

over a period of 100 days (Fig. 1A). Imaging showed marked improvement (Fig. 1C). The white-cell count normalized in parallel with the FGF23 levels. Blood transfusions, which had previously been given every 2 to 3 weeks, were avoided for 7 months.

Ultimately, the side effects necessitated intermittent dose interruptions. The patient's disease progressed, and his functional status deteriorated. Ten months after resuming therapy, and 5 years since the initiation of infliximab, the patient died. Additional details are provided in the Supplementary Appendix.

This case enabled us to identify a much-needed treatment for malignant tumor-induced osteomalacia; in addition, it shows how an approach based on personalized medicine, made possible by molecular diagnostics and mechanistically directed therapy, prolonged this patient's life. The dramatic biochemical and structural response to this treatment confirms the role of FGFR1 signaling in phosphaturic mesenchymal tumor growth and FGF23 production. The metaplastic differentiation from sarcoma to differentiated bone suggests that these tumors are derived from skeletal stem cells with the capacity to differentiate into osteoblast-like cells and that the acquisition of gain-of-function FGFR1 signaling both promotes tumorigenesis and blocks differentiation, a phenomenon that is reversed

by FGFR blockade. Infliximab as a treatment for unresectable or nonlocalized phosphaturic mesenchymal tumors is currently being investigated (NCT03510455).

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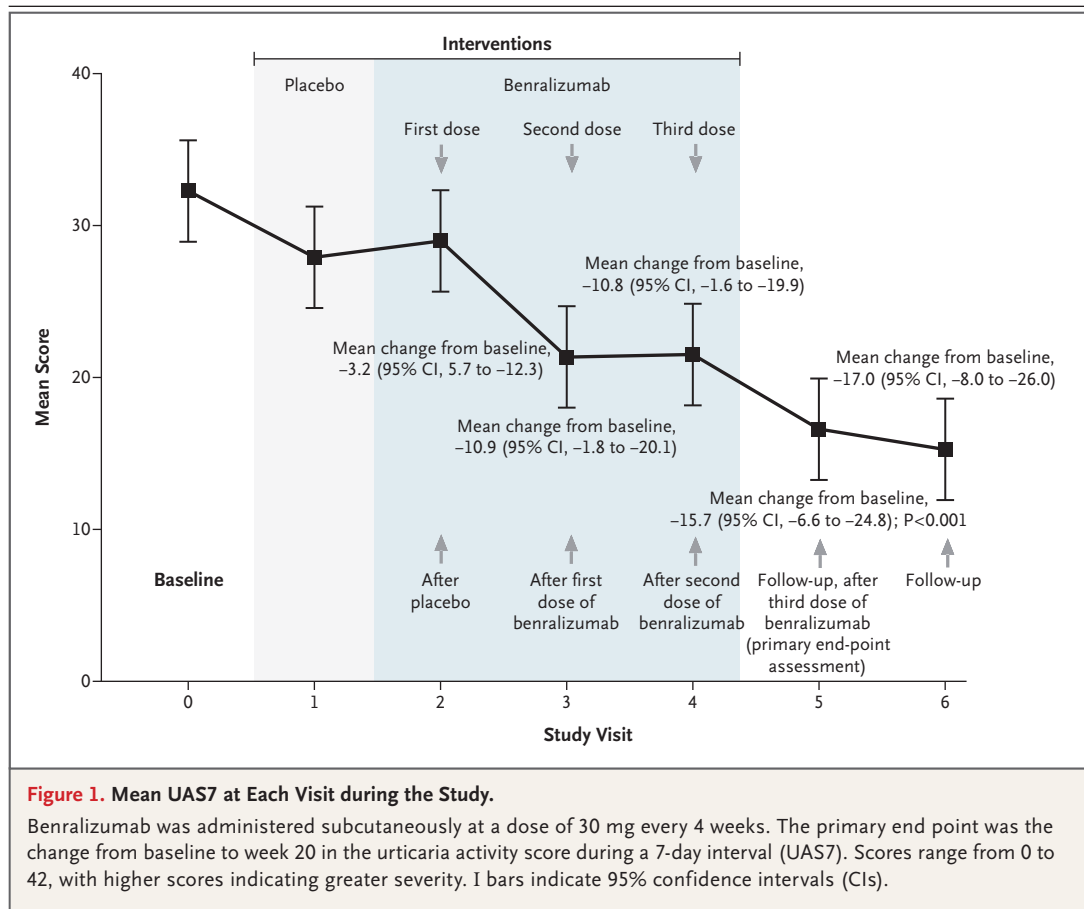
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Benralizumab for Chronic Spontaneous Urticaria

