Ruxolitinib Cream in the Treatment of Necrobiosis Lipoidica

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Protocol version history:

IND Version #	Version Date	Major Changes
1.0	14 January 2020	Initial document
2.0	23 July 2020	Changed time of second biopsy from week 12 to week 4
3.0	30 September 2020	Added RNA Tape Stripping
4.0	28 October 2020	Modified biopsy protocol
5.0	29 January 2021	Removed RNA Tape Stripping and added virtual visits

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
BSA	Body Surface Area
CFR	Code of Federal Regulations
CRF	Case Report Form
DEGs	Differentially Expressed Genes
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GVHD	Graft Versus Host Disease
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IL-1	Interleukin-1
IND	Investigational New Drug Application
IFNα	Interferon Alpha
IFNγ	Interferon Gamma
IRB	Institutional Review Board
JAK	Janus Kinase
NL	Necrobiosis Lipoidica
NRS	Numerical Rating Scale
PGA	Physician Global Assessment
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
DM	Diabetes Mellitus
SCC	Squamous Cell Carcinoma

Study Summary

Title	**Ruxolitinib Cream in the Treatment of Necrobiosis Lipoidica**
Running Title	Ruxolitinib Cream in NL
Protocol Number	
Phase	Phase II
Methodology	Open-Label, Single Arm
Overall Study Duration	17 to 20 weeks
Subject Participation Duration	16 weeks
Single or Multi-Site	Single Site
Objectives	To evaluate the safety and efficacy of ruxolitinib cream in NL as assessed by the change in NL score (0-12) of the index treatment lesions, Pruritus NRS, Skindex-16, Physician Global Assessment (PGA), and BSA. To predict responses through the identification of unique biomarkers of NL at week 0 and utilizing RNA sequencing on responsive and non-responsive tissue at week 12.
Number of Subjects	12
Diagnosis and Main Inclusion Criteria	Non-pregnant adults with cutaneous NL up to 10% of the body surface area (BSA).
Study Product, Dose, Route, Regimen	Ruxolitinib cream 1.5%, topical application, twice daily on lesions of NL.
Duration of Administration	Drug will be administered from Day 0 through Week 12.
Reference therapy	Ruxolitinib cream has been studied in the treatment of plaque psoriasis and is under investigation in treating atopic dermatitis and vitiligo.
Statistical Methodology	The statistical analysis will provide descriptive summary statistics for categorical and continuous outcomes. Categorical variables will be described by their count and proportion of occurrence while continuous, normally distributed variables will be described by their mean and standard deviation; and continuous, non-normally distributed variables will be described by their median and range.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Necrobiosis lipoidica (NL) is a chronic, disfiguring, relapsing-remitting inflammatory disease primarily involving the lower extremities. NL disproportionately afflicts younger patients and is classically associated with diabetes mellitus (DM). Clinically, NL presents with atrophic yellow telangiectatic plaques that ulcerate in 30% of patients.[1-5]

Although multiple therapies have been used to treat NL lesions, experience is largely anecdotal and there is paucity of data to guide effective treatment. Topical, intralesional, and systemic corticosteroids have been effective in a small number of non-DM patients[6]; however, corticosteroids are high-risk treatment option in diabetic patients because they increase blood glucose levels and promote insulin resistance. Various non-steroidal immunosuppressants have been used, including methotrexate, tumor necrosis factor alpha inhibitors, mycophenolate mofetil, and fumaric acid esters, all with mixed success. Better treatment options with acceptable risk/benefit profile are clearly needed.

To characterize NL at the molecular level, we performed RNA sequencing to identify underlying immune mechanisms and possible therapeutic targets. We sequenced 18 representative NL tissues and 5 normal skin tissues, and identified deregulated genes enriched for immune system functions. Independent validation was performed using a Nanostring expression panel (594 immune-specific genes), demonstrating high concordance in the set of genes identified as differentially expressed by both platforms. Upregulated immunologic processes/pathways included those involved in T-helper cell induction, dendritic cell maturation, macrophage stimulation, and autoimmunity, indicating diverse immunological pathways are active in NL.

Importantly, we focused on identifying targetable “drivers” of downstream RNA expression changes. We prioritized such driver genes using by querying multiple drug databases in order to identify clinically targetable drivers. Using stringent criteria, we identified JAK1/2 and IFNγ as key, activated drivers, and ruxolitinib as one of the most highly rated potential therapeutics in this search.

Interestingly, a recent report of an individual with polycythemia vera and NL reported near total resolution of his ulcerated NL upon treatment with oral ruxolitonib[7]. Based upon our discovery of JAK-induced immune upregulation, we propose an open label, single arm study of topical ruxolitinib cream for NL patients to determine the effect of JAK inhibition on NL lesions. The primary objective will be the assessment of the impact of topical ruxolitinib cream on NL in a pilot setting, with the secondary objective being safety of treatment in NL patients. In order to further understand the responses, investigate the molecular effect of JAK inhibition, and explore

potential biomarkers, we propose to RNA sequence tissue and peripheral blood mononuclear cells pre- and post-treatment.

1.2 Investigational Agent

Ruxolitinib is an inhibitor of the JAK family of protein tyrosine kinases. Mitogenic and inflammatory cytokines are strongly implicated in the pathogenesis of psoriasis, alopecia areata, atopic dermatitis, and lichenoid tissue reactions of the skin including NL. Ruxolitinib cream also potently inhibited the phosphorylation of STAT proteins and the production of pro-inflammatory factors induced by cytokines such as IL-23 and IFNγ. (Investigator's Brochure)

Ruxolitinib cream 1.5%, topical application will be used twice daily on lesions of NL.

1.3 Preclinical Data

Ruxolitinib potently (IC50 < 5 nM) inhibits JAK1 and JAK2, yet it does not significantly inhibit (< 30% inhibition) a broad panel of 26 kinases when tested at 200 nM (approximately 100 times the average IC50 value for JAK enzyme inhibition). Moreover, in cell-based assays relevant to the pathogenesis of inflammatory skin diseases, such as IL-2–stimulated phosphorylation of STATs and IL-2–induced proliferation of T cells, ruxolitinib demonstrates excellent potency (IC50 values of 10 to 40 nM). This effect was not due to general cytotoxicity because ruxolitinib (up to 10 μM) had no effect on the survival of resting T cells (without IL-2 stimulation). Ruxolitinib also potently inhibited the phosphorylation of STAT proteins and the production of proinflammatory factors induced by cytokines such as IL-23 and IFN-γ. Topical application of ruxolitinib demonstrated excellent efficacy in an in vivo model of immune-based skin inflammation, the DTH model in mice, including reduced ear swelling, reduced immune cell infiltrates, and normalization of tissue histology. Further, ruxolitinib was also efficacious in the inflammatory phase of the dorsal DTH model when applied in a clinically relevant cream formulation. In summary, pharmacological data obtained with in vitro and in vivo model systems support the use of topically applied ruxolitinib cream in the treatment of psoriasis, AD, vitiligo, and other inflammatory diseases of the skin. For all preclinical data below, please refer to the IB for more details.

The absorption, PK, distribution, and metabolism of ruxolitinib were characterized in CD-1® mice, rats, cynomolgus monkeys, beagle dogs, and Gottingen minipigs. The metabolism of ruxolitinib was characterized in vitro in the rat, dog, and human and in vivo in the CD-1 mouse, rat, dog, minipig, and human. The toxicologic and toxicokinetic profiles of ruxolitinib were characterized in rats, dogs, rabbits and the Gottingen minipig. Additional studies include the mass balance excretion of ^{14}C-ruxolitinib in rats, dogs, and humans after oral dosing, the tissue distribution in rats after an oral dose, and the skin distribution in Gottingen minipigs after daily topical application.

Based on human data, the relative bioavailability of ruxolitinib cream was markedly (~90%) lower than that following oral administration. The primary clearance pathway is via metabolism. *In vitro* metabolism studies show that CYP3A4 is the predominant CYP isozyme responsible for the metabolism of ruxolitinib.

