PART B

STUDY DESCRIPTION

Title of Protocol	An open-label proof of concept study regarding the efficacy and safety of dupilumab in the treatment of keloids
Principal Investigator	Martina Porter, MD

B1. PURPOSE OF PROTOCOL

An open-label proof of concept study regarding the use of dupilumab in patients with keloids may be the first step in elucidating a novel systematic approach to treatment of keloids.

Primary Objectives

To determine changes in Patient and Observer rescue
Scar Assessment Scale (POSAS) from baseline to week 24.

Secondary Objectives

- To determine changes in Vancouver Scar Scale (VSS) from baseline to week 24.
- To determine changes of patient-reported outcomes based on Dermatology Life Quality Index (DLQI).
- Objective changes of Keloid volume and size using Canfield Camera from baseline to week 24
- To determine safety of dupilumab in keloid patients.
- To determine the effects of dupilumab on Th2 pathway molecules (IL-4 and IL-13) and selected indicators and mediators of fibrosis in keloid tissue including collagen 1, TGF-β1, heat shock protein 47 (HSP47), alpha smooth muscle actin (αSMA), and von Willebrand factor (vWF).

Primary Endpoints:

- Changes in overall POSAS from baseline to week 24

Secondary Endpoints:

- Changes in VSS from baseline to week 24
- Changes in pruritus and pain categories of POSAS from baseline to week 24
- Changes in DLQI scores from baseline to week 24
- Changes in size and volume of keloids from baseline to week 24
- Safety evaluations will be conducted through week 24
- Changes in the levels of Th2 pathway (such as IL-4, IL-13), Collagen 1, TGF-β1, HSP47, αSMA, and vWF from skin biopsy samples from keloids between week 0 and 24

Other Study Parameters:

Safety evaluations include adverse event monitoring, physical examinations, and clinical laboratory testing (CBC with differential, BUN/Cr and AST/ALT) as a measure of safety and tolerability for the entire study duration.

The following laboratory tests will be performed according to the study procedures schedule listed in SoA.

- Complete Blood Count (CBC) with differentials: automated WBC, RBC, hemoglobin, hematocrit, platelet count, RBC indices, Neutrophil count and percentage, bands count and percentage, lymphocytes count and percentage, monocytes count and percentage, eosinophils count and percentage, basophils count and percentage, atypical lymphocytes count and percentage
- BUN, Creatinine, ALT, AST
- HIV: HIV1&2 Antibody/Antigen Combo Assay

- Hepatitis B: Hep B Core Ab, Hep B Surface Antigen, and Hep B surface antibody
- Hepatitis C: Hepatitis C antibody
- Tuberculosis: Quantiferon-TB Gold

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Background and Rationale

Current scar treatments have limited efficacy and are often unsatisfactory although over $20 billion dollars are spent annually on the treatment and management of scars[1]. Keloids, an abnormal proliferation of scar tissue, can be disfiguring, functionally impairing, and have dramatic impacts on quality of life[2]. Treatments of keloids include variety of modalities including intralesional steroid injections, silicone gel or sheets, surgery, laser, radiation therapy, cryotherapy, topical imiquimod, and intralesional 5-fluorouracil injections. However, current treatments are limited to primarily localized interventions.

The Th2 cytokines interleukin (IL)-4 and IL-13 gained attention as key mediators in the pathogenesis of fibroproliferative disorders including keloids[3]. IL-4 and IL-13 bind to receptors and activate JAK-STAT signaling pathway, leading to the stimulation of profibrotic pathways and upregulation of transcription of profibrotic genes, such as those encoding type I collagen and transforming growth factor (TGF)-β1[4]. In addition, IL-13 and IL-4 are known to inhibit matrix metalloproteinase (MMP)-3[5]. Therefore, dupilumab may inhibit the overactive signaling of the IL-4/IL-13 pathway that contributes to the initiation and perpetuation of keloid growth.

Recently, anecdotal findings of improvement of keloids by dupilumab were reported in the literature[6]. A 53-year-old male patient with severe atopic dermatitis and two keloids reported shrinkage of the larger keloid and complete disappearance of the smaller keloid after 7 months of treatment with dupilumab for atopic dermatitis. The authors also performed RT-PCR from keloid and healthy tissues and found that IL-4 receptors, directly targeted by dupilumab, and IL-13 were significantly increased in keloid lesions, compared with controls.

These studies suggest a possible role for Th2 cytokines in the pathogenesis of keloids, and raise the possibility that blockade of Th2 cytokines by dupilumab may be a potential therapeutic strategy in patients with keloids. We hypothesize that dupilumab can decrease the size and symptoms of keloids and improve patient's quality of life. An open-label proof of concept study regarding the use of dupilumab in patients with keloids may be the first step in elucidating a novel systematic approach to treatment of keloids.

