A Pilot Study to Assess Safety and Biomarker Responses of the Dual JAK1/TYK2 Inhibitor (Brepocitinib) for Cicatricial Alopecia
PI: Dr. Emma Guttman
NCT05076006
Document Date: 3-28-2023

# A Pilot Study to Assess Safety and Biomarker Responses of the Dual JAK1/TYK2 Inhibitor (Brepocitinib) for Cicatricial Alopecia


Principal Investigator: Emma Guttman-Yassky, MD/PhD

Sub-Investigators: Margaret Snyder, MD
Elizabeth Andrews, MD
Shelley Uppal, MD
Joel Correa da Rosa, PhD

Study Sites: Icahn School of Medicine at Mount Sinai
Departments of Dermatology and the Immunology Institute
5 East 98 Street, 5th Floor, Box 1048
New York, NY 10029

Collaborator: Pfizer, Inc
235 E 42nd Street
New York, NY 10017
US



Protocol Version: July 14, 2020

Amendment #1 February 10, 2021

Amendment #2 April 7, 2022

## LAY SUMMARY (500 words or less)

Alopecia could be subdivided into two main groups of diseases: non-scarring alopecia, such as male pattern baldness, or alopecia areata (AA), in which hair follicles are preserved, yet quiescent, and scarring alopecia, also known as cicatricial alopecia (CA), in which hair follicles are irreversibly destroyed. CA leads to scarred areas, most commonly on the scalp, that cannot re-grow hair. Despite being a long-term condition, that often has significant impact on patients' well-being, available effective treatments for these diseases are lacking. In addition, the molecular abnormalities causing CA are largely unknown. Our research involves administrating patients a new investigational drug (a combined TYK/JAK inhibitor), PF-06700841 (brepocitinib)which has been shown to be safe and well tolerated in clinical studies to date, and is being investigated in other conditions, such as AA. CA patients will be asked to provide small samples of skin and blood throughout the treatment period, to find out how they respond to the drug, and to attempt to better understand these diseases.

## PUBLIC HEALTH IMPACT STATEMENT

Cicatricial Alopecia (CAs) are an increasingly common, progressive, scarring diseases, resulting in permanent hair-loss, affecting quality-of-life.[1-4] The mechanisms of CA are poorly understood,[3,5-9] and treatment options are unsatisfactory.[3,8-12]

We will assess samples of skin and blood from CA patients by molecular techniques to investigate the mechanisms of inflammation and scarring, at baseline and after treatment with the investigational product. These findings will help to identify new treatment targets and direct further investigation for the development of new therapies for these disfiguring diseases.

## ABSTRACT

Cicatricial Alopecias (CAs) are progressive, disfiguring dermatoses, resulting in permanent hair loss. Patients experience anxiety, low self-esteem, and extreme distress. Despite growing incidence, with more than 5.7% of the population affected, CAs are poorly understood. As a result, current therapies are largely lacking, leading to a significant unmet need, that we wish to address. We propose to investigate the most common forms of CA, frontal fibrosing alopecia (FFA)/lichen planopilaris (LPP), and central centrifugal scarring alopecia (CCCA). Previous reports support a role for Th1/IFNγ in LPP. Favorable clinical outcomes following Janus kinase inhibitor therapy for LPP/FFA patients were reported, yet these were not coupled with molecular studies. Work recently submitted for publication by our group revealed strong Th1-skewing in FFA lesions, with concomitant upregulations of IL-23 subunits. For CCCA, the literature is even sparser, with pathogenesis relying on baseline pro-inflammatory state in afro-texture hair, with upregulated IL-1α in scalp sebum. In Phase I of this novel proposed trial, we will treat CA patients with a new dual Janus kinase (JAK)1/ tyrosine kinase (TYK)2 inhibitor, PF-06700841(brepocitinib), still under investigation in various conditions, and analyze their immune profile in scalp and blood. In addition to the much-needed, prospective trial of a new drug for these diseases, we also aim to characterize CA molecularly, and attempt to determine the relationship between inflammation and fibrosis, to ultimately identify new treatment targets, and guide future investigations of therapeutics.

## BACKGROUND

Cicatricial Alopecias (CAs) are a group of progressive, disfiguring skin disorders, resulting in permanent hair loss. The most common subtypes of CA are frontal fibrosing alopecia (FFA)/lichen planopilaris (LPP), and central centrifugal scarring alopecia (CCCA). FFA/LPP more commonly affect women, with FFA being mostly diagnosed in post-menopausal women, comprising up to 17% of new referrals to hair clinic in one study.[13] Despite some clinical differences, the distinction between FFA and LPP is not always clear, and mainly rely on distribution of skin lesions; whereas FFA chiefly involves the frontal hairline and eyebrows, LPP tends to manifest in a patchy or diffuse pattern. The histopathological findings cannot distinguish the two variants.[14] CCCA predominantly affects women of Afro-American origin, and usually manifests as hair loss starting at the vertex of the scalp and progressing circumferentially.[3] Although CCCA was reported as one of the top 5 diagnoses made in patients with skin of color, prominent barriers to care were recently reported due to poor physician-patient interactions.[15,16] CA patients could experience tremendous anxiety, low self-esteem, and extreme psychosocial stress, with reported decreased quality-of-life measures across symptomatic, functional, and global domains.[1-4] Despite being overly common, with more than 5.7% of the population being affected by CA, these diseases are poorly understood.[3,8,9] Previous research efforts mainly focus on histopathologic changes characterizing these diseases, or on the genetic susceptibilities in specific sub-populations, but the inflammatory dysregulations and their effects on hair keratins and the fibrotic process remain unclear.[5-7,17] The elusive pathogenesis of CA prevents the development of safe and efficacious treatments, as there are no studies defining the broad molecular dysregulations of these diseases.[18] As a result, the current therapeutic paradigm for CA is largely lacking,[3,8,9] and chiefly relies on retrospective studies with unsatisfactory results,[10-12] leading to a significant unmet need, that we wish to address.

## RATIONALE

Until recently, the molecular dysregulation underlying the CA has been largely unknown, hindering therapeutic development.[19] Only few studies described a limited number of markers being abnormally modulated in lesional skin from scalp of CA patients. In a study of LPP lesions, immune prevalence collapse was demonstrated in hair follicles, accompanied by a Th1-biased cytotoxic T cell response, and increased expression of interferon (IFN)-inducible chemokines (CXCL9/10/11).[20] Moreover, in skin organ culture, IFNγ was shown to induce hair follicle immune privilege collapse, raising the possibility of blocking IFN signaling and restoring immune privilege as a therapeutic approach in LPP.[20] A recent publication reported successful treatment with a broad immune antagonist, the pan-Janus kinase (JAK) inhibitor, tofacitinib, for LPP and FFA patients, with clinical response in 80% of patients, based on pre-treatment vs post-treatment lichen planopilaris activity index (LPPAI) scores ($p = .0014$).[19] Nevertheless, in this retrospective study, clinical improvements were not coupled with molecular studies.[19] For CCCA, the literature on molecular pathogenesis is even sparser, and we were unable to find data from studies investigating diseased skin with CCCA. Assumptions on CCCA pathogenesis rely on baseline pro-inflammatory state in afro-texture natural hair, where the pro-inflammatory IL-1α was found to be 18 times higher in scalp sebum collected by non-invasive application of adhesive tape, than the anti-inflammatory cytokine IL-1R.[21] A recent genetic study also found a mutation in *PADI3* in CCCA, via whole-exome sequencing that may result in improper formation of hair shaft, leading to the CCCA phenotype.[17]

Our rationale relies primarily on a detailed study of 12 FFA patients, performed at the Laboratory of Inflammatory Skin Diseases at Mount Sinai Hospital, recently submitted for publication, in which gene and protein expression studies were performed in lesional and non-lesional skin of FFA patients. In this study, patients with relatively short duration since FFA initiated (≤7 years) were included in order to elucidate the molecular and cellular fingerprint of FFA. FFA skin samples were compared with skin samples of alopecia areata (AA) patients (a non-scarring alopecia), and controls. Analyses of biomarkers associated with fibrosis, inflammatory pathways, T cell regulation and tissue resident T cell homeostasis shed light on FFA pathogenesis. Robust, significant increases of key Th1 axis markers (IFN-γ, CCL5, CXCL9, CXCL10, IL-12RB1, STAT1) were observed in FFA scalp, not only compared to controls, but also compared to expressions seen in a highly inflammatory condition, alopecia areata (AA), which is a form of non-scarring alopecia, as shown in Figure 1. The IL-23 subunits (IL-23p19, IL-12/23p40) were significantly up-regulated in both AA and FFA compared to control scalp, suggesting pathogenic role for these markers as well. Furthermore, a similar inflammatory profile, but to a lesser extent, was also found in non-lesional scalp of FFA patients compared to scalp lesions. FFA lesions exhibited upregulation of T-regulatory cells and no suppression of hair keratins, in addition to diminished yet detectable stem cells compared with normal scalp, suggesting preservation of the potential for hair re-growth with early intervention.

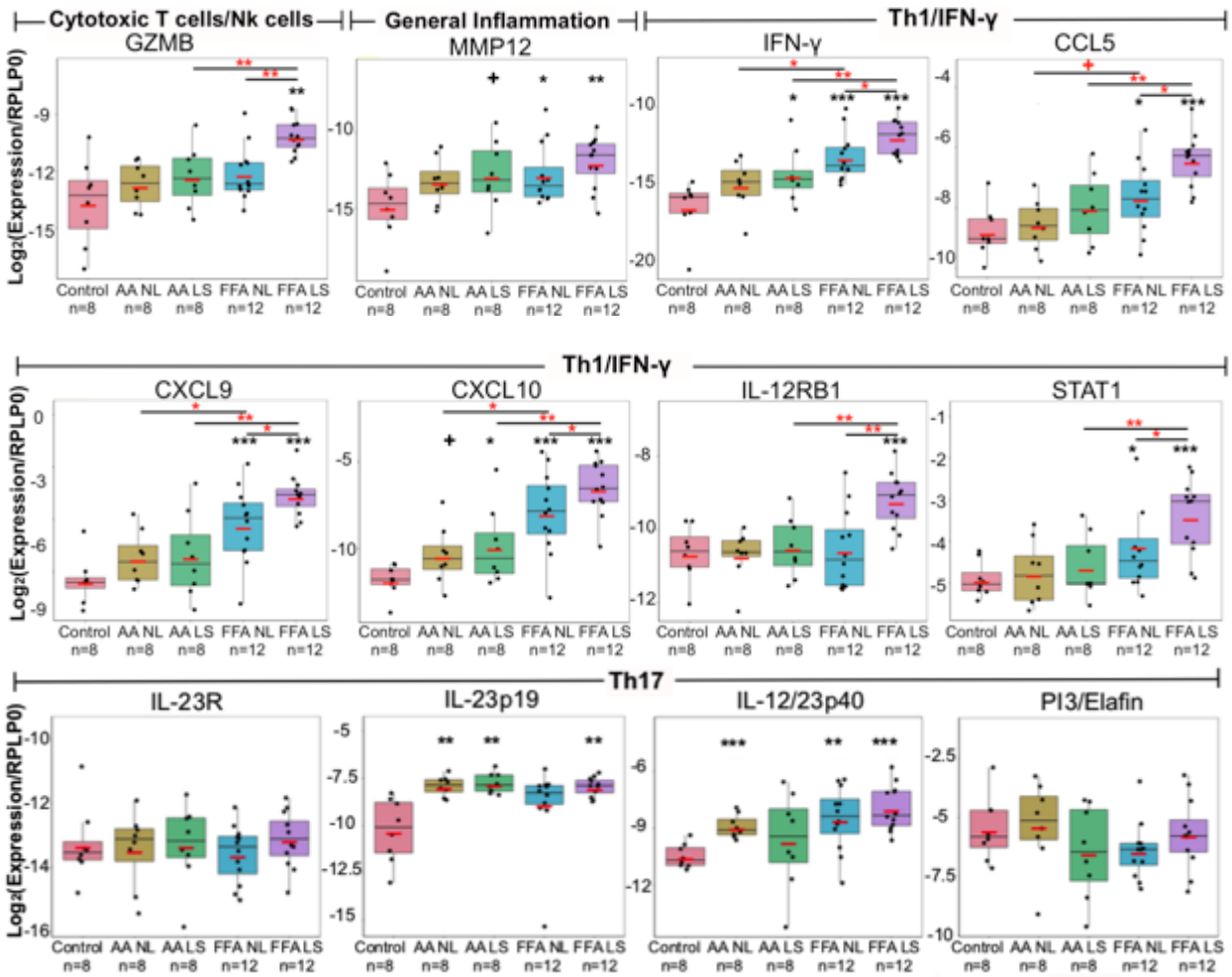
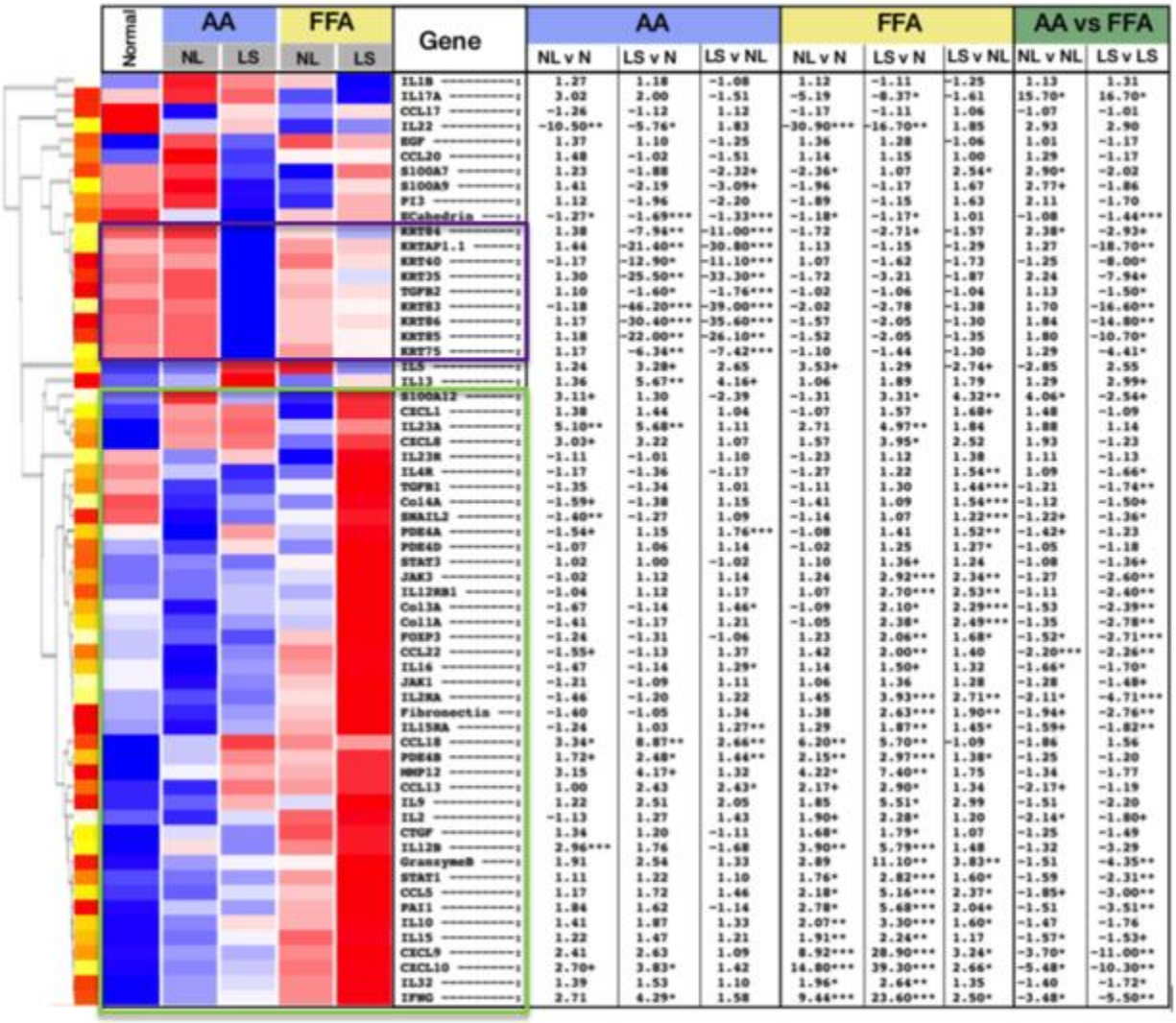


