e.g., relationship to study drug(s) or study procedure or disease progression), occurring after the participant has signed the consent form through the last study visit (or 30 days after the last dose of study drug, whichever is later) must be reported to Incyte Safety within 7 calendar days of learning of the event, via email to SafetyReporting@incyte.com.

Any SAEs occurring more than 30 days after the last dose of study drug should be reported to Incyte only if the investigator suspects a causal relationship to the study drug.

In a study where other medication is used in combination with an Incyte study drug, the relationship to each study drug must be assessed (ie, for the Incyte product(s) and for the other product that is used in combination with the Incyte product).

8.4.7 NIH Intramural IRB Reporting of IND Safety Reports

Not applicable.

8.4.8 Events of Special Interest

Not applicable.

8.4.9 Reporting of Pregnancy

Patients who become pregnant while on study will be withdrawn from this study and the event will be reported to the IRB, the NIAID Clinical Director, and, if appropriate, to Incyte. They will continue to be followed on protocol 94-I-0079 unless they request otherwise, and pregnancy outcome will be documented.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report UPs to the NIH IRB as per HRPP Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 Outcomes

The primary outcome measure for this study is the peripheral blood AEC at 1 month (for imatinib) and 3 months (for ruxolitinib). This will be measured at the same time of day in each participant at specified times before and after treatment. The efficacy of imatinib mesylate or ruxolitinib in reducing eosinophilia will be estimated at 1, 3, 6, 9, and 12 months after the initiation of treatment as the percentage of patients who reach eosinophil counts in the normal range ($<860/mm^3$). Efficacy will also be estimated as the percentage of patients who reach eosinophil levels below $1500/mm^3$, the usual target level for treatment of HES. Outcomes will be measured separately for patients with the myeloid variant of HES and those with non-myeloid variant steroid-refractory disease.

Secondary outcome measurements for HES will include serum levels of eosinophil granule proteins (a surrogate marker of eosinophil activation), expression of surface markers of eosinophil activation, resolution of myelofibrosis and other bone marrow abnormalities, reduction or disappearance of *FIP1L1/PDGFRA* transcript or *JAK2* V617F as detected by standard Clinical Center testing, and progression of clinical disease. In addition, we hope to determine how long remission of disease continues following discontinuation of imatinib therapy.

9.2 Statistical Methods

This study is limited by the availability of patients with this rare disorder. None of the patients are expected to experience decreasing eosinophil counts spontaneously or due to other treatment during the study period. Assuming that 10 patients are enrolled, at least 4 of them will need to reach an eosinophil count in the normal range for the study to show a significant effect at the 95% level (Fisher exact test, 1-tailed). Similar power calculations for efficacy estimates based on eosinophil counts below 1500/mm^3 apply. If only 5 participants are enrolled, 4 of these would have to reach the desired endpoint to achieve the same level of significance.