References

1. Block L, Gosain A, King TW. Emerging Therapies for Scar Prevention. *Adv Wound Care (New Rochelle).* 2015;4(10):607-614.
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6. Diaz A, Tan K, He H, et al. Keloid lesions show increased IL-4/IL-13 signaling and respond to Th2-targeting dupilumab therapy. *J Eur Acad Dermatol Venereol.* 2020;34(4):e161-e164.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Study Design:
This study is a non-randomized, open-label, proof of concept study.

Administration of Study Regimen
Subjects will receive dupilumab as a subcutaneous injection (2 ready-to-use syringes (600 mg) at initial visit, 1 ready-to-use syringe (300 mg) subcutaneously every 2 weeks until week 22) in healthy skin.

The drug will be administered in the Harvard Catalyst Clinical Research Center (CRC) at Beth-Israel Deaconess Medical Center (BIDMC) for the first three times (weeks 0,2,4). Patients and/or caregivers will be trained on drug administration at weeks 2 and 4. Under the supervision of an investigator, patient and/or caregiver will be instructed how to properly administer study medication. Then, the drug will be administered every 2 weeks for weeks 6 to 22. Subjects will receive drug in the CRC administered by study personnel at weeks 8,12,16,20 and will self-administer at home by patients or caregivers at weeks 6,10,14,18,22. If patients or caregivers request, we will provide supervision through video visits. Patient will also be required to maintain a drug diary to ensure compliance.

At requested video visits, patient and/or caregiver will be supervised by qualified study staff as they administer study medication. Study staff will confirm whether patient experienced any AEs since last study visit. The virtual visit will occur over an institutional approved platform, such as Starleaf.

Patient and/or caregiver may request to come on-site to receive study medication, in the event they do not want to or cannot administer study medication at home.

Patient will be given a physical copy of the drug diary at every visit where drug will be dispensed for home administration. At the following visit, the study team will collect the completed form to verify compliance.

Overview of study procedures

	Screen	Wk 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12	Wk 14	Wk 16	Wk 18	Wk 20	Wk 22	Wk 24	Wk 26	FU call Wk 26
Informed Consent	✓															
Inclusion/Exclusion	✓	✓														

Demographics/Medical History	✓															
Keloid History	✓															
Complete Physical Examination including height and weight	✓													✓		
Vital Signs	✓	✓	✓	✓		✓		✓		✓		✓		✓		
HIV, Hepatitis B (Hep B Sag, Hep B Sab, Hep B Cab) and Hepatitis C Screening (Hep C ab), TB Screening	✓															
CBC with differential, BUN/Cr and AST/ALT	✓													✓		
Pregnancy Test (Urine)	✓	✓	✓	✓		✓		✓		✓		✓		✓		
POSAS/VSS assessment, size measurement	✓	✓		✓		✓		✓		✓		✓		✓		
DLQI		✓		✓		✓		✓		✓		✓		✓		
Digital photography of lesions		✓		✓		✓				✓				✓		
Adverse Events			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
Skin biopsy (up to 5 volunteers)		✓												✓		
Suture removal*			✓												✓	
Medication Administration in CRC		✓	✓	✓		✓		✓		✓		✓				
Medication Administration at Home					✓		✓		✓		✓		✓			

* = if suture is placed following skin biopsy

Study Procedures

Prior to performing any study related procedures, the investigator will discuss with each subject the nature of the study, its requirements and its restrictions. A written informed consent form will be obtained from each participant prior to any procedure. Written informed consent will be obtained in accordance with BIDMC IRB policies for informed consent.

Each subject with a signed informed consent form will be screened at the first visit, to ensure all the inclusion criteria are met and none of the exclusion criteria. Study procedures are described in detail below and an overview of the study procedures during each visit can be found in the chart.

Informed Consent

Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed, signed, and dated by the subject, the person who administered the informed consent, and any other signatories according to local requirements. A copy of the signed informed consent will be given to the subject and the original will be placed in the subject's record. An entry must also be made in the subject's dated source documents to confirm that informed consent was obtained prior to any study-related procedures and that the subject received a signed copy.

Medical History

- A complete medical history will be taken at screening. The subject's medical history will be updated at the Baseline visit. This updated medical history will serve as the baseline for clinical assessment.
- Demographic information, including subject self-reported gender, race, age, ethnicity, and child-bearing potential will be recorded.
- Keloid history: Date of onset of keloid, preceding cause of keloid (i.e. acne, surgery, piercing, trauma, etc), family history of keloids, all previous and current medical and procedural treatments for keloid, and reasons for discontinuation or outcomes of previous treatments will be obtained.

Vital Signs

Vital sign determinations of systolic and diastolic blood pressure, pulse rate, and body temperature will be obtained at visits as specified in schedule of activities. Blood pressure and pulse rate should be measured after the subject has been sitting for at least 3 minutes.

Complete Physical Exam, including Height and Weight

Height will be measured at screening only. Body weight will be measured at screening and week 24 visits as specified in SoA. The subject will wear lightweight clothing and no shoes during weighing. A complete physical examination, including cutaneous examination to evaluate for skin malignancies, will be performed at the designated study visits as specified in SoA. The physical examination performed on study day 0 will serve as the baseline physical examination for the entire study. If appropriate, a targeted physical exam should be performed at any other visit (e.g., to evaluate a reported adverse event). Any significant physical examination findings after the first dose will be recorded as adverse events (AE), while any findings prior to the first dose will be recorded as medical history. All findings, whether related to an AE or part of each subject's medical history, will be captured on the appropriate AE report form. At any time, a symptom-directed physical examination can be performed as deemed necessary by the Investigator.