**Figure 1.** Differential expression of inflammatory markers (by pathway) between AA, FFA, and control scalp by qRT-PCR. Box plots show log2 normalized mRNA expression. Black bar in the boxplot indicates median, while the red bar indicates the mean, with each black dot representing a patient. Black star indicates significance as compared to controls, whereas red stars indicate significant differences between two groups as indicated by line. NL=non-lesional, LS=lesional. +p<0.1, *p<0.05, **p<0.01, ***p<0.001.

A summary heatmap of mRNA expressions of all analyzed genes included in this cohort is presented in Figure 2 (found in "other documents"). It reveals the robust up-regulations of multiple inflammatory markers in FFA lesional skin, and, to a lesser extent, non-lesional skin, compared with the relatively moderate upregulations of these markers in AA (green box). On the other hand, as mentioned above, hair keratins were more down-regulated in AA, hinting towards a possible reversibility of the scarring process of early FFA (purple box).



| Normal | AA | | FFA | | Gene | AA | | | FFA | | | AA vs FFA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NL | LS | NL | LS | | NL v N | LS v N | LS v NL | NL v N | LS v N | LS v NL | NL v NL | LS v LS |
| | | | | | IL1B | 1.27 | 1.18 | -1.08 | 1.12 | -1.11 | -1.25 | 1.13 | 1.31 |
| | | | | | IL17A | 3.02 | 2.00 | -1.51 | -5.19 | -8.37+ | -1.61 | 15.70* | 16.70* |
| | | | | | CCL17 | -1.26 | -1.12 | 1.12 | -1.17 | -1.11 | 1.06 | -1.07 | -1.01 |
| | | | | | IL22 | -10.50** | -5.76* | 1.83 | -30.90*** | -16.70** | 1.85 | 2.93 | 2.90 |
| | | | | | EGF | 1.37 | 1.10 | -1.25 | 1.36 | 1.28 | -1.06 | 1.01 | -1.17 |
| | | | | | CCL20 | 1.48 | -1.02 | -1.51 | 1.14 | 1.15 | 1.00 | 1.29 | -1.17 |
| | | | | | S100A7 | 1.23 | -1.88 | -2.32+ | -2.36* | 1.07 | 2.54* | 2.90* | -2.02 |
| | | | | | S100A9 | 1.41 | -2.19 | -3.09+ | -1.96 | -1.17 | 1.67 | 2.77+ | -1.86 |
| | | | | | PI3 | 1.12 | -1.96 | -2.20 | -1.89 | -1.15 | 1.63 | 2.11 | -1.70 |
| | | | | | BCathedrin | -1.27* | -1.69*** | -1.33*** | -1.18* | -1.17+ | 1.01 | -1.08 | -1.44*** |
| | | | | | KRT84 | 1.38 | -7.94** | -11.00*** | -1.72 | -2.71+ | -1.57 | 2.38* | -2.93+ |
| | | | | | KRTAP1.1 | 1.44 | -21.40** | -30.80*** | 1.13 | -1.15 | -1.29 | 1.27 | -18.70** |
| | | | | | KRT40 | -1.17 | -12.90* | -11.10*** | 1.07 | -1.62 | -1.73 | -1.25 | -8.00* |
| | | | | | KRT35 | 1.30 | -25.50** | -33.30** | -1.72 | -3.21 | -1.87 | 2.24 | -7.94+ |
| | | | | | TGFB2 | 1.10 | -1.60* | -1.76*** | -1.02 | -1.06 | -1.04 | 1.13 | -1.50* |
| | | | | | KRT83 | -1.18 | -46.20*** | -39.00*** | -2.02 | -2.78 | -1.38 | 1.70 | -16.60** |
| | | | | | KRT86 | 1.17 | -30.40*** | -35.60*** | -1.57 | -2.05 | -1.30 | 1.84 | -14.80** |
| | | | | | KRT85 | 1.18 | -22.00** | -26.10** | -1.52 | -2.05 | -1.35 | 1.80 | -10.70* |
| | | | | | KRT75 | 1.17 | -6.34** | -7.42*** | -1.10 | -1.44 | -1.30 | 1.29 | -4.41* |
| | | | | | IL5 | 1.24 | 3.28+ | 2.65 | 3.53+ | 1.29 | -2.74+ | -2.85 | 2.55 |
| | | | | | IL13 | 1.36 | 5.67** | 4.16+ | 1.06 | 1.89 | 1.79 | 1.29 | 2.99+ |
| | | | | | S100A12 | 3.11+ | 1.30 | -2.39 | -1.31 | 3.31* | 4.32** | 4.06* | -2.54+ |
| | | | | | CXCL1 | 1.38 | 1.44 | 1.04 | -1.07 | 1.57 | 1.68+ | 1.48 | -1.09 |
| | | | | | IL23A | 5.10** | 5.68** | 1.11 | 2.71 | 4.97** | 1.84 | 1.88 | 1.14 |
| | | | | | CXCL8 | 3.03+ | 3.22 | 1.07 | 1.57 | 3.95* | 2.52 | 1.93 | -1.23 |
| | | | | | IL23R | -1.11 | -1.01 | 1.10 | -1.23 | 1.12 | 1.38 | 1.11 | -1.13 |
| | | | | | IL4R | -1.17 | -1.36 | -1.17 | -1.27 | 1.22 | 1.54** | 1.09 | -1.66* |
| | | | | | TGFB1 | -1.35 | -1.34 | 1.01 | -1.11 | 1.30 | 1.44*** | -1.21 | -1.74** |
| | | | | | Col4A | -1.59+ | -1.38 | 1.15 | -1.41 | 1.09 | 1.54*** | -1.12 | -1.50+ |
| | | | | | SMAIL2 | -1.40** | -1.27 | 1.09 | -1.14 | 1.07 | 1.22*** | -1.22+ | -1.36* |
| | | | | | PDE4A | -1.54+ | 1.15 | 1.76*** | -1.08 | 1.41 | 1.52** | -1.42+ | -1.23 |
| | | | | | PDE4D | -1.07 | 1.06 | 1.14 | -1.02 | 1.25 | 1.27* | -1.05 | -1.18 |
| | | | | | STAT3 | 1.02 | 1.00 | -1.02 | 1.10 | 1.36+ | 1.24 | -1.08 | -1.36+ |
| | | | | | JAK3 | -1.02 | 1.12 | 1.14 | 1.24 | 2.92*** | 2.34** | -1.27 | -2.60** |
| | | | | | IL12RB1 | -1.04 | 1.12 | 1.17 | 1.07 | 2.70*** | 2.53** | -1.11 | -2.40** |
| | | | | | Col3A | -1.67 | -1.14 | 1.46* | -1.09 | 2.10* | 2.29*** | -1.53 | -2.39** |
| | | | | | Col1A | -1.41 | -1.17 | 1.21 | -1.05 | 2.38* | 2.49*** | -1.35 | -2.78** |
| | | | | | FOXP3 | -1.24 | -1.31 | -1.06 | 1.23 | 2.06** | 1.68* | -1.52* | -2.71*** |
| | | | | | CCL22 | -1.55+ | -1.13 | 1.37 | 1.42 | 2.00** | 1.40 | -2.20*** | -2.26** |
| | | | | | IL16 | -1.47 | -1.14 | 1.29* | 1.14 | 1.50+ | 1.32 | -1.66* | -1.70* |
| | | | | | JAK1 | -1.21 | -1.09 | 1.11 | 1.06 | 1.36 | 1.28 | -1.28 | -1.48+ |
| | | | | | IL2RA | -1.46 | -1.20 | 1.22 | 1.45 | 3.93*** | 2.71** | -2.11* | -4.71*** |
| | | | | | Fibronectin | -1.40 | -1.05 | 1.34 | 1.38 | 2.63*** | 1.90** | -1.94+ | -2.76** |
| | | | | | IL15RA | -1.24 | 1.03 | 1.27** | 1.29 | 1.87** | 1.45* | -1.59+ | -1.82** |
| | | | | | CCL18 | 3.34* | 8.87** | 2.66** | 6.20** | 5.70** | -1.09 | -1.86 | 1.56 |
| | | | | | PDE4B | 1.72+ | 2.48* | 1.44** | 2.15** | 2.97*** | 1.38* | -1.25 | -1.20 |
| | | | | | MMP12 | 3.15 | 4.17+ | 1.32 | 4.22* | 7.40** | 1.75 | -1.34 | -1.77 |
| | | | | | CCL13 | 1.00 | 2.43 | 2.43* | 2.17+ | 2.90* | 1.34 | -2.17+ | -1.19 |
| | | | | | IL9 | 1.22 | 2.51 | 2.05 | 1.85 | 5.51* | 2.99 | -1.51 | -2.20 |
| | | | | | IL2 | -1.13 | 1.27 | 1.43 | 1.90+ | 2.28* | 1.20 | -2.14* | -1.80+ |
| | | | | | CTGF | 1.34 | 1.20 | -1.11 | 1.68* | 1.79* | 1.07 | -1.25 | -1.49 |
| | | | | | IL12B | 2.96*** | 1.76 | -1.68 | 3.90** | 5.79*** | 1.48 | -1.32 | -3.29 |
| | | | | | GranzymeB | 1.91 | 2.54 | 1.33 | 2.89 | 11.10** | 3.83** | -1.51 | -4.35** |
| | | | | | STAT1 | 1.11 | 1.22 | 1.10 | 1.76* | 2.82*** | 1.60* | -1.59 | -2.31** |
| | | | | | CCL5 | 1.17 | 1.72 | 1.46 | 2.18* | 5.16*** | 2.37* | -1.85+ | -3.00** |
| | | | | | PAI1 | 1.84 | 1.62 | -1.14 | 2.78* | 5.68*** | 2.04* | -1.51 | -3.51** |
| | | | | | IL10 | 1.41 | 1.87 | 1.33 | 2.07** | 3.30*** | 1.60* | -1.47 | -1.76 |
| | | | | | IL15 | 1.22 | 1.47 | 1.21 | 1.91** | 2.24** | 1.17 | -1.57* | -1.53+ |
| | | | | | CXCL9 | 2.41 | 2.63 | 1.09 | 8.92*** | 28.90*** | 3.24* | -3.70* | -11.00** |
| | | | | | CXCL10 | 2.70+ | 3.83* | 1.42 | 14.80*** | 39.30*** | 2.66* | -5.48* | -10.30** |
| | | | | | IL32 | 1.39 | 1.53 | 1.10 | 1.96* | 2.64** | 1.35 | -1.40 | -1.72* |
| | | | | | IFNG | 2.71 | 4.29* | 1.58 | 9.44*** | 23.60*** | 2.50* | -3.48* | -5.50** |