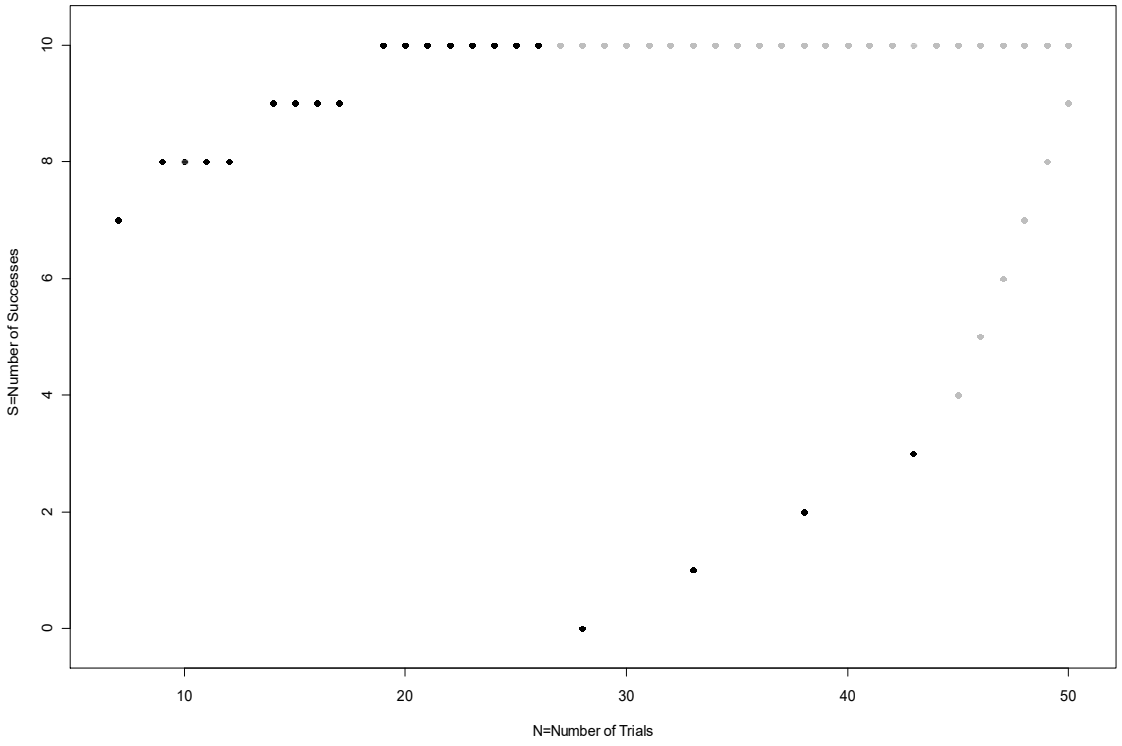
In addition to assessing the effects of imatinib on eosinophilia in *FIP1L1/PDGFRA*-positive patients, patients who are *FIP1L1/PDGFRA*-negative with features of myeloid HES or who do not respond to steroids will also be treated on this protocol. Current data suggest that approximately 50% of such patients will respond to imatinib therapy (21). Because the treatment recommended within this protocol for these patients is within the bounds of acceptable standard of care for such patients, it is reasonable to continue to study such patients as they become available since they could receive this treatment even if they are not enrolled in this study. The advantage of continuing the study this way is that better estimates of the proportion of responders from this population will be made.

To calculate the number of *FIP1L1/PDGFRA*-negative patients for this extension, we do not rely on standard fixed sample size power calculations for the following reasons. First, there is no obvious null hypothesis that should be tested, since we are interested in getting a good estimate of the proportion of responders not showing that the rate is statistically significantly different than some null value. Second, the data structure is ideally suited for a sequential design, because the time until measurement of the response for each patient is relatively quick compared to the waiting time until the next patient becomes available. Because of these reasons, we use a sequential design for this portion of the study.

The sequential design should have the following properties. We want an upper bound on the total number of participants who could enroll to prevent the possibility that the study would continue too long without conclusion. We want to be able to stop and report the proportion of responders if it is very much larger or smaller than the current best estimate of 20%. We want our final confidence interval on the true proportion of responders to have good frequentist properties (i.e., if we repeat similarly designed trials many times, about 95 percent of the time the 95% confidence interval would cover the true proportion). Although, most patients will be treated and have a measured response before the next patient is enrolled, we cannot guarantee this separation in time between patient enrollments; therefore, we cannot design the study as a fully sequential design such that stopping may occur after every patient.

With these properties in mind, we use a working design based on a truncated sequential probability ratio test. We call this a “working” design because we allow adaptations to the design in order to accommodate the last property mentioned in the previous paragraph. We use the B value formulation of this test (45,46), where the maximum sample size is set to 50, the stopping criteria for the B-value are the 0.025 and the 0.975 quantiles of the standard normal distribution, and the null hypothesis is that the true response rate is 20%. We use stage-wise ordering (see (47), p. 179) so that the confidence intervals do not depend on the implementation of the future design if the study is stopped before the maximum of 50 is reached. We use median unbiased

estimators of the true proportion so that they will be consistent with the confidence intervals (see (48), p. 133). If a few patients are enrolled closely together, we allow an adaptation of the working design such that such groups may be analyzed together. The adaptation we use is to simply remove the stopping times for all but the last of the patients that are grouped close together, and then we recalculate the confidence intervals based on the adaptation. This procedure will continue to produce 95% confidence intervals with proper coverage since we can assume the grouping to be unrelated to the response. If other logistical reasons not related to the observed response prevent the checking of the stopping boundary, then similar adaptations could be done without adversely affecting the coverage of the confidence intervals. We plot the stopping points for the working design below. Black points show significant differences from 20%. We see for example that if the first 7 patients respond, then we stop. Or if none of the first 28 patients respond then we stop. The full working sequential design is listed in Appendix A.