TO THE EDITOR: The standard care for chronic spontaneous urticaria includes second-generation H₁-antihistamines, but such treatment is often ineffective, even with four times the dose recommended by the Food and Drug Administration.¹ Urticarial lesions are characterized pathologically by increased lymphocytes with perivascular eosinophilic infiltrates, features that imply a role for interleukin-5.²

We investigated the effect of benralizumab, an anti-interleukin-5-receptor alpha monoclonal antibody, on chronic spontaneous urticaria that was unresponsive to second-generation H₁-antihistamines in a 24-week, single-center, single-blind, repeated-measures study (ClinicalTrials.gov number, NCT03183024). The study proto-

col is available with the full text of this letter at NEJM.org. A total of 12 patients (9 women and 3 men) were enrolled; the mean (±SD) age was 47.3±1.3 years. After a run-in period of 14 days, patients received a single dose of a placebo, followed by three monthly subcutaneous injections of benralizumab (30 mg); after completion of treatment, patients had two monthly follow-up visits. The primary end point was the change from baseline to week 20 (visit 5) in the urticaria activity score during a 7-day interval (UAS7) (scores range from 0 to 42, with higher scores indicating greater severity).³ Three patients (25%) withdrew or were withdrawn from the study after receiving the first dose of benralizumab (visit 3): 1 withdrew because of per-



sonal reasons, 1 withdrew because of nonresponse, and 1 was withdrawn because of loss to follow-up. Among these 3 patients, 2 had an improvement in the UAS7 and 1 did not have a response (and had previously also not had a response to omalizumab). The remaining 9 patients (75%) completed the study.

It was assumed that the urticarial lesions were not self-limiting⁴ and that any improvement in the UAS7 during the study was a result of the intervention. An intention-to-treat analysis, in which multiple imputation was used to take into account missing data for patients who withdrew, was performed with the use of SAS software, version 9.4 (SAS Institute). A generalized linear mixed-model regression analysis with random effects was used to assess the change in the UAS7 from baseline to the first visit after the receipt of placebo (visit 2) and from baseline to each visit after the receipt of the first dose of benralizumab (visits 3 through 6); adjustment

was made for the baseline UAS7 and the duration of disease.

The mean duration of chronic spontaneous urticaria symptoms was 7.0 years (95% confidence interval [CI], 0.2 to 18). Overall, the baseline UAS7 ranged from 16 to 42, indicating baseline disease that was consistent with moderate-to-severe chronic spontaneous urticaria. The change in the UAS7 after the administration of benralizumab was -10.9 points (95% CI, -1.8 to -20.1) at visit 3, -10.8 points (95% CI, -1.6 to -19.9) at visit 4, and -15.7 points (95% CI, -6.6 to -24.8; P<0.001) at visit 5 (the primary end point) (Fig. 1). At the completion of the study, 5 patients had had a complete response (i.e., a UAS7 of 0), and 2 patients had had a UAS7 of 6 or lower, which was considered to be a partial response, at visit 5 or visit 6. There was a sustained mean change in the UAS7 from baseline to week 24 (visit 6) (i.e., 8 weeks after the last dose of benralizumab) (mean change, -17.0 points; 95% CI,

−8.0 to −26.0). Both components of the UAS7 (i.e., pruritus severity and wheal size) decreased to a similar extent. No drug-related adverse events were reported during the study. Similar efficacy results were observed in the initial study that assessed omalizumab (an anti-IgE antibody) for the treatment of chronic spontaneous urticaria.⁵ In addition, the percentage of patients who had a complete response in our study was similar to that in the omalizumab study.⁵

The prolonged clinical effect of benralizumab in the patients who had a complete response after discontinuation of the treatment may be explained by a modification of the biologic pathways that are important for promoting persistent cutaneous inflammation associated with chronic spontaneous urticaria. These observations support the use of benralizumab in the treatment of chronic spontaneous urticaria that is unresponsive to second-generation H₁-antihistamines and provide evidence of a pathogenic role for infiltrating eosinophils. Therefore, a double-blind, placebo-controlled trial in a large patient population is warranted.