**Figure 2.** Summary heatmap illustrating mean mRNA expressions of all analyzed genes by qRT-PCR in AA and FFA versus control scalp. Fold change differences in gene expression between tissues are shown. Red; up-regulated; blue: down-regulated genes. N=normal, NL=non-lesional, LS=lesional. Genes are ordered by unsupervised hierarchical clustering. +p<0.1, *p<0.05, **p<0.01, ***p<0.001.

Due to clinical and histopathological similarities between the 3 commonest forms of scarring alopecia: LPP, FFA and CCCA, as described in many publications, these primary CAs are discussed together.[19,22-24] Furthermore, all three conditions were shown to improve with immune based treatments. LPP/FFA was shown to improve following non-specific, broad anti-inflammatory drugs, such as methotrexate,[25] or pan-JAK inhibitor (tofacitinib),[19] and common clinical practice for CCCA include topical corticosteroids, topical immunomodulators (tacrolimus and pimecrolimus), intralesional corticosteroids, and anti-inflammatory antibiotics.[3,26] We thus postulate that LPP/FFA and CCCA may be driven by similar immune polarity.[26] The findings from our FFA study, as well as previous literature, as detailed above, provide strong rationale for CA treatment that targets Th1, IL-23, and related cytokines and chemokines. We hypothesize that the inflammation driven by Th1-related cytokines and chemokines and IL-23, is causing the characteristic fibrosis of CA, leading to scarring and permanent hair loss. In this proposed trial, we will include two study arms: an arm of LPP/FFA patients, and an arm of CCCA patients.

JAK inhibitors are a group of small molecules, recently emerging as an appealing class of immune modifiers in dermatology.[27] These are antagonists of the various members of the JAK enzymes family, which consists of JAK1, JAK2, JAK3, and tyrosine kinase-2 (TYK2).[28] JAKs enable the binding and activation of the transducer and activator of transcription (STAT), by phosphorylating the cytoplasmic domain of multiple cytokine receptors. This results in translocation of the STAT into the nucleus, which greatly affects transcription. JAK antagonism therefore blocks this signaling through STAT activation,[29-31] targeting Th1/IFN-$\gamma$ as well as common $\gamma_c$ cytokines (shared between IL-2, IL-4, IL-9, IL-7, IL-15 and IL-21), and TYK2 also adds an IL-23 capability.[32-34] Therefore brepocitinib, a dual inhibitor of JAK1 and TYK2, currently being investigated for a number of indications including psoriasis, Crohn's disease, ulcerative colitis , psoriatic arthritis, atopic dermatitis, psoriasis, systemic lupus erythematosus and AA, and which has been shown to be safe and well tolerated, with good safety profile,[35] was chosen for this protocol.

We will evaluate scalp and blood markers of inflammation, hair keratins and fibrosis, and our ultimate goal would be to elucidate the relations between inflammation and tissue scarring. While our study design is specifically powered to detect mechanistic tissue effects of brepocitinib, drug safety and tolerability in this patient population will also be closely monitored.

Our research proposal is novel in that we propose to investigate the immune profile of CA patients in skin and blood, at baseline, as well as during treatment with brepocitinib. In addition to the much-needed, prospective investigation of a new treatment modality for these diseases, we also aim to better characterize these diseases molecularly, and attempt to determine the effects of the inflammatory process on the resultant fibrosis. These findings will help to identify new treatment targets as well as direct further investigation for the development of new therapies for these disfiguring diseases.

The study will be conducted in accordance with our department's Standard Operating Procedures, which are based on US FDA Title 21 Code of Federal Regulations and ICH Good Clinical Practice guidelines.

## HYPOTHESIS

Brepocitinib administration will be safe and tolerable in CA patients and will downregulate biomarkers known to be associated with inflammation and fibrosis.

## OBJECTIVES

Primary Objectives:

To evaluate the safety of brepocitinib and the effects of brepocitinib on biomarkers of inflammation and fibrosis in skin and blood in study participants with CA.

Secondary Objectives:

To characterize the effect of brepocitinib on scalp biomarkers, clinical endpoints, and quality of life.

## OUTCOMES

### Primary Endpoints

- Safety profile and tolerability will be measured by Incidence and severity of Treatment-Emergent adverse events including adverse events of special interest, Serious Adverse Events, clinically significant abnormalities in vital signs and incidence of clinically significant abnormalities in clinical laboratory parameters.
- Changes in CCL5 (a surrogate for IFNγ activity) and biomarkers of fibrosis (e.g. TGFB1/2, vimentin, fibronectin, CTGF) in CA scalp from baseline to week 24.

### Secondary Endpoints

- Evaluate the baseline molecular dysregulations of CA by mechanistic studies on scalp and blood
- Change in scalp biomarkers in response to brepocitinib from baseline at weeks 24 and 48.
- Change in the applicable clinical score (for FFA - Frontal Fibrosing Alopecia Severity Score – FFASI,[36] for LPP – Lichen Planopilaris Activity Index Score – LPPAI,[37] for CCCA - Change in Central Hair Loss Grade – CHLG[38])
- Change in Physician Global Assessment of Improvement (PGA-I) at weeks 24 and 48. PGA-I will range from -4 (significant worsening) to 4 (significant improvement).
- Change in the Dermatology Quality of Life Index (DLQI)
- Percent change of clinical response from baseline to primary and secondary endpoints
- Compare molecular response among different CA subtypes
- Compare clinical response among different CA subtypes (i.e. LPP/FFA and CCCA) by PGA-I response.
- Changes of CCL5 (a surrogate for IFNγ activity) and biomarkers of fibrosis (e.g., TGFB1/2, vimentin, fibronectin, CTGF) in CA scalp in response to PF-06700841 from baseline to week 48.

## STUDY DESIGN AND METHODS

This is a prospective, randomized, double-blind, placebo-controlled clinical trial. Following Phase I of the study (double blind treatment period), Phase II will include an open label period, in which all participants will be treated with the investigational product. Approximately 50 participants will be enrolled into this study and 44 participants are anticipated to complete this trial through Week 24 (approximately 26 in CCCA, and 18 in LPP/FFA disease groups, accounting for an overall 18% drop out rate). Randomization will be stratified by participants' initial diagnosis – LPP/FFA or CCCA. Power calculation rationale is detailed under "Statistical Considerations".

The study will take place at the Icahn School of Medicine at Mount Sinai (ISMMS). Approximately 50 participants will be recruited and enrolled at ISMMS.

All participants will have a recently (less than 7 years since disease onset) diagnosed CA, LPP/FFA or CCCA, at the time of screening.

During Phase I, at the Baseline/Day 0 visit, subjects will be randomized to receive brepocitinib or placebo (3:1). At week 24, primary endpoint, all participants will cross over to Phase II, in which all participants will receive brepocitinib (open-label phase). In Phase I all subjects will receive their 45 mg dosage (PF-06700841 or placebo) as one 25 mg tablet and four 5 mg tablets daily. In Phase II, all subjects will receive brepocitinib as one 25 mg tablet and four 5mg tablets daily.

Clinical and mechanistic evaluations will take place at weeks 0, 12, 24 (primary endpoints), 36 and 48 (secondary endpoints), with additional visits on weeks 4, 8, 16, 20, 28, 32, 40, 44, 48 and 52, for documentation of adverse events as well as clinical observations of hair and skin.

The treatment period will conclude at week 48 (the secondary endpoint), and subjects will be asked to return for a follow up visit at week 52 (end of trial) as shown below, for continued observation of skin and hair changes. Timeline of this trial is presented in Figure 3.

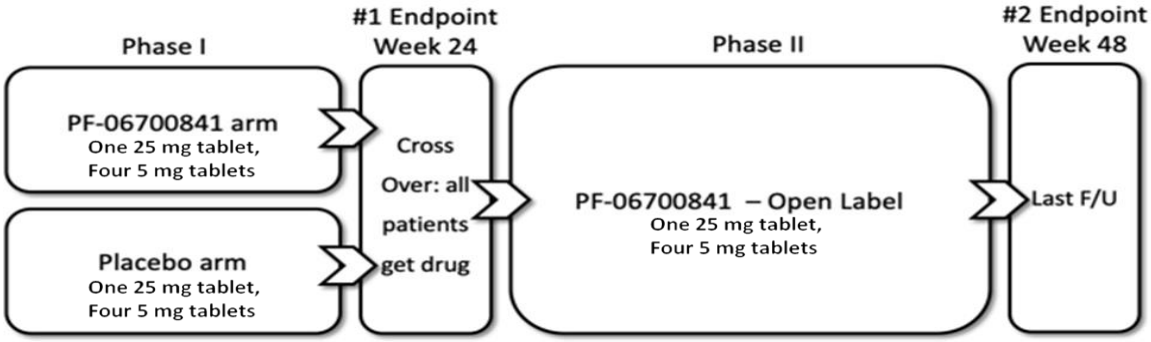



**Figure 3. Study Timeline**

## DOSE JUSTIFICATION

In this Phase 2a study, a single dose of 45 mg of brepocitinib will be administered (taken as one 25 mg tablet and four 5 mg tablets) once a day (QD) for 48 weeks. This dose was selected based on the safety and efficacy observed to date with brepocitinib in healthy participants (B7931001) and participants with active psoriasis (B7931001 and B7931004) and alopecia areata with more than 50% hair loss (B7931005). The overall safety profile to date with brepocitinib has been acceptable with doses up to 60 mg QD for 4 weeks and 30 mg QD for up to 20 weeks in completed studies. Ongoing studies in inflammatory bowel disease (IBD) are assessing the 60 mg QD dose for up to 12 weeks, and an extended period at 30 mg QD for up to 52 weeks. Also, ongoing Phase 2 studies in Systemic Lupus Erythematosus and Psoriatic Arthritis are assessing brepocitinib at 45mg QD dose and 60 mg QD dose, respectively, for up to 52 weeks.

Based on the preliminary population PK analysis with combined data from studies B7931001 in healthy participants and B7931004 in patients with moderate to severe PsO, the expected median steady state exposures ($AUC_{24}$) following 45 mg QD is 2030 ng.h/mL, in a patient population. As a result, the exposure of brepocitinib ($AUC_{24}$) at 45 mg QD in this study are expected to be 56x and 8.6x below the $AUC_{24}$ at the NOAELs observed in the 6-month rat (45 mg/kg/day) and 9-month monkey (20 mg/kg/day) studies, respectively. The pharmacological activity of brepocitinib was assessed by reduction of hsCRP in patients with moderate to severe PsO (B7931004). In study B7931004, the median reduction (percent change from baseline) of hsCRP observed at Week 4 were approximately 61% and 76% with brepocitinib 30 and 60 mg QD treatment, respectively. Study B7931004 also showed that brepocitinib reached maximal effect on hsCRP within approximately 2 weeks after start of treatment and remained constant throughout the 12-week treatment period. The median hsCRP reduction with brepocitinib 45 mg daily dose up to 24 weeks would expect to be between 61% to 76%.

## SUBJECT ELIGIBILITY

### INCLUSION CRITERIA

1. Subjects of any gender, age 18 years or older, at the time of informed consent at Screening

2. Subjects who are willing and able to adhere to the study visit schedule and comply with protocol requirements.

3. Subject self-reports a history of at least 6 months of CA (LPP/FFA or CCCA). Diagnosis will be made clinically (according to the LPPAI[37], FFASI[36] and/or CHLG[38]).

