Protocol with Statistical Analysis Plan Cover Page:

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Investigational Agents *(if applicable)***: N/A**

Drug Name:	Ruxolitinib (Jakafi)
IND Number:	Exempt
Sponsor:	NIH
Manufacturer:	Incyte

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

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

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; 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:

Single Patient Study of JAK/STAT Inhibition in CNS Köhlmeier Degos Disease

Study Description:

This single patient protocol will provide off-label treatment with a JAK/STAT (Janus kinases/ Signal transducer and Activator of Transcription proteins) inhibitor to a patient with Köhlmeier Degos disease (K-D) with neurologic involvement. We hypothesize that ruxolitinib, which targets type I IFN (Interferons) and IFN-γ signaling, will attenuate various neurological manifestations of K-D that are observed clinically, radiologically or in abnormal laboratory findings in our K-D patient. This will help reduce IFN signaling in a manner that may slow or halt the disease progression as measured by the endpoints established below.

Objectives:

Primary Objective: To test the hypothesis that JAK/STAT inhibition by ruxolitinib will delay progression of neuroradiological manifestations of our one patient with neurological involvement of K-D disease.

Exploratory Objectives:

1) Assess changes in immune cell proportion using single cell RNAseq (scRNA-seq) in biospecimens such as skin, cerebrospinal fluid (CSF) and blood after 13 weeks and up to 73 weeks of ruxolitinib treatment.
2) Assess changes in plasma/serum cytokines levels and IFN scores as well as biomarker assays in CSF/ blood samples after 13 weeks and up to 73 weeks of ruxolitinib treatment..
3) To test the hypothesis that ruxolitinib will stabilize or improve clinical neurologic exams in this patient.

Endpoints:

Primary Endpoint:

The primary endpoint is stability or regression of existing enhancing lesions or no development of new enhancing lesions in the brain and spine observed in MRI evaluation after 13 weeks and up to 73 weeks of ruloxitinib (10 mg BID) compared to pre-treatment MRI images.

Exploratory endpoints of this study are clinical and potential surrogate biomarker efficacy data, including:

1. Changes in transcriptome/RNA expression determined by scRNA-seq in skin, CSF and peripheral blood mononuclear cells (PBMCs) between baseline measurements and after 13 weeks and up to 73 weeks of treatment.
2. Attenuation of plasma/serum cytokine levels and IFN scores as well as biomarker assays in CSF and blood samples between the baseline visit and after 13 weeks and up to 73 weeks of treatment.
3. Stability OR improvement of motor and/or sensory function on clinical neurologic exams between the baseline visit and after 13 weeks and up to 73 weeks of treatment.

Study Population:	A 58 year old male with neurological involvement of K-D disease.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	This study will be conducted only at the NIH Clinical Center.
Description of Study Intervention:	Ruxolitinib at 5 mg twice a day (BID) for 1 week and then at 10 mg BID for 13-73 weeks and 1 week of 5 mg BID before stopping ruxolitinib.
Study Duration:	1 year
Participant Duration:	17-77 weeks

1.2 SCHEMA

N/A

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Visit 1: Baseline Research Testing Week 0	Visit 2: Day 7 after start of study drug (+/- 3 days). Telehealth visit.	Visit 3: Day 28 (+/-7 days) Telehealth Visit	Visit 4: End of Week 13 (+/- 7 days)	Visit 5: End of week 14+/- 7 days Telehealth Visit for drug continuation	*Visit 6: End of Week 25 (+/- 7 days)	*Visit 7: End of Week 49 (+/- 7 days)	Visit 8: End of week 61 (+/- 7 days) Telehealth visit	*Continuation Visit 9: Up to the end of week 73 or End of Treatment	**Telephone/or at NIH Safety Visit 4 weeks after last dose of study drug (+/- 7 days) or prn
Informed consent	X	-	-	-		-	-	-	-	-
History/Physical	X	-	X	X		X	X	X	X	X
Concomitant Medication	X	-	X	X		X	X	X	X	-
Vital signs/Wt.	X	-	X	X		X	X	-	X	X
Research Labs	X	-	X	X		X	X	X	X	X
Clinical Labs										
CBC with diff	X	X	X	X		X	X	X	X	X
Mineral panel	X	X	X	X		X	X	X	X	X
Acute care panel	X	X	X	X		X	X	X	X	X
Liver function panel	X	X	X	X		X	X	X	X	X
Lipid panel	X	X	X	X		X	X	X	X	X

Procedures	Visit 1: Baseline Research Testing Week 0	Visit 2: Day 7 after start of study drug (+/- 3 days). Telehealth visit.	Visit 3: Day 28 (+/-7 days) Telehealth Visit	Visit 4: End of Week 13 (+/- 7 days)	Visit 5: End of week 14+/- 7 days Telehealth Visit for drug continuation	*Visit 6: End of Week 25 (+/- 7 days)	*Visit 7: End of Week 49 (+/- 7 days)	Visit 8: End of week 61 (+/- 7 days) Telehealth visit	*Continuation Visit 9: Up to the end of week 73 or End of Treatment	**Telephone/or at NIH Safety Visit 4 weeks after last dose of study drug (+/- 7 days) or prn
Lymphocyte peripheral blood phenotyping	X	X	X	X		X	X		X	X
Oligoclonal banding, serum	X	-	-	X		X	X	-	X	-
IgG Index, serum	X	-	-	X		X	X	-	X	-
Procedures										
Brain /Spine MRI with contrast	X	-	-	X		X	X	-	X***	-
Neurological Consult	X	-	X	X		X	X	-	X	-
CSF collection with LP • Glucose • Protein • Oligoclonal Banding, CSF • L-lactate • Albumin	X	-	-	X		X	X	-	X***	-

Procedures	Visit 1: Baseline Research Testing Week 0	Visit 2: Day 7 after start of study drug (+/- 3 days). Telehealth visit.	Visit 3: Day 28 (+/-7 days) Telehealth Visit	Visit 4: End of Week 13 (+/- 7 days)	Visit 5: End of week 14+/- 7 days Telehealth Visit for drug continuation	*Visit 6: End of Week 25 (+/- 7 days)	*Visit 7: End of Week 49 (+/- 7 days)	Visit 8: End of week 61 (+/- 7 days) Telehealth visit	*Continuation Visit 9: Up to the end of week 73 or End of Treatment	**Telephone/or at NIH Safety Visit 4 weeks after last dose of study drug (+/- 7 days) or prn
• CSF, Cell Count and Diff • IgG Index, CSF										
Skin biopsies	X	-	-	X		X	X	-	X***	-
Begin study medication	X	-	-	-		-	-	-	-	-
Dose increase	-	X	-	-		-	-	-	-	-
Pill count	-	X	X	X		X	X	X	X	-
AE review and collection	-	X	X	X		X	X	X	X	X
Analysis of primary endpoint	-	-	-	X		-	-	-	-	-
Determine if medication continuation or weaning	-	-	-	-	X	-	-	-	-	-

All visits may occur either in person in NIH or via Telehealth platform, based on subject's ability to travel to NIH, except for visits 4, 6, 7, and 9 which should be in person NIH visits.

If the visit occurs via telehealth platform all activities are optional and only minimal risk activities may be performed.

*This visit will only occur if patient continues therapy

**This visit will occur 4 weeks after last dose of study drug (+/- 3 days) and as needed per subjects AE review and safety assessment throughout the study. It may occur via Telephone/Telehealth or in person in NIH. If it happens in NIH all tests/procedures are optional, except for AE review and collection. If subject continues to have AEs during last safety visit (4weeks after last dose), we will see him atin NIH for additional unscheduled safety visits, until all AEs are resolved, return to baseline or are stabilized.

***These tests will be optional at Visit 9

Telehealth **Visit 2** blood draw may take place at local doctor's office or phlebotomy laboratory.

2 INTRODUCTION

2.1 STUDY RATIONALE

Köhlmeier-Degos disease (K-D) disease is a rare small vessel vasculopathy of unknown etiology leading to small blood vessel occlusions in multiple organs including the skin, central nervous system (CNS), eye, gastrointestinal (GI) tract, lungs and heart. Approximately 300 K-D cases have been reported in the literature to date [1-3]. K-D commonly presents as a benign cutaneous form with lesions that appear as erythematous papules and evolve to form a scar with an atrophic, porcelain-white center surrounded by a telangiectatic border. Progression to systemic K-D, or malignant atrophic papulosis, occurs in 2/3 of the patients, is often debilitating and can be fatal (60-70% mortality rate within 2-5 years after diagnosis) due to GI perforations, brain infarcts, spinal lesions, cardiac or pulmonary failure, sepsis or cachexia[3] .