Ruxolitinib cream (up to 1.5%) was well-tolerated when applied daily for up to 9 months in the Gottingen minipig. Observed decreases in lymphocytes were considered nonadverse in the absence of any other findings suggestive of systemic toxicity. Ruxolitinib did not induce contact sensitization, and dermal and ocular irritation was minimal to negligible. Ruxolitinib does not have acute phototoxicity or photoallergic potential. There was no evidence of systemic toxicity following repeated ocular administration to rabbits or dogs.

Ruxolitinib administered as a single oral dose produced adverse effects on respiratory function in rats and blood pressure in dogs at exposures that were several-fold higher than in patients administered ruxolitinib cream.

Administration of oral ruxolitinib to juvenile rats beginning at Days 7, 14, or 21 postpartum (approximately equivalent to a human newborn, 1-year-old, or 2-year old, respectively) resulted in effects on growth and bone. In animals administered ruxolitinib beginning on Day 21 postpartum, the NOAEL was considered to be 15 mg/kg per day. There was no indication of a generalized or persistent effect on bone growth. Systemic exposure at this dose was several-fold higher than that observed in patients administered ruxolitinib cream.

Oral administration of ruxolitinib to pregnant rats and rabbits resulted in maternal toxicity and minimal embryo-fetal toxicity only at the highest doses evaluated. Ruxolitinib was not teratogenic in either species. The NOAEL for both rats and rabbits was 30 mg/kg per day. In an evaluation of fertility and early embryonic development, no effects were noted on reproductive performance or fertility in male or female rats. Increases in postimplantation loss were noted at the higher doses. In a pre- and postnatal development study in rats, there were no adverse effects on embryo-fetal or postnatal survival, growth, and developmental parameters.

Ruxolitinib was not mutagenic or clastogenic, nor did it demonstrate potential for carcinogenicity in a 6-month study in Tg.rasH2 mice or in 2-year studies in mice or rats.

1.4 Clinical Data to Date

Plasma concentrations of ruxolitinib cream were determined following topical application of ruxolitinib cream in plaque psoriasis, AA, AD, and vitiligo, mostly using sparse sampling. The exposures of ruxolitinib were generally strength-dependent with moderate intersubject variability.

In Study INCB 18424-201 in plaque psoriasis, the observed systemic bioavailability of ruxolitinib was independent of formulation strength.

In Study INCB 18424-202 in plaque psoriasis, an intensive sampling scheme was used on the last day of treatment to better characterize the PK following topical application. The systemic PK following topical application was characterized by apparent first-order release, modest peak-to-trough excursion (ratio ~2), estimated relative bioavailability of 3% to 5%, and an $t_{½}$ of approximately 3 days. Systemic bioavailability of ruxolitinib cream was shown to be independent of the strength of the cream formulation and %BSA. The PD results from skin biopsies, including markers for Th1, Th17 pathway activation, reduced levels of inducible nitric oxide synthase, and KRT16 transcript levels, suggest that ruxolitinib cream was pharmacologically active at a local but

not systemic level when administered to participants with active psoriasis for 28 days as either a QD or BID regimen and suggest that key pathologic pathways in the psoriatic lesion are favorably impacted by treatment with ruxolitinib cream.
In Study INCB 18424-204 in AA, ruxolitinib trough concentrations increased as percentage scalp involvement increased.

In Study INCB 18424-206 in AD, trough plasma concentrations increased as the strength of ruxolitinib cream and frequency of applications increased. Participants whose plasma concentrations exceeded 125 nM (equivalent to half of the IC_{50} value based on the effect of ruxolitinib on IL-6) tended to have more extensive skin involvement and highly inflamed AD lesions. However, no correlation was observed between higher ruxolitinib blood levels in these participants and their response to treatment.

In Study INCB 18424-102 in AD patients, steady-state plasma concentrations (based on geometric mean) following BID administration of 1.5% ruxolitinib cream in adolescent participants were similar to that in adult participants.

In Study INCB 18424-211 in vitiligo, plasma concentrations of ruxolitinib increased as the strength and frequency of application of the cream were increased.

The plasma metabolite profile following topical application is generally similar to that observed following oral administration.

Psoriasis
Study INCB 18424-201: a double-blind, vehicle-controlled, increasing strength, safety, tolerability, PK, and preliminary efficacy study of ruxolitinib cream 0.5% QD (n = 6), 1.0% QD (n = 6), and 1.5% BID (n = 6) in Part 1. Efficacy (change from baseline to Day 28 in mean total lesion scores) was consistently shown for the 1.0% QD and 1.5% BID cohorts compared with vehicle. In Part 2, 1.5% BID showed a change in mean total scores for target lesions compared with Dovonex® (calcipotriene) 0.005% (n = 6) and with Diprolene® AF (betamethasone dipropionate) 0.05% (n = 5) at Day 28.

Study INCB 18424-202: an open-label, multicenter, sequential-cohort, increasing strength, safety, tolerability, PK, PD, and preliminary efficacy study of different regimens of ruxolitinib cream applied for 4 weeks (28 days) to participants based on their BSA: 2% to 7% (1.5% BID; n = 5), 8% to 13% (1.5% BID; n = 5), 14% to 20% (1.5% BID; n = 5), 14% to 20% (1.0% BID; n = 5), and 14% to 20% (1.5% QD; n = 5). The results of this study indicated that all 5 of these regimens were effective in decreasing the individual signs of lesion severity, lesion area, and the overall disease severity of psoriatic plaques.

Study INCB 18424-203: a double-blind, randomized, multicenter, parallel-group, vehicle-controlled dose-ranging study to compare the safety and efficacy of ruxolitinib cream 0.5% (n = 51), 1.0% (n = 49), or 1.5% (n = 49) QD with a vehicle cream (n = 50) when applied for 12 weeks (84 days). Overall, 199 participants were enrolled of which 161 (80.9%) completed the Day 84 visit. Within each active treatment group, the mean scores for the individual and total psoriasis lesion assessments, the PASI, and the PGA, as well as the mean treatable percent BSA decreased from baseline to each subsequent assessment, which indicated an overall lessening of disease severity.

Alopecia Areata
Study INCB 18424-204: a 2-part study with ruxolitinib cream 1.5% BID applied for 24 weeks. Part A (n = 12) was open-label and Part B (n = 78) was double-blind, randomized, and vehicle-controlled. Participants enrolled in Part B were to receive ruxolitinib 1.5% cream BID or placebo for 24 weeks. If eligible, participants in Parts A and B could have continued on to an open-label extension period (24 weeks) and long-term extension period (48 weeks). The primary endpoint was not met since no significant difference in SALT50 response in terminal hair was observed between the ruxolitinib cream and vehicle groups at Week 24.

Atopic Dermatitis
Study INCB 18424-206: a double-blind active- and vehicle-controlled study. In total, 306 participants received study treatment in 1 of 6 treatment groups: ruxolitinib cream 0.15% QD (n = 51), 0.5% QD (n = 51), 1.5% QD (n = 51), and 1.5% BID (n = 50), an active comparator group (triamcinolone 0.1% cream BID; n = 51), and a vehicle control group (BID; n = 52). Participants received study drug (ruxolitinib cream or vehicle) for 8 weeks and triamcinolone cream for the first 4 weeks followed by vehicle only for 4 weeks. All participants were offered the option to continue treatment for an additional 4 weeks with ruxolitinib 1.5% cream BID. For the study's primary endpoint (mean percentage change from baseline in EASI score with ruxolitinib 1.5% cream BID at Week 4), ruxolitinib cream 1.5% BID demonstrated a statistically significantly larger mean percentage change (-71.57%) when compared with the vehicle treatment group (-11.90%; $p < 0.0001$). Also, ruxolitinib 1.5% cream QD and 0.5% QD treatment groups showed statistically significant larger mean percentage changes from baseline in EASI score at Week 4 (-66.72% and -52.80%, respectively) compared with the vehicle treatment group (-11.90%; $p < 0.0001$). At Week 8, all 4 ruxolitinib treatment groups (-50.24% to -79.01%) had larger percentage improvements (decreases) from baseline in EASI score than the vehicle treatment group (-21.86%). Ruxolitinib cream 1.5% BID (-71.57%) was noninferior to the triamcinolone treatment group (-59.54%) based on the mean percentage change from baseline in EASI score at Week 4, with a numerical trend toward superiority. At Weeks 4 and 8, all ruxolitinib treatment groups had higher percentages of IGA responders than the vehicle treatment group.