4. Subject has a negative Tuberculin purified protein derivative (PPD) or QuantiFERON TB-Gold test (QFT) at screening.

5. A female participant is eligible to participate if she is not pregnant or breastfeeding, and at least one of the following conditions applies:
   Is not a woman of childbearing potential (WOCBP)
   OR

Is a WOCBP (all female participants, regardless of whether or not they have experienced/reported menarche, are considered WOCBP unless they are permanently sterile or confirmed infertile). A WOCBP who is sexually active must use a contraceptive method that is highly effective, with a failure rate of <1%, during the intervention period and for at least 28 days after the last dose of study intervention.

And if a WOCBP, must have a negative highly sensitive serum pregnancy test at the screening visit and a negative urine pregnancy test at baseline performed before the first dose of study intervention.

6. Subject is judged to be in otherwise good overall health following a detailed medical and medication history, physical examination, and laboratory testing.

**EXCLUSION CRITERIA**

1. The presence of any of the following will exclude a subject from enrollment:

2. Subject's cause of hair loss is indeterminable and/or they have concomitant causes of alopecia, such pregnancy-related, drug-induced, telogen effluvium, or advanced androgenetic alopecia.

3. Subject has a history of CA for ≥ 7 years since their disease onset, severe fibrosing disease, or very rapid hair loss.

4. Subject has a history of moderate to severe keloids on the scalp, as determined by clinical examination at screening.

5. Other scalp disease that may impact assessment (e.g., scalp psoriasis, dermatitis, etc.).

6. Subject is pregnant or breastfeeding.

7. Participation in other studies involving investigational drug(s) within 8 weeks or within 5 half-lives (if known), whichever is longer, prior to study entry and/or during study participation.

8. Active systemic diseases that may cause hair loss (e.g., systemic lupus erythematosus, thyroiditis, systemic sclerosis, etc.).

9. Any Psychiatric condition in the opinion of the investigator precludes participation in the study.

10. Current or recent history of clinically significant severe, progressive, or uncontrolled renal (including but not limited to active renal disease or recent kidney stones), hepatic, hematological, gastrointestinal, metabolic, endocrine (particularly thyroid disease which can be associated with hair loss), pulmonary, cardiovascular, psychiatric,

immunologic/rheumatologic or neurologic disease; or have any other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration, or interfere with the interpretation of study results; or in the opinion of the investigator, the subject is inappropriate for entry into this study, or unwilling/unable to comply with STUDY PROCEDURES.

11. Any present malignancies or history of malignancies with the exception of adequately treated or excised non-metastatic basal cell or squamous cell cancer of the skin or cervical carcinoma in situ.

12. History of any lymphoproliferative disorder such as Epstein Barr Virus (EBV) related lymphoproliferative disorder, history of lymphoma, history of leukemia, or signs and symptoms suggestive of current lymphatic or lymphoid disease.

13. History (single episode) of disseminated herpes zoster or disseminated herpes simplex, or a recurrent (more than one episode of) localized, dermatomal herpes zoster.

14. History of systemic infection requiring hospitalization, parenteral antimicrobial therapy, or as otherwise judged clinically significant by the investigator within 6 months prior to Day 0.

15. Active acute or chronic infection requiring treatment with oral antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals within 4 weeks prior to Day 0 or superficial skin infection within 1 week prior to Day 0.

16. Significant trauma or major surgery within 1 month of signing informed consent.

17. Considered in imminent need for surgery or with elective surgery scheduled to occur during the study.

18. Active hepatitis B, hepatitis C, human immunodeficiency virus (HIV), or positive HIV serology at the time of screening for subjects determined by the investigators to be at high-risk for this disease.

19. **ANY** of the following abnormalities in the clinical laboratory tests at screening, as assessed by the study-specific laboratory and confirmed by a single repeat, if deemed necessary:
    1. Absolute neutrophil count of $<1.2 \times 10^9$/L ($<1200/mm^3$);
    2. Hemoglobin <10.0 g/dL or hematocrit <30%;
       - Platelet count of $<150 \times 10^9$/L ($<150,000/mm^3$);
    3. Absolute lymphocyte count of $<0.50 \times 10^9$ /L ($<500/mm^3$);
    4. Estimated Glomerular Filtration Rate (eGFR) less than 60 mL/ml/min/1.73$m^2$ based on the age appropriate serum creatinine-based calculation and serum cystatin C based calculation;
    5. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) values >2 times the ULN;

6. Total bilirubin ≥ 1.5 times the ULN; participants with a history of Gilbert's syndrome may have a direct bilirubin measured and would be eligible for this study provided the direct bilirubin is ≤ ULN.

20. Have an active history of alcohol or substance abuse within 1 year prior to Day 0.

21. Donation of blood in excess of 500 mL within 8 weeks prior to Day 0.

22. Subject has received a live attenuated vaccine ≤ 30 days prior to study screening.

23. Subject has any uncertain or clinically significant laboratory abnormalities that may affect interpretation of study data or endpoints, at determined by the PI.

24. History of adverse systemic or allergic reactions to components of study drug.

25. Use of systemic immunosuppressive medications, including, but not limited to, cyclosporine, systemic corticosteroids, mycophenolate mofetil, azathioprine, methotrexate, within 8 weeks prior to baseline visit.

26. Use of other systemic agent for CA, including, 5α-reductase inhibitors, hydroxychloroquine, or retinoids, within 4 weeks prior to baseline visit.

27. Use of an intralesional corticosteroids or oral JAK inhibitor (tofacitinib, ruxolitinib, or any JAK1/TYK2 product) within 4 weeks prior to the baseline visit.

28. Subject has used topical corticosteroids, and/or tacrolimus, and/or pimecrolimus or cyclosporine within 1 week before the baseline visit.

29. Subject has been previously treated with biological drugs in the last 12 weeks for other indications.

30. Subjects previously tested with a positive or indeterminable PPD or QFT result, including subjects that completed standard tuberculosis therapy.

31. Screening 12-lead ECG that demonstrates clinically significant abnormalities requiring treatment, e.g. acute myocardial infarction, serious tachy or brady arrhythmias or that are indicative of serious underlying heart disease (e.g. cardiomyopathy, major congenital heart disease, low voltage in all leads, Wolff-Parkinson-White syndrome and other clinically relevant abnormalities which may affect participant safety or interpretation of study results. A history of additional risk factors for Torsades de Pointes (TdP) (e.g. heart failure, hypokalemia, family history of long QT syndrome).

    - If QTcF exceeds 450 ms, or QRS exceeds 120 ms, the ECG should be repeated 2 more times and the average of 3 QTc or QRS values should be used to determine the participants' eligibility. Participants with average screening value QTcF > 450 ms should be excluded.

32. The use of concomitant CYP3A substrates and inducers (Appendix 2) and medications (Appendix 3) that prolong the QT/QTcF interval are exclusionary.

33. The concomitant use of drugs that are substrates for P-gp, BCRP, OCT/MATE transporters with a narrow therapeutic index to minimize any potential significant drug interaction. The substrates that should be prohibited include the following:

| Pgp substrates | BCRP substrates | OCT1 substrates | OCT2 substrates | MATE1 substrates |
|---|---|---|---|---|
| dabigatran | rosuvastatin | dofetilide | pilsicainide | dabigatran |
| digoxin | | | tenofovir | tenofovir |

34. Subjects with a Columbia Suicide Severity Rating Scale (C-SSRS) score = 4 or 5 at Visit 2 (Baseline).

35. History of thromboembolic events including DVT and PE or history of inherited coagulopathies.

## PROCEDURES

### CLINICAL PROCEDURES

After providing informed consent, subjects will be assessed for study eligibility during the screening/washout period (4 weeks prior to baseline), which includes a review of past and current medical conditions, detailed review of past and current medications, a brief physical examination, and clinical assessments (FFAI/LLPAI/CHLG, whichever applies). The following laboratory tests will be performed on all participants: complete blood count (CBC) with differential, complete metabolic panel (CMP), C-reactive protein (CRP), HIV, hepatitis B surface antigen (HbsAg), hepatitis C virus antibodies, urinalysis, and serum pregnancy (where applicable).

Subjects who meet inclusion and exclusion criteria will return for baseline assessments and clinical photographs at Day 0. Blood samples and skin biopsies will be collected for gene expression and proteomic studies. Two 4.5 mm scalp biopsies will be performed at baseline, one from lesional skin (from frontal scalp in FFA patients, and from the edge of lesions in LPP and CCCA patients) and one from non-lesional skin (taken from clinically non-involved scalp areas, ≥5cm from any lesional areas). Thereafter, only lesional biopsies. Blood sampling for mechanistic studies will be collected at weeks 12, 24, 36 and 48.

The following procedures/assessments will take place at each visit:

Visit 1 – Screening (within 4 weeks of baseline)*

- Sign and date an IRB-approved informed consent and HIPPA agreement
- Review Inclusion and Exclusion Criteria
- Record gender, race, ethnicity, and medical history (including personal and family history of CA or other dermatological diseases, and autoimmune diseases)

- Physical exam, including: height (cm), weight (kg), blood pressure, and heart rate
- 12 –lead ECG (in triplicate); if QTcF exceeds 450 ms, the ECG should be repeated 2 more times and the average of the three QTcF used to determine eligibility.
- Record all prior systemic and topical therapies/treatments used for CA
- PPD test or Quantiferon blood test as outlined in Inclusion/Exclusion criteria
- HIV, HBV, and HCV screening. Serum HCG for all female subjects of child-bearing potential to confirm subject is not pregnant
- Safety labs, including: Complete blood count (CBC), Comprehensive metabolic panel (CMP), and C-reactive protein (CRP), urinalysis
- Clinical assessment (for FFA - Frontal Fibrosing Alopecia Severity Score – FFASI,[36] for LPP – Lichen Planopilaris Activity Index Score – LPPAI,[37] for CCCA - Change in Central Hair Loss Grade – CHLG[38]; eyebrow/eyelash score). Record all other concomitant medications and adverse events
- Contraception check (throughout study- all visits)

*****If more than 4 weeks has lapsed between screening and baseline/Visit 2, then all procedures should be repeated except for TB and HIV screening and HBV/HCV serologies.

<u>Visit 2 – Baseline (Day 0)</u>

- Confirm all Inclusion and Exclusion criteria have been met
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Confirm proper contraception is being used
- Obtain vital signs (BP and heart rate)
- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS)
- Clinical photos of scalp and eyebrows
- Collect blood sample for mechanistic endpoints
- Collect blood for genetic assay
- Collect blood for PK
- Collect blood and urine for safety labs (including cystatin-C and CPK)
- Fasting Lipid Panel
- Perform biopsies of lesional and non-lesional skin of the scalp.
- Record concomitant medications and adverse events
- Subjects will undergo randomization to brepocitinib/ placebo (3:1 ratio).
- Dispense and administer study drug

<u>Visit 3, 4, 5, 6, 7 – Weeks 4, 8, 12,16, 20</u>

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs (BP and heart rate)
- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS) (Weeks 8 and 16)
- Collect blood and urine for safety labs (including cystatin-C and CPK)
- Collect blood sample for mechanistic endpoints (Week 12 only)

- Clinical photos of scalp and eyebrows (Weeks 8 and 16)
- 12 –lead ECG (Weeks 8 and 16)
- Columbia Suicide Severity Rating Scale (C-SSRS) (Weeks 8 and 16)
- Dispense/collect study drug
- Confirm proper contraception is being used
- Record concomitant medications and adverse events

Visit 8 – Week 24 – **Primary Endpoint / Study Cross Over**

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs (BP and heart rate)
- 12 –lead ECG
- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS)
- Collect blood and urine for safety labs (including cystatin-C and CPK)
- Clinical photos of scalp and eyebrows
- Collect blood sample for mechanistic endpoints
- Collect blood for PK
- Fasting Lipid Panel
- Perform biopsy of lesional skin of the scalp
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Dispense and collect study drug
- Confirm proper contraception is being used
- Record concomitant medications and adverse events

Visit 9 10, 11, 12, 13 and 14 – Weeks 28, 32, 36, 40, 44

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs (BP and heart rate)
- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS) (Weeks 32, 40 and 48)
- Collect blood and urine for safety labs (including cystatin-C and CPK)
- Clinical photos of scalp and eyebrows (Weeks 32 and 40)
- 12 –lead ECG (Weeks 32 and 40)
- Columbia Suicide Severity Rating Scale (C-SSRS) (Weeks 32 and 40)
- Dispense/collect study drug
- Confirm proper contraception is being used
- Record concomitant medications and adverse events

Visit 14 – Week 48 - **Secondary Endpoint**

- Obtain vital signs (BP and heart rate)
- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS)
- 12 –lead ECG
- Collect blood and urine for safety labs (including cystatin-C and CPK)
- Clinical photos of scalp and eyebrows

- Collect blood sample for mechanistic endpoints
- Collect blood for PK
- Fasting Lipid Panel
- Perform biopsy of lesional skin of the scalp
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Collect unused study drug
- Confirm proper contraception is being used
- Record concomitant medications and adverse events

Visit 11 – Week 52 –Follow-up

- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS)
- Medical photos of scalp and eyebrows
- Record concomitant medications and adverse events

**Early Termination Visit**

In the event a study subject wants to withdraw early from the study or the subject is being discontinued from the study for any reason, the following procedures should be followed:

- Urine pregnancy test for all female subjects of child-bearing potential to confirm subject is not pregnant
- Obtain vital signs (BP and heart rate)
- 12-lead ECG
- Physical examination
- Clinical assessment (FFASI/LPPAI/CHLG; eyebrow/eyelash score)
- Questionnaires (DLQI and VAS)
- Collect blood and urine for safety labs (including cystatin-C and CPK)
- Fasting Lipid Panel
- Clinical photos of scalp and eyebrows
- Collect blood sample for mechanistic endpoints
- Perform biopsy of lesional skin of the scalp
- Confirm proper contraception is being used.
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Collect unused study drug
- Record concomitant medications and adverse events

Monitoring of AEs and SAEs will begin once the participant has provided informed consent for the study. All study procedures will be completed at designated intervals during the study (see Table 1).