10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 Informed Consent Process

10.1.1 Consent/Assent Procedures and Documentation

All participants will provide written informed consent prior to undergoing any study procedures. Most patients will sign consent at the time of their first visit to the NIH Clinical Center. Consent will be obtained by a clinically credentialed investigator on the study team. The study team member obtaining consent will document the signing of the consent form in the participant's medical record and provide copies to medical records and to the participant for their personal records. The study procedures, risks and benefits will be clearly explained, and it will be made

clear that refusing to participate will in no way affect the care that they receive from their outside providers and that they may decide not to enroll or withdraw consent at any time throughout the course of the trial. The consent will be provided to prospective participants to review prior to their enrollment, and they will be permitted as much time as they need to consult with family members, their physician, or others and to ask questions before signing. Questions will be addressed by the study team member obtaining consent and, if requested, by the PI. The consent (and assent document, if applicable) can be printed to sign and date in ink or signed via an approved electronic platform.

Currently enrolled participants who are unable to return to the NIH in a timely manner may provide consent remotely when resigning of the consent form is required by the IRB. When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. Whether in person or remote, the privacy of the participant will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). The informed consent document (and assent document, if applicable) will be sent via secure email, file transfer or mail to the potential participant and parent/guardian, if applicable. An explanation of the study will be provided over the telephone or approved video conference platform after the participant (and parent/guardian, if applicable) has had an opportunity to read the consent/assent form. The participant and parent(s)/guardian(s) can print the appropriate form(s) to sign and date in ink, or they can sign and date electronically via an approved platform.

When an electronic document is used for the documentation of consent at the NIH Clinical Center or remotely, this study permits the use of the DocuSign platform and/or iMED, the latter of which is 21 CFR Part 11–compliant, to obtain the required signatures. The identity of the participant will be determined by a prompt which will require the provision of information from a form of govern issued identification prior to obtaining the signature. During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations. Both the investigator and the participant will sign the document with a hand signature using a finger, stylus mouse or equivalent. Electronic signature will be provided by required parties through system prompts.

Consent Process for Minors/Children (under 18 years of age)

Parental permission for children under 18 years of age to participate in the study will be obtained prior to performing any research procedures. In situations where there is joint custody of a child, both parents must sign consent. If only one parent can be present at NIH, the other parent's consent can be obtained remotely.

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 7-11. Children under the age of 18, but who are age 12 or older will be asked to sign an age-appropriate assent form. Children under the age of 7 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.

10.1.2 Consent for Minors When They Reach the Age of Majority

When a pediatric participant reaches age 18, continued participation (including ongoing interactions with the participant or continued analysis of identifiable data) will require that consent be obtained from 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 45 CFR 46.116(f)(3):

(1) The research involves no more than minimal risk to the participants.
    a. Analysis of samples and data from this study involves no additional risks to participants.
(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) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.
    a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.
(4) The waiver or alteration will not adversely affect the rights and welfare of the participants.
    a. Retention of these samples or data does not affect the welfare of participants.
(5) Whenever appropriate, the participants will be provided with additional pertinent information after participation.
    a. We only request a waiver of consent for those participants who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

10.1.3 Considerations for Consent of NIH Staff or Family Members of Study Team Members

Consent for NIH staff and family members of the study team will be obtained as detailed above and will comply with the requirements of NIH Human Research Protections Program (HRPP) Policy 404 *Research Involving NIH Staff as Subjects*.

Consent from NIH staff for whom this research is taking place within their own work unit or is conducted by any of their supervisors will, when possible, be obtained by an individual in a

non-supervisory relationship with that staff member. When consent of that staff member is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on the staff member. Similarly, for family of the study team, a study team member unrelated to the participant will obtain their informed consent. When consent of that staff member's relative is conducted, a third party will be present to observe the consent process in order to minimize the risk of undue pressure on them.

