Title Role of IgE Bearing Cells in Chronic Idiopathic Urticaria

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PROTOCOL SYNOPSIS

Title	Role of IgE Bearing Cells in Chronic Idiopathic Urticaria
Short Title	Omalizumab in CIU
Protocol Number	*XXXXX Version 1.3*
Study Drug	Omalizumab
Clinical Phase	Phase IV
Principal Investigator	Dr. Sarbjit S. Saini, M.D.
Participating Site	Johns Hopkins Asthma and Allergy Center
Primary Objective	This will be a study of the kinetics of clinical symptom relief during treatment of patients with CIU. The purpose is to determine if the rate of clinical remission is concordant with the rate that IgE-dependent functions of basophil change or mast cell changes during treatment.
Secondary Objectives	1) Basophil surface IgE, FcεRI and Syk by flow cytometry 2) Dendritic cell surface IgE, FcεRI, functions 3) Basophil anti-IgE, anti FcεRI, C5a, FMLP mediated histamine along with sensitivity measures 4) Serum free IgE measures 5) Characterization of presence of serum autoantibody presence ± Syk inhibitors 6) Basophil enumeration 7) Basophil mRNA profiling baseline at 3 timepoints (baseline, meaningful clinical changes, and 30 days) 8) To assess the rate of IgE and FcεRI change in skin mast cells at day 0, 6, 90, via a small punch biopsy as well as the MrgX2 receptor expression

Study Design

This is a Phase IV, single-site study that will examine blood cells or tissue obtained from CIU patients receiving open-label treatment with omalizumab at the current FDA-approved dose of 300 mg/month for 12 weeks in addition to standard therapy with anti-histamines. Results from the 3 Phase III studies in CIU patients provide evidence that a meaningful change in symptoms is apparent at 1-2 wks. The **MID** (**Minimal Important Difference**) is achieved by 70% of patients by 2 wks on multiple background drugs for hives. The goal is to identity the IgE bearing cell type associated with clinical symptom change.

The study will enroll 30 patients and will consist of **three phases**.

Screening Visit (Week -3 to Week-2)-establish compliance with diary and review safety labs.

Standard therapy Run-in (Week -2 to Day 0)

Open-label Treatment Period (Day 0 to Week 12)

During the **screening period** (Week -3 to Week -2), each patient's eligibility for the trial will be established. To be eligible at the **screening visit** (Week **-1**), subjects must:
1) Be 18 years of age or older

2) Have a diagnosis of moderate to severe CIU, as defined by pruritus and hives for > 3 days in a 7-day period for > 6 consecutive weeks despite treatment with H1 antihistamine:

Must have a non-diary based daily urticaria activity score (UAS) score ≥ 2 established in the outpatient setting based on the patient's condition over 12 hours prior to the visit; the UAS is a composite score of pruritus (0–3) and number of hives (0–3) with a maximum value of 6.

This requirement may be met either at screening visit, run-in visit (Week -2), or beginning of treatment (Day 0).

Must have been on an approved dose of an H1 antihistamine for CIU such as loratidine 10 mg once a day or equivalent, for at least 7 days prior to the screening visit. Approved agents include loratadine 10 qd, desloratadine 5 mg qd, fexofenadine 180 mg qd, cetirizine 10 mg qd or levocetirizine 5 mg qd as once daily medications at the

current FDA-approved dose. As per international treatment guidelines, daily doses up to 4-fold of the approved doe of the listed antihistamines will be allowed if the patient demonstrates high disease activity scores based on screening week.

Must be willing to fill out a twice-daily patient-diary to establish the patient's Urticaria Activity Score 7 (UAS7) score. The UAS is a composite diary- recorded score with numeric severity intensity ratings (0 = none to 3 = intense) for 1) the number of wheals (hives); and 2) the intensity of the pruritus (itch). The UAS7 is the sum of the daily average UAS scores (average of a.m. and p.m.) for 7 days. The maximum UAS7 value is 42.

To be eligible to begin the run-in period (Week -2 to Day 0), the patients:

Must have a UAS7 score ≥ 16 established over 7 consecutive days in the screening or run-in period.

Must remain on stable dose of a H1 antihistamines (not including antihistamine rescue medication) as established at the screening visit.

During the **run-in** period, patients will establish their baseline symptom scores using the diary. Patients with a diary-based UAS7 symptom score ≥ 16 during the screening or run-in periods will be eligible to enter the 12-week open treatment period. The baseline UAS7 score at time of treatment (Day 0) must be calculated using the diary scores for 7 days. Only in rare circumstances, (pending lab studies, in clinic UAS score < 2) will a longer screening period be permitted.

On the first day of the **treatment period (Day 0)**, eligible patients will receive 300 mg of omalizumab by sq injection. Baseline blood work, skin biopsy of non-lesional skin will also be collected. The primary endpoint will be measured using the daily diary scoring beginning at Day 0. For the duration of the 12-week, treatment period, patients must remain on stable doses of their pre-determined CIU H1 antihistamine treatment.

At the end of the 12-week treatment period, subjects will have last visit a final visit to collect diary, blood work, non-lesional skin biopsy and safety data.

	All patients will be provided diphenhydramine (25 mg po TID) as rescue medication for pruritus relief on an as-needed basis (to a maximum of three doses in 24 hours). Patients who require treatments other than diphenhydramine (e.g., prednisone) to treat persistent/worsening disease will be discontinued from the study. Patients will also be provided an epipen and trained in its use as per AAAAI guidelines for omalizumab.
Study Duration	15 months (including recruitment)
Primary Endpoint	The time to meaningful change in diary-based clinical symptoms as measured by the Urticaria Activity Score from baseline (Wk -7 to Day -1) to the date at which an MID (5 point change in weekly UAS 7) or achievement of > 50% reduction in daily symptom score for 3 days if in the first week. The UAS score, which is the sum of pruritus and hives, will be used to calculate the UAS7. The UAS7 score obtained 1 week prior to randomization will be used as the baseline.
Secondary Endpoints	Change in the weekly pruritus score from baseline to the 12th week in the treatment period. The pruritus score will be measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (intense). The weekly pruritus score is the sum of average daily pruritus scores over the previous 7 days. Change in the weekly score for number of hives from baseline to the 12th week in the treatment period. The number of hives is measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (> 12 hives, see below). The weekly score of number of hives is the sum of the average daily scores over the previous 7 days. **Scoring for Wheals** 0= None 1= Mild 1-6 hives/12 hours 2= Moderate 7-12 hives/12 hours 3= Severe > 12 hives/12 hours **Scoring for Itch** 0= None 1= Mild 2= Moderate 3= Severe Change in the amount of rescue medication (diphenhydramine 25 mg) from baseline to the 12th week in the treatment period using the question on the rescue medication use in the patient diary.

	Change in *SKINDEX29* quality of life survey instrument with 29 questions on a 5-point Likert scale. It will be given at baseline and again at 90 days.
Safety Outcome Measures	The safety of omalizumab will be assessed using the following outcome measures: incidence and severity of treatment-emergent adverse events and serious adverse events, clinical laboratory measures, and vital signs. In particular we will measure CBC's with differential at Week -3 and Week 12 and CMP at Week -3 and Week 12.
Pharmacodynamic Outcome Measures	PD parameters will be assayed using the blood basophil IgE receptor flow based assay. This assay was successfully used in multiple past omalizumab studies.
Exploratory Endpoints	1) Basophil surface IgE, FcεRI and Syk by flow cytometry- Day 0, 1, 3, 6, 10, 20, 30 and 90 days 2) Dendritic cell surface IgE, FcεRI -Day 0, 1, 3, 6, 10, 20, 30 and 90 days 3) Basophil anti-IgE, anti FcεRI, C5a, FMLP mediated histamine release along with sensitivity measures- Day 0, 1, 3, 6, 10, 20, 30 and 90 days 4) Serum free IgE measures (stored serum) Day 0, 1, 3, 6, 10, 20, 30 and 90 days 5) Characterization of presence of serum autoantibody presence ± Syk inhibitors -Day 0, 1, 3, 6, 10, 20, 30 and 90 days 6) Basophil enumeration by manual counting and blood histamine content - Day 0, 1, 3, 6, 10, 20, 30 and 90. This will determine the presence or absence of basopenia and identify subjects for mRNA profiling studies. 7) Basophil mRNA profiling baseline at 3 timepoints (baseline, meaningful clinical changes, and 30 days) in select subjects with sufficient basophil numbers (>1.5x 106/100 cc blood) 8) Numbers of IgE +, FcεRI + cells in non-lesional skin biopsies at day 0, 6, 90 to monitor skin mast cells well as the MrgX2 receptor expression

9) Change in circulating leukocyte population numbers that are targeted by omalizumab such as blood basophils, eosinophil and lymphocyte counts by automated analysis (week -3 to week 12).

10) Change in urine prostaglandins and eicosanoids pretreatment (Days-21, -14, 0,) relative to post-treatment timepoints 1, 3, 6, 10, 20, 30 and 90.

Inclusion Criteria

Male or female, ages 18 years or older

Females must be surgically sterile or postmenopausal or using a highly effective form of birth control throughout the duration of the study such as an oral contraceptive, or double barrier method contraception condom with spermatocide or IUD. Females in certain categories (not sexually active, vasectomized partner, tubal occlusion) will be admitted at the discretion of the investigator on a case-by-case basis.

Females must have a negative urine pregnancy test at screening and other visits specified in this protocol unless documented to have a hysterectomy or be postmenopausal.

Clinical history of CIU at the time of screening, as defined by pruritus and hives for > 3 days in a 7-day period for > 6 consecutive weeks despite treatment with H1 antihistamine.

CIU diagnosis > 3 months (by history)

No underlying etiology clearly defined for urticaria (main manifestation cannot be physical urticaria).

Non-diary based UAS scores ≥ 2 at either the screening visit (Week -3), at the run-in visit (Week -2), or on Day 1.

A diary-based UAS7 score ≥ 16 established during the screening or run-in periods despite stable doses of H1 antihistamine.

Compliance with study procedures during run-in period (e.g., completion of the study diary).

Exclusion Criteria

Pregnant females, confirmed by a positive Human Chorionic Gonadotropin (HCG) test, or females with plans to become pregnant or breastfeed during the duration of the study.

Recent history of drug or alcohol abuse (within 3 years prior to screening visit).

Inability or unwillingness of a participant to give written informed consent or comply with study protocol.

Use of any investigational drugs within 30 days of screening.

Active atopic dermatitis or other skin disease associated with pruritus during the time of the study, which require treatment with topical corticosteroids.

Clinically relevant major systemic disease (making interpretation of the study results difficult) including a history of anaphylaxis.

Inability to comply with study and follow-up procedures

Patients may not take during treatment period or have been taking within the past one-month any of the following medications/treatments: regular (daily/every other day) hydroxychloroquine, sulfasalazine, dapsone, methotrexate, cyclosporin, IVIG, or other monoclonal antibody therapies.

Patients may not take doxepin during screening, run-in, or treatment period or have been taking doxepin within the past 2 weeks regularly (daily/every other day) prior to screening.

Patients may not use cutaneous corticosteroid (intranasal, inhaled, and ophthalmic steroids are permitted) during the screening, run-in, or treatment phases.

Patients may not use oral or systemic steroids during the study or within 4 weeks prior to enrollment.

Patients may not take H2 antihistamines and leukotriene receptor antagonists within 7 days before screening, during the screening, run-in, or treatment phases. The exception will be if they are already on these medications for the treatment of GERD, asthma or allergic rhinitis.

Contraindications to diphenhydramine

Any clinically relevant abnormal findings in clinical chemistry, hematology, physical examination, pulse, blood pressure at baseline, which, in the opinion of the investigator, could put the patient at risk because of his/her participation in the study.

Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which,

	in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or may compromise the quality of the data obtained from the study.
Safety Plan	Patient will be instructed to recognize the signs and symptoms of any severe hypersensitivity reaction, and to contact a healthcare provider in case of any such symptoms. All will be provided with an epipen as per usual standard of care with omalizumab recipients. Patients with CIU can have angioedema as part of their symptoms. When that angioedema poses a risk to the airway, the emergent use of systemic corticosteroids is indicated. Patients who require treatment with systemic corticosteroids will be discontinued from the study and followed for safety assessment for the remainder of the study. Patients will be provided emergency contact information and advised to contact a physician during the entire trial, in case of angioedema. Safety data including laboratory tests will be reviewed internally on a periodic basis during the conduct of the study.

Investigational Product(s)/Intervention(s), Dosage and Mode of Administration	Patients will receive the study drug (Omalizumab) which is supplied by the manufacturer and is administered subcutaneously as the per package insert.
Study Procedures	Phlebotomy; Skin biopsies.
Statistical Considerations	Our plan is to look at multiple cellular parameters in Omalizumab treated patients and relate cellular changes to the timing of the MID for itch (>5 points) or hives (> 5 points) with the primary outcome being the percentage of patients achieving a 50% rise in basophil anti-IgE functions at the time of reaching a clinical MID. .

Table of Contents

Glossary of Abbreviations

AE	Adverse Event
BHR	Basophil Histamine Release
BID	Two Times a Day
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CIU	Chronic Idiopathic Urticaria
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
FEV1	Forced Expiratory Flow in 1 Second
G6PD	Glucose-6-phosphate Dehydrogenase
GCP	Current Good Clinical Practice
HCG	Human Chorionic Gonadotropin
ICH	International Conference on Harmonization
ICS	Inhaled Corticosteroid
INR	International Normalized Ratio

IRB	Institutional Review Board
IVIG	Intravenous Immunoglobulin
LABA	Long-Acting-beta-2-Agonists
LDH	Lactate Dehydrogenase
MAO	Monoamine Oxidase
MOP	Manual of Operations
PD	Pharmacodynamic
PEF	Peak Expiratory Flow
PI	Principal Investigator
PK	Pharmacokinetic
QD	Once a Day
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TID	Three times a Day
UAS	Urticaria Activity Score
ULN	Upper Limit of Normal

BACKGROUND AND RATIONALE

Background

Overview of CIU

Episodes of urticaria affect up to 25% of the US population during their lifetime (1, 2). While many short-lived, urticarial reactions have identifiable causes such as allergens or infections, there is a large class of reactions for which the underlying cause is unknown (3). Recurrent urticaria for greater than 6 weeks is termed chronic urticaria (CU), and in the vast majority of cases (>90%) no trigger is found and thus is termed chronic idiopathic urticaria (CIU). The annual prevalence of CIU is approximately 1% (4, 5). CIU is a widely recognized disorder in multiple continents (North America, Asia, Europe) and multiple disease guidelines have been issued in these various regions supporting the global burden of this ailment (6-9). CIU also affects both children and adults and nearly twice as many females in the adult population (4, 10-14). Although CIU skin reactions are rarely life-threatening and are limited in duration for an average of 2-3 years in 80% of cases, the pruritus that accompanies the disease has an impact on quality of life that is similar to heart disease (15).

Mechanisms active in CIU

It is well-accepted that CIU subjects suffer from recurrent episodes of hives virtually anywhere on body that are extremely pruritic and related to excessive histamine presence in the skin (16). The pathology observed in CIU lesional skin biopsies, is of mast cells that are degranulated and presence of skin infiltrating basophils, and blood basopenia (17, 18). However, the identifiable triggers for these two cells remain unknown. The skin pathology in CIU lesions closely resemble that found in skin biopsies of allergen triggered skin late-phase reactions but lacks an identifiable trigger (19, 20). Over the last decades, two theories have been explored for the cause of this condition. The first suggests an autoimmune trigger for either mast cells or basophils and the second focuses on the cell that might be controlling the reaction, the mast cell or the basophil. In the 1980s it was noted that there was a higher frequency of thyroid autoantibodies among CIU patients as compared to the normal population (21). This observation gave rise to the idea that autoimmunity may also be a trigger for CIU lesions given the absence of external triggers. Subsequently, a subset of CIU patients was identified with circulating IgG anti-IgE (5-10% of patients) or anti-FcεRI antibodies (35-40 % of patients) (22, 23). This seemed a remarkable discovery since either type of antibody could be the trigger for driving secretion from either mast cells or basophils. However, additional studies have raised a number of issues with this explanation such as (3, 24): 1) the lack of specificity of autoantibodies for CIU given that such IgG antibodies occur in other skin diseases, SLE, RA and healthy subjects 2) antibody titers are stable in remission and do not follow disease symptoms 3) the lack of distinct clinical phenotype, skin biopsy, or therapeutic response in those with autoantibodies 4) the lack of a standardized autoantibody detection assay despite nearly two decades of work. The success of omalizumab (Xolair, anti-IgE antibody) therapy in CIU patients provides support that this disease depends on IgE dependent pathways. However, the clinical response kinetics to omalizumab therapy did not differ among the subset of CIU subjects (25-30%) with serologic evidence of functional autoantibodies (CIU Index assay-basophil histamine release assay), raising questions about the pathogenic role of such autoantibodies (25-27). The second area of

interest is the role of the basophil (28). The blood basophil is selectively recruited to the skin lesions of CIU patients and is capable of secreting histamine that can drive the skin reaction, yet it is hard to reconcile with the long-standing view that the skin mast cell drives the disease (29). But studies have shown that IgE-mediated basophil reactivity tracks well with the remissions and exacerbations of this disease (30-32). We have identified 2 patterns of basophil reactivity based on the degree of histamine release after optimal IgE receptor activation: CIU-non-responders (CIU NR) which release less than 10% of total cellular content and CIU-responders (CIU-R) which release greater than 10% of histamine content (33). We have established the stability of these 2 functional phenotypes in active disease and improvement with disease remission (30, 32). Blood basopenia is a unique feature of this disorder since it was first reported in the 1960's (34). Evidence also exists that blood basopenia is related to disease severity and improves with symptom improvement and in remission (31, 32, 35). Indeed, the concordance between basophil functionality and the disease is far better than the relationship between the presence of autoantibodies and disease (30). Further, systemic corticosteroids rapidly suppress CIU disease symptoms and all classes of basophil mediators release, but not skin mast cell degranulation. Systemic corticosteroids therapy leads to a rise in blood basophil numbers in CIU whereas in normal subjects basophil numbers fall (35), favoring the idea that steroids impair basophil migration to the CIU skin lesions.

While skin mast cell degranulation is generally accepted in CIU, the trigger remains unclear. Evidence supports that skin mast cell numbers are not increased in skin biopsies (18, 36-38) and serum tryptase levels, an indicator of total body mast cell number, are within the normal range (39, 40). Older studies support that increased skin mast cell releasability to stimuli such as 48/80 and codeine (41, 42) occurs in CIU. Recently, MrgX2 has been identified as the receptor for numerous stimuli (48/80, codeine, and multiple drugs) on human and murine mast cells (43). A recent report has identified increased MrgX2 in skin mast cells in CIU(38).

Without a clear mechanism or knowledge of the principle cell type responsible for CIU disease, treatments are relegated to symptomatic relief (44). ***The purpose of this proposed research is to determine what cell type(s) strongly influence expression of this disease***. This will be done by using an observation made possible by the recent introduction of omalizumab, a monoclonal IgG anti-IgE therapeutic antibody, into the treatment regimen for CIU (25-27, 45).

Omalizumab therapy in CIU

Omalizumab has shown success in H1 refractory CIU cases and was approved by the FDA in April 2014 for this indication. It has undergone 3 Phase III studies that support a fixed dose of 150 mg or 300 mg every 4 weeks is effective in 70% of anti-histamine refractory CIU recipients (25-27). However, it carries the high costs of a biologic agent, requires subcutaneous injection in a physician's office, and requires an EpiPen prescription due to the risk of drug-related anaphylaxis of 0.2%. The efficacy of omalizumab therapy in CIU has raised questions as to the role of IgE in this non-allergic skin disease. A recent study has shown changes in blood basophils but not skin mast cells at an early timepoint associated with clinical symptom improvement. The results for omalizumab have been surprising. The surprise occurs at two levels, first, that an anti-IgE therapeutic would have such dramatic effects in this disease and second, that the effects occur so rapidly (25-27, 45, 46).

Results from the 3 Phase III studies in CIU provide evidence that a meaningful change in symptoms is apparent at 1-2 weeks (25-27). Based on pooled Phase III trial data, approximately

70% of CIU subjects treated with 300 mg/month omalizumab for 12 weeks will have a 70% reduction in their pretreatment UAS7 (Urticaria Activity Score for 7 days: scale is 0-3 for itch and 0-3 for number of hives; maximal daily score is 6 (average of AM and PM scores), and maximum UAS7 is 42); 60% will have an 80% reduction in symptoms (UAS7< 6), and 40% are fully asymptomatic (UAS7=0) (47). The **MID** (**Minimal Important Difference**) is achieved by 70% of patients by 2 weeks in patients on multiple drugs for hives versus a median time of 4-5 weeks on placebo injections (26). Therefore, this protocol is designed to make an assessment of the clinical symptoms at a high frequency in the early 2 weeks during treatment along with the blood and skin tissue biopsy studies.

Current approaches to CIU per guidelines and unmet needs

Based on recent guidelines, Omalizumab is the only FDA-approved adjunctive agent beyond first-line oral, non-sedating antihistamines (48). This is important because up to 50% of patients fail to respond to a non-sedating oral antihistamine (49). Current guideline recommended add-on therapies include H2 blockers, leukotriene receptor antagonists, older generation sedating antihistamines (48). In more severe CIU cases, systemic steroids are recommended and in steroid-dependent cases, there are several other agents that have been reported to be beneficial such as cyclosporine, sulfasalazine, dapsone, plaquenil, and mycophenolate mofetil but all carry significant toxicities and require frequent, costly laboratory monitoring (CBC, renal function, G6PD)(50, 51). Thus, there is an unmet need to improve the understanding of the pathogenesis of CIU to lead to improved, less toxic therapies.

Rationale for Selection of Study Population

Results from the 3 Phase III studies in CIU provide evidence that a meaningful change in symptoms is apparent at 1-2 weeks (25-27). The **MID** (**Minimal Important Difference**) is achieved by 70% of patients by 2 weeks in patients on multiple drugs for hives versus a median time of 4-5 weeks on placebo injections (26). Therefore, this protocol is designed to make an assessment of the clinical symptoms at a high frequency in the early 2 weeks during treatment along with the blood and skin tissue biopsy studies. The goal is to identify ***which cell type(s) strongly influence expression of this disease*** using a monoclonal therapeutic anti-IgE antibody that is FDA approved in CIU subjects not controlled on treatment with anti-histamines.

Intervention(s)

Omalizumab is a recombinant DNA-derived humanized IgG1κ monoclonal antibody that selectively binds to human immunoglobulin E (IgE). The antibody has a molecular weight of approximately 149 kiloDaltons. Xolair is produced by a Chinese hamster ovary cell suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product.

Rationale for Selection of Intervention(s) and Regimen

We propose a single-site study with 12 weeks of open label therapy with omalizumab 300 mg/month with no placebo arm. We favor the higher dose of approved drug given it's faster onset of clinical benefit and open label therapy to gain the greatest possible participation from eligible subjects given the widespread knowledge of the efficacy of omalizumab in CIU. Omalizumab is currently FDA approved, and a standard of care option for patients who have anti-histamine refractory urticaria. We propose to treat for 12 weeks as there is ample evidence for clinical benefit within this time frame and the likelihood to impact skin mast cell IgE receptors expression based on prior studies after 8 weeks (52).

This is a study of the kinetics of clinical symptom relief during treatment with omalizumab of patients with chronic idiopathic urticaria. The purpose is to determine whether the rate of clinical remission is concordant with the rate that IgE-dependent functions of basophil change during treatment. In addition, skin mast cell expression of FcεRI and IgE in the non-lesional skin will be monitored to determine if the rate of change in these two parameters is consistent with prior measurements (52) or uniquely faster in these individuals. Previous studies of omalizumab therapy in CIU have not examined the changes in cellular functions with a temporal granularity being proposed in this study. Notably, the earliest assessment of basophil functions in previous studies occurred at 1 month, a time point in which basophil function has generally decreased in most patients (53). We are interested in judging whether the kinetics for early clinical symptoms matches the changes to the blood basophil phenotype.