**MECHANISTIC PROCEDURES**

Blood Analyses

- OLINK MSD and Singulex platforms will be used to assess for biomarkers in blood. These platforms are able to evaluate more than 200 analytes, including all the inflammatory markers that are analyzed in skin, as well as cardiovascular markers, and neuro-

immunological markers, including cytokines and chemokines related to Th1, Th2, Th17, Treg, PDE-related markers, and more.

- PAX DNA for genetic analyses. DNA samples will be collected from all participants on Day 0 and archived for future analysis. Based on recent publications, we will seek known genetic variations strongly associated with FFA or CCCA.[7,17] for FFA, genome-wide significant association were found at four genomic loci: 2p22.2, 6p21.1, 8q24.22 and 15q2.1.[7] For CCCA, a whole-exome sequencing study found a mutation in *PADI3*, which encodes peptidyl arginine deiminase, type III, an enzyme that post-translationally modifies other proteins that are essential to hair-shaft formation. *PADI3* mutation may result in improper formation of hair shaft, leading to the CCCA phenotype.[17] Of note, the CCCA-related variant was identified in a group of 16 CCCA patients,[17] underscoring the great potential of such data collection. These susceptibility loci, as well as future possible variants to be published, will be explored, and we also aim to correlate genetic variants with molecular and clinical findings/responses.

Skin Analyses

We will perform gene expression studies using RT-PCR and RNAseq, as well as protein expression studies using immunohistochemistry, and immunofluorescence studies. We will also perform OLINK proteomic studies on scalp biopsies.

Immunohistochemistry (IHC) and immunofluorescence (IF) will be performed as described on frozen sections,[39,40] using purified mouse anti-human monoclonal antibodies. Cell counts will be quantified using Image J V1.42 software (National Institutes of Health, Bethesda, MD).

RNA will be extracted for Quantitative real-time PCR (qRT-PCR) with the miRNAeasy Mini Kit (Qiagen, Hilden, Germany). Reverse transcription to cDNA from RNA will be performed using the High Capacity cDNA Reverse Transcription Kit, and cDNA will be amplified with TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA). TaqMan Low Density Array/TLDA cards will be used for qRT-PCR. Eukaryotic 18S rRNA will be used as an endogenous control. Expression values (threshold cycle [Ct]) will be normalized to Rplp0.

We will also perform single cell analyses on 8 patients with CCCA and 8 with FFA/LPP (a total of 16 patients), with the same number of biopsies per patient (16x4-3 lesional and one non lesional=64 total biopsies), at similar weeks as other biopsies. Biopsies will be divided as follows: ¼ for single cell, ¼ for gene expression and half for OCT.

## CLINICAL OVERVIEW OF POTENTIAL RISKS:

Based on the current clinical and nonclinical experience with brepocitinib and other information from other approved oral JAK inhibitors (e.g. Xeljanz (tofacitinib), Jakafi (ruxolitinib), Olumiant (baricitinib) and Rinvoq (upadacitinib), the potential risks for brepocitinib include (1) viral reactivation, (2) serious infection and opportunistic infections, (3) malignancy and lymphoproliferative disorders(4) hematological abnormalities and other alterations in laboratory parameters; decreased neutrophil counts, changes in lymphocyte counts, decreased hemoglobin

level, decreased platelet counts, and elevation of hepatic transaminases (5) alteration in the lipid profile; (6) increases in serum creatinine; (7) increases in creatine phosphokinase, (8) QT prolongation, (9) bone changes, and (10) thromboembolism. .

In general, the safety profile of brepocitinib may be similar to that observed with other JAK inhibitors with regard to effects related to immunosuppression. Other potential effects related to decreased counts of hematopoietic cells and changes in lipid profile may be different based on the specific pattern of JAK inhibition.

Major adverse cardiovascular events (MACE) have been reported with tofacitinib with RA patients aged 50 years or older with at least 1 additional cardiovascular risk factor.

Events of pulmonary embolism have been reported with JAK inhibitors approved in inflammatory diseases, including tofacitinib, baricitinib, and upadacitinib. In the brepocitinib development program, there has been an adverse event of pulmonary embolism in B7981007, an ongoing blinded study in Crohn's disease. One participant in the completed B7931023 study in psoriasis (a topical 1% formulation of the study drug) experienced a serious adverse event of pulmonary embolism; the participant received appropriate medical treatment for the event which subsequently resolved.

One participant in the 30-10 mg group in Phase 2 psoriasis study B7931004 was found to have a positive urine human chorionic gonadotrophin test at week 6 (Day 42) and on Day 165, an obstetrical ultrasound demonstrated a right-sided cleft lip in the fetus, with no definite cleft palate. Due to the investigational nature of this product, brepocitinib should not be administered to pregnant or breastfeeding women or women of childbearing potential who are unwilling or unable to use contraception as defined in the protocol. Pregnancy testing is required for female participants of child-bearing potential at baseline, each study visit and as defined in the protocol and at the end of study treatment, as well as whenever a menstrual cycle is missed, to rule out pregnancy. Male participants are not required to use contraception to minimize partner exposure to brepocitinib.

For further safety information on brepocitinib, refer to the Investigator's Brochure.

**PRIOR TREATMENT:**

All relevant treatment received by the subject within 30 days before screening will be recorded. Over-the-counter drugs (e.g. vitamins, acetaminophen) taken by the subject within 14 days before screening will also be recorded.

**CONCOMITANT TREATMENT:**

Concomitant medications are permitted for the treatment of stable, chronic illness. Use of such concomitant medication or other medications that may be required throughout the study will be recorded (including the reason for treatment and name, dose, unit, route, and the date of treatment as appropriate) until week 52 or Early Termination.

## PROHIBITED TREATMENT:

All treatments prohibited during the screening period are also prohibited throughout the course of the study. No live attenuated vaccinations are permitted through the study.

## MEASURES TO MINIMIZE/AVOID BIAS;

### Subject Identification

Subjects are numbered sequentially. Each subject will be assigned a unique number and will keep this number for the duration of the study. Subject numbers will not be reassigned or reused for any reason. Subjects who discontinue or withdraw from the study before receiving a treatment assignment code, who re-enroll at a later time must be assigned a new subject number. Subjects should be identified only by their assigned identified code number. The investigator must maintain a subject master log linking the subject number to the subject's name. The investigator must follow all applicable privacy laws in order to protect a subject's privacy and confidentiality.

### Randomization and Blinding

A subject who meets all the inclusion and exclusion criteria will be assigned a randomization number pre-dose on day 0/baseline. The randomization number is distinct from the subject screening number.

After fulfilling the enrollment criteria, the subjects enrolled will be randomized in a 3:1 ratio of brepocitinib to placebo.

The randomization for all sites will be performed by the designated Research Pharmacist/personnel at the Icahn School of Medicine utilizing the Web site http://www.randomization.com (or similar). The method uses random block sizes and randomly permuted blocks.

Subjects will be assigned a randomization number on day 0/Baseline. The randomization schema will be kept confidentially after generation.

## PHARMACOKINETICS

Blood samples will be collected into appropriately labeled tubes containing $K_2$EDTA at times specified in the Schedule of Events (Table 1) section of the protocol for measurement of brepocitinib . All efforts will be made to obtain the PK samples at the exact nominal time relative to dosing. The date and exact time of the sample collection is to be noted on the source document and data collection tool (e.g., CRF). Samples obtained outside the windows specified in the Schedule of Events (Table 1) may be considered a protocol deviation.

- Further details regarding the collection, processing, storage, and shipping of the blood samples will be provided in the lab manual.
- Samples will be analyzed using a validated analytical method in compliance with Pfizer standard operating procedures.
- As part of understanding the PK of the IP, samples may be used for metabolite identification and/or evaluation of the bioanalytical method. These data will be used for

internal exploratory purposes and will not be included in the clinical report. The PK samples must be processed and shipped as indicated in the instructions provided to the investigator site to maintain sample integrity. Any deviations from the PK sample handling procedure (e.g. sample collection and processing steps, interim storage, or shipping conditions), including any actions taken, must be documented and reported to Pfizer. On a case-by-case basis, Pfizer may make a determination as to whether sample integrity has been compromised.

## SAFETY REPORTING

### Definition of an Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Events meeting the AE definition include:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital sign measurements, changes or worsening in physical examination findings), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator.
- Any abnormal laboratory test results that meet any of the conditions below must be recorded as an AE:
- Is associated with accompanying clinical symptoms.
- Requires additional diagnostic testing or medical/surgical intervention.
- Leads to a change in study dosing (outside of any protocol-specified dose adjustments) or discontinuation from the study, significant additional concomitant drug treatment, or other therapy.

NOTE: Merely repeating an abnormal laboratory test in the absence of any of the above conditions does not constitute an AE. Any abnormal test that is determined to be an error does not require recording as an AE.

- Exacerbation of a chronic or intermittent preexisting condition including either an increase in frequency and/or intensity of the condition. Note that anticipated day-to day fluctuations of pre-existing disease or conditions present at the start of the study that **do not worsen** do not meet AE criteria.
- New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an

AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses must be reported regardless of sequelae.

**Definition of a Serious Adverse Event**

An SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life- threatening (immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization. In general hospitalization signifies that the participant has been detained (usually an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Hospitalization for elective treatment of a preexisting treatment that did not worsen from baseline is not considered an AE.
- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);
- Results in a congenital anomaly/birth defect.

Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.

**Method of Detecting AEs and SAEs**

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the participant is the preferred method to inquire about AE occurrences. Examples of non-directive questions include: "How have you felt since your last clinical visit?" and "have you had any new or changed health problems since you were last here?"

**Time period and frequency for collecting AE and SAE information**

The time period for actively eliciting and collecting AEs and SAEs ("active collection period") for each participant begins from the time the participant provides informed consent, which is obtained before the participant's participation in the study (i.e., before undergoing any study-related procedure and/or receiving study intervention), through and including a minimum of 28 calendar days after the last administration of the study intervention or until study completion or withdrawal, whichever is longer.

Follow-up by the investigator continues throughout and after the active collection period and until the AE or SAE or its sequelae resolve or stabilize at a level acceptable to the investigator and Pfizer concurs with that assessment.

For participants who are screen failures, the active collection period ends when screen failure status is determined.

If the participant withdraws from the study and also withdraws consent for the collection of future information, the active collection period ends when consent is withdrawn.
If a participant definitively discontinues or temporarily discontinues study intervention because of an AE or SAE, the AE or SAE must be recorded on the Case Report Form (CRF) and the SAE reported using the Pfizer CT SAE Report Form.

Investigators are not obligated to actively seek AEs or SAEs after the participant has concluded study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has completed the study, and he/she considers the event to be reasonably related to the study intervention, the investigator must promptly report the SAE to Pfizer using the CT SAE Report Form.

**Reporting SAEs to Pfizer Safety**
All SAEs occurring in a participant during the active collection period are to be reported to Pfizer Safety on the CT SAE Report Form immediately upon awareness and under no circumstance should this exceed 24 hours. The investigator will submit any updated SAE data to Pfizer within 24 hours of it being available. It is very important that the investigator always makes an assessment of causality for every event before the initial transmission of the SAE data to Pfizer Safety.

**Follow up of AEs and SAEs**
After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. For each event, the investigator must pursue and obtain adequate information until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

In general, follow-up information will include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Any information relevant to the event, such as concomitant medications and illnesses, must be provided. In the case of a participant death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer Safety.