Photographs

Digital photographs will be taken of the keloid (close and distant) by the investigator or research personnel at weeks 0, 4, 8, 16, and 24. The photos will try not to contain any identifiable areas if possible. No facial identification will be captured in the photos. Identification marks (i.e tattoos, moles, birth marks, scars, piercings etc.) located near keloids may be captured in the photos. The photos will be used to measure keloid lesion volume. Subject photos will be taken with a professional camera provided by Canfield and sent to Canfield for analysis. We are using canfield camera and their Vectra H1 software for the image processing. The Mirror and VECTRA software is pre-installed on laptop provided by canfield. The VECTRA H1 system enables high quality 3D image capture of small- to medium-field subjects. The 3D images appear in the designated VECTRA® patient chart and may be viewed and analyzed using the included VECTRA Analysis Module (VAM) software and optional Sculptor® software. The H1 camera is shipped completely configured and ready to use. Photos will be stored for atleast 6years and destroyed upon PI's request from the canfield database.

Efficacy Assessment

Patient Scar Assessment Scale (PSAS) of POSAS and VSS of disease severity will be performed by the investigator at designated study visits. Patient-reported outcomes, including Observer Scar

Assessment Scale (OSAS) of POSAS and DLQI, will be administered to subjects as subject questionnaires prior to any assessment of disease severity or laboratory testing. The size and volume of keloids, pigmentation and erythema will measurement by Canfield Camera.will be measured . The degree of will be measured by Mexameter and the degree of pliability will be measured by Cutometer.

Laboratory Testing
Blood samples will be collected at scheduled visits as listed in SoA. A certified laboratory at BIDMC or Quest Diagnostics will be utilized to process and provide results for the clinical laboratory tests.

Laboratory reference ranges will be obtained prior to the initiation of the study. The baseline laboratory test results for clinical assessment for a particular test will be defined as the last measurement prior to the initial dose of study drug.

Whether action needs to be taken to address notable laboratory values will be decided by the investigator, taking into account the overall status of the subject. No specific action is foreseen as part of the study protocol.

The following laboratory tests will be performed according to the study procedures schedule listed in schedule of activities:

- Complete Blood Count (CBC) with differentials: automated WBC, RBC, hemoglobin, hematocrit, platelet count, RBC indices, Neutrophil count and percentage, bands count and percentage, lymphocytes count and percentage, monocytes count and percentage, eosinophils count and percentage, basophils count and percentage, atypical lymphocytes count and percentage
- BUN, Creatinine, ALT, AST
- HIV: HIV1&2 Antibody/Antigen Combo Assay
- Hepatitis B: Hep B Core Ab (Hep B Cab), Hep B Surface Antigen (Hep B Sag), and Hep B surface antibody (Hep B Sab)
- Hepatitis C: Hepatitis C antibody
- Tuberculosis: Quantiferon-TB Gold

Incidental Findings:
Potential incidental findings: HIV, Hepatitis B, Hepatitis C, TB, and pregnancy
Plan for communicating the incidental findings: If subjects are found to have infectious diseases, such as HIV, Hepatitis B, Hepatitis C, or tuberculosis, they will not be eligible for enrollment in the study. These patients will be referred to infectious disease. HIV positive patients will also be reported to the Massachusetts Department of Public Health as required by state law. Subjects who are found to be pregnant will not be eligible for the study. These subjects will be referred to appropriate OB/GYN or primary care providers. Depression and quality of life scores will be calculated through Dermatology Life Quality Index. These will be reviewed at the time of the patient visit. Referral to appropriate mental health providers and subject's primary care provider will be performed, if needed. Any abnormal laboratory findings will be reported to the subject's primary care provider, if warranted.

Skin Biopsy
Up to 10 subjects will undergo optional punch biopsies at baseline and week 24. A 4 mm punch biopsy will be obtained from the lesional keloid skin, only in subjects (up to 10) who consent to optional skin biopsy for the sub-study. Skin biopsy location will be determined by the investigator

and standard procedures for skin biopsy will be employed. Prior to the biopsy, the study doctor will wipe skin with alcohol and inject numbing medication (lidocaine with epinephrine, up to 1.5mL per biopsy).Subjects may have lesions closed with suture or gelfoam, depending on nature of lesion and biopsy. The specimen will be sent to BIDMC Research Core Facilities for further processing.

Keloids may become larger as a result of biopsy. In cases deemed appropriate by the investigator, the subject will be given rescue treatment.

Rescue Treatment:
Subjects will only be given rescue treatment for keloids that have grown larger than the original keloid lesion. Patients will only be followed throughout the duration of the study for rescue treatment eligibility. The rescue treatment will follow procedures for standard of care treatment, intralesional steroid injections.