Other IgE-bearing cell types known to be impacted by omalizumab treatment include the peripheral blood dendritic cell. We have previously shown that these cells change in allergic patients in a time-frame similar to basophils. IgE receptor function of dendritic cells includes impacting their ability to release various cytokines induced by toll receptor activation. To date, TLR9 receptors activation of pDCs is impaired in subjects with CIU(54).

In addition, we will examine whether IgE receptor alteration of skin mast cells via omalizumab has an impact on MrgX2 expression. Our proposed hypothesis is that omalizumab in CIU will reduce IgE levels and IgE receptor bearing leukocyte expression in a time dependent fashion with clinical symptom improvement and most rapidly on those cells (basophils) implicated in disease pathogenesis thus reducing the signs and symptoms in CIU refractory to control with antihistamines.

Clinical Studies

Omalizumab has undergone extensive clinical trials in subjects with allergic asthma and chronic idiopathic urticaria. It is currently FDA approved for both of these indications with allergic asthma approval occurring in 2003 and CIU in 2014. In the clinical studies completed in subjects with chronic uriticaria, omalizumab was generally well tolerated(25-27). Dose ranging studies identified that flat dosing schedule was efficacious with maximal efficacy seen at 300 mg as compared to either a single dose of 75 mg or 600 mg. The clinical efficacy of omalizumab in CIU has been evaluated in 3 phase III randomized, double-blind, placebo-controlled studies. Two of the studies evaluated CIU subjects on standard dosed antihistamines with doses of 75

mg, 150 mg and 300 mg injected every 4 weeks for either 12 or 24 weeks. The third study tested only the 300 mg dose or placebo injection every 4 weeks for 24-week studies in patients refractory to usual CIU care per guidelines (up to 4 –fold standard dose of non-sedating antihistamines, ± H2 blocker, ± leukotriene receptor antagonists.). All three studies demonstrated efficacy in reducing the UAS7 score and weekly itch score. Differences between treatment groups for secondary variables (weekly hive score, angioedema) also favored either the 150 mg or 300 mg dose of omalizumab . As per the PI, the mechanism of action, besides lowering IgE receptors through binding of IgE, remains unknown.

Risks

Risks of Intervention(s)

Omalizumab: In CIU patients ≥12 years of age, the most commonly observed adverse reactions: from 3 placebo-controlled CIU studies for omalizumab 150 mg and 300 mg, respectively, were: headache (12%, 6%), nasopharyngitis (9%, 7%), arthralgia (3%, 3%), viral upper respiratory infection (2%, 1%), nausea (1%, 3%), sinusitis (1%, 5%), upper respiratory tract infection (1%, 3%), and cough (1%, 2%). Injection site reactions of any severity occurred during the trials in more omalizumab treated patients;[2.7%] at 300 mg, [0.6%] at 150 mg) compared with 0.8% in placebo-treated patients.

Anaphylaxis has been reported to occur after administration of omalizumab in asthma clinical trials and in postmarketing spontaneous reports. The frequency of anaphylaxis was estimated to at least 0.2%. A case-control study showed that among XOLAIR users, patients with a history of anaphylaxis to foods, medications, or other causes were at increased risk of anaphylaxis associated with XOLAIR, compared to those with no prior history of anaphylaxis. Based on this, AAAAI guidelines support monitoring patients for 2 hours for the first 3 injections of xolair and the prescription of an epipen injector.

Malignant neoplasms were observed in 20 of 4127 (0.5%) omalizumab treated patients compared with 5 of 2236 (0.2%) control patients in clinical studies of adults and adolescents (≥12 years of age) with asthma and other allergic disorders. The observed malignancies in XOLAIR-treated patients were a variety of types, with breast, non-melanoma skin, prostate, melanoma, and parotid occurring more than once, and five other types occurring once each. A subsequent 5-year observational study of 5007 XOLAIR-treated and 2829 non- XOLAIR -treated patients with moderate to severe persistent asthma and a positive skin test reaction or in vitro reactivity to a perennial aeroallergen found that the incidence rates of primary malignancies (per 1000 patient years) were similar in both groups (12.3 vs 13.0, respectively) (Package Insert).

Risk of Study Procedures

Phlebotomy: pain, bruising, infection, light-headedness and fainting. The American Red Cross recommends that no more than 480 mL of blood be donated in a 2 month time period. The maximum blood that would be drawn in a 2-month period in the primary outcome study is 436 mL over 9 separate blood draws, whereas the total volume drawn is 515 over 90 days. At no

time will Red Cross guidelines be exceeded. Further, CBCs will be drawn at baseline for safety monitoring and at the end of week 12.

Skin biopsy: Risks of skin punch biopsy include pain, swelling, bleeding, bruising, infection, and scarring at the site of the biopsy. Allergic reactions to lidocaine are rare, but may cause hives, itching, asthma, hay fever, or a drop in blood pressure. Significant bleeding from biopsy sites is rare. Infection of a biopsy site is unusual, but may occur. A scar the size of a pencil eraser will result at the biopsy site. This scar will be slightly raised or depressed below the surface of the skin and will be a little lighter or darker than the rest of your skin.

Risk of Concomitant Medications, Prophylactic Medications and Rescue Medications

Antihistamines (concomitant medications allowed during study) may cause sedation, dizziness, coordination problems, and dried mucous membranes.

Diphenhydramine (Rescue medication) may cause sedation.

Epipen (Rescue medicine). The most common side effects include increase in heart rate, stronger or irregular heartbeat, sweating, nausea and vomiting, difficulty breathing, paleness, dizziness, weakness or shakiness, headache, apprehension, nervousness or anxiety. These side effects usually go away quickly.

Benefits of Intervention(s)

The patients treated with omalizumab are expected to experience decreased urticarial symptoms. These benefits are not expected to be maintained after treatment discontinuation. The benefits may begin at any point during treatment. There are no known benefits of the study procedures in the general population or in subjects with CIU.

OBJECTIVES

Primary Objective(s)

This is a study of the kinetics of clinical symptom relief during treatment of patients with CIU with omalizumab. The purpose is to determine if the rate of clinical remission is concordant with the rate that IgE-dependent functions of basophil change. We propose as the primary outcome as the time for change in the functional response from baseline of basophil in vitro anti-IgE for histamine release (defined as a 2-fold rise at suboptimal concentrations of anti-IgE) relative to the time to reach MID of > 5 point change in either itch of hive. We expect subjects with baseline basophil CIU-NR (non-responder) phenotype to become Responders and baseline Responder phenotype to demonstrate a rise the in suboptimal anti-IgE response.

Secondary Objective(s)

1.Change in the weekly pruritus score from baseline to the 12th week in the treatment period. The pruritus score will be measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (intense). The weekly pruritus score is the sum of average daily pruritus scores over the previous 7 days.

2. Change in the weekly score for number of hives from baseline to the 12th week in the treatment period. The number of hives is measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (> 12 hives, see below). The weekly score of number of hives is the sum of the average daily scores over the previous 7 days.

Scoring for Wheals
0= None
1= Mild 1-6 hives/12 hours
2= Moderate 7-12 hives/12 hours
3= Severe > 12 hives/12 hours

Scoring for Itch
0= None
1= Mild
2= Moderate
3= Severe

3. Change in the amount of rescue medication (diphenhydramine 25 mg) from baseline to the 12th week in the treatment period using the question on the rescue medication use in the patient diary.

4. Change in *SKINDEX29* quality of life survey instrument with 29 questions on a 5-point Likert will be given at baseline and again at 90 days.

Exploratory Objective(s)

1) Change in basophil surface IgE, FcεRI and Syk by flow cytometry- Day 0, 1, 3, 6, 10, 20, 30 and 90 days

2) Change in Dendritic cell surface IgE, FcεRI -Day 0, 1, 3, 6, 10, 20, 30 and 90 days

3) Change in Basophil anti-IgE, anti FcεRI, C5a, FMLP mediated histamine along with sensitivity measures- Day 0, 1, 3, 6, 10, 20, 30 and 90 days

4) Serum free IgE measures -(stored serum) Day 0, 1, 3, 6, 10, 20, 30 and 90 days

5) Characterization of presence of serum autoantibody presence ± Syk inhibitors -Day 0, 1, 3, 6, 10, 20, 30 and 90 days

6) Basophil enumeration by manual counting and blood histamine content - Day 0, 1, 3, 6, 10, 20, 30 and 90 days. This will determine the presence or absence of basopenia and identify subjects for mRNA profiling studies.

7) Basophil mRNA profiling baseline at 3 timepoints (baseline, meaningful clinical changes, and 30 days) in select subjects with sufficient basophil numbers

8) Numbers of IgE +, FcεRI + cells in non-lesional skin biopsies at day 0, 6, 90 to monitor skin mast cells well as the MrgX2 receptor expression

9) Change in circulating leukocyte population numbers that are targeted by omalizumab such as blood basophils, eosinophil and lymphocyte counts by automated analysis (week -3 and week 12).

10) Measurement of urine prostaglandins and eicosanoids at Day -14, 0, 1, 3, 6, 10, 20, 30 and 90.

STUDY DESIGN

This is a Phase IV, single-center, open-label 12 week omalizumab treatment study of CIU subjects refractory to standard of care to examine to identify disease relevant IgE bearing cells that are altered concordant with the onset of clinical symptom relief . CIU patients will receiving open-label treatment with omalizumab at the current FDA-approved dose of 300 mg/month for 12 weeks. We favor this approach to gain the greatest participation from eligible subjects given the widespread knowledge of the efficacy of omalizumab in CIU. Results from the 3 Phase III studies in CIU provide evidence that a meaningful change in symptoms is apparent at 1-2 wks. The **MID** (**Minimal Important Difference**) is achieved by 70% of patients by 2 wks on multiple background drugs for hives. All subjects will have CIU for 3 months, and will be age 18 or older. All subjects will have a baseline UAS score of 2 or greater despite treatment with daily doses of non-sedating H1 antihistamines. The UAS is a composite score of pruritus (0-3) and hives (0-3) with a maximum value of 6. This study will include a 2-week run-in. The study will enroll 30 patients.

Figure 1: Overall Study Design

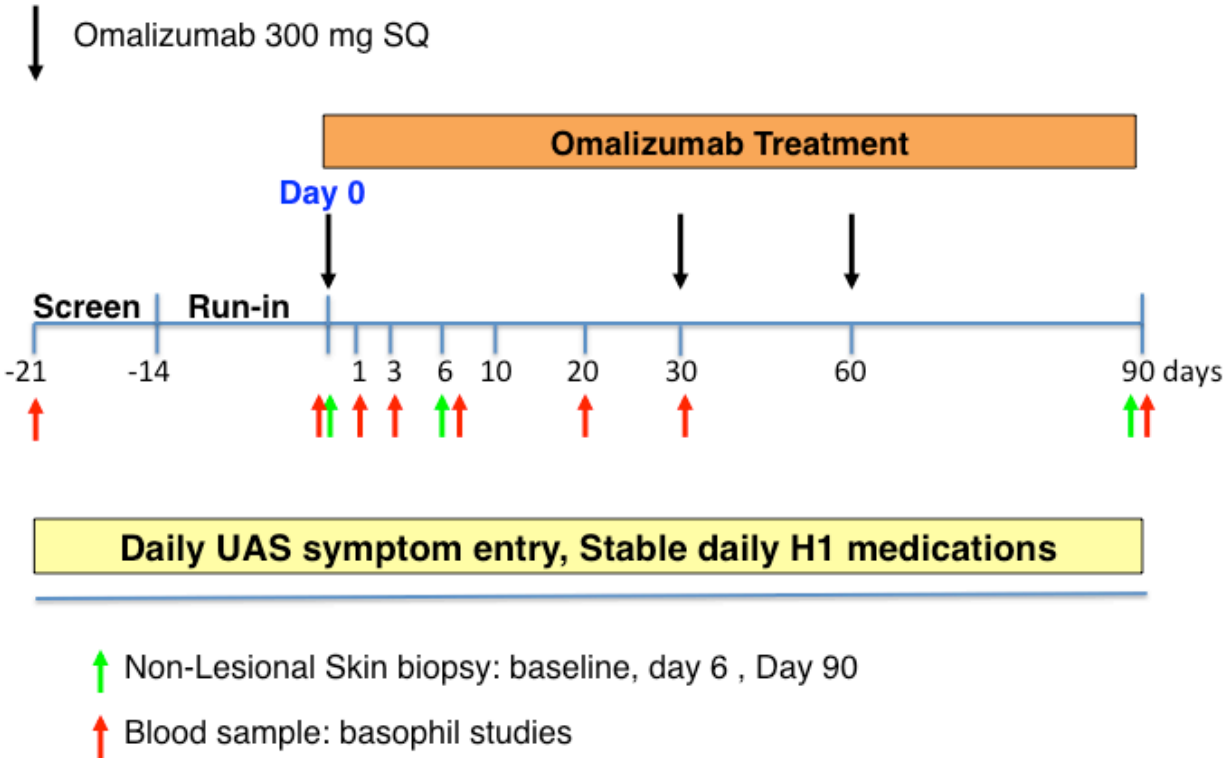

Figure 1: Study Schema

The study will enroll 30 patients and will consist of **three phases**.