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Amoxicillin Course Length or Use in Childhood Pneumonia in Underserved Areas

TO THE EDITOR: The trials by Ginsburg et al.¹ and Jehan et al.² (July 2 issue) question the recommended treatment of nonsevere pneumonia in children younger than 5 years of age. The trial conducted by Ginsburg et al. in Malawi showed that a 3-day course of amoxicillin was noninferior to a 5-day course, despite the former regimen having been shown to be insufficient in patients with radiologically confirmed pneumonia in a previous trial in Israel.³ The other trial, conducted by Jehan et al. in Pakistan (Randomized Trial of Amoxicillin Versus Placebo for [Fast Breathing] Pneumonia [RETAPP]), did not show noninferiority of placebo to a 3-day course of amoxicillin in patients with fast-breathing pneumonia.

More than 93% of the Pakistani children who

had been randomly assigned to receive placebo recovered rapidly without relapse. The World Health Organization (WHO) criteria for diagnosing pneumonia probably capture a high proportion of viral illness,⁴ and both trials are limited by the lack of radiologic or laboratory assessment.

The identified predictors of treatment failure could guide new diagnostic definitions. A subgroup analysis of treatment-failure rates among nonvaccinated children would also be helpful, given that both trials reported vaccine coverage that was up to 30% lower than the average national rates.⁵

The effect of overtreatment on the community should not be neglected in the era of increasing antimicrobial multiresistance. Therefore, the critical question is more likely to be

Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Bernstein JA, Singh U, Rao MB, et al. Benralizumab for chronic spontaneous urticaria. *N Engl J Med* 2020;383:1389-91. DOI: 10.1056/NEJMc2016395

Supplement

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan (**section 8**, p33 of **Original protocol**), final statistical analysis plan, summary of changes (**section 8**, p33 of **Final protocol**)

ORIGINAL PROTOCOL

includes **Statistical Analysis Plan** (section 8, page 33)

Clinical Study Protocol

Drug Substance

Study Code

Edition Number 1

Date 21 December 2016

Treatment of chronic urticaria unresponsive to H1-antihistamines with an anti-IL5Ralpha monoclonal antibody

Sponsor:

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment
Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

PROTOCOL SYNOPSIS

Treatment of chronic urticaria unresponsive to H1-antihistamines with an anti-IL5Ralpha monoclonal antibody

Principal Investigator

Jonathan A. Bernstein

Study site(s)

Bernstein Clinical Research Center (office visits and sample collection) **Number of subjects planned**

18 human subjects (12 chronic urticaria cases, 6 normal controls)

Study period		Phase of development
Estimated date of first subject enrolled	2 nd Quarter, 2017	Phase 0
Estimated date of last subject completed	1 st Quarter, 2018	

Study design

This exploratory study will assess the efficacy of Benralizumab, anti-IL5 receptor α monoclonal antibody, in the treatment of chronic urticaria (CU) refractory to H1-antihistamines. This is a single center, single-blind, case-controlled, uniform dose multi-treatment (Benralizumab 30mg $\times$ 3 treatments), repeated measures study. The study will enrol up to 18 subjects (12 CU cases, 6 normal controls). The study duration is about one year.

Eligible subjects will be male or non-pregnant/non-lactating female subjects, age >18 and ≤ 70 years, with CU unresponsive to up to 4 $\times$ the FDA approved doses of H1-antihistamines and age, gender-matched non-CU healthy controls.

There will be 7 office visits. During screening visit 0, after signing an informed consent, CU subjects meeting inclusion/exclusion criteria with a Urticaria Activity Score 7 (UAS7) ≥ 4 , will be enrolled and asked to complete daily diaries for 2 weeks prior to visit 1 to ensure $>70\%$ compliance with diaries. During visit 1, subjects will be administered a placebo subcutaneously. All subjects will return after 4 weeks and those subjects still with a UAS7 ≥ 4 at visit 2 will be switched to Benralizumab 30mg subcutaneously and continue to receive Benralizumab at visits 3 and 4 at one month intervals. Subjects will be assessed after completing the medication at visit 5 and after being off medication for 60 days at visit 6.

Clinical outcomes, i.e. UAS7 and urticarial control test (UCT) score and the urticaria quality of life questionnaire, will be assessed at every (screening visit and visits 1-6) visit. Blood samples and skin biopsies for efficacy assessment will be collected at baseline, 4-weeks post-placebo and 4-weeks after the third dose of Benralizumab. Blood samples in such assessments

will be used for quantification of anti-FCER1, anti-TPO and skin biopsies for determining fold-changes in gene expression and immunohistochemistry.