In INCB 18424-303, the most frequently reported TEAEs were upper respiratory tract infection in 3 participants (1.6%) and dermatitis atopic, ear infection, gastroenteritis, headache, papule, pruritus, and respiratory tract congestion in 2 participants each (1.0% each). In INCB 18424-304, the most frequently reported TEAEs were application site pain in 5 participants (1.5%), headache and nasopharyngitis in 4 participants each (1.2% each), and application site pruritus, oropharyngeal pain, and upper respiratory tract infection in 3 participants each (0.9% each).

No fatal TEAEs were reported, and few serious TEAEs occurred across the program.

1.5 Dose Rationale

Topical Dosing:

In addition to the safety pharmacology and toxicology studies that were completed to support development of ruxolitinib cream. The relative bioavailability of ruxolitinib cream was markedly (~ 90%) lower than that following oral administration. In vitro metabolism studies strongly suggest that cytochrome P450 (CYP) 3A4 is the predominant CYP isozyme responsible for the metabolism of Ruxolitinib cream. 1.5% ruxolitinib cream BID was generally found to be the most efficacious regimen in studies with AD and psoriasis (see below) and hence this regimen is also proposed for this trial.

Ruxolitinib cream has been evaluated in over 200 subjects with plaque psoriasis in 3 clinical studies with dosing of 1 to 3 months' duration. Study INCB 18424-201 was a double-blind, vehicle-controlled, rising-dose, safety, tolerability, PK, and preliminary efficacy study of INCB01824 1% cream applied QD and 1.5% cream applied BID. All adverse events (AEs) were mild to moderate in intensity and most judged unrelated to study medication, with no associated serious AEs (SAEs) or withdrawals. Laboratory and ECG evaluations did not suggest any safety issues, specifically no instances of neutropenia, thrombocytopenia, or leukopenia.

INCB 18424-202 was an open-label, multicenter, sequential-cohort, dose-escalation, safety, tolerability, PK, pharmacodynamics (PD), and preliminary efficacy study of Ruxolitinib cream 1.0% or 1.5% applied to 2% to 20% BSA QD or BID for 4 weeks. Application area increased sequentially, and alternative dosing paradigms were explored at the highest application area. The efficacy analyses collectively demonstrated efficacy of all 5 regimens of Ruxolitinib cream in psoriasis. The incidence of all reported AEs, the clinical laboratory results, vital signs, and ECG findings showed no confirmed safety signals or trends. Mean topical bioavailability ranged from 3.4% to 5.2% with no significant inhibition of cohort mean PD. Overall, Ruxolitinib cream (1.0% or 1.5%) was demonstrated to be safe and well tolerated when applied QD or BID for 28 days to plaque psoriasis affecting 2% to 20% of the BSA.

INCB 18424-203 was a double-blind, randomized, multicenter, parallel group, vehicle-controlled dose-ranging study with application of Ruxolitinib cream or vehicle in subjects with stable plaque psoriasis. Overall, Ruxolitinib cream (0.5%, 1.0%, or 1.5%) was demonstrated to be safe and well tolerated when applied QD for 12 weeks to plaque psoriasis affecting 2% to 20% of the BSA."

Study INCB 18424-206 was a double-blind active- and vehicle-controlled study in adults with AD. Three hundred seven participants were randomized, and 306 participants received blinded study treatment. Those who received treatment in the 1.5% ruxolitinib cream BID arm achieved a significantly larger improvement from baseline when compared to the vehicle treatment group. Thus, we propose to use that same dosing regimen in this trial as it has demonstrated efficacy in treatment of AD and psoriasis in other trials.

1.6 Risks and Benefits

Benefits:

Others with NL may benefit in the future from what we learn in this research study. It is possible their symptoms could also improve while being treated with this study drug

Risks:
The following adverse events were reported as very common Side effects associated with the use of Ruxolitinib cream:

- Very common (ccurring in greater than or equal to 10% of subjects), common (occurring in 1-10% of subjects), and rare (occurring in 0.1% of subjects) side effects occurring in subjects. As of the clinical data cutoff date, 793 unique participants have been exposed to ruxolitinib cream and 515 participants have been randomized in ongoing blinded studies in AD, where participants receive 0.75% ruxolitinib or 1.5% ruxolitinib or vehicle in a 2:2:1 ratio. The most frequently reported TEAEs in healthy participants and participants with psoriasis, AA, AD, and vitiligo are listed below.

Indication	N	TEAEs by PT (≥ 6 participants)
Healthy participants	41	No TEAEs at the time of data cutoff
Psoriasis	203	Upper respiratory tract infection, nasopharyngitis, application site irritation, headache, application site pruritus, abnormal ECG QT interval, pruritus, sinusitis
AA	83	Nasopharyngitis, pruritus, sinusitis
AD	314	Nasopharyngitis, upper respiratory tract infection, headache, atopic dermatitis
Vitiligo	152	Acne, viral upper respiratory tract infection, application site pruritus, pruritus, upper respiratory tract infection, sinusitis, bronchitis, influenza, oral herpes, application site erythema, diarrhea, headache, skin exfoliation

Very Common (affecting more than 10 in every 100 patients)
Pruritus (itching)
Upper respiratory tract infection / nasopharyngitis (common cold)
Dry Skin
Headache
Acne
Application site redness / irritation

Common (affecting less than 10 in every 100 patients)	
Change in ECG tracing	Abdominal pain (stomach pain)
Sinusitis (sinus infection)	Muscle strain
Back Pain	Throat Pain
Diarrhea	Psoriasis (skin disorder under treatment)
Liver enzyme increased	Blood sugar increased
Gastroenteritis (vomiting/diarrhea caused by virus)	Cholelithiasis (stone in gall bladder)
	Cough / Bronchitis
Influenza (flu)	Cystitis (irritation of bladder)
Pneumonia (lung infection)	Bulging disk in back
Fever	Blood cell count decreased
Skin peeling	Mouth pain
Ear infection	Urinary tract infection
Atopic Dermatitis (treatable skin condition)	Tendonitis
Bursitis	Oral herpes

Rare but Serious
Asthma
Psychosis exacerbation (worsening delusions, paranoia)

Photosensitivity:
There may be a risk of skin reaction to the combined exposure of ruxolitinib and sunlight.

Hematologic abnormalities:
Ruxolitinib taken systemically can inhibit the growth of blood cells. Low blood cells can make subjects more susceptible to infections by bacteria, virus, and fungi. The risk is low with systemic therapy and was not noted with topical therapy but subjects will be monitored for any signs of inhibition of their blood counts.

Infection risk:
There is a theoretical increase in risk of infection with topical use of ruxolitinib cream. This will be monitored for.

Ruxolitinib may increase risk of infections and reactivation of latent infections such as Tuberculosis or Valley Fever. Serious bacterial, fungal and viral infections were observed in patients receiving ruxolitinib. Please seek medical advice if signs or symptoms suggestive of infection occur.

Allergy:
It is possible that some people could have an allergic reaction to ruxolitinib cream.

Cancer Risk:
Ruxolitinib cream may have the potential to affect subject's immune system; they may be at increased risk for infections and possibly cancer. Live vaccines should not be given concurrently with ruxolitinib cream.

Pregnancy Risk:
The effect of the study drug on a fetus (developing baby still in the womb), or on a breastfeeding infant, is unknown and may be harmful. Because of these risks, women cannot take part in this study if they are pregnant or breastfeeding.

Skin biopsy:
A skin biopsy is generally a safe procedure, but some potential risks may include local pain, mild local bruising, bleeding, scarring, and an infection at the site where the skin biopsy was performed. If a topical antibiotic is used afterwards, then there is a small risk of an allergic reaction.