Screening (Day -21 to day -14)

During the **screening period**, each patient's eligibility for the trial will be established. To be eligible at the screening visit (Week -3), patients:

Must have a non-diary based daily urticaria activity score (UAS) score ≥ 2 established in the outpatient setting based on the patient's condition over 12 hours prior to the visit; the UAS is a composite score of pruritus (0–3) and number of hives (0–3) with a maximum value of 6. This requirement may be met either at screening day, run-in visit (Day -14), or Day 0.

Must have been on an approved dose of an H1 antihistamine for CIU such as loratadine 10 mg once a day or equivalent, for at least 7 days prior to the screening visit. Approved agents include loratadine 10 qd, desloratadine 5 mg qd, fexofenadine 180 mg qd, cetirizine 10 mg qd or levocetirizine 5 mg qd as once daily medications at the current FDA-approved dose. If necessary for symptom control, per international guidelines, patients who use up to 4-fold the standard daily dose will be allowed in the study.

Must be willing to fill out a twice-daily patient-diary to establish the patient's Urticaria Activity Score 7 (UAS7) score. The UAS is a composite diary- recorded score with numeric severity intensity ratings (0 = none to 3 = intense) for 1) the number of wheals (hives); and 2) the intensity of the pruritus (itch). The UAS7 is the sum of the daily average UAS scores (average of a.m. and p.m.) for 7 days. The maximum UAS7 value is 42.

Run-in (Day -14 to Day 0)

To be eligible to begin the **run-in period (Day -14 to 0)**, patients:

Must have a UAS7 score ≥ 16 established in the screening week or subsequent run-in period.

Must remain on stable dose of a single H1 antihistamine at approved dose (not including antihistamine rescue medication) as established at the screening visit.

During the run-in period, patients will establish their baseline symptom scores using the diary. Patients with a diary-based UAS7 symptom score ≥ 16 during the screening or run-in periods will be eligible to enter the 12-week open label treatment period. The baseline UAS7 score at time of drug treatment (Day 0) must be calculated using the diary scores for 7 days. The UAS7 score will be calculated on the last 7 days captured in the diary prior to the first drug treatment visit. Only in rare circumstances, (pending lab studies, in clinic UAS score < 2) will a longer screening period be permitted.

Open Label Treatment Period (Day 0 to Week 12)

At Day 0 of treatment, eligible patients will be give 300 mg of omalizumab SQ every 4 weeks for a total of 12 weeks (3 injections) . Efficacy, safety, and pharmacodynamic (PD) data will be collected. The primary endpoint will be measured at the time of meaningful change in UAS7 expected to occur within 1-2 weeks of first dose of omalizumab. For the duration of the 12-week treatment period, patients must remain on stable doses of their pre-determined CIU H1 antihistamine treatment. All patients will be provided diphenhydramine (25 mg po TID) as rescue medication for pruritus relief on an as-

needed basis (to a maximum of three doses in 24 hours). Patients who require treatments other than diphenhydramine (e.g., prednisone) to treat persistent/worsening disease will be discontinued from the study. All patients will be provided with an epipen as per AAAAI guidelines for omalizumab recipients.

Study Endpoints

Primary Endpoint(s)

We propose as the primary outcome as the time for change in the functional response from baseline of basophil in vitro anti-IgE for histamine release (defined as a 2-fold rise at suboptimal concentrations of anti-IgE) relative to the time to reach MID of > 5 point change in either itch of hive. We expect subjects with baseline basophil CIU-NR (non-responder) phenotype to become Responders and baseline Responder phenotype to demonstrate a rise the in suboptimal anti-IgE response.

Secondary Endpoint(s)

Change in the weekly pruritus score from baseline to the 12th week in the treatment period. The pruritus score will be measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (intense). The weekly pruritus score is the sum of average daily pruritus scores over the previous 7 days.

Change in the weekly score for number of hives from baseline to the 12th week in the treatment period. The number of hives is measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (> 12 hives, see Table 1). The weekly score of number of hives is the sum of the average daily scores over the previous 7 days.

Table 1.

Twice Daily Assessment of Disease Activity in Patients with CIU (UAS Scale)

Score	Wheals (Hives)	Pruritus (Itch)
0	None	None
1	Mild (1–6 hives/12 hour)	Mild
2	Moderate (7–12 hives/12 hour)	Moderate
3	Intense (> 12 hives/12 hour)	Severe

Change in the amount of rescue medication (diphenhydramine 25 mg) from baseline to the 12th week in the treatment period using the question on the rescue medication use in the patient diary.

Change in *SKINDEX29* quality of life survey instrument with 29 questions on a 5-point Likert will be given at baseline and again at 90 days (Appendix 5).

Exploratory Endpoint(s)

1) Change in basophil surface IgE, FcεRI and Syk by flow cytometry from baseline at 1, 3, 6, 10, 20, 30 and 90

2) Change in Dendritic cell surface IgE, FcεRI, from baseline at Days 1, 3, 6, 10, 20, 30 and 90

3) Change in Basophil anti-IgE, anti FcεRI, C5a, FMLP mediated histamine along with sensitivity measures from baseline at 1, 3, 6, 10, 20, 30 and 90 days

4) Change in serum free IgE measures from baseline at days 1, 3, 6, 10, 20, 30 and 90

5) Characterization of presence of serum autoantibody presence ± Syk inhibitors at baseline and at days 1, 3, 6, 10, 20, 30 and 90

6) Change in basophil enumeration by manual counting and blood histamine content from baseline at days 1, 3, 6, 10, 20, 30 and 90. This will determine the presence or absence of basopenia and identify subjects for mRNA profiling studies.

7) Change in basophil mRNA profiling baseline at 3 timepoints (baseline, meaningful clinical changes, and 30 days) in select subjects with sufficient basophil numbers as identified at baseline measure.

8) Change in numbers of IgE +, FcεRI + cells in non-lesional skin biopsies from baseline to days 6 and 90 to monitor skin mast cells well as the MrgX2 receptor expression

9) Change in circulating leukocyte population numbers that are targeted by omalizumab such as blood basophils, eosinophil and lymphocyte counts by automated analysis (week -3 to week 12).

10) Change in urine prostaglandins and eicosanoids at Pre-treatment (Day, -21 -14, 0,) 1, 3, 6, 10, 20, 30 and 90.

Study Completion

This study will be considered "completed" when the primary, secondary and/or exploratory objectives have been met. This includes the analysis of all the data required to meet the chosen objectives.

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Inclusion Criteria

Male or female, age 18years or older

Females must be surgically sterile or postmenopausal or using a highly effective form of birth control throughout the duration of the study such as an oral contraceptive, or double barrier method contraception condom with spermatocide or IUD. Females in certain categories (not sexually active, vasectomized partner, tubal occlusion) will be admitted at the discretion of the investigator on a case-by-case basis.

Females must have a negative urine pregnancy test at screening and other visits specified in this protocol unless documented to have a hysterectomy or post-menopausal.

Clinical history of moderate to severe CIU at the time of screening, as defined by pruritus and hives for > 3 days in a 7-day period for > 6 consecutive weeks despite treatment with H1 antihistamine.

CIU diagnosis > 3 months (by history)

No underlying etiology clearly defined for urticaria (main manifestation cannot be physical urticaria).

Non-diary based UAS scores ≥ 2 at either the screening visit (Week -1), at the run-in visit (Day 1), or on Day 15.

A diary-based UAS7 score ≥ 16 during the screening or run-in periods despite stable doses of H1 antihistamine.

Compliance with study procedures during run-in period (e.g., completion of the study diary).

Exclusion Criteria

1. Pregnant females, confirmed by a positive Human Chorionic Gonadotropin (HCG) test, or females with plans to become pregnant or breastfeed during the duration of the study.

2. Recent history of drug or alcohol abuse (within 3 years prior to screening visit).

3. Inability or unwillingness of a participant to give written informed consent or comply with study protocol.

4. Use of any investigational drugs within 30 days of screening.

5. Active atopic dermatitis or other skin disease associated with pruritus during the time of the study, which require treatment with topical corticosteroids.

6. Clinically relevant major systemic disease (making interpretation of the study results difficult) including a history of anaphylaxis.

7. Inability to comply with study and follow-up procedures

8. Patients may not take during treatment period or have been taking within the past one-month any of the following medications/treatments: regular (daily/every other day) hydroxychloroquine, sulfasalazine, dapsone, methotrexate, cyclosporin, IVIG, or other monoclonal antibody therapies.

9. Patients may not take doxepin during screening, run-in, or treatment period or have been taking doxepin within the past 2 weeks regularly (daily/every other day) prior to screening.

10. Patients may not use cutaneous corticosteroid (intranasal, inhaled, and ophthalmic steroids are permitted) during the screening, run-in, or treatment phases.

11. Patients may not use oral or systemic steroids during the study or within 4 weeks prior to enrollment.

12. Patients may not take H2 antihistamines and leukotriene receptor antagonists within 7 days before screening, during the screening, run-in, or treatment phases. The exception will be if they are already on these medications for the treatment of GERD, asthma or allergic rhinitis.

13. Contraindications to diphenhydramine.

14. Any clinically relevant abnormal findings in clinical chemistry, hematology, physical examination, pulse, blood pressure at baseline, which, in the opinion of the investigator, could put the patient at risk because of his/her participation in the study.

15. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or may compromise the quality of the data obtained from the study.

Participant Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

The participant elects to withdraw consent from all future study activities, including follow-up.

Patient decision. The patient is at any time free to discontinue treatment, without prejudice to further treatment

The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).

The participant dies.

The participant develops a medical condition or is initiated on new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the

participant's ability to comply with study requirements or may compromise the quality of the data obtained from the study.

The participant meets any of the individual stopping rules as delineated in Section 8.

Patient is non-compliant with study protocol in the judgment of the PI.

Management of Participant Withdrawal

Patients are at any time free to withdraw from study, without prejudice to further treatment (withdrawal of consent). Such patients will always be asked about the reason(s) and the presence of any adverse events. Adverse events will be followed up (See Sections 0 and 0); the patient should return patient diaries, questionnaires, and all study drugs. If possible, they will be seen and assessed by an investigator.

For patients withdrawn due to incorrect inclusion, no further assessments will be performed except for the completion of the Follow-up Visit. This is done out of concern for the patients' safety.

If a patient prematurely discontinues the study after drug treatment the patient will be asked to complete the final visit at the time of discontinuation. For patient withdrawn due to study specific discontinuation criteria, see Section 4.3.