Follow up Phone Call at Week 26 (4 weeks after week 22)
Study personnel will call subjects to collect information regarding the status of any ongoing adverse effects (AEs) /serious adverse effects (SAEs) or the occurrence of any new AEs/SAEs since last study visit.

Data/Specimen Use
Unstained slides from a formalin fixed paraffin embedded tissue of keloid skin biopsy samples will be sent to Thomas Jefferson University for collaboration. Slides will be coded so as not be associated with subject's name.

The BIDMC Histologic core's recommended standard practice is to give the processed samples to PI and they can store the slides and paraffin block in a locked cabinet in their work space. The slides and paraffin blocks will be stored on Gryzmish 5th floor in PI's locked cabinet. They will be stored for 10 years.

Assessment

Clinical Assessment
Clinical efficacy assessments will be performed at the screening/baseline visit, and weeks 4, 8, 12, 16, 20, 24 to calculate POSAS score and VSS. During visits, patients will also be asked to complete the DLQI. There are several methodologies for efficacy assessment in current clinical scar research. Among them, POSAS and VSS are the most commonly used, validated, and high-quality published scar scales. We will use POSAS for the primary method because this scale incorporates the patient-reported outcomes on aspects of scarring, such as pain and pruritus.

Patient and Observer Scar Assessment Scale (POSAS)
The POSAS measures scar quality by evaluating visual (e.g. color), tactile (e.g. pliability) and sensory (e.g. itch) characteristics of the scar from the perspective of the observer (investigator) and patients. The first version of the POSAS has been introduced in 2004[9] and the second version was published with minor adjustments in 2005[10]. POSAS is comprised of two numeric scales: the Patient Scar Assessment Scale (PSAS, patient scale measuring pain, pruritus, color, stiffness, thickness, bumpiness) and the Observer Scar Assessment Scale (OSAS, observer scale measuring vascularity, pigmentation, thickness, relief, pliability, surface area). Both scales contain six items that are scored numerically on a 1-10 scale. A score of "1" being "no, not at all" and a score of "10" being "yes, very

much". Together, they make up the total score (range of 12-120) of the PSAS (range of 6-60) and OSAS (range of 6-60). We would like to use POSAS version 2 and 3 validated online.

Vancouver Scar Scale (VSS)

The VSS measures four parameters of scars: vascularity, pigmentation, pliability, and height. Each parameter contained ranked subscales that may be summed to obtain a total score ranging from 0 (representing normal skin) to 13 (representing worst scar imaginable).

- Vascularity: assessed by looking at the scar at resting and by blanching the scar and observing the rate and amount of blood return
  1) Score 0: normal color and capillary refill
  2) Score 1: pink scar with a slight increase in the local blood supply
  3) Score 2: red scar with a significant increase in the local blood supply
  4) Score 3: purple scar with excess local blood supply, scars which are congested and refill slowly or cannot be completely blanched
- Pigmentation: The skin will be blanched with a piece of plastic to eliminate the effect of vascularity on skin color and compared with normal skin
  1) Score 0: normal skin
  2) Score 1: hypopigmented skin
  3) Score 2: hyperpigmented skin
- Pliability
  1) Score 0: normal skin
  2) Score 1: supple scar with minimal resistance
  3) Score 2: yielding scar that gives way to pressure while offering a moderate resistance but does not behave as a solid, inflexible unit
  4) Score 3: firm scar that moves as a solid, and inflexible unit
  5) Score 4: banding producing ropes of scar that blanch on stretching but do not limit range of motion
  6) Score 5: contracture of any type of scar that limits range of motion
- Height
  1) Score 0: flat scar
  2) Score 1: < 2 mm

Dermatology Life Quality Index

The DLQI is a validated general dermatology questionnaire that consists of 10 items that assess subject health-related quality of life (daily activities, personal relationships, symptoms and feelings, leisure, work and school, and treatment). It has been extensively used in dermatology clinical trials for atopic dermatitis. The DLQI is a psychometrically valid and reliable instrument that has been translated into several languages, and the DLQI total scores have been shown to be responsive to change. The minimally important difference for the DLQI has been estimated as a 2 to 5 point change from baseline.

Objective measurement of scar qualityWe will use canfield camera at the screening/baseline visit, and weeks 4, 8, 12, 16, 20, 24.

Molecular Assessment

As cellular mechanisms of action of dupilumab are well understood, we will focus on potential effects of this antibody on selected indicators of fibrosis and parameters that characterize the collagen-rich

keloid tissue. In a specific experimental setting we propose here, we will focus on changes in Th2 pathway and selected protein indicators of fibrosis.