**Exposure During Pregnancy or Breastfeeding, and Occupational Exposure**
Exposure to the study intervention under study during pregnancy or breastfeeding and occupational exposure are reportable to Pfizer Safety within 24 hours of investigator awareness, irrespective of whether they are accompanied by an AE/SAE. Occupational exposure occurs when a person comes in direct, unplanned contact with the investigational product, during the performance of job duties.

**Adverse Events of Special Interest**
Adverse events of special interest for brepocitinib include:
(1) viral reactivation
(2) serious infection and opportunistic infections
(3) malignancy and lymphoproliferative disorders

(4) hematological abnormalities and other alterations in laboratory parameters: decreased neutrophil counts, change in lymphocyte counts, decreased hemoglobin level, decreased platelet counts, and elevation of hepatic transaminases
(5) alterations in the lipid profile
(6) increases in serum creatinine
(7) increases in creatine phosphokinase
(8) QT prolongation
(9) Thromboembolism

## INFORMED CONSENT:

Prior to the initiation of any study related procedures, the potential subjects will be given a copy of the most recent IRB stamped and approved informed consent to read. Additionally, the PI or study staff member who has been designated to consent will discuss the specifics of the study including but not limited to the purpose of the research, procedures, time commitment, required tasks, test article or device, alternative treatments, benefits, risks, confidentiality etc. in a comprehensible (non-scientific) manner, using language readily understandable by the subject. Subjects will be told that participation is voluntary and that, if they do not consent, they will not be penalized. The person consenting will assure the voluntariness of the subject.

## DISCONTINUATION OF TREATMENT AND WITHDRAWAL OF SUBJECTS:

The reasons why a subject may discontinue or be withdrawn from the study include, but are not limited to the following: subject request, protocol violation, loss to follow up, subject non-compliance, study termination by investigators, and a confirmed grade 3 or higher adverse event, which is suspected to be related to test article administration. Subjects that elect to withdraw from the trial due to lack of efficacy and/or exacerbation of disease will undergo the procedures required at an early termination visit.

**Stopping Rules**
Patients will be permanently discontinued from study treatment in the event of:

- Anaphylactic reaction or other severe systemic reaction to study drug injection
- Diagnosis of a malignancy during study, excluding carcinoma in situ of the cervix, or squamous or basal cell carcinoma of the skin
- Evidence of pregnancy
- Any infection that is opportunistic, such as active TB and other infections whose nature or course may suggest an immuno-compromised status
- Treatment with any prohibited concomitant medication or procedure
- Other reasons that may lead to the permanent discontinuation of study drug include certain AEs deemed related to the study drug.
- Columbia Suicide Severity Rating Scale (C-SSRS) score = 4 or 5.
- If an individual participant demonstrates CONCOMITANT serum Cr based AND serum Cystatin C based eGFR decline of at least 30% (≥ 30%) compared to the subject's baseline eGFR then the participant should not be further dosed and adequate, immediate, supportive measures including immediate evaluation by a nephrologist (preferably within 24 hours) with appropriate management and treatment as clinically indicated. Results

should be repeated as indicated by the nephrologist or weekly at a minimum until the eGFR returns to baseline ±15%, or the renal parameters are deemed to be stable by the nephrologist and/or PI. If the subject cannot be seen by a nephrologist within 24 hours (as described above), then the subject should be sent to a local emergency room for evaluation and treatment as clinically indicated.

- If the following lab results are abnormal, participants will be retested in a window of 7-10 days and reassessed. If the test results remain abnormal, participants will be discontinued from the study:
  Hematology
  Absolute Neutrophil Count: $<1000/mm^3$; $<1.0 \times 10^9/L$
  Hemoglobin: <8.0 g/dL; <4.96 mmol/L; <80 g/L
  Hemoglobin drop ≥ 2 g/dL from baseline on two consecutive blood draws with associated symptoms; **or** hemoglobin drop ≥ 3 g/dL from baseline on two consecutive blood draws without associated symptoms.
  Platelet count: $<75{,}000/mm^3$; $<75.0 \times 10^9/L$
  Lymphocytes: $<500/mm^3$; $<0.5 \times 10^9/L$
  Chemistry
  AST >3.0x ULN
  ALT >3.0x ULN
  Total bilirubin >1.5x ULN
  CPK >10x ULN
  Total bilirubin ≥1.5 x ULN; subjects with a history of Gilbert's syndrome may have a direct bilirubin measured and would be eligible for this study provided the direct bilirubin is ≤ ULN
- Other intercurrent illnesses or major surgery
- An infection that requires systemic treatment with antibiotic, antifungal, antiviral, anti-parasitic, or anti-protozoal agents or requires oral treatment with such agents for longer than 2 weeks
- Treatment with systemic corticosteroids or non-steroidal immunosuppressive/ immunomodulating medications (e.g., cyclosporine, methotrexate, azathioprine, mycophenalate-mofetil, Janus kinase inhibitors, biologic agents, etc.).

Duration of study drug suspension should be reviewed on a case-by-case basis and can be discussed among the PIs.

**Unblinding**

In case of an emergency, when knowledge of the test article assignment is required for the medical management of an individual subject, the subject will be unblinded. The code should be broken only in the event of a medical emergency, when knowing the treatment assignment is absolutely necessary. The investigators will notify the IRB, FDA, and any other regulatory group, within 24 hours after determining that it is necessary to unblind the treatment assignment. The investigator must also indicate in source documents and in the CRF that the blind was broken and provide the date, time, and reason for breaking the blind. Any AE or SAE associated with breaking the blind must be recorded and reported as specified in this protocol.

A clinical data manager will assemble all the patient data by groups (treatment versus placebo) and the biostatistician may request the unblinding of subject treatment assignment for analyzing safety data, upon the appearance of unequal adverse events.

## STATISTICAL CONSIDERATIONS:

### Sample Size Calculation

A sample size of 44 completers (3:1 ratio Brepocitinib to placebo) will provide 92% power at 5% significance to detect a difference of -1.47 in mean RT-PCR-derived CCL5 log2 fold-change from baseline between Brepocitinib and Placebo arms, with a one-sided unpaired t-test. We assume a standard deviation (SD) of 1.4 for both arms, based on preliminary data of 16 alopecia areata (AA) participants treated with JAK1/TYK2 and 12 treated with placebo. The same formulation provides 90% power at 5% significance to detect a difference between arms of -1.52 in mean CXCR3 log2 fold-change from baseline, assuming SD = 1.5 according to a recently published study that observed 12 FFA and 8 healthy controls. Both calculations were performed in G*Power 3.1.

### Data Analyses

An Analysis of Covariance will be used to analyze the change from baseline, induced by brepocitinib, in CCL5 (a surrogate for IFN$\gamma$ activity) expression level at week 24. Treatment, CA subtypes (LPP/FFA and CCCA), and tissue (lesional/non-lesional) will be included as fixed factors. Baseline CCL5 will be included as a covariate. Least square means for each treatment and difference between treatments will be provided, together with their 90% confidence intervals (CI). The CCL5 expression level will be log2 transformed before the analysis, and least-square means estimates will be back-transformed into corresponding estimates for the ratio to baseline. Changes in CXCR3, a biomarker of fibrosis, and other biomarkers measured by qRT-PCR will be analyzed similarly. Change from baseline in clinical and quality of life scores between study arms (Brepocitinib and Placebo) will be analyzed with non-parametric tests (Wilcoxon-Mann-Whitney) performed within CA subtypes. We will use the Spearman correlation coefficient to correlate changes in clinical and quality of life scores with up- and down-regulated biomarkers. Multiple imputation based on Expectation-Maximization (EM) algorithm may be used to handle missing data, depending on their proportion and patterns. In Phase II, paired t-tests will analyze the change in biomarkers level from week 24 to week 48 promoted by Brepocitinib. An integrated analysis of Phase I and Phase II will compare combined treatment arms: Placebo + Brepocitinib vs. single Brepocitinib. A linear mixed-effects model will be used with the combined treatment arm, time point, and tissue as fixed factors and a random term for each subject. This model allows the formulation of several contrasts of interest to be estimated with 90% CI.

All research data will be identified using a unique identifier assigned to each study participant upon study enrollment; study participants' names will not be associated with any research data. The personal computers used to store and analyze the research data will be used by the PI and his designees and will be encrypted and password protected. The data will be periodically backed-up using a backup system. These safety checks will ensure that the risks to subjects' confidentiality are minimal.

**Table 1. Schedule of Events (+/- 3 day window is allowed for visits 3 through 15)**

| Visit Week | (-4 to 0) | 0 | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 | 44 | 48 | 52 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | Early Termina-tion |
| Visit Type | Screening | BL | | | | | | Cross Over | | | | | | EOT | End of F/U | |
| Informed Consent | X | | | | | | | | | | | | | | | |
| Inc/Exc Criteria | X | X | | | | | | | | | | | | | | |
| Demographics and Medical Hx | X | | | | | | | | | | | | | | | |
| TB Screening[1] | X | | | | | | | | | | | | | | | |
| HIV[2], HBV, HCV[3] | X | | | | | | | | | | | | | | | |
| Safety Labs (Blood and Urine) | X | X | X | X | X | X | X | X | | X | X | X | X | X | | X |
| Fasting Lipid Panel | | X | | | | | | X | | | | | | X | | X |
| Cystatin C (and eGFR) and CPK | | X | X | X | X | X | X | X | | X | X | X | X | X | | X |
| Pregnancy Test[4] | X | X | X | X | X | X | X | X | | X | X | X | X | X | | X |
| Brief Physical Exam[9] | X | | | | | | | | | | | | | | | |
| Vitals[5] | X | X | X | X | X | X | X | X | | X | X | X | X | X | | X |
| Drug Administration (QD)[6] | | X | X | X | X | X | X | X | X | X | X | X | X | X | | |
| CA Clinical Assessments | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| ECGs [7] | X | | | X | | X | | X | | X | | X | | X | | |
| Blood for Gene Analyses | | X | | | | | | | | | | | | | | |
| Blood for Mechanistic Studies | | X | | | X | | | X | | | X | | | X | | X |
| Blood for PK[8] | | X | | | | | | X | | | | | | X | | |
| C-SSRS | | X | | X | | X | | X | | X | | X | | X | | X |
| Questionnaires (DLQI and VAS) | | X | | X | | X | | X | | X | | X | | X | X | X |
| Medical Photos of Scalp and Eyebrows | | X | | X | | X | | X | | X | | X | | X | X | X |
| Lesional Scalp Biopsy | | X | | | | | | | | | | | | | | |
| sional Scalp Biopsy | | X | | | | | | X | | | | | | X | | X |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dispense Study Drug | | X | X | X | X | X | X | X | X | X | X | X | X | | | |
| Adverse Events | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

[1] PPD or QuantiFERON TB-Gold test;
[2] HIV testing, either in blood or POCT, consent form prior to test;
[3] HBS-ag and HCV-ab;
[4] Where applicable, serum BHCG at screening, urine tests at all other visits to be performed prior to dosing female participants of childbearing potential with Investigational Product (IP). Two negative pregnancy tests are required before receiving IP (1 negative Serum Pregnancy Test at Screening and 1 negative Urine pregnancy test at the baseline visit before IP administration).
[5] Includes height and weight (only at screening), blood pressure, and pulse;
[6] Drug administered once daily (QD) from Week 0 through Week 48
[7] ECG collected in triplicate at screening 2-4 minutes apart. The mean of the triplicates will be the baseline for the participant. Single ECGs will be collected at all other time points.
[8] Blood for PK will be collected as follows: Week 0- prior to dosing; and at Week 24 and Week 48- at pre-dose and at 0.5 hours, 1 hour and 2 hours post dose.
[9] Brief physical will include General Appearance, Skin, HEENT, Heart, Chest & Lung, and Abdomen

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## APPENDIX 1: CLINICAL ASSESSMENTS/SCORING

## DLQI

| The aim of this questionnaire is to measure how much your skin problem has affected your life over the last week. Please check one box for each question. | | | |
|---|---|---|---|
| 1. | Over the last week, how itchy, sore, painful, or stinging has your skin been? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 2. | Over the last week, how embarrassed or self-conscious have you been because of your skin? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 3. | Over the last week, how much has your skin interfered with you going shopping or looking after your home or garden? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 4. | Over the last week, how much has your skin influenced the clothes you wear? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 5. | Over the last week, how much has your skin affected any social or leisure activities? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 6. | Over the last week, how much has your skin made it difficult for you to do any sport? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 7. | Over the last week, has your skin prevented you from working or studying? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 8. | Over the last week, how much has your skin created problems with your partner or any of your close friends or relatives? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 9. | Over the last week, how much has your skin caused any sexual difficulties? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |
| 10. | Over the last week, how much of a problem has the treatment for your skin been, for example by making your home messy, or by taking up time? | Very much<br>A lot<br>A little<br>Not at all | ☐<br>☐<br>☐<br>☐ |