Withdrawn subjects will not be replaced.

INTERVENTION MATERIALS

Investigational PRODUCT (s)/INTERVENTION (s)

Omalizumab is a sterile, white, preservative free, lyophilized powder contained in a single use vial that is reconstituted with Sterile Water for Injection (SWFI), USP, and administered as a subcutaneous (SC) injection. Each 202.5 mg vial of omalizumab also contains L-histidine (1.8 mg), L-histidine hydrochloride monohydrate (2.8 mg), polysorbate 20 (0.5 mg) and sucrose (145.5 mg) and is designed to deliver 150 mg of omalizumab in 1.2 mL after reconstitution with 1.4 mL SWFI, USP.

Packaging and Labeling

Omalizumab will be supplied by the manufacturer Novartis.

Preparation, Administration, and Dosage

Omalizumab will be prepared for administration according to the FDA approved package insert. A pharmacist at the research pharmacy located within the Johns Hopkins Asthma and Allergy Center will dispense the study drug and rescue medications to subjects. The drug will be administered by the research nurse typically in the upper arm region. Subjects will be observed for 2 hours post each of the 3 omalizumab monthly injections and provided with an epipen. Subjects will be trained in the appropriate use of the epipen.

Accountability of Investigational Product(s)/ Intervention(s)

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the intervention material(s), including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any intervention material(s) accidentally or deliberately destroyed.

The study site will maintain records for receipt, storage, use, and disposition. A dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of the intervention material(s) dispensed.

All records regarding the disposition of the investigational product(s)/intervention material(s) will be available for inspection. All residual product(s) will be destroyed or retained for future in vitro studies.

Assessment of Compliance with Intervention Material(s)

All study drug will be supplied on site at the study site. Monitoring of basophil IgE receptor levels will be performed as a measure of PD. The pharmacy will keep records of doses dispensed and returned pills will be counted.

Discontinuation of Intervention Material(s)

Refer to Section 0 and Section 0.

OTHER MEDICATIONS

Concomitant Medications

Concomitant medications may be used as follows: loratadine 10 qd, desloratadine 5 mg qd, fexofenadine 180 mg qd, cetirizine 10 mg qd or levocetirizine 5 mg qd as once daily medications at the current FDA-approved dose. Patients taking H2 blockers for diseases other than CIU (e.g. Gastroesophageal Reflux Disease) will be permitted to continue their use during the study. Continued use of leukotriene receptor antagonists will be allowed only if sole indication is for the treatment of asthma and/or allergic rhinitis. Inhaled asthma controllers, including corticosteroids, are also permitted during the study. These diseases must be recorded as part of the medical history collected during the screening period. All concomitant medication and changes in treatment will be recorded in appropriate section.

Prophylactic Medications

None

Rescue Medications

Diphenhydramine 25 mg will be provided as a rescue medication, which may be used up to three times a day as needed.

Epipens are issued as a rescue medicine for the risk of anaphylaxis with omalizumab.

Prohibited Medications

Oral or cutaneous corticosteroids or other immunosuppressive medications (e.g. hydroxychloroquine, methotrexate, sulfasalazine, dapsone, cyclophosphamide) will be prohibited at enrollment but oral corticosteroids may be allowed as a rescue medication. In this case, the subjects would be discontinued from the study.

The use of leukotriene receptor antagonists (e.g. montelukast) will not be allowed within 7 days of screening, during the screening, run-in, or treatment phases. The only exception will be the use of leukotriene receptor antagonists for asthma or allergic rhinitis (Section 0). Doxepin may not be taken during screening, run-in, treatment, or within the past 2 weeks regularly (daily/every other day) prior to screening.

STUDY VISITS AND PROCEDURES

Enrollment and Randomization

A dedicated clinical study coordinator will be responsible for advertising, screening and providing consents to the study subjects. This research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any screening study procedures. Participants who are deemed eligible for the study (see Section 4.1, 4.2, and 4.3) will be enrolled and assigned a unique participant number.

Screening Visit(s)

Visit 1, (Week -3)

Obtain informed consent document signed by subject and assign number.

Review inclusion and exclusion checklist.

Demographic information, medical and medication history including diagnosis of CIU for 3 months.

Urine pregnancy test for all females unless surgically sterile or postmenopausal.

Comprehensive physical exam including vital signs, body weight and height, and skin exam.

Complete In Clinic UAS: Must have a non-diary based daily urticaria activity score (UAS) score ≥ 2 established in the outpatient setting based on the patient's condition over 12 hours prior to the visit; the UAS is a composite score of pruritus (0–3) and number of hives (0–3) with a maximum value of 6.This requirement may be met either at screen, run-in visit (Day 1), or Day 15.

Must have been on an approved dose of an H1 antihistamine for CIU such as loratadine 10 mg once a day or equivalent, for at least 7 days prior to the screening visit. Approved agents include loratadine 10 mg qd, desloratadine 5 mg qd, fexofenadine 180 mg qd, cetirizine 10 mg qd or levocetirizine 5 mg qd as once daily medications at the current FDA-approved dose. If needed for symptom control, up to 4 fold standard dosing is allowed.

Obtain Safety labs: Complete blood count (including platelet count) with differential glucose, BUN, bicarbonate, phosphorus, magnesium, creatinine, total and direct bilirubin, alkaline phosphatase, total protein, albumin, AST, (ALT), LDH, and calcium, store serum tube for baseline IgE measures and autoantibody testing (15 mL blood). Urine collection for measurement of urine prostaglandins and eicosanoids up to 10cc per collection.

Must be willing to fill out a twice daily patient diary to establish the patient's seven-day urticaria activity score (UAS7)

Dispense one week diary with instructions (Appendix 2)

Assessment of adverse events during study visit

Schedule next visit (in 1 week).

Visit 2, (Day -14 ± 2 days): Start of Run-In Visit

Assessment of adverse events that may have occurred since the previous visit

Confirmation of inclusion/exclusion criteria

Review of safety lab values and confirm that they are within normal range.

Review UAS diary reporting. To be eligible to begin the **run-in period (Week -2 to 0)**, the patients:

Must have a UAS7 score ≥ 16 established in the screening week with no missing entries.

Must remain on stable dose of H1 antihistamine at dose (not including antihistamine rescue medication) established at the screening visit.

Complete In Clinic UAS.

Brief physical exam (Vital Signs, Skin Exam, Cardiovascular Exam, Respiratory Exam).

Draw blood for basophil enumeration, BHR, flow, and serum studies (32 ml)

Whole blood for basophil flow assays (4 mL green top tube)

Tube for BHR, Blood histamine content, basophil enumeration to determine if patient meets criteria for mRNA profiling studies(> 1.5 x106 cells/100 cc) (28 mL EDTA syringe)

Urine collection for measurement of urine prostaglandins and eicosanoids

Dispense 2 week UAS diary for patient symptom recording and rescue medicine Benadryl supplements.

Schedule the next visit (in 2 weeks).

Treatment Study Visit(s)

Visit 3 (Day 0): First treatment visit

Assessment of adverse events that may have occurred since the previous visit.

Confirmation of inclusion/exclusion criteria.

Complete In Clinic UAS.

Urine pregnancy test for all females unless surgically sterile or postmenopausal.

Review diary information to confirm compliance (90% filled) and adequate disease activity for drug treatment in the latter 7 days (Day-7 to Day-1) to meet entry criteria (UAS score ≥ 16 during screening or run-in periods). Record Benadryl supplement use.

Focused physical exam of the head and neck, heart, lungs and skin including vital signs

Complete Skindex survey.

Draw blood for mechanistic outcomes (87 mL)

Serum sample for free IgE measure/autoantibody testing (5 mL stored)

Whole blood for basophil flow assays (4 mL green top tube)

Tube for BHR and Blood histamine content, basophil enumeration (28 mL EDTA syringe)

Blood sample for dendritic cell functional study ± basophil mRNA profiling (50 mls) only in subjects with basophils > 15,000 basophils/ml).

Urine collection for measurement of urine prostaglandins and eicosanoids

Perform non-lesional skin biopsy. Provide instructions for biopsy site care.

Inject subject with omalizumab 300 mg. Observe for 2 hours for signs of anaphylaxis..

Dispense epipen and educated patient on use. Provide rescue medicine (25 mg diphenhydramine TID prn, 1 month supply).

Remind patient to complete twice daily UAS diary for symptom recording.

Schedule the next visit the next day.

Visit 4 (Day 1): Post 1st Treatment ,Week 0

Assessment of adverse events that may have occurred since the previous visit.

Complete In Clinic UAS.

Review diary information to confirm compliance.

4. Draw blood for mechanistic outcomes (32 mL)
    a. Whole blood for basophil flow assays (4 mL green top tube)
    b. Tube for BHR, Blood histamine content, baso count (28 mL EDTA syringe)
5. Urine collection for measurement of urine prostaglandins and eicosanoids
6. Schedule the next visit in 2±1 days

Visit 5 (Day 3 ±1): Post 1st Treatment, Week 0

1. Assessment of adverse events that may have occurred since the previous visit
2. Complete In Clinic UAS.
3. Review diary information to confirm compliance (90% filled, Day 0 to date).
4. Draw blood for mechanistic outcomes (32 mL)
    a.Whole blood for basophil flow assays (4 mL green top tube)
    b.Tube for BHR and Blood histamine content (28 mL EDTA syringe)
5. Urine collection for measurement of urine prostaglandins and eicosanoids
6. Schedule the next visit in 3 days ±1

Visit 6 (Day 6 ±1): Post 1st Treatment, Week 1

1. Assessment of adverse events that may have occurred since the previous visit

2. Complete In Clinic UAS.

3. Review diary information to confirm compliance (90% filled, Day 0 to date). Determine if UAS7 scores to date have declined by MID > 5 from baseline. If so perform mid study non- lesional biopsy and basophil mRNA profiling (if appropriate subject).

4. Focused physical exam of the head and neck, heart, lungs and skin including vital signs

5. Draw blood for mechanistic outcomes (37 - 87 mL)

a. Serum sample for free IgE measure/autoantibody testing (4 mL stored)

b. Whole blood for basophil flow assays (5 mL green top tube)

c. Tube for BHR and Blood histamine content (28 mL EDTA syringe)

d. If indicated -Blood sample for dendritic cell functional study ± basophil mRNA profiling (50 mls) only in subjects with basophils > 15,000 basophils/ml). Perform only one time during treatment period.

6. If indicated by UAS7 decline, perform non-lesional skin biopsy. Provide instructions for biopsy site care.

7. Urine collection for measurement of urine prostaglandins and eicosanoids

8. Schedule the next visit in 4 days ±2

Visit 7 (Day 10 ±2): Post 1st Treatment, Week 2

1. Assessment of adverse events that may have occurred since the previous visit

2. Complete In Clinic UAS.

3. Review diary information to confirm compliance (90% filled, Day 0 to date). Determine if UAS7 scores to date have declined by MID > 5 from baseline. If so perform mid study non- lesional biopsy and basophil mRNA profiling (if appropriate subject).

4. Focused physical exam of the head and neck, heart, lungs and skin including vital signs

5. Draw blood for mechanistic outcomes (32 - 82 mL)

a. Whole blood for basophil flow assays (4 mL green top tube)

c. Tube for BHR and Blood histamine content (28 mL EDTA syringe)

d. If indicated -Blood sample for dendritic cell functional study ± basophil mRNA profiling (50 mls) only in subjects with basophils > 15,000 basophils/ml). Perform only one time during treatment period.

6. If indicated by UAS7 decline, perform non-lesional skin biopsy. Provide instructions for biopsy site care.

7. Urine collection for measurement of urine prostaglandins and eicosanoids

8. Schedule the next visit in 10 days ±2.

Visit 8 (Day 20 ±2): Post 1st Treatment, Week 3

1. Assessment of adverse events that may have occurred since the previous visit

2. Complete In Clinic UAS.

3. Review diary information to confirm compliance (90% filled, Day 0 to date). Determine if UAS7 scores to date have declined by MID > 5 from baseline. If so perform mid study non- lesional biopsy and basophil mRNA profiling (if appropriate subject).