Skin biopsies will be sent to BIDMC Histology Core Facility for tissue processing, sectioning and H&E staining. The FFPE (formalin fixed paraffin embedded) tissue sections will be processed for immunohistochemistry (IHC) or immunofluorescent (IF) analysis by BIDMC confocal imaging and IHC core facility as below. Samples will be preserved in formalin jars and sent to the histology lab to be processed. The paraffin blocks will be sectioned and stained at the BIDMC histology, confocal imaging and IHC core facilities, and then blocks and slides will be returned to the BIDMC, Department of Dermatology and stored securely at Department of Dermatology. Samples are retained for the duration of the project, through publication. If all members of the study team deem they are no longer needed, they will then be destroyed.

Immunohistochemistry and immunofluorescent staining
Each biopsy will be fixed in formalin, bisected vertically, dehydrated by routine histologic methods, and embedded in a paraffin block. 5-µm-thick surface sections will be cut, mounted on glass slides, and baked for 60 minutes at 60C. The first slide will be stained with Hematoxylin and Eosin. The next slide will be undergo antigen retrieved by boiling in antigen retrieval buffer for 10 minutes. It will then incubated with primary antibodies followed by secondary antibodies using standard immunostaining protocols. After appropriate washes, antibody detection will be performed using DAB detection kit for immunohistochemistry staining. Slides will be developed in DAB, then dehydrated and mounted with coverslips. For Immunofluorescent staining, the antigen labeling will be detected with secondary antibodies conjugated with fluorescent dyes. The immunofluorescent staining will be analyzed by confocal imaging with Zeiss LSM 880 confocal system.

We will assay the Th2 pathway markers (IL-4 and IL-13), collagen 1, and following fibrotic markers.

- TGF-β1: upregulation of this growth factor is an indicative of accelerated fibrotic processes, including those in keloids. Decrease in tissue TGF-β1 suggests attenuation of broad fibrotic processes that drive excessive scarring.
- HSP47: upregulation of this collagen-specific chaperone protein is a reliable marker of accelerated synthesis of collagen in all fibrotic tissues, including keloids. Its expression correlates well with biosynthesis of collagens[13,14].
- αSMA: this marker indicates a presence of pro-fibrotic myofibroblasts. Assays of αSMA-positive cells in keloid tissues will provide reliable indication for the direction of changes due to the dupilumab treatment.
- vWF: assays of this marker will determine changes in the vascularization of keloids.

Evaluation of immunohistochemistry or immunofluorescent staining
Once the IHC and/or IF staining is completed, images will be taken by using Zeiss AxioImager microscope for IHC staining and Zeiss LSM 880 confocal system for IF staining. The intensity of staining will be analyzed by using NIH Image J or Imaris software.

<u>Evaluation of Immunohistochemistry or Immunofluorescent staining</u>
Intensity of expression of the markers will be measured by an image analysis program at BIDMC Histology Core Facility. Pre- and post-treatment expression levels will be compared using paired T test.

Study Endpoint Criteria

Subject Discontinuation

Subjects are free to leave the study at any time for any reason without any consequences with a written notification addressed to the principal investigator (PI).

Subject Termination

Investigator(s) can terminate subjects for urgent medical reasons as well as changes in their medical status (that may lead to compromised safety of subjects), no longer meeting inclusion/exclusion criteria, serious adverse events, serious acute illness and/or worsening of a current illness, protocol violations (such as non-compliance), and subjects lost to follow-up.

Study Termination

Investigator(s) will terminate the study if it is determined that the study has serious safety concerns and/or has falsified any data. If any safety concerns, patient should be seen on a short notice and be provided with suitable medical care and/or be referred to an appropriate physician.

Study Completion

The study will be considered complete, when data from all 20 subjects are collected.

Confidentiality of Records

All subjects will be assigned a subject number at their screening visit. Only investigators and study staff at BIDMC will have access to the subject's identifiable health information. To ensure appropriate privacy and confidentiality, any paper source documentation and questionnaires will be stored in a locked cabinet. Only the PI, sub-investigator(s), and study staff will have access to this locked cabinet. This documentation will all be coded. Subject's identifiable health information will be kept separate from coded documentation. Any electronic data containing PHI will be stored on a password protected computer in a locked office and on a secure server behind the BIDMC firewall.

The investigators will maintain adequate records for the study, including results, source documentation, informed consent documents, drug dispensing and disposition records, safety reports, information regarding participants who discontinued, adverse events, and other pertinent data. All essential documents will be retained by the investigators for at least 2 years in a long-term storage facility as arranged through the Clinical Trials Office at BIDMC.

Delegation of Investigator Responsibilities

The PI will plan, execute, and supervise the entire study and train all necessary research staff. The PI will also be in charge of reporting any study deviations and amendments to the Sponsor as well as to the BIDMC CCI (or direct appropriate individuals to do so). The PI, sub-investigator(s), and research staff (including any students and trainees under the supervision of the PI) will conduct study visits, collect data, and be active participants in data analyses and any future publications resulting from the study.

Adverse Events and Reporting

Safety will be assessed from reported adverse experiences and clinical assessments. Prior to enrollment, subjects will be screened for eligibility, at which time a complete medical history and clinical diagnosis and assessment of keloids will be performed. Subjects who do not fulfill the inclusion criteria will not be enrolled. Subjects whose histories are outlined in the exclusion criteria

will also not be enrolled. At each study visit, one of the investigators will seek information on adverse events by questioning the subject and as appropriate by examining the subject.