Chest X-ray:
Subjects will be exposed to radiation from the chest x-ray. The amount of radiation has a low risk of harmful effects.

Blood draw:
The risks of drawing blood include pain, bruising, lightheadedness, and/or fainting, or rarely, infection at the site of the needle stick.

Other:
As with all research, there is a chance that confidentiality could be compromised; however, we take precautions to minimize this risk.

2 Study Objectives

Primary Objective:
To determine the efficacy of ruxolitinib cream as measured by the change in NL score (0-12) of the index treatment lesions (weeks 0 and 12). In prior psoriasis studies including Study INCB 18424-201 and Study INCB 18424-202, preliminary efficacy (change from baseline) was evident by Day 28. However, the single case report of NL treated with systemic ruxolitinib required 3 months of therapy. Therefore, we will request 12 weeks of therapy.

Secondary Objective:
To determine the efficacy of INCB018424 and the duration of response after discontinuation as measured by the change in 24 hour recall of Pruritus NRS, (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in Skindex-16 (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in Physician Global Assessment (PGA) (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to week 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in BSA (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in NL Lesion Score (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16).

Exploratory Objectives:
To predict responses and examine the pharmacodynamics of treatment through the identification of unique biomarkers and transcriptomic changes of NL at week 0 and utilizing RNA sequencing on responsive and non-responsive tissue at week 4 To correlate these biomarkers with measures of global response: NL Lesion Score, PGA, and BSA.

3 Study Design

3.1 *General Description*

This is a single center, exploratory, open-label, single-arm design study of 12 patients. Treatment naïve and treatment refractory patients with NL will be treated with ruxolitinib cream. Patients who are non-responders, to physician choice standard of care, such as topical, intralesional, and systemic or various non-steroidal including methotrexate, tumor necrosis factor alpha inhibitors, mycophenolate mofetil and fumaric acid esters will undergo a washout period and will be enrolled in the study.

Multiple safety studies have been conducted with ruxolitinib cream (see Safety) and a treatment regimen of 1.5% ruxolitinib cream BID on up to 20% BSA is deemed safe (Investigator's Brochure). Due to the thinned skin and potential for ulceration of NL, we will only treat up to 10% BSA Therefore, we propose a single center, exploratory, open-label, single-arm design study of 12 patients. Treatment naïve and treatment refractory patients with NL will be treated with ruxolitinib cream.

Individuals with NL will be eligible. All lesions will be treated, annotated, photographed, and scored. Prior treatment will be allowed; however, a washout period of 2 weeks for topical and 4 weeks for systemic agents which may alter disease course is required. At the washout period, individuals will undergo evaluation with a PGA, BSA calculation and NL lesion score lesion. Additional Pruritus measures using the NRS and Skindex-16 will be collected at that time.

Individuals will then initiate treatment on all lesions of NL BID and will be evaluated weekly and assessed by PGA, BSA, NL lesion score, Pruritus NRS, and Skindex-16 between weeks 0-4 (see Appendix). At week 12 will be the primary endpoint; however therapy will be continued for an additional 4 weeks. Therapy will be stopped and the individuals will be evaluated at week 12 and assessed by PGA, BSA, NL lesion score, Pruritus NRS, and Skindex-16. Laboratory and safety monitoring will occur at weeks 1, 2, 3, 4, 8, 12, 16. 3D photographs will be taken at weeks 0, 1, 2, 3, 4, 8, 12, 16. Individual lesions will be circled at that time and the exact volume of each lesion will be measured through use of 3D images. Up close photos will be taken of the disease and normal tissues attained at weeks 0 & 12.

Tissue will be collected for RNA sequencing at week 0 and 4. Blood collection will include the isolation at week 0, 2, 4, 8, 12. 5mL vials of blood which will be separated and the serum and cell

pellet will be stored for future analysis. Three skin biopsies will be taken during the study. At week 0, one 3mm tissue biopsy will be taken from lesional skin and one biopsy will be taken from non-lesional (adjacent but normal-appearing a minimum of 2 cm from the primary lesion but within the same anatomical subunit) skin. At week 4, a 3mm biopsy will be taken from a treated lesion (Ideally the same lesion as the initial biopsy, separated by a minmum of 1cm, or a similar lesion in the same anatomical subunit). The lesions will be chosen using full body photographs taken at week 0. From the biopsy specimen, a small piece of tissue (approximately 1 mm) will be cut and placed in a bottle labeled for RNAseq. The larger specimen will be placed in a bottle labeled for biobank storage. Both samples will be snap frozen and stored in the biobank. Once sample collection is completed for all samples, the tissues labeled for biobank storage will continue to be stored in the biobank and RNA sequencing will be performed on the tissue samples labeled for RNAseq (up to 36 tissue samples) and analysis will be performed. Paired analysis of treatment responsive and refractory lesions will be made as well as treated and untreated lesions.

Nucleic Acid Extractions: RNA will be extracted from a total of 50 um of fresh tissue using the Qiagen FFPE RNeasy Micro extraction Kit according to manufacturer's recommendation.

RNA Transcriptome Sequencing: RNA transcriptome sequencing will be carried out using commercially available techniques. Briefly, RNA libraries will be created using up to 100ng of RNA as starting material using the Illumina RNA TruSeq Library kit according to manufacturer's recommendation. Libraries will be QCed for quality and quantity using the Illumina TapeStation High Sensitivity tape. Paired-end sequencing will then be carried out on the Illumina HiSeq 4000 using 101bp insert fragments.

Primary and Secondary Measures:
All efficacy assessments will be performed prior to the administration of study treatment at each visit. The recommended order and the overall outline of measurements for the efficacy assessments are described below.

Efficacy measures:
PGA, BSA, NL Lesion Score, Pruritus NRS, Skindex-16

Primary Outcome Measures:
To determine the efficacy of ruxolitinib cream as measured by the change in NL score (0-12) of the index treatment lesions (weeks 0 and 12).

Secondary Outcome Measures:
To determine the efficacy of ruxolitinib and the duration of response after discontinuation as measured by the change in Pruritus NRS, (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in Skindex-16 (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in Physician Global Assessment (PGA) (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in affected BSA% (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4

to 8, weeks 8 to 12, weeks 12 to 16), Change in NL Lesion Score (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16). Change in lesional volume (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16).

Exploratory Outcome Measures:
To predict responses and examine the pharmacodynamics of treatment through the identification of unique biomarkers and transcriptomic changes of NL at week 0 and utilizing RNA sequencing on responsive and non-responsive tissue at week 12 if data allows to correlate these biomarkers with measures of global response: NL Lesion Score, PGA, and BSA.

3.2 Number of Subjects

12 subjects will be enrolled in this study.

3.3 Duration of Participation

The study consists of 3 treatment regimens screening/washout period (of at least 1 week and up to 4 weeks), treatment (of 12 weeks from screen/washout), and follow up (of 4 weeks). The screening and washout period will allow for treatment naïve/ new diagnosis NL to undergo evaluation and diagnosis and for treatment refractory to undergo a washout. The total duration of the study will be 17-20 weeks.

Tables:

Table-1: Prohibited treatment

Prohibited treatments[†, ‡]	**Washout period (before initiation of trial medication)**
Any concomitant oral or topical JAK inhibitor	Prohibited
Any biologic drug for NL[*]	3 months or 5x half-life (whichever is longer)
Immunomodulation treatments for NL[§] [e.g., methotrexate, cyclosporine A, corticosteroids (oral, i.v., intramuscular, s.c., intra-articular, transdermal), mycophenolate mofetil, azathioprine]	4 weeks
Topical treatment that is likely to impact signs and symptoms of NL (e.g., pimecrolimus, tacrolimus) if applied onto NL lesions	2 weeks
Non-immunosuppressive, anti-inflammatory agents (tetracycline antibiotics & niacinamide)	2 weeks

Prohibited regimen of Topical Corticosteroids (TCS)	
TCS on any location on body (including face, scalp and/or genitoanal area)	2 weeks

*Treatments with biologics for other indications permitted at 3 months or 5x half-life (whichever is longer)
† If the prohibited treatment is being used during the study for any indication, the subject must discontinue use of the prohibited treatment if he/she wishes to continue in the study.
‡ In case of undue safety risk for the subject, the subject should discontinue study treatment at the discretion of the investigator/qualified site staff. If the subject received a live virus vaccination during the study, the subject must discontinue study treatment.
§Inhalative CS with only a topical effect (e.g., to treat asthma) are not considered "systemic immunomodulation treatments" and are therefore acceptable as co-medication. Immunosuppressive medication for conditions other than NL will be allowed.