Among patients with systemic involvement, the GI tract is affected in more than 70%, followed by CNS involvement (40-50%). CNS manifestations have been present in more than 60 % of K-D patients with GI involvement [4]. In part due to the lack of insight into K-D pathophysiology, no standard of care exists and there are currently no clinical trials available for K-D patients. Eculizumab and treprostinil have been used as off-label treatment for systemic K-D with GI involvement and have shown some moderate-term symptom control[4-6]. However, there are no known effective treatments for CNS K-D, driving poor outcomes and high mortality rates in these patients.

The patient selected for this study is a 58 year-old male with a history of systemic K-D disease with GI and CNS manifestations, who is currently being treated with eculizumab and treprostinil.

He had no significant past medical history until 2008 when he developed his first K-D skin lesion, although he was not diagnosed with K-D disease until 2021 based on a skin biopsy. This delay in diagnosis is due to lack of knowledge and specific diagnostic tests of this rare disease. He began to develop neurologic symptoms of peripheral neuropathy in 2018, which rapidly progressed resulting in bowel and bladder incontinence necessitating self-catheterization and bowel evacuation, and leg weakness (left worse than right) affecting ambulation. The patient has had an extensive infectious, immunological work-up and all the reports were negative. Cerebrospinal fluid analysis showed elevated protein, but the other labs were within normal limits.

He additionally developed unilateral sensorineural hearing loss and cognitive complaints. A brain MRI in September of 2021 revealed multiple leptomeningeal enhancing lesions, most significantly in the left anterior frontal lobe as well as multifocal enhancements along the lumbosacral cauda equina. He was first seen at NIH in 2022 and CSF collected with a lumbar puncture (LP) demonstrated elevated CSF protein, albumin, IgG, neopterin levels, IL-2 Rsol and IL-6. At that time, he was started on eculizumab and treprostinil. Nevertheless, his neurologic symptoms have continued to worsen, and he is now wheelchair-bound. Along with these worsening symptoms, he has developed new areas of leptomeningeal enhancement in the brain and spinal cord, consistent with the progression of K-D disease.

2.2 BACKGROUND

The pathogenesis of K-D disease remains unknown, but it is diagnosed by biopsy of skin lesions, which on pathology review reveal a wedge-shaped ischemia-driven fibrosis with obliterative intimal arteriopathy and adjacent vascular ectasia. Magro et al described a thickening of venules and capillary basement membrane and pauci-inflammatory thrombogenic microangiopathy with endothelial cell injury and mucin deposition within the dermis and subcutaneous fat[6,7]. An increase in interferon-alpha (IFN-α) activity in the pathogenesis of the K-D lesions by increased Myxovirus resistance protein 1 (MxA) protein deposits and Rho Associated Coiled-Coil Containing Protein Kinase 2 (ROCK-2) upregulation in endothelium has been previously reported [7,8].

The pathology of gastrointestinal and neurological K-D lesions are less clearly defined. GI lesions appear grossly similar to cutaneous lesions and they present with extravascular sclerosis with arteriopathy limited to the sub serosal fat[4]. Information on the pathology of CNS lesions is even more limited, but case reports describe thickened leptomeningeal vessels with perivascular inflammation. Cortical vessels with fibrin thrombus and vessel wall inflammation are present [9]. It is known that the complement pathway plays an essential role in endothelial cell injury in systemic and cutaneous lesions[10]. While the contribution of inflammation in K-D remains undetermined, dysregulation of type I IFN-α response has been reported in skin biopsies [8,10]. Our preliminary studies using scRNA-seq analyses have shown increased interferon γ (IFN-γ) activity in skin, blood and cerebral spinal fluid (CSF) of one patient with neurological involvement of K-D. This appears to be driven by CD8 T cells, suggesting a role for adaptive immunity facilitated by IFN-γ in patients with systemic K-D. Additionally, we have observed a chronic elevation of the type I

IFN and IFN-γ response genes in blood measured with NanoString technology and ten increased cytokines in plasma samples of systemic K-D patients.

JAK Inhibitors in Neurologic Disease

Ruxolitinib interferes with the signaling of several cytokines and growth factors that are important for immune function as noted in the ruxolitinib package insert. JAK signaling involves recruitment of signal transducers and activators of transcription (STATs) to cytokine receptors, activation, and subsequent localization of STATs to the nucleus leading to modulation of gene expression. Dysregulation of the JAK/STAT pathway has been associated with multiple autoimmune and autoinflammatory disorders with CNS involvement[38] including various interferonopathies: Aicardi-Goutières syndrome[39,40] [39,41] and STING-associated vasculopathy with onset in infancy (SAVI [42]**.** Aicardi-Goutières syndrome is a rare autosomal recessive leukodystrophy, in which type I interferon plays a pivotal role were ruloxitinib was used with a favorable outcome.

In stroke animal models, ruxolitinib treatment improved neurological score, decreased infarct size and cerebral edema [43] and has neuroprotective effect on traumatic brain injury[44]. Secondary-hemophagocytic lymphohistiocytosis (HLH) is a life-threatening clinical syndrome that can lead to rapid mortality. Ruxolitinib has been used in several HLH patients, including those with CNS involvement and resulted in improvement in overall survival[45]. Ruloxitinib can cause weight gain in patients with myeloproliferative neoplasms by blocking leptin signaling in the brain[46].Taken together, data from these studies suggests that ruloxitinib can be used in this patient with CNS K-D.

JAK Inhibitors in Autoimmune Diseases

JAK inhibitors have shown efficacy in a number of autoimmune disorders and are currently being used in clinical practice. To date, ruxolitinib has not been extensively studied or licensed in patients without hematologic or oncologic diagnoses. In rheumatoid arthritis, three JAK inhibitors are currently FDA approved: tofacitinib (JAK1/JAK3 and mild JAK2 inhibition), baricitinib (JAK1/JAK2 + some moderate TYK2 inhibition), and upadacitinib (JAK1 with some mild JAK2/JAK3 inhibition). JAK inhibitors show efficacy in patients with active rheumatoid arthritis that does not inadequately respond to methotrexate compared with placebo or a standard-of-care disease modifying antirheumatic drug (DMARD), adalimumab, a TNF inhibitor. [47].

Preliminary Results:

Laboratory Evaluation of CSF Cytokines/Biomarkers:

This neurological K-D patient has had four lumbar punctures, taken over the course of 28 months while enrolled in one of our natural history protocols, 18-H-0108 (Vascular Disease Discovery Protocol, VDDP). We examined a panel of 13 cytokines in these CSF samples (IL-2, Interleukin 2 Receptor Soluble, IFN-γ, IL-10, IL-12, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-13, IL-17, and TNF-α)

with a CLIA certified quantitative multiplex bead assay. Through this assay, we found increased levels of soluble IL-2 receptor and IL-6 in the CSF. In fact, high CSF levels of both cytokines have been previously reported in various infectious, inflammatory, and degenerative CNS disorders, where these molecules have been shown to correlate with disease activity status and expanded disability status scale score [11,12]. High levels of neopterin were also noted in the CSF with a mean value of 70 nmol/L with 2.5 fold higher levels than those observed in healthy volunteers (8-28 nmol/L normal values in CSF). Neopterin is an immune activation biomarker produced by microglia and astrocytes after the immune activation of IFN-γ. The elevation of neopterin in CSF has also been reported in multiple diseases including acute viral and bacterial infections, multiple sclerosis, Parkinson disease, autoimmune encephalitis, and CNS lymphoma [13].Further, high neopterin values have been previously reported as a valuable marker to differentiate between inflammatory and non-inflammatory neurological disorders, but doesn't appear to be a specific marker that can differentiate between various neuroinflammatory diseases. From other CSF standard parameters, we found an increase in protein (mean 106 mg/dL, normal <40 mg/dL) and albumin quotient (22.13 ratio, normal <9) in our neurological K-D patient over a period of 18 months.