10.1.4 Consent of Participants Who Are or Become Decisionally Impaired

Adults unable to provide consent may enroll in the protocol at the discretion of the PI. In addition, it is possible that participants enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. The capacity to consent and the identity of the legally authorized representative (LAR) will be determined by the PI in both of these situations based on available documentation. If documentation is unclear or no documentation is available, the NIH Ability to Consent Assessment Team may be consulted for formal independent assessment of the ongoing capacity of a participant to consent for themselves in the setting of a major neurologic event or at the request of the PI or other member of the study team. For adult participants that lack decision-making capacity to consent, the procedures described in NIH HRPP Policy 403 *Research with Subjects Lacking Capacity to Consent* for identifying an LAR to consent on their behalf at the NIH Clinical Center will be followed. The validity of the LAR will be checked to be consistent with Policy 403, and their responsibilities as a representative of the participant will be discussed. When an LAR is used to provide initial consent or re-consent, the same process used to obtain consent from participants (described above) will be used.

10.1.5 Request of Waiver of Consent for Screening Activities

Prior to the patient signing the consent for this study, screening activities listed in section 8.1 may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the potential participants. A waiver will not adversely affect the rights and welfare of the participants given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the waiver as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The potential participants will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the PI. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and Incyte (the supplier of ruxolitinib), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Demonstration of efficacy that would warrant stopping.
- Insufficient compliance to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination that the primary endpoint has been met.
- Determination of futility.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB and, if applicable, Incyte.

10.3 Confidentiality and Privacy

The ability to link longitudinal clinical data with research samples is crucial to the objectives of this study. Consequently, data and samples collected under this protocol will be linked to identifiers with the exception of coded and/or de-identified data and samples sent to research collaborators (see below). All data will be kept in password-protected computers. Only investigators or their designees will have access to samples and data. Samples will be tracked using Biological Specimen Inventory System.

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the drug supplier. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the PI.

All research activities will be conducted in as private a setting as possible.

Representatives of the IRB and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB and institutional policies.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 Future Use of Stored Specimens and Data

Data collected as part of this protocol will be stored in secure databases on password-protected computers. Biological samples (serum, cells, and tissue samples) will be stored indefinitely for

future use in the laboratory of Dr. Amy Klion. Serum will be stored in a –80°C freezer, cells in liquid nitrogen, and tissue samples at room temperature or frozen as appropriate.

Other investigators (at NIH and elsewhere) may wish to study these specimens and data. If the planned research falls within the category of "human subjects research" on the part of the investigators, NIH IRB review and approval or exemption will be obtained. This includes the investigators sending out coded and linked specimens or data and getting results that they can link back to their participants.

Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) that meets the definition of a reportable event will be reported to the NIH IRB per HRPP Policy 801. Additionally, participants may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the participant and to the IRB. This decision may not affect the participant's participation in this protocol or any other protocols at NIH.

10.5 Safety Oversight

The clinical research team will meet on a regular basis (approximately weekly) when participants are being actively enrolled/evaluated on the study to discuss each participant.

All data will be collected in a timely manner and reviewed by the PI or a lead associate investigator. Events meeting requirements for expedited reporting as described in HRPP Policy 801 will be submitted within the required timelines.

The PI will review all data on each subject to ensure safety and data accuracy. The PI will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10.6 Clinical Monitoring

Monitoring for this study will be performed by the PI.

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

Monitoring of data and samples collected on this protocol will be conducted by the PI on an ad hoc basis. Verification and documentation of stored research samples will occur yearly as part of NIH biospecimen inventory annual reporting requirements.

10.7 Quality Assurance and Quality Control

The study team will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into CRIMSON, a 21 CFR Part 11-compliant data capture system provided by NIAID. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. CRIMSON will also serve as the primary source document for the clinical data collected in this study.

10.8.2 Study Records Retention

Study documents should be retained as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the PI.

10.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH IRB as per HRPP Policy 801. All deviations must be addressed in study source documents. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the participant, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 Reporting to the NIAID Clinical Director

Major protocol deviations, UPs, and deaths will be reported to the NIAID clinical director according to institutional timelines.

10.11 Publication and Data Sharing Policy

10.11.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

The NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers at any time after the completion of the primary endpoint by contacting the PI.

10.11.2 Genomic Data Sharing Compliance

This protocol does not include collection of large-scale human or non-human genomic data.

10.12 Collaborative Agreements

10.12.1 Agreement Type

A collaborative research and development agreement is currently in place with Incyte for provision of ruxolitinib for this study.