Table-2: Screening and Visits (+/- 3 day window)

	Screening	Day 0	Week 1	Week 2	Week 3	Week 4	Week 8	Week 12	Week 16
Ruxolitinib*		X	X	X	X	X	X		
Physical Exam	X	X	X	X	X	X	X	X	X
Adverse Event Assessment		X	X	X	X	X	X	X	X
Chest X-ray	X								
Assessments: PGA, BSA, lesional number, NL Lesion, Skindex-16, Pruritus NRS		X	X	X	X	X	X	X	X
Photographer		X	X	X	X	X	X	X	X
Skin Biopsy (tissue/serum bank)		X				X			
Urine Pregnancy Test	X								
Venipuncture	X	X	X	X	X	X	X	X	X
Biomarker/RNAseq blood (tissue/serum bank)		X		X		X	X	X	
CMP	X		X	X	X	X	X	X	X
CBC	X		X	X	X	X	X	X	X
Quantiferon Gold	X								
Hepatitis B	X								
Hepatitis C	X								
HIV	X								
Coccidioidomycosis	X								

*Patients will receive 1.5% ruxolitin b cream tubes to apply cream at home BID inbetween visits to be applied from Day 0 through Week 12. Patients will not receive 1.5% ruxolitinib cream at Week 12 visit as this begins their follow up period.

3.4 Primary Study Endpoints

Change in NL score (0-12) of the index treatment lesions (weeks 0 and 12).

3.5 Secondary Study Endpoints

Change in Pruritus NRS, (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in Skindex-16 (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in Physician Global Assessment (PGA) (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in BSA (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16), Change in NL Lesion Score (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16). Change in lesional volume (week 0 to week 4, week 0 to week 8, week 0 to week 12, week 0 to 16, weeks 4 to 8, weeks 8 to 12, weeks 12 to 16).

3.6 Primary Safety Endpoints

A thorough baseline screening will be followed for all patients and is outlined in Table-2. A detailed list of the methods in which baseline screening will be performed is outlined in Supplemental 2. All blood draws and safety assessments must be performed prior to study treatment administration. Appropriate safety assessments (e.g., evaluation of AEs and SAEs) should be repeated after dosing with study treatment. A physical examination, including general appearance and vital signs, will be performed as indicated in Table-2. If indicated, based on medical history and/or symptoms, additional exams will be performed at the discretion of the investigator. If possible, the same member of the study site staff throughout the study will perform assessments for an individual subject. Information for all physical examinations will be included in the source documentation at the study site. Significant findings that are present prior to the subject signing informed consent will be included in the Medical History. Significant findings made after the signing of the informed consent, which meet the definition of an AE, must be recorded as an AE. Vital signs (blood pressure, pulse, height, weight) will be assessed at each physical examination as indicated in Table-2 (see Supplemental 2 for details on how to acquire vital signs). Whether action needs to be taken to address notable vital signs will be decided by the investigator, considering the overall status of the subject. Laboratory studies will be drawn as indicated in Table-2. Whether action needs to be taken to address notable laboratory values will be decided by the investigator, considering the overall status of the subject. Hematology assessments will be measured at all scheduled study visits specified in Table-2. Serum chemistry will be a comprehensive metabolic panel will be measured at all scheduled study visits specified in Table-2.

If the prohibited treatment is being used during the study for any indication, the subject must discontinue use of the prohibited treatment if he/she wishes to continue in the study. In case of undue safety risk for the subject, the subject should discontinue study treatment at the discretion of the investigator/qualified site staff. If the subject received a live virus vaccination during the study, the subject must discontinue study treatment.

3.7 Identification of Source Data

All data in the study will be captured in the case report forms including:

- Safety measures
- Efficacy measures
- Laboratory studies
- Vital Signs
- Exploratory measures

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

Subjects eligible for inclusion in this study must fulfill **all** the following criteria:

- Subjects must be able to understand and comply with the requirements of the study and communicate with the investigator. Subjects must give written, signed, and dated informed consent before any study related activity is performed. When appropriate, a legal representative will sign the informed consent according to local laws and regulation
- Both men and women must be at least 18 years of age at the time of screening
- Subjects must have clinical and histological features of NL
- Subjects must have at least one NL lesion measuring at least 1.7 cm
- NL must not affect greater than 10% BSA

4.2 Exclusion Criteria

Subjects fulfilling **any** of the following criteria are not eligible for inclusion in this study. To ensure the recruitment of a representative sample of all eligible subjects, the investigator may apply no additional exclusions.

- On excluded therapies, not on a stable dose of a therapy, or incompletely washed out for a therapy (Table-1).
- Known hypersensitivity to Ruxolitinib formulation.
- Pregnant or nursing (lactating) women (pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin (hCG) laboratory test).
- Women of childbearing potential [Post-menopausal or not of child-bearing potential is defined by: 1 year of natural (spontaneous) amenorrhea or Surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks ago. Oophorectomy alone must be confirmed by follow up hormone level assessment to be considered not of child-bearing potential, defined as all women physiologically capable of
- becoming pregnant, unless they are using medically acceptable methods of contraception which includes:
-
    -
    - Total abstinence (periodic abstinence and withdrawal are not acceptable methods of contraception).
    - Female sterilization (bilateral oophorectomy with or without hysterectomy), total hysterectomy, or tubal ligation at least 6 weeks before taking study treatment. Oophorectomy alone requires follow up hormone level assessment for fertility.
    - Male sterilization (at least 6 months prior to screening). The vasectomized male partner should be the sole partner for that subject.
    - Barrier methods of contraception: condom or occlusive cap.
    - Use of oral, injected or implanted hormonal methods of contraception or other forms or hormonal contraception that have complete efficacy (failure <1%). (The dose of the contraceptive should be stable for 3 months).
- Active ongoing inflammatory diseases of the skin other than NL that might confound the evaluation of the benefit of ruxolitinib cream.
- Underlying condition (including, but not limited to metabolic, hematologic, renal, hepatic, pulmonary, neurologic, endocrine, cardiac, infectious or gastrointestinal conditions) which, in the opinion of the investigator, significantly immunocompromised the subject and/or places the subject at unacceptable risk for receiving an immunomodulatory therapy.
- Active systemic infections during the 2 weeks prior to initiating trial medication (common cold viruses not included) or any infection that reoccurs on a regular basis.
- Current severe progressive or uncontrolled disease which the investigator renders the subject unsuitable for the trial or puts the subject at increased risk.

4.3 Subject Recruitment, Enrollment and Screening

- From the Principal Investigator or Co-Investigator clinical practices
- Screening requirements or qualifying lab values
- Evaluation and documentation of inclusion/exclusion criteria

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

- Subject safety issues
- Failure of subject to adhere to protocol requirements
- Disease progression
- Subject decision to withdraw from the study (withdrawal of consent)

Subjects who withdraw from the study for any reason will have their information recorded at the time of withdrawal. At the time of withdrawal, the subject will be considered at the final treatment date and will move into the treatment observation phase (4 weeks). Subjects will not be replaced. Follow up for subjects will continue to follow the normal follow up (Table-2)

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

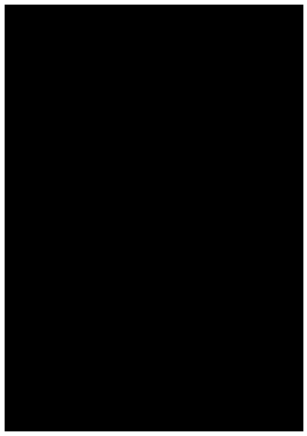
At the time of withdrawal, the reason for withdrawal will be recorded in the CRF. Individuals who withdraw will go into the observation phase for 4 weeks. If a subject withdraws consent, attempts will be made to obtain permissions to collect follow up information.