4. Focused physical exam of the head and neck, heart, lungs and skin including vital signs

5. Draw blood for mechanistic outcomes (32 - 82 mL)

a. Whole blood for basophil flow assays (4 mL green top tube)

b. Tube for BHR and Blood histamine content (28mL EDTA syringe)

c. If indicated -Blood sample for dendritic cell functional study ± basophil mRNA profiling (50 mls) only in subjects with basophils > 15,000 basophils/ml). Perform only one time during treatment period.

6. If indicated by UAS7 decline, perform non-lesional skin biopsy. Provide instructions for biopsy site care.

7. Urine collection for measurement of urine prostaglandins and eicosanoids

8. Schedule the next visit in 10 days ±4

Visit 9 (Day 30 ±2): Second Treatment Visit, Week 4

1. Assessment of adverse events that may have occurred since the previous visit

2. Complete In Clinic UAS.

3. Review diary information to confirm compliance (90% filled, Day 0 to date). Determine if UAS7 scores to date have declined by MID > 5 from baseline. If so perform mid study non- lesional biopsy and basophil mRNA profiling (if appropriate subject). If MID has not been reached, perform non-lesional biopsy and basophil mRNA profiling (if appropriate subject)

4. Focused physical exam of the head and neck, heart, lungs and skin including vital signs

5. Draw blood for mechanistic outcomes (37 - 87 mL)

a. Serum sample for free IgE measure/autoantibody testing (5 mL stored)

b. Whole blood for basophil flow assays (4 mL green top tube)

c. Tube for BHR and Blood histamine content (28 mL EDTA syringe)

d. If indicated -Blood sample for dendritic cell functional study ± basophil mRNA profiling (50 mls) only in subjects with basophils > 15,000 basophils/ml). Perform only one time during treatment period.

6. If indicated by UAS7 decline or study visit, perform non-lesional skin biopsy. Provide instructions for biopsy site care.

7. Urine collection for measurement of urine prostaglandins and eicosanoids

8. Inject subject with omalizumab 300 mg. Observe for 2 hours for signs of anaphylaxis..

9. Dispense rescue medicine (25 mg diphenhydramine TID prn, 1 month supply).

10. Schedule the next visit in 30 days ±7

Visit 10 (Day 60 ±7): Third Treatment Visit, Week 8

1. Assessment of adverse events that may have occurred since the previous visit
2. Complete In Clinic UAS.
3. Review diary information to confirm compliance (90% filled, Day 0 to date).
4. Focused physical exam of the head and neck, heart, lungs and skin including vital signs
5. Draw blood for mechanistic outcomes (32 mL)
    a. Whole blood for basophil flow assays (4 mL green top tube)
    b. Tube for BHR and Blood histamine content (28 mL EDTA syringe)
6. Urine collection for measurement of urine prostaglandins and eicosanoids
7. Inject subject with omalizumab 300 mg. Observe for 2 hours for signs of anaphylaxis..
8. Dispense rescue medicine (25 mg diphenhydramine TID prn, 1 month supply).
9. Schedule the next visit in 30 days ±7

Visit 11 (Day 90 ±7): Final visit Week 12

1. Assessment of adverse events that may have occurred since the previous visit
2. Complete In Clinic UAS.
3. Comprehensive physical exam including vital signs, body weight and height, and skin exam.
4. Review diary information to confirm compliance (80% filled, Day 0 to date).
5. Complete Skindex survey
6. Draw blood for mechanistic outcomes (47 mL) and safety labs
    a. Serum sample for free IgE measure/autoantibody testing (5 mL stored)
    b. Whole blood for basophil flow assays (4 mL green top tube)
    c. Tube for BHR and Blood histamine content (28 mL EDTA syringe)
    d. Obtain Safety labs: Complete blood count (including platelet count) with differential and CMP (10 mL blood).
7. Urine collection for measurement of urine prostaglandins and eicosanoids
8. Perform non-lesional skin biopsy. Provide instructions for biopsy site care. Arrange contact for follow-up call.
9. Collect final diary for patient symptom recording.

As needed visits

Subjects may come in at any time at the discretion of the investigator or the subject for follow up.

Early discontinued patients will be required to report for safety follow-up visit within a month of study discontinuation.

7.4 Visit Windows

Study visits should take place within the time limits below:

As detailed above, most visits have a ± 2 day window except as indicated for visits in the first week ±1, and study visits 9-11 (±4-7).

SAFETY PROCEDURES

Stopping Rules

Study Stopping Rules

Study enrollment and ongoing study procedures will be suspended pending expedited review of all pertinent data by the institutional review board (IRB), and medical monitor if any one of the following occurs:

A serious adverse event (Section 0)

Individual Stopping Rules

A study participant will be discharged from the study if he/she suffers:

A serious adverse event. (Section 0)

A severe, unanticipated, drug-related event (Section 0)

Pregnancy (Section 0)

Premature Discontinuation of Intervention(s) with continued study participation/follow-up

Continued follow up if the intervention were discontinued for safety reasons would be based on the visit number in the protocol at the time of drug discontinuation and the possibility of evaluating outcome.

Premature Termination Follow-up

Participants who are prematurely terminated from the study will be followed to monitor safety for a minimum of 30 days or until resolution of the disqualifying event whichever is longer or until the Medical Monitor and the Principal Investigator determine that the follow-up is complete.

Participant Replacement

Participants who prematurely terminate from this study will not be replaced.

Adverse Events

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting* and *ICH E6: Guideline for Good Clinical Practice,* and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events version 3.0* (Aug. 9, 2006). The Investigators conducting this trial have reviewed these criteria and consider them appropriate for this subject population.

Steps included in the protocol to enhance patient safety are as follows: Subjects will receive omalizumab in room 3B. 46 at JHAAC, which is equipped with an anaphylaxis treatment kit. The PI and study team have previously conducted several studies of CIU with omalizumab in this research space and are experienced in this administration and safety monitoring. In clinical care, omalizumab is approved since 2003 and has been given to > 100,000 pts using a similar practice of administration. The PI and/ or study nurse is present during the dosing observation period of 2 hours to manage anaphylaxis or symptoms suggestive of anaphylaxis if they occur. In this case, we would administer epinephrine via an epipen. All subjects are also dispensed epipens as well and trained on their proper use. We will treat subjects at the first signs of anaphylaxis as per AAAAI guidelines and practice parameters.

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product. The XOLAIR(r) (omalizumab) Events of Special Interest are:

- Anaphylaxis
- Drug-induced liver injury
- Suspected transmission of an infectious agent by the study drug

Adverse Event Definition

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, abnormal laboratory finding, or disease that is temporally associated with participation in this study whether considered related to the study or not.

Any adverse event that occurs from the moment the subject has signed the consent form will be recorded and is reportable. An adverse event will be followed until any of the following takes place: a) it is resolved, b) participant is stable, c) a minimum of 30 days after participant is terminated from the study or d) the Medical Monitor and the Principal Investigator determine that follow-up is complete.

Serious Adverse Event (SAE) Definitions

An SAE is defined as "any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution." This includes but is not limited to any of the following events:

Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of study will be reported whether it is considered to be study-related or not.

A life-threatening event. A life-threatening event is any adverse experience that, in the view of the investigator, places the study participant at immediate risk of death from the reaction as it occurred.

An inpatient hospitalization or prolongation of existing hospitalization.

Persistent or significant disability.

An event that required intervention to prevent permanent impairment or damage.

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Congenital anomaly or birth defect.

Regardless of the relationship of the adverse event to the study, the event will be reported as an SAE if it meets any of the above definitions.

'Expected' verses 'Unexpected' Adverse Event Definition

A suspected adverse reaction is considered "expected" when it is listed in the investigator brochure, the package insert or the protocol. An adverse event is considered "unexpected" when its nature or severity is not consistent with the information that is provided in the Package insert, and/or this protocol (Section 0).

Management of Adverse Events

Collecting Procedure

Adverse events may be discovered through any of these methods:

Observing the participant.

Questioning the participant, with standardized questions at the beginning (except Visit 1) and the end of all study visits.

Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event.

Recording and Reporting Procedure

Throughout the study the Principal Investigator will record **all** adverse events on appropriate adverse event case report forms regardless of their severity or relation to the study.

NOTIFYING THE MEDICAL MONITOR

The Principal Investigator will ensure the timely dissemination of AE information to the Medical Monitor.

NOTIFYING THE INSTITUTIONAL REVIEW BOARD

The Principal Investigator will ensure the timely dissemination of AE information to the IRB in accordance with applicable regulations and guidelines.

Grading and Attribution

GRADING CRITERIA

In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 3.0* (published Aug. 9, 2006). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

Adverse events listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = Mild adverse event.

Grade 2 = Moderate adverse event.

Grade 3 = Severe and undesirable adverse event.

Grade 4 = Life-threatening or disabling adverse event.

Grade 5 = Death.

Adverse events not included in the NCI-CTCAE listing, which have relative specificity for this protocol will be recorded and graded 1 to 5 according to the grade definition provided below:

For anaphylaxis:

Grade 1= Oral urticaria or erythema

Grade 2= Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis

Grade 3= Marked dysphagia, hoarseness, and/or stridor; shortness of breath, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness

Grade 4= Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

Grade 5= Death

Other adverse events not included in the NCI-CTCAE listing, will be recorded and graded 1 to 5 according to the General Grade Definition provided in the Table below:

Grade 1	**Mild**	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, e.g., aspirin for simple headache, acetaminophen for post-surgical pain).
Grade 2	**Moderate**	Mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 3	**Severe**	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible.
Grade 4	**Life-threatening**	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required hospitalization or hospice care probable.
Grade 5	**Death**	Death.

DEFINITION OF ATTRIBUTION

The relationship, or attribution, of an adverse event to the study will be determined by the Principal Investigator or designee by using the descriptors provided in the following table. The Principal Investigator or designee will record the determination of attribution on the appropriate adverse event or serious adverse event form. The relationship of an adverse event

to the investigational drug(s)/intervention(s)/procedure(s) or other study drug will be further determined.

NCI-CTCAE attribution of adverse events

Code	**Descriptor**	**Definition**
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related to study
RELATED CATEGORIES		
2	Unlikely	The adverse event is doubtfully related to study
3	Possible	The adverse event may be related to study
4	Probable	The adverse event is likely related to study
5	Definite	The adverse event is clearly related to study

(For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE website: http://ctep.cancer.gov/reporting/ctc.html)

Management of Serious Adverse Events

SAE Collecting Procedure

Serious adverse events will be collected described for adverse events in Section 0.

SAE Recording and Reporting Procedure

Serious adverse events will be recorded on the **serious adverse event case report form** (Appendix 4) and will include a narrative of the event signed and dated by the Principal Investigator and the site Medical Monitor. In addition, the **FDA MedWatch 3500A form** will be filled out.

REPORTING PREGNANCY

The Principal Investigator will counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant will continue until the conclusion of the pregnancy, and a follow-up adverse event case report form detailing

the outcome of the pregnancy will be submitted. Follow-up information detailing the outcome of the pregnancy should be reported to the Medical Monitor as it becomes available. Any premature termination of the pregnancy will be reported. Any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE.

UNEXPECTED, NON-SERIOUS ADVERSE EVENTS

An unexpected, non-serious adverse event that is of Grade 2 severity or higher and study related will be recorded and reported to the site Medical Monitor under the serious adverse event reporting procedure outlined below in Sections 0, and 0.

SAE REPORTING CRITERIA AND PROCEDURES

The Principal Investigator will be notified by the staff no later than 24 hours after a staff member becomes aware of the SAE.

The site Medical Monitor will be notified by the Principal Investigator no later than 24 hours after the Principal Investigator becomes aware of the SAE.

Within another 24 hours, the site Medical Monitor will discuss with the Principal Investigator the impact of the SAE on the participant and on the study and will decide whether standard or expedited reporting will be applied. A finalized, initial **SAE case report form** (Appendix 4) and a **MedWatch 3500A form** will be generated by the Principal Investigator.