More than 15 patients with cutaneous and systemic K-D are currently enrolled in our VDDP and Biospecimen Procurement and Analysis (15-H-0190) protocols since 2013. Most of these patients are evaluated annually at the NIH Clinical Center for comprehensive clinical and laboratory assessments. We have analyzed their samples with a BioRad Bio-Plex Pro Human Chemokine 40-plex Panel (No. #171AK99MR2) to identify plasma cytokines that are increased in K-D. Among 40 chemokines tested in this panel, four (CCL19, CCL23, CCL17 and CCL25) were elevated as compared to healthy volunteers at a P<0.05 significance level using a two-tailed t-test and an additional six (IFN-γ, IL-6, CCL11,CCL26, CCL7, and CXCL9) were increased at P<0.1 significance level (Fig 1A). Consistent with chronic elevation of the type I IFN and IFN-γ responses, signaling network analysis performed by MetaCore software (Clarivate) (figure 1B) demonstrated that these ten cytokines lie within an IFN-γ and IFN-α-dependent signaling network downstream of JAK1 and JAK2 (figure 1C). This result suggests that JAK1/JAK2-dependent signaling may be upregulated in K-D patients and we plan to use these cytokines to monitor the efficacy of ruxolitinib treatment.

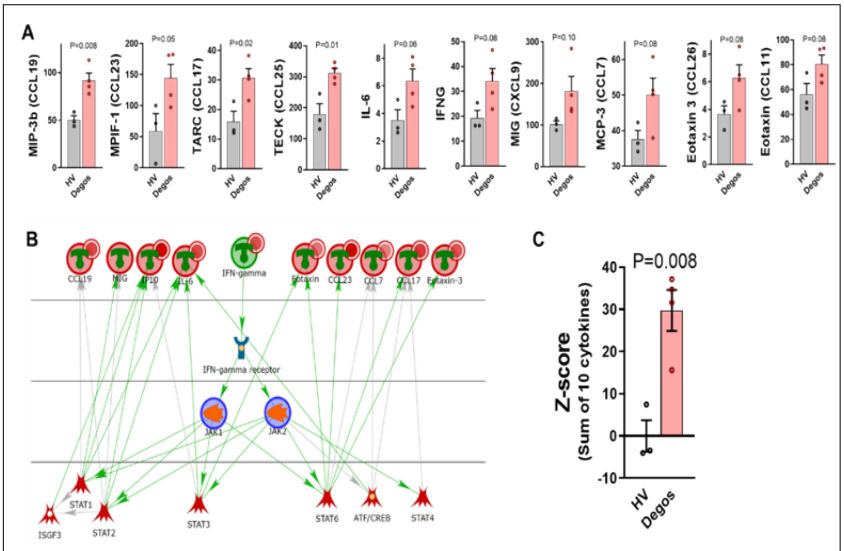

Figure 1. Elevated JAK/STAT–dependent cytokine levels in K-D patient plasma . A) Ten cytokines with increased levels in K-D patients, B) Signaling network demonstrating that 10 cytokines are upregulated by IFN/JAK/STAT signaling, C) Combined Z-score derived from 10 cytokines, calculated relative to mean and standard deviation (SD) of healthy volunteer (HV) group.

We also evaluated a standardized 28 gene interferon score (IFNs) previously developed for CANDLE (chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature) and SAVI patients [14,15] in 5 of our own patients with systemic K-D and 2 with cutaneous K-D. We found that the IFN signatures with increased expression of IFN regulated genes in peripheral blood were significantly higher in patients with systemic K-D than those with cutaneous K-D, which appeared to be similar to healthy control samples (Figure 2). Taken together, this data shows enhanced IFN responses in K-D patients confirmed by multiple different approaches.

In order to characterize the immune responses at the single-cell level, we examined the K-D transcriptome from 4 patients using scRNA-seq (Figure 3). Major skin cell subsets were successfully captured, and comparison of cell compositions revealed that K-D skin was enriched in the lymphoid cluster, consistent with what was observed in histology. Importantly, similar to blood and CSF, we found that K-D CD8 T cells upregulated IFN-γ. Furthermore, analysis of non-T cells showed enrichment of JAK/STAT-associated genes across multiple cell types. In particular, *STAT1* and *JAK1* were upregulated in keratinocytes, vascular endothelial cells (EC), fibroblasts and dendritic cells (DCs) (Figure 3). These results demonstrate directly in affected tissue that K-D is characterized by an inflammatory milieu driven by type I IFN and IFN-γ responses with enhanced JAK-STAT pathways, supporting potential benefit of ruxolitinib in these patients.

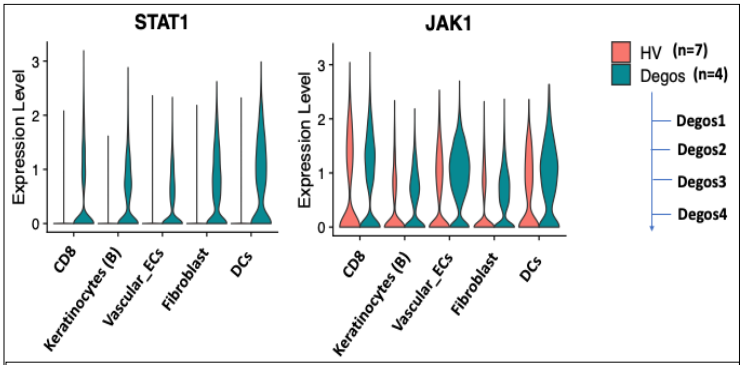

Figure 3. Upregulated JAK1-STAT1 molecules in non-immune cell (Keratinocytes, Vacular_ECs, Fibroblast) and immune cells (CD8, DCs) of K-D skin, supporting a Type I and II IFN response and suggesting an inflammatory milieu within tissue micro-environment.

Notably, scRNA-seq analyses on PBMC from 10 patients revealed that circulating K-D immune cells were characterized by type I and II (IFN-γ) signatures. In particular, whereas both cutaneous and systemic K-D harbored CD8 T cells with upregulated IFN-γ, those from systemic K-D displayed prominent upregulation of type I IFN-related genes such as *MX1* and *IRF7* and cytotoxic molecules encoding granzymes and perforins (Figure 4A). Furthermore, scRNAseq analysis of CSF from one patient with K-D has demonstrated activated JAK-STAT pathways not only in PBMCs, but also in CSF as revealed by increased *STAT1* and *JAK2* expressions. *CXCL10,* an IFN-γ-induced chemokine was also upregulated in both PBMCs and CSF of K-D patients, suggestive of their enhanced responses against type I IFN and IFN-γ (Figure 4B). These findings are supplemented by significantly elevated neopterin levels, soluble IL-2 receptor and IL-6 in CSF samples from four different lumbar punctures of one CNS K-D patient. Taken together, our data highlight an inflammatory milieu in tissues from K-D patients that is potentially driven by type I and IFN-γ responses, pointing to these cytokines and their receptor signaling as attractive therapeutic targets for ruxolitinib.

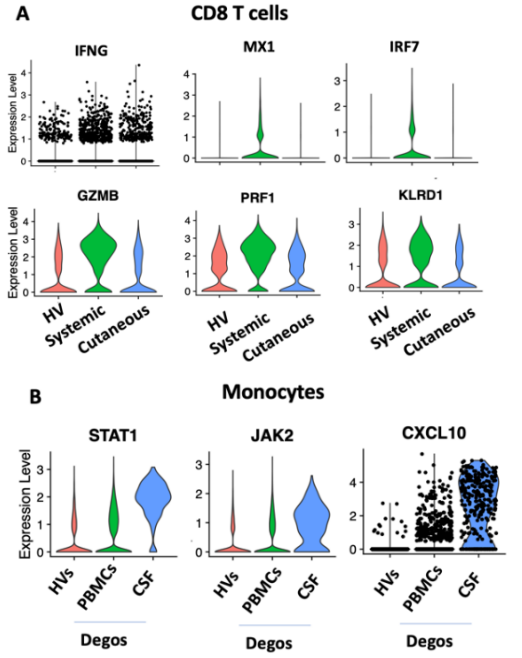

Figure 4: Upregulated Interferon responsive genes in K-D suggesting an activated JAK-STAT signaling both in peripheral blood and CSF immune cells.