5 Study Drug

5.1 Description

The chemical name of INCB018424 phosphate is [redacted] (Figure 1). INCB018424 phosphate has a molecular formula of [redacted] and a molecular weight of [redacted]

INCB018424 Phosphate Structural Formula



INCB018424 phosphate drug substance is a white to off-white to light pink powder. Ruxolitinib cream has been formulated in 5 strengths ( ) that are actively being investigated. All excipients in both the ruxolitinib and vehicle cream formulations are compendial grade or are approved for use in topical products.

Ruxolitinib cream is a topical formulation of an investigational product under development for the treatment of patients with psoriasis, alopecia areata, atopic dermatitis, and other potential autoimmune diseases of the skin. INCB018424 phosphate (ruxolitinib) is an inhibitor of the Janus kinase (JAK) family of protein tyrosine kinases. ruxolitinib inhibits Janus Associated Kinases (JAKs) JAK1 and JAK2 which mediate the signaling of a number of cytokines and growth factors that are important for hematopoiesis and immune function. JAK signaling involves recruitment of STATs (signal transducers and activators of transcription) to cytokine receptors, activation and subsequent localization of STATs to the nucleus leading to modulation of gene expression. Mitogenic and inflammatory cytokines are strongly implicated in the pathogenesis of psoriasis, alopecia areata, atopic dermatitis, and other potential autoimmune diseases of the skin. Inhibition of specific cytokine function using antibodies directed against the common p40 subunit of interleukin (IL)-12 and IL-23 has demonstrated proof-of-concept validating cytokine signaling as a therapeutic target for the treatment of psoriasis. For details regarding pharmacodynamics and pharmacokinetics, please see IB.

5.2 Treatment Regimen

Subjects will apply the study drug topically to NL lesions twice daily. Treatment will take place from Day 0 to Week 12.

5.3 Preparation and Administration of Study Drug

The study drug will be supplied by Incyte to the Mayo Clinic Pharmacy, attn. , . The study drug will be stored in the Mayo Clinic Pharmacy. The study drug will be labelled in the Mayo Clinic Pharmacy and will be dispensed to the subjects. The subjects will be given the appropriate amount of topical cream to apply to the disease. Instructions on proper use will be provided to each subject.

5.4 Subject Compliance Monitoring

Compliance will be assessed through direct questioning of subjects as well as through drug use diaries. Drug compliance is defined as 80-100% as the ratio between actual and prescribed applications between individual study visits The drug tubes will be weighed, and weight will be documented at each visit to determine usage.

5.5 Prior and Concomitant Therapy

Individuals on stable doses of medications for chronic illnesses will be allowed. Individuals on immunosuppressive agents for NL will not be allowed; however, individuals on stable doses of immunosuppressant for other conditions will be allowed if deemed to be safe by the treating physicians. Additional exclusionary drugs are included in Table-1.

Individuals using Ruxolitinib cream should use topical broad spectrum sunscreens with a minimum of SPF30, avoid excess sunlight, and wear sun protective clothing. Sunscreens should not be applied onto treated NL lesions within 4 hours of the planned use of study drug and within one hour thereafter.

5.6 Packaging

The drug will be packaged in 15 gram single tubes and bubble wrapped for shipment. The entire quantity needed for the study will be provided in one shipment. The study drug tubes will be labeled with a diaper label that fully surrounds the tube. All applicable US FDA required text will be included on the label.

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

The drug will be obtained or delivered from Incyte Corporation to the pharmacy at each investigative site.

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipping invoice. Any discrepancies, damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The sponsor-investigator must be notified immediately of any discrepancies, damaged or unusable products that are received.

5.7.2 Storage

The drug product (ruxolitinib) and placebo cream should be stored between 15°C and 30°C (59°F and 86°F). The supplies will be stored in the Mayo Clinic Pharmacy.

5.7.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.7.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

Due to COVID-19 transmission concerns, traveling patients will have the option to participate in virtual visits in lieu of in-person clinic visits at the discretion of the Principal Investigator. Laboratory studies that can be performed off-site will be permitted at the discretion of the Principal Investigator. Study drug will be given to the patient at in-person visits that will last them the appropriate time until their next in-person clinic visit. Patients residing outside of Arizona will be eligible for travel reimbursement and will give the research coordinator the receipts of purchased flights, hotel, and meals as needed. Patients will also have to option to do remote-consenting. This will be done via phone and a copy will be mailed to the patient.

6.1 Visit 1

Screening visit:
During this visit, we will do some tests and procedures to see if subjects are eligible to take part in this research study. The study staff will review the results of these tests and procedures. If subjects aren't eligible, the Principal Investigator will tell them why. At this visit we will:

- Ask about medical history
- Perform a physical exam, including height, weight, and "vital signs" (blood pressure, temperature, heart and breathing rates)
- Perform a chest x-ray to screen for underlying coccidioidomycosis
- Draw a blood sample
- We may take swabs to test for certain fungal and bacterial infections
- Test urine for pregnancy if female subject is able to become pregnant

If it isn't known if subject has HIV, Hepatitis B or C, blood tests will need to be done.

6.2 Visit 2

Day 0 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes

- Take photographs of skin lesions to document any changes
- Draw a blood sample
- Perform a skin biopsy (2 tissue samples will be taken)
- Dispense study drug

6.3 Visit 3

Week 1 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
- Draw a blood sample
-
- Dispense study drug

6.4 *Visit 4*

Week 2 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
- Draw a blood sample
-
- Dispense study drug

6.5 *Visit 5*

Week 3 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
- Draw a blood sample
- Dispense study drug

6.6 *Visit 6*

Week 4 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
- Draw a blood sample
- Perform a skin biopsy (1 tissue sample will be taken)
- Dispense study drug

6.7 *Visit 7*

Week 8 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
- Draw a blood sample
- Dispense study drug

6.8 *Visit 8*

Week 12 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
-
- Draw a blood sample

6.9 *Visit 9*

Week 16 Visit we will:

- Perform a physical exam, check vital signs and ask about side effects or health problems since last visit
- Exam skin lesions to note any changes
- Take photographs of skin lesions to document any changes
- Draw a blood sample.

7 Statistical Plan

7.1 Sample Size Determination

Data analysis: Due to the costs associated with a study, we propose a single armed study of 12 subjects with corollary science including RNA sequencing. This will reduce costs and provide

biomarkers predictive of response. Effect sizes will be estimated for the appropriate statistical tests that will be used for group comparisons in future studies. This will enable more accurate power and sample size estimates moving forward.

7.2 Statistical Methods

Descriptive Statistics
Sample Size Computation and Power Analysis:
The sample size for this pilot study is set at 12 subjects for logistical and financial reasons. This is similar in size to other exploratory studies and will provide adequate data for estimation purposes for planning future studies. With 12 subjects, the study is intended to be for estimation purposes only.

Statistical Analysis Plan:
Data Analysis - The statistical analysis will provide descriptive summary statistics for categorical and continuous outcomes. Categorical variables will be described by their count and proportion of occurrence while continuous, normally distributed variables will be described by their mean and standard deviation; and continuous, non-normally distributed variables will be described by their median and range. The paired or unpaired Wilcoxon tests will be used to quantify differences in numerical outcomes while the Fisher's exact test and the McNemar's or Bowker's tests will be used to quantify changes in categorical variables.

If missingness occurs at random a mixed model will be utilized to assess the change in the lesion over time. A sensitivity analysis will be performed using the last observation carried forward and the results will be compared to that of the mixed-model.

Effect sizes will be estimated for the appropriate statistical tests that will be used for group comparisons in future studies. This will enable more accurate power and sample size estimates moving forward.