Adverse events (AEs): Adverse events are defined as any undesirable experience occurring to a subject during the study. All adverse events observed by investigator or staff members or reported by the subject will be recorded.
An AE also includes any worsening (ie, any clinically significant change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the use of the study drug.

Serious Adverse Events (SAEs): An adverse event is classified as a serious adverse event, when the subject's outcome is:

- Death
- Life-threatening
- Hospitalization (initial or prolonged)
- Disability or permanent damage
- Congenital anomaly and/or birth defect
- Required intervention to prevent permanent impairment or damage
- Important medical events that do not fit the other outcomes, but require medical or surgical intervention in order to prevent one of the other outcomes.

All SAEs will be reported to BIDMC IRB within 24 hours after taking knowledge of the events.

All SAEs, regardless of assessment of causal relationship to study drug, will be reported to Regeneron within 24 hours. Faxed SAE/CIOMS or MedWatch Reports to: 914-345-7476 or form emailed to medical.safety@regeneron.com
Information not available at the time of the initial report will be documented in a follow-up report. Substantiating data such as relevant hospital or medical records and diagnostic test reports may also be requested by Regeneron .

Other Events that require accelerated reporting to Regeneron:

Symptomatic Overdose of Study Drug: Accidental or intentional overdose of at least 2 times the intended dose of study drug within the intended therapeutic window, if associated with an AE, Pregnancy: Although pregnancy is not considered an AE, it is the responsibility of the investigator to report to Regeneron (or designee), within 15 days of identification, any pregnancy occurring in a female or female partner of a male, during the study or within days of the last dose of study drug. Any complication of pregnancy affecting a female study patient or female partner of a male study patient, and/or fetus and/or newborn that meets the SAE criteria must be reported as an SAE. Outcome for all pregnancies will be reported to Regeneron.

Follow-up of Adverse Events: The investigators will follow adverse events until the time the adverse event either ceases permanently or is clinically stabilized. All adverse events will be reported according BIDMC Committee on Clinical Investigations (CCI) guidelines.

Withdrawal of individual subjects

- Subjects are free to leave the study at any time for any reason without any consequences. The investigator can decide to withdraw a subject from the study, due to urgent medical reasons. Other criteria for withdrawal of a subject are listed below:

- Changes in the patients' medical status that lead to compromised safety of the patient or no longer meeting all the inclusion criteria.
- Serious adverse events
- Serious or acute illness or worsening of a current illness
- Protocol violations, such as non-compliance to study procedures and subject lost to follow-up

Data and specimen collected from subjects prior to withdrawal will be utilized unless subject request for destruction of data and specimen. Subject will need to send the PI a letter requesting destruction of collected samples and data.

B. Statistical Considerations

Sample Size Justification:

Twenty subjects with keloids who meet study inclusion/exclusion criteria will be recruited into the study. While there is no study measuring minimal clinically important differences (MCID) specifically in keloids, MCID was estimated to be 15 of 100 points of POSAS in linear scar[7]. If we use this estimation for keloids with POSAS of 120 points, it is calculated to be 18. Assuming significance level of 5%, correlation of 0.1 between baseline and 6 months (conservative estimate), and standard deviation of 15 based on another study of keloids[8] with possible loss to follow-up of 20%, a study of 20 patients will give a power of 90% to detect an MCID of 18.

Data Analysis:

Primary study parameter(s):

We will examine the distribution of POSAS score. If it is normally distributed, we will summarize the mean and standard deviation of the changes of scores between baseline and week 24 and use a paired T test. If it is not normally distributed, we will summarize the median and interquartile range and use a sign test.

Secondary study parameter(s):

We will examine the distribution of VSS score and DLQI score and the changes of pigmentation, erythema, size and volume. We will use a paired T test or sign test based on the distribution.

Other study parameter(s):

Patient characteristics and demographics data will be presented using descriptive statistics. At every visit, adverse events and changes in medications will be registered.

C. Subject Selection

Inclusion Criteria

- Men and women between the ages of 18 and 65 at the time of dupilumab initiation.
- Subjects must have either one keloid with ≥ 2 cm length-wise or at least two keloids with ≥ 0.4 cm (width) x 0.4 cm (length)
- Subjects must be able to understand and communicate with the investigator and comply with the requirements of the study and must give a written, signed and dated informed consent before any study related activity is performed.

Exclusion Criteria

- History of an ongoing, chronic or recurrent infectious disease, or evidence of tuberculosis (Tb) infection as defined by a positive QuantiFERON TB-Gold test at screening.
- Known infection with HIV, hepatitis B or hepatitis C at screening.