Our preliminary data shows that the spinal fluid of this patient with neurological involvement contains high levels of STAT1 and JAK 2 (Figure 4), suggesting that JAK/STAT signaling pathway is activated in the spinal cord. Further, our data highlights an inflammatory milieu in tissues from K-D patients, which appears to be driven by type α/β and IFN-γ responses, pointing to these cytokines as attractive therapeutic targets. JAK/STAT inhibitors, like ruxolitinib can reduce both type α/β IFN and IFN-γ signaling and have been used in various immune-mediated conditions, including monogenic interferonopathies and are often used to treat patients with various inflammatory diseases [16]. We hypothesize that inhibition of JAK/STAT signaling using ruxolitinib has potential utility in reducing IFN signaling in our patient with neurological involvement of K-D.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Ruxolitinib:

Ruxolitinib is an orally administered small molecule inhibitor of Janus kinase JAK1/2. Ruxolitinib is FDA approved for the treatment of intermediate or high-risk myelofibrosis (MF) in adults, refractory acute and chronic graft-versus-host disease (GVHD) and polycythemia vera (PV) at dose ranges of 5mg-20mg. It has also been safely and effectively used in children with type I interferonopathy[17]. It has an established safety profile that includes more than 550 healthy volunteers and subjects with various degrees of renal (n=32) or hepatic (n=24) impairment. In healthy volunteers, the most frequent Adverse Events (AEs) reported were headache (13.0% overall), diarrhea (13.0% overall), and blood sampling catheter site hemorrhage (13.0% overall). AEs were mild and resolved without interventions[18-26]. There was no dose dependency for the frequency of AE with 25 mg BID and 100 mg QD established as the maximum tolerated doses.

Further, the AE profile of ruxolitinib has been assessed in over 14,000 patients with myelofibrosis, polycythemia vera, graft-versus-host disease, COVID-19, essential thrombocythemia, advanced malignancies, and other immune mediated inflammatory diseases[18-26]. The primary clinical risks with ruxolitinib treatment in these populations are the potential sequelae of decreased hematopoietic proliferation attributable to the inhibition of growth factor pathways associated with JAK inhibition.

In two pivotal studies, discontinuation of ruloxitinib due to AEs (regardless of causality) was observed in 8-10 % of patients[27,28]. The most frequently reported adverse drug reactions (ADRs) were thrombocytopenia and anemia. Hematological adverse reactions of grade 3 or 4 in patients with myelofibrosis included anemia (45.2% versus 19.2 %in placebo), thrombocytopenia (12.9% versus 1.3 %placebo) and neutropenia (7.1% versus 2 % placebo). Anemia, thrombocytopenia and neutropenia were dose-related effects. The three most frequent non-hematological adverse reactions were bruising (18.7%), dizziness (14.8 %) and headache (14.8%). The three most frequent non-hematological laboratory abnormalities were raised alanine aminotransferase (ALT) (27.2%), raised aspartate aminotransferase (AST) (18.6%) and hypercholesterolemia (16.9%)[29-31].

In patients with steroid-refractory acute graft versus host disease (SR-aGVHD) (REACH 1 study), ruxolitinib 10 mg BID was well tolerated when added to corticosteroid-based treatment. Treatment-emergent AEs led to discontinuation of ruxolitinib treatment in 32.4% of patients, with the most common being sepsis (5.6%), and thrombocytopenia, acute kidney injury, and respiratory failure (2.8% each). The most frequently reported ADRs were thrombocytopenia (33% versus 18 % in control group), anemia (30%), and neutropenia (16%). The incidence of cytomegalovirus infection occurred in 26% of patients receiving ruxolitinib versus 21% in control[32].

In healthy volunteers, rheumatoid arthritis patients, and patients with pancreatic cancer or hormone-refractory prostate cancer, the effects on hematopoietic proliferation are less pronounced, presumably because of greater bone marrow reserve[33,34]

The most frequent non-hematologic AEs were mild, reversible increases in ALT and AST ; bruising; hypercholesterolemia; dizziness; headache; and urinary tract infections. Tuberculosis has been infrequently reported in patients receiving ruxolitinib to treat

myelofibrosis. The symptoms of tuberculosis include chronic cough with blood-tinged sputum, fever, night sweats, and weight loss.

There may also be risks associated with rapid discontinuation of ruxolitinib. Patients with myelofibrosis, particularly those who have stopped taking ruxolitinib suddenly, have reported return of MF symptoms such as fatigue, bone pain, fever, pruritus, night sweats, symptomatic splenomegaly and weight loss[35]. In very few patients, respiratory distress, disseminated intravascular coagulation (DIC), multiorgan failure have been reported [35].

A rare disease called progressive multifocal leuko-encephalopathy (PML), has been reported with ruxolitinib[36]. It is important to note that PML and infections are complications associated with MF that has been previously described in the absence of ruxolitinib. Additionally, nonmelanoma skin cancers (NMSCs), including basal cell, squamous cell, and a rare and aggressive type of skin cancer called merkel cell carcinoma has been reported in patients who took ruxolitinib, it is unknown whether this was due to ruxolitinib treatment [37].

Ruxolitinib has no known contraindications. Detailed information about ruxolitinib is available in the Package Insert.

In summary, the expected risks of ruxolitinib treatment in our patient are:

- Thrombocytopenia, anemia and neutropenia
- Risk of infection
- Symptom exacerbation following interruption or discontinuation of treatment with ruxolitinib
- Non-melanoma skin cancer
- Lipid elevations
- Major Adverse Cardiovascular Events (MACE)
- Thrombosis
- Secondary malignancies

History and Physical Exam:

No known risks

Phlebotomy:

Standard precautions for obtaining human blood samples will be taken. There are no major risks involved with blood draws. Minor complications include bleeding, pain, bruising at the site of phlebotomy, vasovagal reactions or infections may rarely occur. This protocol will follow the NIH Clinical Center MAS policy M95-9 guidelines for limits of blood drawn for research purpose in the Clinical Center.

Skin Biopsy:

Discomfort at the biopsy site is usually mild and transient. This can be treated with minor analgesics. Most common risks include a reaction to the local anesthetic and the slight possibility of local bleeding or infection. A stitch may be placed at the discretion of the performing clinician to minimize bleeding in susceptible individuals. Scarring always occurs at the biopsy site.

Lumbar Puncture:

Adverse effects associated with lumbar punctures include brief pain or tingling radiating down the lower extremities due to the needle brushing against a nerve. Should this occur, the needle can be repositioned. Mild lower back pain at the site of needle insertion following the procedure can occur and can be managed with over the counter non-steroidal anti-inflammatory agents if needed. In approximately one third of patients, a post-dural puncture headache may develop and persist for a few days. In 1 in 50 to 200 lumbar punctures, the post-dural puncture headache can last longer than 7 days. Generally, this headache is not severe and resolves spontaneously within days to 2 weeks. Should the headache persist or be severe, a blood patch can be performed. Extremely rare complications of lumbar puncture include temporary double vision related to abducens nerve palsy, and infection. Strict aseptic technique will be followed. In adults, CSF replaces itself at a rate of approximately 21.5cc/hour, or 500cc/24hours. Thus, the maximum volume of 20cc of CSF that would be collected will be replenished in its entirety within approximately 1-1.5 hours after collection. Headache and mild low back pain are expected and will not be reported or collected in database unless Grade 2 or higher.

Magnetic Resonance Imaging of the Brain and Spine:

There are no known adverse effects from having an MRI scan performed. Standard precautions will be taken including review of any implanted devices. We will use gadolinium-based (e.g., gadobutrol) agents.

Line placement:

The placement of a peripheral intravenous line may result in mild discomfort, vasovagal reactions or bruising.

Gadolinium-based contrast agents:

The contrast agents that may be used are commercially available and are routinely employed in hospitals and radiology practices. The most commonly used are gadolinium-based contrast agents. Experience with many subjects has shown that commercially available gadolinium chelates are safe and without side effects in the majority (>98%) of subjects. When side effects

do occur, they are usually mild and transient. These include coldness in the arm during the injection, a metallic taste, headache and nausea. More severe reactions (shortness of breath, wheezing, or hypotension) are extremely rare.

Gadolinium-based contrast agents (GBCAs) are injected medications used to change the image contrast in MRI and thus providing information not available without GBCA. Most patients experience a metallic taste when gadolinium contrast is injected. Some (2%) report mild symptoms such as headache, nausea or vomiting, or a rash near the injection site. Rarely (<0.1%) patients experience severe symptoms such as wheezing, shortness of breath, and low blood pressure as part of an allergic (anaphylactoid) reaction that may require emergency medical treatment. In a few cases per million, usually in patients with severe kidney disease, gadolinium contrast can cause a rare, debilitating or even fatal, skin disease called Nephrogenic Systemic Fibrosis (NSF) that cause thickening of the skin and other organs. Since physicians became aware of the disease and began screening patients at risk of kidney disease, they switched to safer ("macrocyclic") forms of gadolinium contrast. New reports of NSF are much rarer.