## VAS

Init:_____ ID # _____

**Date:** _____

**Subject VAS:**

**Draw a vertical line across the present line to represent the average for the last 3 days or nights regarding how much itching and sleep loss you have experienced:**

**Itching:**

**Sleep Loss:**

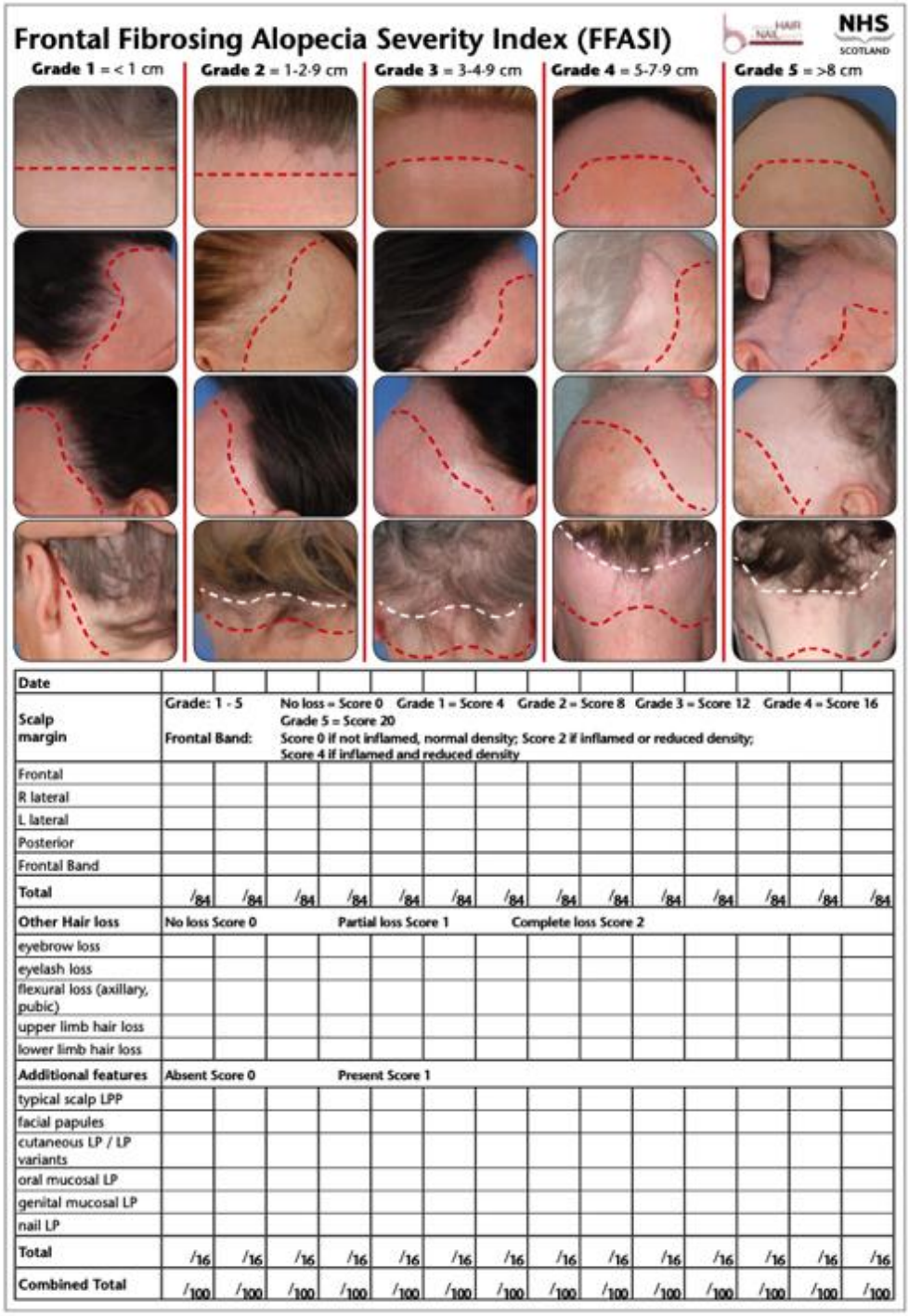
**LPPAI**[37]

| **Date of visit** | | | | |
|---|---|---|---|---|
| **LPPAI (see below)** | | | | |
| **SCALE: 0-3**<br>A Pruritus | | | | |
| B Pain | | | | |
| C Burning | | | | |
| D Erythema | | | | |
| E Perifollic erythema | | | | |
| F Perifollicular scale | | | | |
| Crusting | | | | |
| Pustules | | | | |
| Pull test: Anagen/Total **(0=-, 1=+)** | | | | |
| Spreading? **no (0) ? (1) yes (2)** | | | | |
| Dimensions / Extent | | | | |
| Loss follicular mark | | | | |
| Tufting | | | | |
| Telangiectasia | | | | |
| Atrophy | | | | |
| Pigment change | | | | |
| Other skin, nail, mucous membrane | | | | |
| **Labs**<br>CBC | | | | |
| AST/ALT/Alk Phos | | | | |
| G6PD | | | | |
| Eye exam | | | | |
| BUN/CR | | | | |
| Blood Pressure | | | | |
| **Culture & Sensitivities** | | | | |
| **Treatment/ Comments** | | | | |
| **Biopsy** | | | | |
| **Photographs** | | | | |
| **F/u visit:** | | | | |

LPPAI (0-10) = (A+B+C+D+E+F)/3 + 2.5(pull test) + 1.5(spread/2)

Scale: **0** = negative **1**= +/- **2** = + **3** = ++,+++

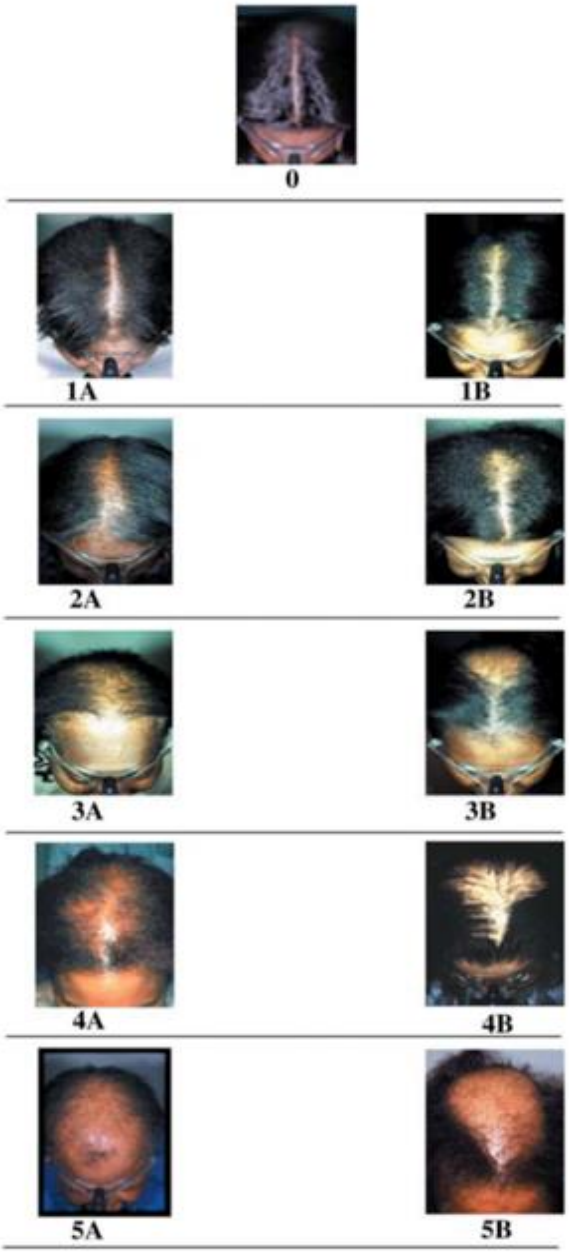
**FFASI3[6]**




| Date | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scalp margin | Grade: 1 - 5<br>Frontal Band: | | No loss = Score 0 Grade 1 = Score 4 Grade 2 = Score 8 Grade 3 = Score 12 Grade 4 = Score 16 Grade 5 = Score 20<br>Score 0 if not inflamed, normal density; Score 2 if inflamed or reduced density; Score 4 if inflamed and reduced density | | | | | | | | | | | |
| Frontal | | | | | | | | | | | | | | |
| R lateral | | | | | | | | | | | | | | |
| L lateral | | | | | | | | | | | | | | |
| Posterior | | | | | | | | | | | | | | |
| Frontal Band | | | | | | | | | | | | | | |
| **Total** | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 | /84 |
| **Other Hair loss** | No loss Score 0 | | Partial loss Score 1 | | | | Complete loss Score 2 | | | | | | | |
| eyebrow loss | | | | | | | | | | | | | | |
| eyelash loss | | | | | | | | | | | | | | |
| flexural loss (axillary, pubic) | | | | | | | | | | | | | | |
| upper limb hair loss | | | | | | | | | | | | | | |
| lower limb hair loss | | | | | | | | | | | | | | |
| **Additional features** | Absent Score 0 | | Present Score 1 | | | | | | | | | | | |
| typical scalp LPP | | | | | | | | | | | | | | |
| facial papules | | | | | | | | | | | | | | |
| cutaneous LP / LP variants | | | | | | | | | | | | | | |
| oral mucosal LP | | | | | | | | | | | | | | |
| genital mucosal LP | | | | | | | | | | | | | | |
| nail LP | | | | | | | | | | | | | | |
| **Total** | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 | /16 |
| **Combined Total** | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 | /100 |

Fig 1. Frontal Fibrosing Alopecia Severity Index (FFASI).

**CHLG[38]**




Eyelash/Eyebrow Assessment Score

0= None
1= Minimal eyelashes/eyebrows
2= Moderate eyelashes/eyebrows
3= Prominent eyelashes/eyebrows
4= Very prominent eyelashes/eyebrows

## APPENDIX 2: PROHIBITED CONCOMITANT MEDICATIONS

This is not an all-inclusive list. Study personnel should stay current and consult with their pharmacy to exclude all concomitant medications that are moderate to potent CYP3A inducers or sensitive or moderate sensitive CYP3A substrates. Chinese traditional medicines with unknown CYP3A metabolism should be excluded. If a medication is a sensitive or moderate sensitive CYP3A substrate and is not listed below as prohibited or permitted, consultation with Pfizer is required. The list of drugs prohibited for potential DDI concerns with the IMP may be revised during the course of the study with written notification from Pfizer, to include or exclude specific drugs or drug categories for various reasons (e.g., emerging DDI results for the IMP, availability of new information in literature on the DDI potential of other drugs).

Investigators should consult the product label for any other medication used during the study for information regarding medication that is prohibited for concomitant use.

| ***CYP3A4 Inhibitors*** | ***CYP3A4 Inhibitors con't*** | ***CYP3A4 Inducers*** | ***Sensitive CYP3A Substrates*** | ***Moderate Sensitive CYP3A Substrates*** |
|---|---|---|---|---|
| Amiodarone | Indinavir | Avasimibe | Dasatinib | Aprepitant |
| Amprenavir | Isavuconazole | Barbiturates | Dronedarone | Eliglustat |
| Aprepitant | Itraconazole | Bosentan | Ebastine | Pimozide |
| Atazanavir | Ketoconazole | Carbamazepine | Lomitapide | Rilpivirine |
| Boceprevir | Lopinavir | Efavirenz | Nisoldipine | |
| Casopitant | Mibefradil | Enzalutamide | Sirolimus | |
| Cimetidine | Mifepristone (RU486) | Etravirine | Tacrolimus | |
| Ciprofloxacin | Nefazodone | Genistein | Tolvaptan | |
| Clarithromycin | Nelfinavir | Lersivirine | | |
| Cobicistat | Netupitant | Lopinavir | | |
| Conivaptan | Nilotinib | Mitotane | | |
| Crizotinib | Norfloxacin | Modafinil | | |
| Cyclosporine | Norfluoxetine | Nafcillin | | |
| Danoprevir | Posaconazole | Phenobarbital | | |
| Darunavir | Ritonavir | Phenytoin | | |
| Delavirdine | Saquinavir | Rifabutin | | |
| Diltiazem | Schisandra sphenanthera | Rifampin | | |
| Dronedarone | Telaprevir | Ritonavir | | |
| Elvitegravir | Telithromycin | Semagacestat | | |
| Erythromycin | Tipranavir | St. John's Wort | | |
| Faldaprevir | Tofisopam | Talviraline | | |
| Fluconazole | Troleandomycin | Teriflunomide | | |
| Gestodene | Verapamil | Thioridazine | | |
| Grapefruit Juice, marmalade | Viekira pak | Troglitazone | | |
| Idelalisib | Voriconazole | | | |
| Imatinib | | | | |

All prohibited drugs that are CYP3A inhibitors require at least a 7 day or 5 half-lives (whichever is longer) prior to the first dose of study drug. Note: Amiodarone requires discontinuation at least 290 days (~5 half-lives, half-life averages ~58 days) prior to the first dose of IP.