10.13 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with NIAID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse Event
AEC	Absolute Eosinophil Count
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CML	Chronic Myelogenous Leukemia
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRIMSON	Clinical Research Information Management System of the NIAID
CRIS	Clinical Research Information System
CYP	Cytochrome P450
DNA	Deoxyribonucleic Acid

EKG	Electrocardiogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GIST	Gastrointestinal Stromal Tumor
GvHD	Graft-versus-Host Disease
HES	Hypereosinophilic Syndrome
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protection Program
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
Ig	Immunoglobulin
IL	Interleukin
IND	Investigational New Drug Application
IRB	Institutional Review Board
JAK	Janus-associated Kinase
LAR	Legally Authorized Representative
LDH	Lactate Dehydrogenase
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural Killer
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PO	Oral(ly)
PV	Polycythemia Vera
QC	Quality Control
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
UP	Unanticipated Problem

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Appendix A. Working Sequential Design, with 95% Confidence Intervals, and Median Unbiased Estimates of the Proportion of Responders (N=total sample at stopping time, S=number of responders at stopping time)

N	S	Estimate	95% C.I.	p-value
7	7	0.9057	(0.5904,1.0000)	<.0001
9	8	0.8617	(0.5110,0.9964)	0.0001
10	8	0.7791	(0.4424,0.9715)	0.0002
11	8	0.7079	(0.3898,0.9331)	0.0005
12	8	0.6483	(0.3487,0.8906)	0.0012
14	9	0.6070	(0.3352,0.8483)	0.0014
15	9	0.5765	(0.3157,0.8181)	0.0021
16	9	0.5440	(0.2955,0.7849)	0.0034
17	9	0.5133	(0.2765,0.7523)	0.0055
19	10	0.4922	(0.2711,0.7212)	0.0062
20	10	0.4766	(0.2621,0.7025)	0.0076
21	10	0.4582	(0.2516,0.6800)	0.0101
22	10	0.4396	(0.2406,0.6571)	0.0139
23	10	0.4216	(0.2300,0.6349)	0.0192
24	10	0.4046	(0.2199,0.6136)	0.0263
25	10	0.3886	(0.2105,0.5935)	0.0355
26	10	0.3738	(0.2018,0.5744)	0.0472
27	10	0.3600	(0.1937,0.5565)	0.0614
28	0	0.0245	(0.0000,0.1234)	0.0039
28	10	0.3472	(0.1862,0.5394)	0.0786
29	10	0.3352	(0.1793,0.5234)	0.0989
30	10	0.3240	(0.1728,0.5083)	0.1225
31	10	0.3135	(0.1668,0.4939)	0.1495
32	10	0.3037	(0.1611,0.4803)	0.1799
33	1	0.0373	(0.0009,0.1594)	0.0127
33	10	0.2945	(0.1559,0.4674)	0.2137
34	10	0.2858	(0.1510,0.4552)	0.2510
35	10	0.2776	(0.1463,0.4436)	0.2916
36	10	0.2698	(0.1420,0.4325)	0.3352

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 37 10   0.2625 (0.1379,0.4220)  0.3818
 38  2   0.0610 (0.0075,0.1811)  0.0262
 38 10   0.2556 (0.1340,0.4120)  0.4311
 39 10   0.2490 (0.1304,0.4024)  0.4828
 40 10   0.2427 (0.1269,0.3933)  0.5365
 41 10   0.2368 (0.1236,0.3845)  0.5919
 42 10   0.2311 (0.1205,0.3761)  0.6488
 43  3   0.0789 (0.0169,0.1958)  0.0433
 43 10   0.2257 (0.1176,0.3681)  0.7067
 44 10   0.2206 (0.1147,0.3604)  0.7652
 45  4   0.0953 (0.0265,0.2145)  0.0819
 45 10   0.2157 (0.1120,0.3530)  0.8241
 46  5   0.1133 (0.0374,0.2363)  0.1619
 46 10   0.2110 (0.1095,0.3460)  0.8830
 47  6   0.1318 (0.0495,0.2576)  0.2881
 47 10   0.2065 (0.1070,0.3392)  0.9416
 48  7   0.1498 (0.0621,0.2777)  0.4587
 48 10   0.2021 (0.1047,0.3326)  0.9995
 49  8   0.1672 (0.0748,0.2966)  0.6639
 49 10   0.1980 (0.1025,0.3263)  1.0000
 50  9   0.1839 (0.0876,0.3144)  0.8875
 50 10   0.1940 (0.1003,0.3202)  0.9434
p-values test whether proportion is different (two-sided) from
0.2
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