In accordance with the FDA Drug Safety Communication of 05/16/2018, the Medication Guide for gadobutrol (or other macrocyclic gadolinium contrast agents if applicable) will be made available to outpatients with scans that will involve gadolinium-based contrast agent administration.

2.3.2 Known Potential Benefits

The efficacy of ruxolitinib in subjects with K-D has not yet been established. However, available pre-clinical data provide rationale for the use of JAK inhibitors in K-D. The JAK-STAT pathway plays an important role in immune cell development and function, including antigen presenting cells, B and T cells, and its activation leads to a cascade promoting a proinflammatory cytokine milieu. Ruxolitinib is an oral JAK1/JAK2 inhibitor, with an established safety and efficacy profile in the treatment of myeloproliferative neoplasms (myelofibrosis) and in acute and chronic GVHD.

Multiple studies are ongoing looking at JAK inhibitors in various autoimmune disorders. It is thus noteworthy that many of the cytokines elevated in K-D, such an IFN-γ, IL-2 R, IL-6, signal through the JAK/STAT pathway[48] . By dampening signaling downstream of these cytokines, the interruption of the JAK-STAT pathway holds promise to effectively lessen K-D associated immunopathology. There are no current standard of care treatment for patients with K-D. If successful, this study will provide preliminary evidence which may provide a new therapy for K-D where there remains a clear unmet need.

2.3.3 Assessment of Potential Risks and Benefits

Despite the aggressive nature of systemic K-D disease and more than 70 years since the first description of the disease there has been no cure with limited therapy options and no clinical trials conducted. The pathophysiology of K-D disease is poorly understood and there are no known markers of disease activity. There is a high mortality rate in K-D patients with systemic disease, including pediatric K-D patients.

This study will evaluate the effect of ruxolitinib 10 mg BID in a patient with progressive and unremitting neurological manifestation of K-D disease despite treatment with eculizumab and treprostinil. The data from this study is intended to inform the benefit-risk relationship of ruxolitinib in K-D patients. We seek to gain crucial insight into the potential efficacy of JAK/STAT inhibitors in ameliorating neurologic manifestations of disease and attenuating immunoinflammatory parameters in the skin, blood and CSF. If this profile is found to mirror disease activity, this expression profile could be used in future clinical trials.

This patient has baseline lymphopenia, which we believe is related to the disease. Serious infection is a risk with ruxolitinib and this subject will be on concomitant therapy with eculizumab and steroids, which may also increase the risk of infection. We will continuously monitor for infection and ruxolitinib treatment will be interrupted if a serious infection develops. The patient will receive either the Shingrix vaccine or viral prophylaxis with acyclovir prior to initiation of ruxolitinib, and has already received meningitis vaccination.

Although bowel perforation is not listed in the package insert as a risk for ruxolitinib, it has been reported in clinical studies of other JAK inhibitors. This patient has K-D lesions on his intestines and is at-risk of perforation. For this reason, there may be an additional risk of perforation with the use of ruxolitinib and we will monitor closely for symptoms of impending perforation.

Overall, we believe the benefits will outweigh the risks in this patient with a rare, life-threatening and untreatable disease based on our current research findings and the safety profile of ruxolitinib.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To test the hypothesis that JAK/STAT inhibition by ruxolitinib will delay progression of neuroradiological manifestations of one patient with neurological involvement of K-D disease.	The primary endpoint is stability or regression of existing enhancing lesions or no development of new enhancing lesions the brain and spine on MRI after 13 weeks of 10 mg BID ruloxitinib treatment compared to pre-treatment images.	This subject has experienced progressive neurological decline in parallel with development new lesions on brain and spine MRI so we hypothesize that these can be followed as markers of K-D disease progression.
Tertiary/Exploratory		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Assess changes in immune cell proportion using scRNA-seq analysis in skin, CSF and blood after 13 weeks or up to 73 weeks of ruxolitinib treatment.	Changes in transcriptome/RNA expression determined by scRNA-seq analysis in skin, CSF and peripheral blood mononuclear cells (PBMCs) between baseline measurements and after 13 weeks or up to 73weeks of treatment.	Increased levels of type I and II interferon signature are present in skin, blood and CSF in patients with K-D disease.
Assess changes in plasma/serum cytokine levels and IFN scores as well as biomarker assays in CSF and blood samples after treatment with ruxolitinib.	Attenuation of plasma/serum cytokine levels and IFN scores as well as biomarker levels in CSF and blood samples.	Observed improvement in upregulation of blood cytokines and 28 gene interferon scores.
To test the hypothesis that ruxolitinib will stabilize or improve the clinical neurologic exam in this patient.	Stability OR improvement of motor and/or sensory function on clinical neurologic exam.	Functional improvement is an indicator of reduction in disease burden.

4 STUDY DESIGN

This is a single patient treatment protocol to assess effectiveness of 13, 25, 49, and up to 73 weeks of JAK/STAT inhibition on progression of CNS K-D disease.

4.1 OVERALL DESIGN

Referring to the SOA.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

N/A

4.3 JUSTIFICATION FOR DOSE

It is unknown what the effective dose of ruxolitinib would be in K-D disease. Considering the subject will also be on a concomitant immunosuppressing agent eculizumab and has baseline lymphopenia, we will start with a low dose (5 mg BID) and escalate to a maximum dose of 10 mg BID while closely monitoring for toxicity.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

The study design was constructed to treat one subject, a 58 year old male with CNS Kohlmeier Degos Disease. Therefore, there are no specific inclusion criteria.

5.2 EXCLUSION CRITERIA

- Active life-threatening infections
- Hemoglobin <7 g/dL
- Platelet counts < 50 K /mcL
- Neutropenia (ANC <0.5 x k/mcL)
- Lymphopenia (ALC <0.2x k/mcL)
- LFTs (liver function test) > 3x time upper limit
- eGFR/CreatCr < 30 mL/min

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

N/A

5.3.1 Participation of NIH Staff or family members of study team members

N/A

5.4 INCLUSION OF PREGNANT WOMEN, FETUSES OR NEONATES

N/A

5.5 LIFESTYLE CONSIDERATIONS

N/A

5.6 SCREEN FAILURES

N/A

5.7 STRATEGIES FOR RECRUITMENT AND RETENTION

N/A

5.7.1 Costs

Study related procedures and any clinical testing provided at the NIH CC will be provided free of charge. Medications will be supplied by the NIH CC on this protocol.

5.7.2 Compensation

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects policy or institutional guidelines.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS(S) ADMINISTRATION

6.1.1 Study Intervention Description

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 an IND exemption as this investigation is not intended to support a new indication for use or any other significant change to the ruxolitinib labeling. This drug is already approved and marketed and the investigation is not intended to support a significant change in advertising. Further, 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.

6.1.2 Dosing and Administration

Ruxolitinib will be administered to maximum dose as follows:

- 5 mg BID x 1 week
- 10 mg BID x 13-up to 73 weeks
- 5mg BID x 1 week

The patient will have clinical labs checked to assess for hematologic toxicity prior to dosage increase. The dose will be increased to the maximum of 10 mg BID if no toxicity is observed based on criteria below:

Definition of Hematologic Toxicity:

- Hemoglobin less than 6 g/dL (60 g/L)
- Reduction in peripheral cytopenias >50% compared to pre-treatment levels in patients with a pre-treatment ANC >500 or platelets >50

6.1.2.1 DOSE ESCALATION

See section **6.1.2** above.

6.1.2.2 DOSE LIMITING TOXICITY

N/A

6.1.2.3 DOSE MODIFICATIONS

Hematologic toxicity is defined in section 6.1.2

If hematologic toxicity develops while on 10 mg BID, patient will decrease dose to 5 mg BID.

If hematologic toxicity develops while on 5 mg BID, patient will decrease dose to 5 mg QD.

If hematologic toxicity develops while on 5 mg QD, patient will stop drug.

6.1.2.4 DRUG ADMINISTRATION

Ruxolitinib is dosed orally and can be administered with or without food. If a dose is missed, the subject should not take an additional dose, but should take the next usual prescribed dose.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

Refer to package insert.

6.2.1 Acquisition and Accountability

The study drug will be obtained and dispensed by the NIH pharmacy. The tablets for this study are available as 5 and 10 mg tablets.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Ruxolitinib (Jakafi) tablets are available as follows:

Jakafi Trade Presentations

NDC Number	Strength	Description	Tablets per Bottle
50881-005-60	5 mg	Round tablet with "INCY" on one side and "5" on the other	60
50881-010-60	10 mg	Round tablet with "INCY" on one side and "10" on the other	60

6.2.3 Product Storage and Stability

Store at room temperature, 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C and 30°C (59°F and 86°F).