All prohibited drugs that are CYP3A inducers require at least a 28 day or 5 half-lives (whichever is longer) prior to the first dose of IP.

| ***CYP3A4 Inhibitors*** | ***CYP3A4 Inhibitors con't*** | ***CYP3A4 Inducers*** | ***Sensitive CYP3A Substrates*** | ***Moderate Sensitive CYP3A Substrates*** |
|---|---|---|---|---|

**In a situation where appropriate medical care of a subject requires the use of a prohibited CYP3A inhibitor or inducer:**

Moderate to potent inhibitors and inducers of CYP3A are not permitted in the study EXCEPT in emergency situations requiring no more than one day of administration. ***Note: Amiodarone and mitotane are not permitted for any duration due to their long half-lives.*** Topical (including skin or mucous membranes) application of antimicrobial and antifungal medications is permitted.

## Concomitant Medications to be Used with Caution

| **BCRP Substrates (Use with caution)*** | |
|---|---|
| Pibrentasvir | |
| Glecaprevir | |

*As per their respective labels, the concentration of these compounds may increase when co-administered with BCRP inhibitors (e.g., brepocitinib).

## Permitted Concomitant CYP3A Substrates

| **Sensitive CYP3A Substrates**[a] | **Moderate Sensitive CYP3A Substrates**[b] |
|---|---|
| Alfentanil | Alprazolam |
| Avanafil | Atorvastatin |
| Buspirone | Colchicine |
| Darifenacin | Rivaroxaban |
| Eletriptan | Tadalafil |
| Eplerenone | |
| Felodipine | |
| Lovastatin | |
| Lurasidone | |
| Midazolam | |
| Naloxegol | |
| Quetiapine | |
| Sildenafil | |
| Simvastatin | |
| Ticagrelor | |
| Triazolam | |
| Vardenafil | |

a. Sensitive CYP3A substrates are drugs that demonstrate an increase in concentration-time curve (AUC) of ≥5-fold with strong index inhibitors of a given metabolic pathway in clinical drug-drug interaction (DDI) studies. Topical (including skin or mucous membranes) application of antimicrobial and antifungal medications is permitted.

b. Moderate sensitive substrates are drugs that demonstrate an increase in AUC of ≥2 to <5-fold.

## APPENDIX 3: MEDICATIONS PROLONGING QT INTERVAL

This is not an all-inclusive list. Study personnel should stay current and consult with their pharmacy to exclude all concomitant medications that fall into the category to prolong the QT interval.

Aclarubicin/Aclacin
Amiodarone/Cordarone
Anagrelide/Agrylin
Arsenic trioxide/Trisenox
Artemether/Lumefantrine
Astemizole/Hismanal
Azithromycin/Zithromax
Bedaquiline/ Sirturo
Bepridil /Vascor
Cesium Chloride
Chloroquine /Aralen
Chlorpromazine/Thorazine
Chlorprothixene/Truxal
Cilostazol /Pletal
Ciprofloxacin/Cipro
Cisapride /Propulsid
Citalopram/Celexa
Clarithromycin/Biaxin/
Cocaine
Disopyramide/Norpace
Dofetilide/ Tikosyn
Domperidone/Motilium
Donepezil / Aricept
Dronedarone /Multaq
Grepafloxacin / Raxar
Halofantrine/Halfan
Haloperidol /Haldol
Hydroquinidine (Dihydroquinidine)/Serecor
Hydroxychloroquine /Plaquenil
Ibogaine
Ibutilide /Corvert
Levofloxacin / Levaquin
Levomepromazine (Methotrimeprazine)/Nosinan
Levomethadyl acetate / Orlaam
Levosulpiride /Lesuride /
Mesoridazine / Serentil
Methadone /Dolophine
Moxifloxacin / Avelox
Nifekalant / Shinbit
Ondansetron /Zofran
Oxaliplatin /Eloxatin
Papaverine HCl (Intracoronary)
Pentamidine/Pentam
Pimozide/Orap
Probucol /Lorelco
Procainamide/Pronestyl
Propofol /Diprivan
Quinidine / Quinaglute
Roxithromycin / Rulide
Sevoflurane / Ultane
Sotalol / Betapace
Sparfloxacin / Zagam
Sulpiride /Dogmatil
Sultopride / Barnetil
Terfenadine / Seldane
Terlipressin / Teripress
Terodiline / Micturin
Thioridazine / Mellaril
Vandetanib / Caprelsa

## APPENDIX 4: COMPONENTS OF SAFETY LAB TESTING

### CBC w/Diff

- White blood cell
- Red blood cell
- Hemoglobin
- Hematocrit
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Red cell distribution width (RDW)
- Platelet
- Mean Platelet Volume
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils

### Complete Metabolic Panel

- Glucose
- Sodium
- Potassium
- Chloride
- CO2 Total
- Anion Gap
- Urea Nitrogen
- Creatinine
- GFR Estimate
- Calcium
- Albumin
- Alk Phosphatase
- ALT(SGPT)
- AST(SGOT)
- Bilirubin Total
- Total Protein

### Lipid Panel

- Cholesterol
- Triglycerides
- HDL Cholesterol
- LDL Cholesterol
- Chol/HDL Chol Ratio

## APPENDIX 5: SUMMARY OF PROTOCOL AMENDMENT CHANGES

**AMENDMENT # 1** FEBRUARY 10, 2021

- Cover Page:
  - Title– added (Brepocitinib)
  - Sub-Investigators – Andrew Alexis, MD was removed and replaced with Margaret Snyder, MD, Avi Bitterman, MD and Gerardo Russo, MD
  - Collaborator "Pfizer" added
  - Amendment # 1 January 11, 2021 added
- Page 8 Study Design and Methods:
  - "Part A" changed to "Phase I" and "Part B" and "Period II" changed to "Phase II" for consistency.
  - The following was added to clarify dosing: "In Phase I all subjects will receive their 45 mg dosage (PF-06700841 or placebo) as one 25 mg tablet and four 5 mg tablets daily. In Phase II all subjects will receive PF-06700841 as one 25 mg tablet and four 5mg tablets daily."
  - Figure 3 was replaced with a new image and labeled with "**Figure 3. Study Timeline"**
- Page 9 Dose Justification:
  - Added "(taken as one 25 mg tablet and four 5 mg tablets)" to clarify dosing.
  - Added additional information from ongoing studies: "Also, ongoing Phase 2 studies in Systemic Lupus Erythematosus and Psoriatic Arthritis are assessing PF-06700841 at 45mg QD dose and 60 mg QD dose, respectively, for up to 52 weeks."
- Page 10 Inclusion Criteria:
  - Criterion #3: added (according to the LPPAI37, FFASI36 and/or CHLG38).
- Page 11 Exclusion Criteria:
  - Criterion # 19 (4) was replaced with the following: Estimated Glomerular Filtration Rate (eGFR) less than 60 mL/ml/min/1.73m2 based on the age appropriate serum creatinine-based calculation and serum cystatin C based calculation.
- Page 12 Exclusion Criteria:
  - Criterion # 27: added use of "any Jak1/Tyk2 product" as an exclusionary JAK inhibitor drug.
- Page 13 Exclusion Criteria:
  - Criteria #31 and #32 were combined and the following criteria were renumbered.
  - Criterion # 33 added "The concomitant use of drugs that are substrates for P-gp, BCRP, OCT/MATE transporters with a narrow therapeutic index to minimize any potential significant drug interaction. The substrates that should be prohibited include the following:"

| Pgp substrates | BCRP substrates | OCT1 substrates | OCT2 substrates | MATE1 substrates |
|---|---|---|---|---|
| dabigatran | rosuvastatin | dofetilide | pilsicainide | dabigatran |
| digoxin | | | tenofovir | tenofovir |

  - Criterion # 34 added "Subjects with a Columbia Suicide Severity Rating Scale (C-SSRS) score = 4 or 5 at visit 2 (Baseline)."

- Page 14 Clinical Procedures:
  Added: Columbia Suicide Severity Rating Scale (C-SSRS) to Baseline Visit; Weeks 8, 16, 24, 32, 40 and 48; and Early Termination visit.
- Page 20 Pharmacokinetics: "sponsor" was changed to "Pfizer."
- Page 23 Stopping rules: The following were added:
  - Columbia Suicide Severity Rating Scale (C-SSRS) score = 4 or 5.
  - If an individual participant demonstrates CONCOMITANT serum Cr based AND serum Cystatin C based eGFR decline of at least 30% (≥ 30%) compared to the subject's baseline eGFR then the participant should not be further dosed and adequate, immediate, supportive measures including immediate evaluation by a nephrologist (preferably within 24 hours) with appropriate management and treatment as clinically indicated. Results should be repeated as indicated by the nephrologist or weekly at a minimum until the eGFR returns to baseline ±15%, or the renal parameters are deemed to be stable by the nephrologist and/or PI. If the subject cannot be seen by a nephrologist within 24 hours (as described above), then the subject should be sent to a local emergency room for evaluation and treatment as clinically indicated.
- Page 27 Schedule of Events Table 1: C-SSRS assessments added and ECG corrected to Screening Visit instead of Baseline Visit, and added at Early Termination Visit.
- Page 28 Schedule of Events Table 1: ECG changed from Baseline to Screening.

**AMENDMENT # 2 APRIL 7, 2022**

PF-06700841 was replaced with brepocitinib throughout the document.

- Cover Page:
  - Sub-Investigators – Avi Bitterman, MD and Gerardo Russo, MD were removed and replaced with Joel Correa da Rosa, Elizabeth Andrews, MD and Shelley Uppal, MD.
  - Amendment # 2 April 7, 2022 was added
- Page 8 Study Design and Methods
  - The section has been updated with a clarification of how many subjects are planned to be enrolled vs. how many are expected to complete.
- Page 12-13 Subject Eligibility - Exclusion Criteria
  - Cyclosporine was added to exclusion criterion # 28
    - Subject has used topical corticosteroids, and/or tacrolimus, and/or pimecrolimus or cyclosporine within 1 week before the baseline visit.
  - The word inhibitors was replaced with the word substrates in criterion #32
    - The use of concomitant CYP3A substrates and inducers (Appendix 2) and medications (Appendix 3) that prolong the QT/QTcF interval are exclusionary.
  - One exclusion criterion was added:
    - #35 History of thromboembolic events including DVT and PE or history of inherited coagulopathies.
- Page 14-16 Study Procedures
  - "Confirm proper contraception is being used" was added to each visit
  - "Collect blood and urine for safety labs" was changed to "Collect blood and urine for

safety labs (including cystatin-C and CPK)" for clarification to each visit from baseline to Early Termination
    - Visit 5 was deleted and incorporated into Visit 3, 4, 5, 6, 7 – Weeks 4, 8, 12,16, 20
    - Visit 11 was deleted and incorporated into Visits 9 10, 11, 12, 13 and 14 – Weeks 28, 32, 36, 40, 44
- Page 17 Blood sampling for PAX RNA was deleted as it is not being analyzed in blood.
- Page 18 Clinical Overview of Potential Risks
    - Data from another study with a topical formulation of brepocitinib was added.
- Page 19 Randomization and Blinding – statement regarding enrollment numbers was deleted as it is duplicated in other sections of the protocol.
- Page 19 Pharmacokinetics – "SoA" was revised to "Schedule of Events"
- Page 24 Stopping Rules – "CK" was revised to "CPK" and a stopping rule regarding drop in hemoglobin was added:
    - Hemoglobin drop ≥ 2 g/dL from baseline on two consecutive blood draws with associated symptoms; **or** hemoglobin drop ≥ 3 g/dL from baseline on two consecutive blood draws without associated symptoms
- Page 25 Sample Size Calculation and Data Analyses sections were updated to match the statistical analysis plan.
- Page 26-27 Table 1 Schedule of Events
    - A Visit Window +/- 3 days was added for visits 3-15.
    - Cystatin C (and eGFR) was revised to Cystatin C (and eGFR) "and CPK"
    - Clarifications were added to footers 6 and 8.
- Page 36-37 Appendix 2 Prohibited Concomitant Medications
    - Prohibited Sensitive CYP3A Substrates and Moderate Sensitive CYP3A Substrates were added to the first table. A table for Permitted concomitant CYP3A substrates was added.
- Page 39 APPENDIX 4: COMPONENTS F SAFETY LAB TESTING was added
- Page 40 SUMMARY OF PROTOCOL AMENDMENT CHANGES was changed from Appendix 4 to Appendix 5.