NOTIFYING THE MEDICAL MONITOR

The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the Medical Monitor.

NOTIFYING THE INSTITUTIONAL REVIEW BOARD

The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB in accordance with IRB regulations and guidelines. In addition, all SAEs will be reported to the study drug manufacturer, Novartis.

Protocol Deviations

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:

Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

Quality Assurance and Quality Control, section 5.1.1

Noncompliance sections 5.20.1, and 5.20.2.

Protocol Deviation Definition

A protocol deviation is any noncompliance with the protocol, Good Clinical Practice (GCP), or the study's Manual of Operations (MOP). Noncompliance may be either on the part of the participant, the investigator, or the study staff.

Management of Protocol Deviations

DETECTING PROTOCOL DEVIATIONS

Protocol deviations will be detected by direct questioning of participants and weekly review of CRFs to determine if visits take place in appropriate windows.

REPORTING PROTOCOL DEVIATIONS

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator and b) and will begin completing the Protocol Deviation Form (Appendix). The Principal Investigator will complete and sign the Protocol Deviation form and submit it to the site IRB, per IRB regulations.

SAMPLE SIZE CALCULATIONS AND STATISTICAL PLAN

Sample Size and Power Calculations

The sample size will be determined based on power calculations for the primary endpoint. The primary endpoint for the study utilizes the Urticaria Activity Score 7. This is a composite of a daily score of 0-3 for hives and 0-3 for itch with a maximal daily score of 6 and maximum weekly score of 42. Based on the Phase III study results with omalizumab in CIU, the baseline scores were UAS7=30 with a standard deviation of 7 in approximately 300 subjects completing run-in evaluation (25). Given that the minimally important difference based on this scoring system is 5 (55), we estimated the number of subjects needed for the present study. In that study, the 1-week test-retest correlation coefficient for UAS7 was 0.74. We have thus conservatively estimated the 4-week test-retest correlation to be 0.5. A sample size of 24 subject is adequate to detect a clinically relevant difference of 5 units of UAS7 with 80% power and 5% alpha error.

We are guided in our design by the pooled efficacy reported in Phase III CIU studies, past studies of kinetics and function reported for basophil receptor changes, and our knowledge of two equally distributed functional basophil subsets.

Based on pooled Phase III CIU trial results with omalizumab for 300 mg/monthly for 12 weeks (Maurer EAACI 2014), approximately 70% of CIU subjects have a 70% reduction in UAS7 and 40% are fully asymptomatic (UAS7=0). The median time to achieve an MID response is 1 week for those on standard dose anti-histamine therapy (25, 27), and 2 weeks for patients maintaining multiple therapies (4-fold standard dose antihistamines, ± LTRA, ± H2 blockers)(26) as compared to a median of 4-5 weeks for those treated with placebo. Overall, 70% of treated subjects across all phase III studies achieved an MID for itch score at week 2.

The worst-case scenario is that 30% percent of our omalizumab treated CIU subjects will not have a meaningful change in symptoms by 2 weeks. If we enroll 30 subjects and have a 20% dropout, we would expect 17 of 24 to have a significant clinical response (roughly 50/50 distribution for basophil subsets, CIU R and NR). Our plan is to look at multiple cellular parameters in treated patients and relate cellular changes to the timing of the MID for itch (>5 points) or hives (> 5 points) with the primary outcome being the percentage achieving a 50% rise in basophil anti-IgE functions at the time of reaching a clinical MID. We have also considered the concerns of basopenia, which may interfere with meaningful assessments through the study or allow reasonable collection of basophils for mRNA studies. Our longitudinal study involving active CIU subjects (n=164, with repeated sampling of basophil measures) reveals that 12-15% of individuals had extreme basopenia (56). We also expect that omalizumab therapy will yield an increase in circulating blood basophil presence. We have documented a 40-50% increase in blood basophils in Phase III study participants after 12 weeks therapy with monthly 300 mg injections (Saini, 2014 AAAAI meeting).

Data Analysis

Study Participant Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner:

Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.

Categorical data (i.e., sex and race) will be presented as enumerations and percentages.

The Wilcoxon signed rank test will be used to before and after treatment if a non-Gaussian distributed of data is generated; otherwise, if the data are normally distributed, and paired t test will be used. Wilcoxon singed rank tests will be used to compare continuous variables and Fisher's exact tests will be used to compare categorical variables. The level of significance employed will be 0.05. Relationships between the primary efficacy variable and various in vitro and in vivo variables in will be explored using the Pearson product-moment correlation coefficient or the Spearman rank correlation coefficient, as appropriate.

Summary statistics will be reported for all secondary efficacy variables. Continuous variables will be summarized by sample size, mean, median, standard deviation, minimum, and maximum. Discrete variables will be summarized by frequencies and percentages.

Primary Endpoint Analysis

The primary endpoint analysis is treated patients and relate cellular changes to the timing of the MID for itch (>5 points) or hives (> 5 points) with the primary outcome being the percentage achieving a 50% rise in basophil anti-IgE functions at the time of reaching a clinical MID.

Diary entries for itch and/or hive a.m./p.m. scores may be missing for some days. These missing scores will be imputed using the average of available corresponding scores in that week.

Patients whose post-baseline diaries are completely missing will not be included in the analysis.

Secondary Endpoint Analyses

This study is not designed or powered to perform hypothesis testing on secondary endpoints. All secondary analyses will be treated as supportive. P-values will be presented for the secondary endpoints but will not be adjusted for multiplicity and should be interpreted with caution.

Change in the weekly pruritus score from baseline to the 12 th week in the treatment period.

The pruritus score will be measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (intense). The weekly pruritus score is the sum of average daily pruritus scores over the previous 7 days.

Change in the weekly score for number of hives from baseline to the 12th week in the treatment period.

The number of hives is measured twice daily (a.m. and p.m.), on a scale of 0 (none) to 3 (>12). The weekly score of number of hives is the sum of the average daily scores over the previous 7 days.

Change in the amount of rescue medication (diphenhydramine 25 mg) from baseline to the 12th week in the treatment period using the question on the rescue medication use in the patient diary.

4. Change in *SKINDEX29* quality of life survey instrument with 29 questions on a 5-point Likert will be given at baseline and again at 90 days.

Exploratory Endpoint Analyses

Exploratory endpoints related to mechanisms of immune modulation and tolerance assessed prior to, during and after therapy, including those related to:

1) Change in basophil surface IgE, FcεRI and Syk by flow cytometry from baseline at 1, 3, 6, 10, 20, 30 and 90

2) Change in Dendritic cell surface IgE, FcεRI, and IgE receptor related functions from baseline at Days 1, 3, 6, 10, 20, 30 and 90

3) Change in Basophil anti-IgE, anti FcεRI, C5a, FMLP mediated histamine along with sensitivity measures from baseline at 1, 3, 6, 10, 20, 30 and 90 days

4) Change in serum free IgE measures from baseline at days 1, 3, 6, 10, 20, 30 and 90

5) Characterization of presence of serum autoantibody presence ± Syk inhibitors at baseline and at days 1, 3, 6, 10, 20, 30 and 90

6) Change in basophil enumeration by manual counting and blood histamine content from baseline at days 1, 3, 6, 10, 20, 30 and 90. This will determine the presence or absence of basopenia and identify subjects for mRNA profiling studies.

7) Change in basophil mRNA profiling baseline at 3 timepoints (baseline, meaningful clinical changes, and 30 days) in select subjects with sufficient basophil numbers as identified at

baseline measure.

8) Change in numbers of IgE +, FcεRI + cells in non-lesional skin biopsies from baseline to days 6 and 90 to monitor skin mast cells well as the MrgX2 receptor expression

9) Change in circulating leukocyte population numbers that are targeted by omalizumab such as blood basophils, eosinophil and lymphocyte counts by automated analysis (week -3 to week 12).

10) Changes in urine prostaglandins and eicosanoids at baseline Day (-21, -14, 0) relative to post -treatment (1, 3, 6, 10, 20, 30 and 90).

Safety Analysis

Safety analyses will include AEs, SAEs, laboratory abnormalities, and physical examination abnormalities. All participants in the safety sample will be included in all safety analyses. Frequency of AEs will be tabulated by system organ class and preferred term, as well as by seriousness, severity, and treatment relatedness. Frequency of SAEs will be tabulated by system organ class and preferred term. For pertinent laboratory measurements, mean and mean change from baseline values will be presented by treatment group and visit. Frequency of physical examination abnormalities will be tabulated by treatment group.

Pharmacodynamic Data Analyses

Primary, secondary, and exploratory endpoints may be further analyzed based on the pharmacodynamic parameters assayed using blood basophil surface IgE and FceRI change flow cytometry assay. This assay was successfully used in several past omalizumab studies.

Medical History

Medical history within the past 12 months—including the existence of current signs and symptoms—will be collected for each body system.

Medication Use

All medications taken by or administered to study participants beginning 30 days before enrollment and continuing throughout the study will be collected. All medications used will be coded according to the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

Study Completion

The percent of participants who complete the study, losses to follow-up, times to lost to follow-up, and reasons for discontinuation (e.g., adverse events) will be presented.

Interim Analyses

Given the small study size, no interim data analysis is planned.

Deviations from Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment.

IDENTIFICATION AND ACCESS TO SOURCE DATA

Identifying Source Data

The investigator will keep accurate records to ensure that the conduct of the study is fully documented. Data derived from source documents will be transferred to protocol-specific CRFs. The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records and the data will be transferred to clinical CRFs, as applicable.

Updating Source Documentation

Documents describing the safety profile of an investigational product(s)/intervention material(s), such as the investigator's brochure and the package insert, will be amended as needed by the investigational product(s)/intervention material(s) manufacturer to ensure that the description of safety information adequately reflects any new clinical findings.

The Principal Investigator will provide the site Medical Monitor, and the IRB with the most up-to-date versions of the above documents as soon as the Principal Investigator becomes aware of any changes. For purchased investigational product(s)/intervention material(s), the Principal Investigator will confirm that there are no changes to the package insert every 3 months. In case of package insert changes, the Principal Investigator will notify the site Medical Monitor and the IRB.

Permitting Access to Source Data

The investigational site participating in this study will maintain the highest degree of confidentiality for the clinical and research information obtained from the participants in this clinical trial. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site will permit authorized representatives of the sponsor(s) and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information will be removed). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals.

QUALITY CONTROL AND QUALITY ASSURANCE

The Principal Investigator will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and participant study files are legible and complete for every participant. When the CRFs are complete, they will be reviewed by the Principal Investigator. All discrepancies identified will be resolved with the Principal Investigator and the CRFs will be amended as needed.

ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 Statement of Compliance

This study was designed to ensure the protection of subjects according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human subjects. This clinical study will be conducted using good clinical practice (GCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according to the criteria specified in this study protocol. Before study initiation, the protocol, the informed consent documents will be reviewed and approved by IRB, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

12.2 Informed Consent and Assent

The informed consent form will provide information about the study to a prospective participant to allow for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form prior to study participation.

The informed consent form will be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The Principal Investigator or an approved designee will discuss the consent with the prospective participant and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

12.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used to collect, store, and report participant information.