*Shipping:*This medication is on formulary.

6.2.4 Preparation

N/A

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

N/A

6.4 STUDY INTERVENTION COMPLIANCE

No formal assessment will take place. The study subject will be asked to bring any remaining study medications to landmark visits, during which tablets will be counted.

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 the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications, and supplements.

All medications, administered after the participant was enrolled into the study must be recorded on the appropriate electronic Case Report Forms (eCRFs). The patient must be told to notify the Treating Physician about any new medications he/she takes after the start of study drug.

Patient may receive: anti-virals, inhaled or systemic corticosteroids, treprostinil, eculizumab, heparin, low molecular weight heparin (LMWH), direct oral anticoagulants, anti-emetics, calcineurin inhibitors, azole fungal prophylaxis (acyclovir prophylaxis), broad spectrum antibiotics (either semi-synthetic penicillin or third generation cephalosporin with vancomycin, gentamycin or equivalent), bactrim prophylaxis, steroid pre-meds prior to RBC/platelet transfusions, narcotics, and sedatives warranting close monitoring of potential drug-drug interaction effects of these concurrent drugs.

If the patient is started on warfarin, heparin, LMWH, or direct oral anticoagulants, the degree of thrombocytopenia should be considered, platelet counts and coagulation parameters will be monitored, and the dose of anti-coagulant or non-steroidal anti-inflammatory drug adjusted accordingly.

If the patient is started on any drugs that affect platelet function/count, platelet counts and coagulation parameters will be monitored, and the dose of drug adjusted accordingly. In the presence of potent CYP3A4 inhibitors, there is the possibility of increased exposure to ruxolitinib and more frequent monitoring of hematology parameters is recommended, although no automatic dose adjustment is required. See Appendix 1 for a list of cytochrome P450 3A4 (CYP3A4) inhibitors and inducers.

The following medications are prohibited until treatment discontinuation:

- Concomitant use of another JAK inhibitor
- Aspirin in doses >150 mg/day
- Fluconazole > 200 mg daily

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from ruxolitinib does not mean discontinuation from the study, and the 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 adverse event (AE).

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

- CBC + blood smear
- Mineral panel
- Acute care panel
- Liver function tests

If subject is permanently discontinued from study medication than we will also collect:

- Brain and spine MRI
- LP with CSF collection
- Research blood
- Skin biopsies
- Neurologic consult

Study drug discontinuation may be temporary due to hematologic toxicity, acute infection or other clinically-indicated reasons at the PI's discretion. Before reinitiation of ruxolitinib, we will use the same eligibility criteria as in 5.2, and if the patient meets criteria will restart the ruxolitinib at 10 mg bid (without titration at 5 mg bid) and continue treatment until the patient has completed the full 13 or 24 or 49 or up to 73 weeks course actually on therapy.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Prior to removal from study, every effort must be made to have the subject complete a safety visit approximately 2 weeks following the last dose of study therapy.

The participant is free to withdraw from participation in the study at any time upon request.

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

- Disease progression which requires discontinuation of the study intervention.
- If any clinical adverse event (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.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

7.3 LOST TO FOLLOW-UP

N/A

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 subject has signed a consent include the following:

- Email, written communication, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images.
- Review of existing photographs or videos.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

8.1.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent this study. 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 screening tests and procedures must be performed within 60 days prior to enrollment unless a time period is specifically mentioned.

- CBC with differential.

8.2 STUDY EVALUATIONS & PROCEDURES

The following activities will be performed based on the SOA, section 1.2.

Physical Examination and Medical History: Medical history and physical examination including vital signs and biophysical data (including but not limited to blood pressure, heart rate, respiratory rate, temperature, and weight) may be collected at each visit.

Phlebotomy: Blood draw for clinical and research assays. Up to 150 mL of research blood may be collected at the time of protocol enrollment, at each visit at the clinical center, and research blood collected will be under 550 ml or 10.5mL/kg subject body weight within 8 weeks.

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Brain and spine MRI: Standard test run in non-research mode to answer research questions.

Lumbar Puncture: Lumbar puncture (LP) may be obtained to collect cerebrospinal fluid (CSF) during any scheduled Clinical Center visit. The LP will be performed at the Clinical Center as an inpatient, or at the outpatient day hospital, or in interventional radiology if done under fluoroscopy. We will collect up to 20 cc of CSF per lumbar puncture. Sedation with low-dose benzodiazepines may be used for subjects with expressing anxiety during the LP. Analysis of CSF may include cell count, total protein, albumin , glucose, oligoclonal band, IgG index, cytokine assays, neopterin etc. CSF will also be aliquoted and stored for future use. Additionally, specialized laboratory testing such as detection of autoantibodies to novel antigens, proteomic analysis of the fluid, markers of neurodegeneration like neurofilament, culture and functional assays on the cells in the CSF and microRNA profile, proteomics, scRNA-seq will be performed as needed.

8.2.1 Biospecimen Evaluations

Blood Samples: In addition to blood sent to the DLM laboratory for evaluation, blood may be collected for isolation of plasma, serum, RNA and cells (such as PBMCs or platelets) for analysis. Cell lines derived from blood may be used to generate transformed cell lines, for fluorescence activated cell sorting (FACS) analysis, cell biological and molecular analysis Additional specialized laboratory tests may be performed to study disease processes and specific testing will be tailored to the patient's clinical findings. Research blood may be collected to study components of plasma and serum, including but not limited to biomarkers. Blood samples will be processed using standard procedures currently used at the NIH CC.

Skin Biopsy:

Biopsies of K-D lesions may be obtained. Separate signed procedure informed consent forms will be utilized. Up to two skin biopsies may be obtained with local anesthetic at baseline and again at end of treatment with a maximum of six total skin biopsies allowed. Standard skin biopsies (e.g. punch biopsy, excision) will be obtained with a sterile cylindrical or straight blade as determined by a credentialed care provider. Individual biospecimens will measure approximately 3-4mm in width as is routinely collected in Dermatology clinic. Biopsy specimens may be split prior to processing. Samples of K-D lesions will be used for histology/microscopy, cultured for the isolation of distinct cell populations for subsequent biochemical, cell biological and molecular analyses, and scRNA-seq analysis.

Lumbar Puncture: Analysis of CSF may include (but will not be limited to) cell count, total protein, glucose, Immunofixation electrophoresis, oligoclonal band, IgG index, cytokine assays, etc. CSF will also be aliquoted and stored for future use. Additionally, specialized laboratory testing such as detection of autoantibodies to novel antigens, proteomic analysis of the fluid, markers of neurodegeneration like neurofilament, culture and functional assays on

the cells in the CSF and microRNA profile, proteomics, scRNA-seq will be performed as needed.

Single Cell RNA Sequencing: To ensure that high quality data is generated from scRNAseq, rigorous quality control (QC) efforts will be performed for this study for both the experimental procedures and computational analysis.

Experimental QC: Tissue dissociation will be performed using an optimized protocol to ensure a standardized procedure. Skin biopsy, blood (PBMC) and CSF fluid will be processed in a rapid manner so that the profile of cellular RNA will remain as authentic as possible. By the end of the process, every sample will be assessed for cellular viability, the presence of ambient debris, and cell number. This QC test will determine if the sample is ready for further processing for sequencing or if additional steps are needed to improve the quality of the sample. If the quality of the sample needs to be improved, it will be treated with a magnetic bead-based method to enrich for viable cells, deplete debris, and reduce ambient RNA. If a second QC test confirms improvement in the quality of the sample, the cells will then be further processed. Quality control measures will be included for library construction. Bulk RNAseq will be performed on samples and the RNA integrity number (RIN) scores will be checked.