- Are currently pregnant, breastfeeding, or planning to get pregnant during the study.
- Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unwilling to use effective contraception during the study and for 12 weeks after stopping treatment. Methods of acceptable birth control are listed below under "Women of Childbearing Potential"
- Previous hypersensitivity reaction to dupilumab.
- Patients with acute asthma, acute bronchospasm or status asthmaticus.
- Patients with known helminth infections.
- Currently on any other immunosuppressant systemic medication or within 28 days of baseline visit.
- Has had a live vaccine within 60 days
- Underlying condition (including, but not limited to metabolic, hematologic, renal, hepatic, pulmonary, neurologic, endocrine, cardiac, infectious or gastrointestinal) which in the opinion of the investigator significantly immunocompromises the subject and/or places the subject at unacceptable risk for receiving an immunomodulatory therapy.
- Are participating in another study using an investigational agent or procedure during participation in this study or within 28 days prior to baseline visit.
- Any other treatment for keloids with 28 days prior to baseline visit, including silicone gel/sheets, laser therapy, intralesional steroid or 5-fluorouracil injections, topical steroid, cryotherapy, surgery, or radiation therapy.

Women of Childbearing Potential

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unwilling to use effective contraception during the study and for 12 weeks after stopping treatment. Effective contraception is defined as one of the following:

- Combined (estrogen and progestogen containing) hormonal birth control (oral, intravaginal, transdermal) associated with inhibition of ovulation
- Progestogen-only hormonal birth control (oral, injectable, transdermal) associated with inhibition of ovulation
- Bilateral tubal occlusion/ligation
- Intrauterine device
- Intrauterine hormone-releasing system
- Vasectomized sexual partner(s) (the vasectomized partner should have received medical assessment of the surgical success and is the sole sexual partner of the trial participant)
- True abstinence: Refraining from heterosexual intercourse when this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable)

Early Termination (ET) Visit

Study participants withdrawing from the study or stopping the study medication who are not continuing for all scheduled visits, will undergo an early termination visit. The early termination visit will comprise of vital signs, blood draw (CBC with differential, BUN/Cr, AST/ALT), urine pregnancy test, POSAS/VSS assessment, size measurement, DLQI, and adverse events.
If patients have completed at least 12 weeks of the study, a repeat optional biopsy will be offered to the patient. Photographs will be taken if the ET visit is around the date of planned scheduled photograph.

B4. POSSIBLE BENEFITS

There will be no benefit to subjects who participate in the study. However, in the future, others are expected to benefit from the findings of this study.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

More Common

- Immunologic: Antibody development (5% to 16%; neutralizing: 2% to 5%)
- Local: Injection site reaction (6% to 18%)

Less Common

- Central nervous system: Insomnia (1%)
- Gastrointestinal: Oral herpes simplex infection (4%), gastritis (2%), toothache (1%)
- Hematologic & oncologic: Eosinophilia (≤2%)
- Infection: Herpes simplex infection (2%)
- Neuromuscular & skeletal: Arthralgia (3%)
- Ophthalmic: Conjunctivitis (2% to 10%), eye pruritus (1%)
- Respiratory: Oropharyngeal pain (2%)

Rare

<1%, postmarketing, and/or case reports: Anaphylaxis, dry eye syndrome, eosinophilic granulomatosis with polyangiitis, eosinophilic pneumonitis, erythema nodosum, hypersensitivity reaction, keratitis, serum sickness, serum sickness-like reaction, significant cardiovascular event, vasculitis

Skin Tissue Collection

During the skin tissue collection process (via 4 mm punch method), subjects may feel mild to moderate pain and/or burning on the area where the skin is anesthetized with lidocaine with Epi injection, lasting no longer than 1-2 minutes and quickly resolve on its own. There is a risk of allergic reaction in subjects who have never been injected with lidocaine/epinephrine in the past. After the local anesthesia wears off, subjects may feel mild to moderate pain at the site that generally requires no intervention, and rarely needs to be self-managed with OTC ibuprofen or acetaminophen. Subjects may also experience re-bleeding at the site that generally requires applying pressure with gauze at home for 10 minutes to stop the re-bleeding. Rarely, subjects will need to seek urgent care, requiring cauterization to stop the re-bleeding. Over the course of 7-10 days post collection, there is a minimal risk of local skin infection that generally will respond to oral antibiotic therapy for 10-14 days. Rarely (if ever), it can lead to deeper soft tissue infection. We will take steps to minimize the risk of skin infection by strictly adhering to the sterile protocol of skin biopsy. Additionally, the biopsy site will likely develop a small scar. Overall, this protocol poses minimal risk to subjects.

Venous Blood Draw

The risks and discomforts of blood drawing from a vein include the possibility of pain or bruising at the site of the blood draw, occasional feeling of lightheadedness, and rarely, infection at the site of the blood draw.

Reproductive Risks of Dupilumab
Available data from case reports and case series with DUPIXENT use in pregnant women have not identified a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. Human IgG antibodies are known to cross the placental barrier; therefore, DUPIXENT may be transmitted from the mother to the developing fetus. There are adverse effects on maternal and fetal outcomes associated with asthma in pregnancy. In an enhanced pre- and post-natal developmental study, no adverse developmental effects were observed in offspring born to pregnant monkeys after subcutaneous administration of a homologous antibody against interleukin-4-receptor alpha during organogenesis through parturition at doses up to 10-times the maximum recommended human dose.