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Appendix 1. Schedule of Events

	Screen	Run-in	Treatment								
Day	-21	-14	0	1	3	6	10	20	30	60	90
Week	-3	-2	0	0	0	1	2	3	4	8	12
Visit #	1	2	3	4	5	6	7	8	9	10	11
Visit Window (Days)		± 2	± 2	0	±1	±1	± 2	± 2	± 4	±7	±7
General Assessments											
Informed consent, screening	X										
Inclusion/Exclusion Criteria	X	X	X								
Demographic Data	X										
Medical and Surgical History	X										
Vital Signs (Blood pressure, Pulse)	X	X	X			X			X	X	X
Weight, Height	X										
Comprehensive Physical Exam	X										X
Focused history and physical exam		X	X			X	X	X	X	X	
Physician In Clinic UAS Scoring	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication Use	X	X	X	X	X	X	X	X	X	X	X
Urine pregnancy test[1]	X		X						X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	X	X
Study Medications											
Omalizumab			X						X	X	
Primary Outcome Data											
Patient Recorded UAS7- Diary		X	X	X	X	X	X	X	X	X	X
Basophil HR-C5a, FMLP, anti-IgE, sensitivity, enumeration		X	X	X	X	X	X	X	X	X	X
Secondary Endpoint Data											
Skindex			X								X

	Screen	Run-in	Treatment								
Day	-21	-14	0	1	3	6	10	20	30	60	90
Week	-3	-2	0	0	0	1	2	3	4	8	12
Visit #	1	2	3	4	5	6	7	8	9	10	11
Safety Outcome Measures											
CBC with differential and platelets[3]	X										X
Comprehensive Metabolic Panel[4]	X										X
Pharmacodynamic Measures											
Basophil Surface IgE FceRI Assay, Syk		X	X	X	X	X	X	X	X	X	X
Exploratory Outcome Measures											
Dendritic Cell studies-Flow			X	X	X	X	X	X	X	X	X
Dendritic Cell function			X			X[5, 6]			X		
Basophil mRNA profile			X[5]			X[5, 6]			X[5]		
Total Blood Histamine Content		X	X	X	X	X	X	X	X	X	X
Non-lesional skin biopsy			X			X[6]					X
Urine Prostaglandins and Eicosanoids	X	X	X	X	X	X	X	X	X	X	X
Other Measures											
Serum studies- free IgE, autoantibodies[7]	X[7]		X[7]	X[7]	X[7]	X	X[7]	X[7]	X[7]	X[7]	X[7]

UAS = urticaria activity score.

[1] All women of childbearing potential (including those who have had a tubal ligation) will have a urine pregnancy test at screening and prior to study drug administration. If the urine pregnancy test results are positive, dosing should be held and a serum pregnancy test performed by the central laboratory. Urine pregnancy tests will be performed at the site.

[2] Daily diary completed twice a day by the patient. Includes UAS7 (itch score, number of hives), largest size of hives, rescue medication (diphenhydramine) use, angioedema episodes, and number of calls to doctor or nurse.

[3] Hemoglobin, hematocrit, platelet count, RBC count, WBC count, percent and absolute differential count (neutrophils, bands, eosinophils, lymphocytes, monocytes, basophils, other cells).

[4] Sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, magnesium, total and direct bilirubin, total protein, albumin, ALT, AST.

[5] Basophil profiling will only occur in subjects with sufficient basophil number at baseline examination.

[6] Biopsy, DC function assays, and mRNA profiling (if indicated) will be timed to onset of MID symptom change.

[7] Participants will have serum banked for free IgE measurements and autoantibody testing. Purple top stored at Visit 1.

Appendix 2: Patient Diary

Patient Diary

General Instructions

Please answer each question to the best of your ability.

There are no right or wrong answers.

For each question, please choose the response that describes your experience.

Please pay close attention to the timeframe of interest. Some questions ask about the **past 12 hours**, while others ask about the **past 24 hours**.

Instructions for Counting the Number of Hives

Count each hive separately even if you have more than one hive grouped together with other hives.

Patient Diary

Today's Date

		-				-				

Day **Month** **Year**

Please complete this section <u>every morning</u> throughout the duration of the study. (Please circle only one response.)

1. Thinking about the **<u>past 12 hours</u>**, please record the severity of itch and the number of hives you may have had associated with your skin condition. **<u>Please count each hive separately</u>** even if you have more than one hive grouped together with other hives.

Itch (severity)	Hives (number)
0 = none 1 = mild 2 = moderate 3 = severe	0 = none 1 = between 1 and 6 hives 2 = between 7 and 12 hives 3 = greater than 12 hives

2. Thinking about the **<u>past 12 hours</u>**, please record the severity of itch and the number of hives you may have had associated with your skin condition. **<u>Please count each hive separately</u>** even if you have more one than one hive grouped together with other hives.

Itch (severity)	Hives (number)
0 = none 1 = mild 2 = moderate 3 = severe	0 = none 1 = between 1 and 6 hives 2 = between 7 and 12 hives 3 = greater than 12 hives

Patient Diary

Today's Date

		-				-				

Day Month Year

Please complete this section <u>once each day</u> throughout the duration of the study (preferably at the same time each day).

(Please circle only one response.)

3. Please rate how much your hives or itch interfered with your sleep during the **<u>past 24 hours</u>**.

0 No interference

1 Mild, little interference with sleep

2 Moderate, awoke occasionally, some interference with sleep

3 Substantial, woke up often, severe interference with sleep

4. Please rate how much your hives or itch interfered with your daily activities during **<u>the past 24 hours</u>**. This could include work, school, sports, hobbies, and activities with friends and family.

0 No interference

1 Mild, little interference with daily activities

2 Moderate, some interference with daily activities

3 Substantial, severe interference with daily activities

Patient Diary

These next questions are about your symptoms and how you managed them during the past 24 hours.

5. During the **past 24 hours**, how many pills of diphenhydramine 25 mg did you use in order to control symptoms of your skin condition such as itch or hives?

0 = 0 pills
1 = 1 pill
2 = 2 pills
3 = 3 pills

6a. During the **past 24 hours**, did you have any rapid swelling on your face, (especially your eyelids or lips), inside your mouth (including your throat or tongue), or elsewhere on your body? This rapid swelling, also called angioedema, is at a deeper level under your skin than hives.

0 = No **(GO TO Question 7)** 1 = Yes

6b. If Yes, how did you treat this rapid swelling? **(Circle all that apply.)**

0 Did nothing (**GO TO Question 7**)
1 Took some prescription or non-prescription medication
2 Called my doctor, nurse or nurse practitioner
3 Went to see my doctor, nurse or nurse practitioner
4 Went to the emergency room at the hospital
5 Was hospitalized

7. During the **past 24 hours**, did you or someone else call your doctor, nurse or nurse practitioner because of your skin condition?

0 = No 1 = Yes

Appendix 3: In Clinic UAS

In-Clinic Urticaria Activity Score (Max 6)

The physician or the person he or she designates will provide the sum of the score of the patient's urticaria lesions (number of hives) and pruritus (itch) reflective of the patient's condition over the 12 hours prior to the visit using the following rating scale:

Pruritus:

0 = None

1 = Mild – minimal awareness, easily tolerated

2 = Moderate – definite awareness, bothersome but tolerable

3 = Severe – difficult to tolerate

Number of Hives:

0 = none

1= 1–6

2= 7–12

3= >12

Appendix 4: Serious Adverse Event Report Form

Serious Adverse Event (SAE) Report Form

STUDY NAME

Protocol Number:__

Site Number:__

Pt_ID:__

Visit Date:

__ __ / __ __ __ / __ __ __ __
d d m m m y y y y

SAE onset date: __ __ / __ __ __ / __ __ __ __
d d m m m y y y y

SAE stop date: __ __ / __ __ __ / __ __ __ __
d d m m m y y y y

Location of serious adverse event:_____

Was this an unexpected adverse event? ☐ Yes ☐ No

Brief description of participants with no personal identifiers:

Sex: ☐ F ☐ M Age: _____

Diagnosis for study participation:_____

_____

Brief description of the nature of the serious adverse event (attach description if more space is needed):

_____

_____

Category of the serious adverse event:

☐ Date of death ___/___/___ (dd/mmm/yyyy)
☐ Life threatening
☐ Hospitalization – initial or prolonged
☐ Disability/incapacity
☐ Congenital anomaly/birth defect
☐ Required intervention to prevent permanent impairment
☐ Other:_

Intervention type:

- ☐ Medication or nutritional supplement (specify):_____
- ☐ Device (specify):_____
- ☐ Surgery (specify):_____
- ☐ Behavioral/lifestyle (specify):_____

Relationship of event to intervention:

- ☐ Unrelated (clearly not related to the intervention)
- ☐ Possible (may be related to intervention)
- ☐ Definite (clearly related to intervention)

Was study intervention discontinued due to event? ☐ Yes ☐ No

What medications or other steps were taken to treat the serious adverse event?

_____

List any relevant tests, laboratory data, and history, including preexisting medical conditions:

_____

Type of report:

- ☐ Initial
- ☐ Follow-up
- ☐ Final

Signature of Principal Investigator: _____ Date: _____

Appendix 5: Skindex questionnaire

30.	I am angry about my skin condition.	☐1	☐2	☐3	☐4	☐5
31.	Water brothers my skin condition (bathing, washing, hands)	☐1	☐2	☐3	☐4	☐5
32.	My skin condition makes showing affection difficult.	☐1	☐2	☐3	☐4	☐5
33.	My skin is irritated.	☐1	☐2	☐3	☐4	☐5
34.	My skin condition affects my interactions with others.	☐1	☐2	☐3	☐4	☐5
35.	I am embarrassed by my skin condition.	☐1	☐2	☐3	☐4	☐5
36.	My skin condition is a problem for the people I love.	☐1	☐2	☐3	☐4	☐5
37.	I am frustrated by my skin condition.	☐1	☐2	☐3	☐4	☐5
38.	My skin is sensitive.	☐1	☐2	☐3	☐4	☐5
39.	My skin condition affects my desire to be with people.	☐1	☐2	☐3	☐4	☐5
40.	I am humiliated by my skin condition.	☐1	☐2	☐3	☐4	☐5
41.	My skin condition bleeds.	☐1	☐2	☐3	☐4	☐5
42.	I am annoyed by my skin condition.	☐1	☐2	☐3	☐4	☐5
43.	My skin condition interferes with my sex life.	☐1	☐2	☐3	☐4	☐5
44.	My skin condition makes me tired	☐1	☐2	☐3	☐4	☐5

Time ____:_____

How often over the past 3 months, do these statements apply to you?		ق1 Never	ا2 Rarely	ق3 Sometimes	ا4 Often	ق5 All the time
16.	My skin hurts.	ق1	ا2	ق3	ا4	ق5
17.	My skin condition affects how well I sleep.	ق1	ا2	ق3	ا4	ق5
18.	I worry that my skin condition may be serious.	ق1	ا2	ق3	ا4	ق5
19.	My skin condition makes it hard to work or do hobbies.	ق1	ا2	ق3	ا4	ق5
20.	My skin condition affects my social life.	ق1	ا2	ق3	ا4	ق5
21.	My skin condition makes me feel depressed.	ق1	ا2	ق3	ا4	ق5
22.	My skin condition burns or stings.	ق1	ا2	ق3	ا4	ق5
23.	I tend to stay at home because of my skin condition.	ق1	ا2	ق3	ا4	ق5
24.	I worry about getting scars from my skin condition.	ق1	ا2	ق3	ا4	ق5
25.	My skin itches.	ق1	ا2	ق3	ا4	ق5
26.	My skin condition affects how close I can be with those I love.	ق1	ا2	ق3	ا4	ق5
27.	I am ashamed of my skin condition.	ق1	ا2	ق3	ا4	ق5
28.	I worry that my skin condition may get worse.	ق1	ا2	ق3	ا4	ق5
29.	I tend to do things by myself because of my skin condition.	ق1	ا2	ق3	ا4	ق5

Appendix 6: Protocol deviation forms

JOHNS HOPKINS MEDICINE SCHOOL OF MEDICINE	**FORM R.F.4: PROTOCOL DEVIATION SUMMARY SHEET** Use to report **administrative and minor** departures from the IRB approved protocol that "does not affect the scientific soundness of the plan or the rights, safety, or welfare of human subjects." See IRB Guidance at: http://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/guidelines/protocol_deviations.html. Submit to JHM IRB: Use this form to answer the question 3.1 in the eIRB Continuing Review.

JHM IRB Application Number:
Principal Investigator:
Protocol Expiration Date:
Sponsor:
Sponsor Protocol Number:
Date Submitted to the IRB:

Date of Deviation	Study ID (No PHI, please)	Description of Deviation (attach extra pages, if needed)	Reason for Deviation and Corrective Action Plan	Sponsor Notification Date (required for IND/IDE studies)