Computational QC: scRNAseq data is noisy, and includes false negatives ("drop-outs"): genes expressed in the cell but not detected by the assay. The Klarman Cell Observatory (KCO) at the Broad Institute has developed and leveraged state of the art tools, many developed within the laboratory as well as from other groups, for pre-processing and QC of scRNAseq data. The KCO pipeline uses RNAseq analysis tools such as Trinity, Tuxedo, and RSEM to align reads, transcript reconstruction/annotation, abundance estimation, read quality trimming, and sample quality analysis. Library quality is scored based on alignment rates to the genome and transcriptome, the proportion of ribosomal (rRNA) reads, the uniformity of coverage, 3′ and 5′ biases, biases associated with length or sequence composition, library complexity, reproducibility of expression estimates, and the relationship between single cell and bulk population profiles. The pipeline is optimized for plate, single nucleus sequencing (sNuc-Seq), Droplet-sequencing (Drop-Seq), DroNc-Seq, and 10x Genomics data, addressing each data type's unique characteristics (e.g., unique molecular identifiers [UMIs], 3′-directed libraries, etc.). Tools will be incorporated that increase efficiency by pseudo-alignment, and address unique quality issues that arise at polymerase chain reaction, such as cell barcode chimeras. To calculate expression levels, alternatives to standard approaches that typically rely on assumptions that are violated in single cell profiles of heterogeneous cell populations will be considered. For example, in a population of T-cells, some may be active and others "quiescent" with the former having radically larger and more complex transcriptomes. This is addressed by introducing a scaling factor reflecting the expected number of transcripts (or UMIs) in each condition and by revising differential expression tests, to account for the varying number of transcripts and genes in each condition.

8.2.2 Correlative Studies for Research/Pharmacokinetic Studies

N/A

8.2.3 Samples for Genetic/Genomic Analysis

8.2.3.1 DESCRIPTION OF THE SCOPE OF GENETIC/GENOMIC ANALYSIS

Single cell RNA-seq analysis from skin, CSF, and PBMC will be employed to determine changes in transcriptome/RNA expression and will allow us to to study individual cells at a molecular level. We will be able identify what specific genes that are changes in each type of cell from the tisses mentione above ;characterize cell states and understand how cells interact in various tissues in our patient with neuro KD disease.

8.2.3.2 DESCRIPTION OF HOW PRIVACY AND CONFIDENTIALITY OF MEDICAL INFORMATION/BIOLOGICAL SPECIMENS WILL BE MAXIMIZED

Samples will be coded and stored in the laboratory of the Accountable Investigator with limited access. Patient identification will only be available through locked password –protected database repository archives. Access to the password and hard copy files will be only provided to the PI and the research staff.

8.2.3.3 MANAGEMENT OF PRIMARY RESULTS

Single-cell RNA-seq data will have no immediate use in the care or well-being of the study participant. Therefore, no genetic information will be conveyed to the participant.

8.2.3.4 RETURN OF SECONDARY GENOMIC RESEARCH RESULTS

No secondary genomic information is being collected through the analysis of scRNA-seq.

8.2.3.5 GENETIC COUNSELING

N/A

8.3 SAFETY AND OTHER ASSESSMENTS

Physical examination

Physical examination including vital signs and medication reviews with interval histories will be performed at baseline, and at the times described in the protocol (per SOA) will be conducted in this patient while on study.Physical exam will be performed and may include height, weight, BP, heart rate, respiratory rate, oxygen saturation, eye exam for scleral icterus, cardiopulmonary exam (including heart and lung exam), abdominal exam (including presence/absence of hepatosplenomegaly), and neurologic exam (including cranial nerve exam and assessment of strength, sensation, and reflexes in upper and lower extremities). Performance status may also be assessed and recorded.

Vital signs: Vital signs will be monitored during each study visit.

Blood draw: Blood specimens for the measurement and evaluation of serum chemistries and hematology will be collected at each visit.

Assessment of Adverse Events: Assessment of adverse events will be performed during study visits by the study team. Adverse events that have not resolved after completion of study drug administration will be followed until 14 days after the last dose.

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Definition of Adverse Event: Adverse event 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)).

Non-hematologic abnormal laboratory findings used to evaluate the safety of this protocol regimen will include any change from baseline laboratory assessments that result in a progression to a grade 3 or 4 laboratory toxicity or are characterized by any of the following:

- Results in discontinuation from the study.
- Is associated with clinical signs or symptoms.
- Requires treatment or any other therapeutic intervention.
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact.
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Hematologic toxicity will be assessed as described in Section 6.1 and will be subject to independent assessment and stopping rules compared to non-hematologic AE/SAE. Grade 1 and Grade 2 lab abnormalities that are not associated with any clinical symptoms will not be considered an AE.

8.4.1 Definition of Adverse Event

Adverse event 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 adverse event (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 graded by severity utilizing the Common Terminology Criteria for Adverse Events (CTCAE). For internal consistency, the most current version at the writing of this protocol (version 5.0, published November 27, 2017) shall be used for the duration of this study and its analysis. The CTCAE can be downloaded at the above link or through the CTEP homepage at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm. For AEs not specified by the CTCAE, the following guidelines will be used to describe severity:

General classification of AEs

Grade	Category	Description
1	Mild	Mild; asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)1
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL2
4	Life threatening	Life-threatening consequences; urgent intervention indicated
5	Death	Death related to AE

[1] Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[2] Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

For adverse events (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

- **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.
- **Possibly 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 or designated AI will be responsible for determining whether an adverse event (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 in the package insert. The subject will be receiving two additional FDA-approved medications for treatment of KD disease and AE's associated with these medications as reported in their package inserts will be considered when determining AE expectedness.

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

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

Grade 2 or greater AEs, including local and systemic reactions not meeting the criteria for SAEs and all SAEs, will be captured on the appropriate case report form (CRF). 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.

All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE should be grouped together and recorded as a single diagnosis. If the AE is a laboratory abnormality that is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than the individual laboratory abnormality.

Trained member of the study team will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 15 days (for SAEs) after the last day of study drug. 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

See section 8.4.6 below.

8.4.6 Serious Adverse Event Reporting

In consultation with the PI, a trained member of the study team will be responsible for conducting an evaluation of all adverse events and shall report the results of such evaluation to the NIH Institutional Review Board (IRB) as per Policy 801.

All events listed in section will be reported to the Principal Investigator of this study:

Cornelia Cudrici M.D.

Translational Vascular Medicine Branch

NHLBI Institute

Building 10, Rm 53232

9000 Rockville Pike

Bethesda, MD 20892

Phone: 240-515-5540

E-mail: cudricicd@mail.nih.gov

8.4.7 NIH Intramural IRB and NHLBI Clinical Director Reporting

Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per HRPP Policy 801 "Reporting Research Events".

Reports to the NIH Intramural IRB at the Time of Continuing Review (CR)

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the NHLBI Clinical Director (CD)

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

8.4.8 Events of Special Interest

N/A

8.4.9 Reporting of Pregnancy

N/A

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

8.5.2 Unanticipated Problem Reporting

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 Institutional Review Board (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.

8.5.3 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

We hypothesize that blocking type I IFN and IFN-γ signaling for 13 weeks with ruxolitinib has potential utility in improving clinical, neurological and CNS radiological manifestations in our K-D patient. Only descriptive analysis will be conducted given the nature of the study.

Primary Endpoint: Stability OR regression of existing enhancing lesions OR no development of new enhancing lesions in the brain and spine on MRI after 13 weeks of ruxolitinib treatment. This will be determined by a neuroradiologist experienced in Degos disease MRI evaluation.

Exploratory Endpoint(s): (a) changes in transcriptome/RNA expression determined by single scRNA-seq in skin, CSF and peripheral blood mononuclear cells (PBMCs) between baseline measurements and 13- up to 73 weeks of treatment, (b) attenuation of plasma/serum cytokine assays and IFN scores as well as biomarker assays in CSF and blood samples and (c) improvement or stabilization of motor and sensory function on clinical neurologic exam.

9.2 Sample Size Determination

This protocol is for treatment of one patient protocol.

9.3 Populations for Analyses

N/A

9.3.1 Evaluable for toxicity

The study participant will be evaluated for toxicity during the duration of treatment with ruxolitinib.

9.3.2 Evaluable for objective response

N/A

9.3.3 Evaluable Non-Target Disease Response

N/A

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

With a single study participant, we will report the baseline measurements and 13 week treatment period changes of primary and exploratory endpoints.

9.4.2 Analysis of the Primary Endpoints

The primary endpoint is stability OR regression OR no development of new enhancing lesions of existing enhancing lesions in the brain and spine on MRI after 13 weeks of ruxolitinib treatment. We will report the baseline measurements and 13 week treatment period changes of brain and spine lesions.

9.4.3 Analysis of the Secondary Endpoint(s)

Analysis of the Secondary Endpoint(s): There will be no secondary endpoints. We will report the baseline to 13-up to 73 weeks change of the various exploratory endpoints.

9.4.4 Safety Analyses

The subject will be monitored for the study duration and all grade 2 or greater AEs will be collected. We will also monitor the occurrence of a specified set of treatment related serious adverse events (TRSAEs) during the trial.