Bioinformatic Analysis:
RNA-seq analysis: We will use our recently developed LinNorm program [redacted] to process RNA-seq data and detect differentially expressed genes (DEGs) by comparing the RNA-seq profiles. We will first find DEG between responsive and non-responsive samples from the same individual by using paired or unpaired Wilcoxon rank-sum test (depending on how the samples are collected). We will then rank each gene in the DEGs based on the occurrence frequencies in all 12 subjects to find common DEGs for responsiveness (use permutation test to determine the p value). By comparing the normal tissue with the pathogenic tissue (responsive + non-responsible) using the above approach, we will detect a common DEGs for NL pathogenesis. Hierarchical clustering [8] and Principal Component Analysis (PCA) [9] will be applied to samples for their hierarchical relationship and clustering properties. Ideally normal, responsive and non-responsible tissues should form three distinct groups. The PCA analysis will provide us a set of gene signatures that can distinguish these three types of tissues. The common DEGs for responsiveness and pathogenesis, as well as gene signature from PCA will be used for our pathogenesis or survival

prediction. Additionally, we will perform de-convolutional analysis, to compare the immune profiles of responsive and non-responsive tissue and to correlate immune profiles. Predictive biomarkers will be correlated with lesional and global responses.

7.3 Subject Population(s) for Analysis

- All-completed population: All subjects that receive at least one dose will be considered for analysis.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)

Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event

An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

A thorough baseline screening will be followed for all subjects and is outlined in Table-2. A detailed list of the methods in which baseline screening will be performed is outlined in Supplemental 2. All blood draws and safety assessments must be performed **prior** to study treatment administration. Appropriate safety assessments (e.g., evaluation of AEs and SAEs) should be repeated after dosing with study treatment. A physical examination, including general appearance and vital signs, will be performed as indicated in Table-2. If indicated, based on medical history and/or symptoms, additional exams will be performed at the discretion of the investigator. If possible, the same member of the study site staff throughout the study will perform assessments for an individual subject. Information for all physical examinations will be included in the source documentation at the study site. Significant findings that are present prior to the subject signing informed consent will be included in the Medical History. Significant findings made after the signing of the informed consent, which meet the definition of an AE, must be recorded as an AE. Vital signs (blood pressure, pulse, height, weight) will be assessed at each physical examination as indicated in Table-2 (see Supplemental 2 for details on how to acquire vital signs). Whether action needs to be taken to address notable vital signs will be decided by the investigator, considering the overall status of the subject.

Supplemental 1: Appropriateness of Measures

Skin Scoring:
There are no validated skin scoring systems for NL. One prior publication developed a non-validated NL skin scoring system (0-12) which examines erythema, infiltration, ulceration, and pain[10]. We will use other simple measures of BSA which is objective and PGA which is well validated in other inflammatory skin conditions[11-13]. Additionally, we will simply annotate the number of lesions on the individual as a marker of total disease burden.

Itch Scoring:

The NRS and Skindex-16 are all well validated measure of itch[14,15]. The First score measure focuses in upon a gestalt of itch. The later scoring system focuses in upon the itch and its impact upon quality of life.

Supplemental 2: Safety Measures

Baseline Screening:

A serum β-hCG test will be performed in all pre-menopausal women as indicated. All pre-menopausal women who are not sterile at screening will also have a urine pregnancy test performed locally as indicated. Any woman with a confirmed positive pregnancy test during screening is not eligible for the study. A positive urine pregnancy test during the treatment periods of the study requires immediate interruption of study treatment until serum β-hCG is performed and found to be negative. If the serum β-hCG test is positive, study treatment must be definitively discontinued.

Blood Pressure and Pulse:

Height and Weight:
Height and body weight will be measured in indoor clothing, but without shoes. If possible, body weight assessments should be performed by the same study site staff member and using the same scale throughout the study.

Blood Draws:
Subjects should avoid smoking within the hour preceding the blood draws. All laboratory studies will be conducted within the Mayo Clinic Health Systems (Mayo Clinic Arizona and Mayo Clinic Rochester). Details on the collections, shipment of samples and reporting of results will follow Mayo Clinic's current protocols. For the identification of notable values, the Mayo Clinic reference laboratory should be consulted.

Supplemental 3: Safety Monitoring

Infection monitoring:
Study subjects will be evaluated at each visit for signs or symptoms of infection.

- Vitals signs as well as constitutional symptoms will be assessed.
- Assessment for common infections such as cellulitis as well as oral, vaginal, and cutaneous candidiasis will be performed

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if

- they induce clinical signs or symptoms,
- they are considered clinically significant,
- they require therapy.

Laboratory studies will be drawn as indicated in Table-2. Whether action needs to be taken to address notable laboratory values will be decided by the investigator, considering the overall status of the subject. Hematology assessments will be measured at all scheduled study visits specified in Table-2. Serum chemistry will be a comprehensive metabolic panel will be measured at all scheduled study visits specified in Table-2.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events

should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting

Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Action taken regarding treatment

AE Action:
All adverse events should be treated appropriately. Treatment may include one or more of the following:

- No action taken (i.e. further observation only)
- [study/investigational] treatment dosage adjusted/temporarily interrupted
- [study/investigational] treatment permanently discontinued due to this adverse event
- concomitant medication given
- non-drug therapy given
- patient hospitalized/patient's hospitalization prolonged

AE Outcome:

- All AE outcomes should be recorded (not recovered/not resolved; recovered/resolved; recovering/resolving, recovered/resolved with sequelae; fatal; or unknown)

Serious Adverse Events (SAE)

An SAE is defined as any adverse event (appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s) or medical conditions(s) which meets any one of the following criteria (Life-threatening in the context of a SAE refers to a reaction in which the patient was at risk of death at the time of the reaction; it does not refer to a reaction that hypothetically might have caused death if more severe.):

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
-
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Is medically significant, i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention.

SAE Reporting:

To ensure patient safety, every SAE, regardless of causality, occurring after the patient has provided informed consent and until 30-days after the subject stopped study participation must be reported to the Incyte as soon as possible but no later than 5 days from learning of its occurrence according to the Mayo Clinic IRB policy. Any SAEs experienced after the 30-days period should only be reported to Incyte and the Mayo Clinic IRB if the investigator suspects a causal relationship to study treatment. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. This report must be submitted as soon as possible but no later than 5 days from the investigator receiving the follow-up information. SAE should be followed up until resolution or until it is judged to be permanent. An SAE that is considered completely unrelated to a previously reported one should be reported separately as a new event.

Information collected on the adverse event worksheet (*and entered in the research database)*:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:

- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

AE Reporting:
Its relationship to the:
- Study treatment (no/yes), or
- Investigational treatment (no/yes), or

- The other study treatment (non-investigational) (no/yes), or
- Both or indistinguishable

The relationship will be categorized as follows:
- Unrelated- Clearly due only to extraneous causes, and does not meet criteria listed under possible or probable.
- Unlikely- Does not follow a reasonable temporal sequence from administration. May have been produced by the patient's clinical state or by environmental factors or other therapies administered.
- Possible- Follows a reasonable temporal sequence from administration, but may have been also produced by the patient's clinical state, environmental factors or other therapies administered.
- Probable- Clear-cut temporal association with administration with improvement on cessation of investigational medicinal product or reduction in dose. Reappears upon rechallenge. Follows a known pattern of response to the investigational medicinal product.

Its duration (start and end dates) or if the event is ongoing an outcome of not recovered/not resolved should be reported.
Whether it constitutes a serious adverse event(SAE)

Adverse Events (AE):
The severity grade/Common Toxicity Criteria (CTC) AE Version 5.0 grade
- Mild: usually transient in nature and generally not interfering with normal activities
- Moderate: sufficiently discomforting to interfere with normal activities
- Severe: prevents normal activities

If CTCAE grading does not exist for an adverse event, use
1=mild, 2=moderate, 3=severe, 4=life-threatening, CTCAE Grade 5 (death) is not used, but is collected in other CRFs (Study Completion, Death/Survival).

8.3.2 Sponsor-Investigator reporting: Notifying the FDA and Funding Sponsor

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

SAE Reporting to the Sponsor (Incyte)

Incyte needs to be notified within 24 hours of learning of an event. Incyte also needs to be provided a completed SAE form via email. SAE reporting for each subject begins the day the informed consent is signed by the patient and within 30 days after subject has completed or discontinued from the study or has taken last dose of the study drug, or as described in the IST protocol.