Live Vaccine Risks: Live vaccines should not be given while you are taking dupilumab.

Responsibilities of the Investigator
Adherence to the Study Protocol
The PI has the sole responsibility to ensure adherence to the study protocol and comply with the BIDMC Committee on Clinical Investigations (CCI) guidelines.

Data Handling and Record Keeping
The PI, sub-investigator(s), and designated research staff will be involved in data handling and record keeping under the supervision of the PI.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment
Dermatologists from Beth Israel Deaconess Medical Center, Massachusetts General Hospital, Brigham and Women's, Lahey Health, Tufts Medical Center, Boston Medical Center, Umass will be informed using IRB-approved recruitment email of this study and asked to refer eligible patients to our research unit. These letters will explain the purpose of the research, including a brief description of the nature and extent of involvement, and the investigator's email and phone number will be included. A research study advertisement will also be posted in the dermatology clinic.

We will also advertise this study to local, non-Harvard Medical School affiliated college students through their school's respective employment portals for off-campus employers, such as BU Quick Jobs, Northeastern University Student Employment, Berklee College of Music, etc. We will also post research opportunities on Craigslist. These advertisements will use IRB-approved language from the "college classifieds flyer." A list of potential subjects will be compiled from BIDMC Dermatologist's clinical patients with ICD codes for keloids. These identified patients will be sent recruitment letters informing them of the study and to contact one of the study doctors if they are interested. The study will also be placed on skinstudies.com. Skinstudies.com, twitter will use the social media text/image recruitment material. Additionally, we will also work with a vendor, BuildClinical, to post online patient recruitment advertisements on social media.

Consent

The PI, sub-investigator(s), and study staff with a valid U.S. medical license will be allowed to obtain informed consent from each subject participating in the study. The potential subject will be informed of the study purpose, procedures, expected duration, and the benefits and risks involved in study participation. At the time of the study entrance, subject will be given the opportunity to ask questions and be informed of their right to study withdrawal. After this explanation and before any study-specific procedures have been performed, the potential subject may voluntarily sign and date the informed consent form, thereby giving permission for the subject to enter the study. If, for any reason, at the time of the study visit the subject desires more time to consider the decision, the subject will be given a copy of the unsigned consent form for reference and be instructed to call the office if they decide to participate in the study. A copy of the signed consent form will be provided to the subject for his/her record.

Subject Protection

In order to address the issue of patients feeling pressured to participate, a standard approach was developed and implemented years ago. This gives the patient the opportunity to actively demonstrate their interest before enrolling in the study. If a discussion about the study presents itself during a clinical encounter, the patient is offered to take the consent home and read and then asked to follow up with the clinic if they are interested. It is also attempted to have a physician who is not involved in their clinical care go over their consent form in order to provide further separation.

B7. STUDY LOCATION

Privacy

Subject visits will be conducted in the CRC at BIDMC or, rarely, in private patient exam rooms in the dermatology clinic if the CRC is not available at the time of subject visits. Both locations should ensure adequate privacy for patients. Patients will complete all study related procedures in these private settings, including questionnaires. Questionnaires will be in written form and returned directly to the investigators or study staff.

Pre-screening telephone calls and subject visits will be limited only to the minimum amount of data necessary to accomplish the research purposes. No sensitive questions will be discussed by telephone.

Physical Setting

Subject visits will be conducted in the CRC at BIDMC or, rarely, in private patient exam rooms in the dermatology clinic if the CRC is not available at the time of subject visits. Both locations should ensure adequate privacy for patients. Patients will complete all study related procedures in these private settings, including questionnaires.

B8. DATA SECURITY

All subjects will be assigned a subject number at their screening visit. Only investigators and study staff at BIDMC will have access to the subject's identifiable health information. To ensure appropriate privacy and confidentiality, any paper source documentation and questionnaires will be stored in a locked cabinet. Only the PI, sub-investigator, and study staff will have access to this locked cabinet. This documentation will all be coded. Subject's identifiable health information will be kept separate from coded documentation. Any electronic data containing PHI will be stored on a password protected computer in a locked office and on a secure server behind the BIDMC firewall.

The Investigator will maintain adequate records for the study, including source documentation, Informed Consent documents, drug dispensing and disposition records, information regarding participants who discontinued, adverse events, and other pertinent data.

Direct Access to Source Data/Documents
Only investigators and the study staff at BIDMC will have direct access to source data and documents related to this study.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☒ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☒ No

B10 Dissemination of Research Results

Subjects will be thanked for their participation in the study immediately following their last visit. Because we are not able to anticipate the final completion date of the study, which may take years, and because we are not primarily responsible for analyzing and publishing the data, it will not be feasible for investigators to provide results to individual subjects. Subjects may contact the PI following their completion of the study to inquire about the final findings of the study, if available to the investigators, at that time.

Planned dissemination of research results may include abstract presentations at dermatology conferences and submission of data for publication in academic journals as an original research article.