The following TRSAEs will be monitored for early stopping of the study:

- Death considered to be probably or definitely related to ruxolitinib.
- Any grade 4 toxicity (except cytopenia) considered to be probably or definitely related to ruxolitinib.
- For cytopenias, a TRSAE will be considered if the patient permanently stops ruxolitinib due to hematologic toxicity.

The study will be monitored using the stopping rules as outlined above for early stopping if the subject in the study will develop one or more of the above specified TRSAEs. TRSAEs are those attributed as definitely or probably related to ruxolitinib.

9.4.5 Baseline Descriptive Statistics

N/A

9.4.6 Planned Interim Analyses

With one patient, there will be no formal interim analyses.

9.4.7 Sub-Group Analyses

With one patient, there will be no formal interim analyses.

9.4.8 Tabulation of individual Participant Data

This data will be captured in the analysis of the patient's primary and exploratory endpoints.

9.4.9 Exploratory Analyses

The exploratory analyses will include

1. Changes in transcriptome/RNA expression determined by single cell RNAseq (scRNA-seq) in skin, CSF and peripheral blood mononuclear cells (PBMCs) between baseline measurements and 13 weeks and up to73 weeks after start of treatment.
2. Attenuation of plasma/serum cytokine assays and IFN scores as well as biomarker assays in CSF and blood samples.
3. Stability OR improvement of motor and /or sensory function on clinical neurologic exam.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

Informed consent will be conducted following OHSRP Policy 301- Informed Consent. An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting. The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, the participant and investigator may view the same approved consent document simultaneously when the participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic, both the investigator and the participant will sign the document with a hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic).

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed consent document.

Following the enrolment of the subject, the research coordinator will be informed of the enrolment. The research coordinator will conduct a Quality Control (QC) review of the consent process and documentation periodically to confirm compliance.

10.1.2 Consent for minors when they reach the age of majority

N/A

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

N/A

10.1.4 Consent of Subjects who are, or become, decisionally impaired

This study offers the potential to improve or delay progression of the subject's neurological manifestations. The subject will be evaluated for his cognitive ability at the time of consent and if needed, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **10.1.1**.

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 study participant, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform the study participant, the Institutional Review Board (IRB), and Clinical Director of NHLBI and will provide the reason(s) for the termination or suspension. The study participant 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 the participant
- 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 sponsor, IRB and, as applicable, the Food and Drug Administration (FDA).

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to the participant. 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 sponsor.

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

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (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 participant in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each 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, Institutional policies, or sponsor requirements.

To further protect the privacy of the study participant, a Certificate of Confidentiality has been issued by the National Institutes of Health (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 the research participant, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to the participant.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

We may share specimens and data with other researchers for future use.

Stored Specimens: Following analyses of biospecimens for primary research purposes, remaining samples suitable for future research will be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB approval, as applicable. Biospecimens may be destroyed only when permitted by the clinical director and the IRB. Any future research use of identifiable biospecimens not defined in the research protocol will occur only after IRB review and approval.

10.5 SAFETY OVERSIGHT

The principal investigator will be responsible for the safety oversite of the study.

10.6 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of the trial participant 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 International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Monitoring for this study will be performed by Clinical Research Associates (CRAs)/Monitors working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol.

The objectives of a monitoring visit will be:

1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject;

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

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

4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

10.7 Quality Assurance and Quality Control

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.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

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.

Data recorded in the electronic case report form (eCRF) in password protected excel database derived from source documents should be consistent with the data recorded on the source documents. Data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the

written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

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 Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to NHLBI Program Official. 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 subject, 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 HUMAN DATA SHARING, INCLUDING GENOMIC DATA SHARING, AND PUBLICATION

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

National Institutes of Health (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 1 year after the completion of the primary endpoint by contacting Cornelia Cudrici, MD, NHLBI.

Research data will be deposited in BioData Catalyst (BDC) at the time of study publication or closure, whichever comes first. BDC is a controlled access repository and in order to access the data, an investigator must have an approved Data Access Request (DAR) through dbGaP. Data will be available for access indefinitely.

10.10.1 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy Compliance

N/A

10.10.2 NIH Public Access Policy Compliance

N/A

10.11 COLLABORATIVE AGREEMENTS

N/A

10.11.1 Agreement Type

N/A

10.12 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 NHLBI 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

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
CANDLE	Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature
CFR	Code of Federal Regulations
CMP	Clinical Monitoring Plan
CNS	Central Nervous System
COC	Certificate of Confidentiality
CRF	Case Report Form
CSF	Cerebrospinal Fluid
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services

DIC	Disseminated iIntravascular Coagulation
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
GBCAs	Gadolinium-based contrast agents
GCP	Good Clinical Practice
GI	Gastro Intestinal
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GVHD	Graft-versus-host disease
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IFN	Interferons
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
JAKs	Janus kinases
KD	Kohlmeier Degos disease
LP	Lumbar Puncture
MACE	Major Adverse Cardiovascular Events
MedDRA	Medical Dictionary for Regulatory Activities
MF	Myelofibrosis
MOP	Manual of Procedures
MxA	Myxovirus resistance protein 1
NCT	National Clinical Trial
NIH	National Institutes of Health

NIH IC	NIH Institute or Center
NMSCs	Nonmelanoma skin cancers
NSF	Nephrogenic Systemic Fibrosis
OHRP	Office for Human Research Protections
PBMCs	Peripheral blood mononuclear cells
PI	Principal Investigator
PML	Progressive Multifocal Leuko-encephalopathy
QA	Quality Assurance
QC	Quality Control
ROCK-2	Rho Associated Coiled-Coil Containing Protein Kinase 2
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAVI	STING-associated vasculopathy with onset in infancy
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SR-aGVHD	Steroid-refractory acute graft versus host disease
STAT	Signal transducer and Activator of Transcription proteins
UP	Unanticipated Problem
US	United States

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12.1.1 Appendix 1

List of CYP3A4 inhibitors and inducers

Dual CYP2C9/CYP3A4 inhibitor:

Fluconazole: Avoid the concomitant use of ruxolitinib with fluconazole doses ≥ 200 mg daily;

If clinically necessary to use doses ≥ 200 mg daily will consult at the pharmacy and the company

Category	Drug name
Strong inhibitors[a] of CYP3A	boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir/ritonavir, eltegravir/ritonavir, grapefruit juice[1], idelalisib, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, LCL161, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, sequinavir/ritonavir, telaprevir, telithromycin, voriconazole, indinavir/ritonavir, tipranoavir/ritonavir, troleandomycin,
Moderate inhibitors[b] of CYP3A	amprenavir, aprepitant, atazanavir, atazanavir/ritonavir,, casopitant, cimetidine, ciprofloxacin, crizotinib, cyclosporin, duranavir, darunavir/ritonavir, diltiazem, dronedarone, erythromycin, faldaprevilr, fluconazole[2], fosamprenavir, grapefruit juice[1], imatinib, lomitapide, netupitant,nilotinib, schisandra sphenanthera[3], tofisopam, verapamil
Strong inducers[c] of CYP3A	avasimibe, carbamazepine, enzalutamide, mitotane,phenytoin, rifampin, St. John's wort[3], rifabutin, phenobarbital,
Moderate inducers[d] of CYP3A	bosentan, efavirenz, etravirine, genistein[3], lersivirine, lopinavir, modafinil, nafcillin, ritonavir,

	thioridazine, tipranavir,

The list of CYP inhibitors and inducers was compiled from the FDA's "Guidance for Industry, Drug Interaction Studies;" from the Indiana University School of Medicine's "Clinically Relevant" Table and from the University of Washington's Drug Interaction Database. Note that this may not be an exhaustive list. For a complete and most updated drug list, please check the website https://.crediblemeds.org/healthcareproviders/drug-list.

1 Effect seems to be due to CYP2C19 inhibition by ethinyl estradiol.

2 Fluconazole is a dual CYP3A4 and CYP2C9 inhibitor. Fluconazole is a strong CYP2C9 inhibitor based on the AUC ratio of omeprazole, which is also metabolized by CYP3A; fluconazole is a moderate CYP3A inhibitor.

3 Herbal product.

a. A strong inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by equal or more than 5-fold.

b. A moderate inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 5-fold but equal to or more than 2-fold

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c A strong inducer for a specific CYP is defined as an inducer that decreases the AUC of a sensitive substrate for that CYP by equal or more than 80%.

d. A moderate inducer for a specific CYP is defined as an inducer that decreases the AUC of a substrate for that CYP by 50-80%.

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