SAEs, occurring using Incyte Study drug, are reported in accordance with the effective protocol. SAEs occurring with any another commercial drug are reported to manufacturer of that drug in accordance with regulations and protocol.

Initial Serious Adverse events (SAEs) and/or subsequent follow-up reports should be reported via email to: [redacted] or fax [redacted]. SAE reports should be for a single subject. SAE forms will be e-mailed with a cover sheet and any additional attachments.

All adverse event information is reported to Incyte on the Principal Investigator's/Institution's Adverse Event Report Form, or a CIOMS-I or MedWatch Form FDA 3500A, or on an Adverse Event Report Form which may be provided by Incyte upon request. The Principal Investigator does not provide medical records (e.g., discharge summary) to Incyte, unless specifically requested.

Copies of IND safety reports submitted to the FDA by the Institution will be shared with the contact above so that these reports can be evaluated and included in investigator brochure or

Incyte IND safety submissions as required to ensure safety of other patients who are receiving the product from Incyte for sponsored trials.

Procedure for Reporting of Pregnancy and Lactation to the Sponsor (Incyte)

An "Initial Pregnancy Report" or equivalent must be completed in full and emailed to [redacted], fax [redacted] within 24 hours of discovery of a pregnancy of a subject who has taken the Incyte product or the pregnancy of a partner for a

subject who has taken the Incyte product. The "Follow-up Pregnancy Report Form" or equivalent must be completed and emailed to [redacted], fax [redacted] within 30 days after delivery, so that Incyte is provided with information regarding the outcome of the pregnancy. If the pregnancy results in any events which meet the serious criteria (i.e., miscarriage or termination), the SAE reporting process needs to be followed and the timelines associated with an SAE should be followed.

Stopping Rules

The stopping rules specified below are based on the knowledge available at study development. The stopping rule applies to the overall study. We note that the Adverse Event Stopping Rule may be adjusted in the event of either (1) the study re-opening to accrual or (2) at any time during the conduct of the trial and in consideration of newly acquired information regarding the adverse event profile of the treatment(s) under investigation. The study team may choose to suspend accrual because of unexpected adverse event profiles that have not crossed the specified rule below.

Accrual will be temporarily suspended to this study if at any time we observe events considered at least possibly related to study treatment (i.e. an adverse event with attribute specified as "possible", "probable", or "definite") that satisfy the following:

• If 2 or more patients in the first 6 treated patients (or 30% after the first 6 treated patients have been accrued) experience a grade 3 or higher non-hematologic adverse event.

We note that we will review grade 4 and 5 adverse events deemed "unrelated" or "unlikely to be related", to verify their attribution and to monitor the emergence of a previously unrecognized treatment-related adverse event.

8.4 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

The occurrence of adverse events will be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when the patient volunteers them during or between visits or through physical examination, laboratory test, or other assessments. Please see Supplemental 3 for a detailed description of safety monitoring. Clinically significant abnormal laboratory values or test results will be identified through a review of values outside of normal ranges/clinically notable ranges, significant changes from baseline or the previous visit, or values which are non-typical in patient with underlying disease. Investigators have the responsibility for managing the safety of individual patient and identifying adverse

events. Alert ranges for labs and other test abnormalities are included determined by the Mayo Clinic Arizona and Mayo Medical Laboratory. Adverse events will be recorded in the Adverse Events Case Report Form (CRF) under the signs, symptoms or diagnosis associated with them, and severity. All adverse events will be treated appropriately. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome (see Supplemental 3). Information about common side effects already known about the investigational drug can be found in the package insert. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed. The investigator will also instruct each patient to report any new adverse event (beyond the protocol observation period) that the patient, or the patient's personal physician, believes might reasonably be related to study treatment. This information should be recorded in the investigator's source documents, however, if the AE meets the criteria of an SAE. To ensure patient safety, every SAE (see Supplemental 3 for definition), regardless of suspected causality, occurring after the patient has provided informed consent and after the patient begins taking study drug and until 30 days after the patient has stopped study participation will be recorded and reported to Incyte. Any SAEs experienced after this 30-day period should only be reported to Incyte if the investigator suspects a causal relationship to the study drug. All malignant neoplasms will be assessed as serious under "medically significant" if other seriousness criteria are not met. Medical and scientific judgment will be exercised in deciding whether other situations should be considered serious reactions, such as important medical events that might not be immediately life threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the outcomes listed in SAE. Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction. All AEs (serious and non-serious) are captured and recorded, SAEs also require individual reporting (see Supplemental 3). To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to the sponsor-investigator within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment. Any SAE experienced during the pregnancy and unrelated to the pregnancy must be reported on a SAE form.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information

The rights of a research subject to revoke their authorization for use of their PHI. Study data will be securely stored on a password protected computer that only the research study team will have access to. Any study related paper documents will be stored in a locked cabinet that only the research study team will have access to.
In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

Data Security and Confidentiality

All study data will be collected by the research team, reviewed by the PI, and stored in secure, locked files and/or databases in order to protect it from inadvertent loss or improper access. All laboratory specimens, evaluation forms, reports, and other records will be identified by coded number only to maintain subject confidentiality. Information gained from this study that can be linked to the subject's identity will not be released to anyone other than the investigators, the subject and the subject's physician. All the information obtained in connection with these studies

will remain confidential as far as possible within state and federal law. The results of these studies will be published in scientific journals without identifying the subjects by name.

Data Quality Assurance

Source document verification will be performed to ensure that the database accurately reflects data on the CRFs.

Data Clarification Process

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents. These will include subject case histories and regulatory documents. There will be a subject code master list that will be stored so as to protect subjects'confidentiality. Case Report Forms will be coded. There will be no subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted] Whichever is longer

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

Incyte Corporation is funding this research study.

12.2 Subject Stipends or Payments

Subjects will receive $50 for each biopsy visit they complete (Day 0 and Wk 4). They will receive $25 for all other visits that they complete. If they complete all study visits they will receive a total of $275.

13 Publication Plan

The key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov. In addition, upon study completion and finalization of the study report the results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results.

14 Attachments

Appendix:

Physician Global Assessment: [16,17]
Grade 0 Completely clear: no evidence of disease (100% improvement) CCR
Grade 1 Almost clear: very significant clearance (≥90% to <100%) PR
Grade 2 Marked Improvement: significant improvement (≥75% to <90%)PR
Grade 3 Moderate improvement: intermediate between slight and marked (≥50% to <75%) PR
Grade 4 Slight improvement: some improvement (≥25% to <50%); however, significant evidence of disease remains SD
Grade 5 No change; disease has not changed from baseline condition (+/-<25%) SD
Grade 6 Worse, disease is worse than at baseline evaluation by (≥25%) or more PD

CCR- Complete clinical response
PR- Partial response
SD- Stable disease
PD- Progressive Disease

NL Lesion Score[10]:

Score 0-12
Erythema (0, none; 1, slight; 2, moderate; 3, severe)
Infiltration(0, none; 1, slight; 2, moderate; 3, severe)
Ulceration (0, none; 1, slight; 2, moderate; 3, severe)
Pain (0, none; 1, slight; 2, moderate; 3, severe).

Itch-
Numerical Rating Scale (NRS)[11,12] (bottom)-

0- No itch
1-4 Mild itch
4-7 Moderate itch
7-9 Severe itch
10- Very severe itch

Skindex-16-

Skindex 16-

Scoring[14,15] (0=never bothered to 6=always bothered), Total 0 to 96

Symptom Subscale
1. Skin itching
2. Skin burning or stinging
3. Skin hurting
4. Skin irritated

Emotional Subscale
5. Persistence or recurrence of condition
6. Worry about condition
7. Appearance of skin
8. Frustration about skin
9. Embarrassment about skin
10. Annoyed about skin
11. Feeling depressed

Functional Subscale
12. Effect of skin on interaction with others
13. Effect of skin on desire to be with people
14. Skin making it hard to show affection
15. Effect of skin on daily activity
16. Skin making it hard to work/have enjoyment

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