Repeated measures statistical analysis will be performed on the outcomes to determine the clinical efficacy of Benralizumab in CU that can be interpreted in view of the molecular pathways predicted by gene expression assays and expression of cellular proteins before and after treatment with Benralizumab and placebo. Safety of the drug in the treatment of CU will be measured by hematological tests (total and differential count) and assessment of vital sign parameters at each clinical visit including visit 5 which is one month after receiving the last dose of Benralizumab.

Clinical Assessment

- For subjects meeting inclusion criteria, chronic medications not permitted in the study will be discontinued at the screening visit; in these circumstances visit 1 will take place at least 30 days after the screening visit.
- Placebo will be administered at week 4 and subjects will enter a 4 week run in period followed by treatment with Benralizumab 30mg subcutaneously monthly for 3 months (a total of 3 active doses).
- All patients will be blinded to treatment.
- Daily diaries of urticarial signs and symptoms will be maintained.
- Mean UAS7 and UCT scores will be calculated at monthly visits; urticaria QOL will also be assessed at each visit
- Therapeutic response will be determined at each montlymonthly. Response will be defined as well-controlled urticaria ($UAS7 \leq 6$) or complete response ($UAS7 = 0$)
- Rescue medications include; hydroxyzine 25mg or less up to four times daily and up to two short courses of oral corticosteroids (5-10 days) for severe symptoms during the study.

Objectives

Primary Objective	Outcome Measure
<i>To determine the clinical efficacy of Benralizumab when compared to placebo in patients with chronic urticaria who are refractory to treatment with H1-antihistamines.</i>	1. Urticarial Activity Score averaged over 7 days (UAS7) 2. Urticaria control (UCT) scores
Secondary Objective	Outcome Measure
<i>To investigate possible influence of Benralizumab on pathophysiology of CU in patients refractory to treatment with H1-antihistamines.</i>	1. Blood anti-FCER1, anti-TPO antibody, ECP and eotaxin levels 2. Gene expression profile using RNA-sequencing in skin biopsy samples (lesional vs. non-lesional skin); PCR validation for the top genes 3. Inflammatory cell count and cytokine quantification in skin biopsy using immunocytochemistry
Safety Objective	Outcome Measure
<i>To evaluate the safety and tolerability of Benralizumab in CU patients in relation to H1-antihistamines</i>	1. Adverse Events/Serious Adverse Events 2. Vital signs 3. Collection of clinical chemistry/hematology parameters
Exploratory Objective:	Outcome Measure :
<i>To demonstrate an improvement in health-related quality of life in the study subjects in subjects treated with Benralizumab when compared to placebo.</i>	<i>Health Status as determined by Chronic Urticaria Quality of Life Questionnaire (CU-QoL)</i>

Target subject population

Human subjects aged >18-70 yrs, having urticarial lesions for >6 weeks of unknown cause and hives present most days of the week despite treatment with 4× the FDA approved H1-antihistamines.

Duration of treatment

For completion of the study protocols the subjects will need to be enrolled in the study for up to 7 months.

Investigational product, dosage and mode of administration

- Benralizumab 30mg every 4 weeks ×3 months subcutaneously
- Placebo for Benralizumab ×one month subcutaneously

Statistical methods

Efficacy Variables in Clinical Assessments:

1. **Primary Variable** – Overall change from baseline mean UAS i.e., averaged 4-week score during placebo treatment – averaged final 4-week score during Benralizumab treatment.
2. **Secondary Variable**

- a. Percentage of responders (percentage of subjects demonstrating a change from baseline/post-placebo in mean UAS of $\geq 50\%$).
- b. Log2-fold change in gene expression profile post-Benralizumab vs. baseline/post-placebo
- c. Differences in counts of cell infiltrates/cytokine levels in skin biopsies post-Benralizumab vs. baseline/post-placebo.

	PAGE
TITLE PAGE	1
PROTOCOL SYNOPSIS.....	2
ETABLE OF CONTENTS	6
LIST OF ABBREVIATIONS AND DEFINITION OF TERMS	11
1. INTRODUCTION	12
1.1 Background and rationale for conducting this study	12
1.1. Research hypothesis.....	13
1.2. Rationale for study design, doses and control groups.....	13
1.3. Study Design.....	14
2. STUDY OBJECTIVES.....	15
2.1 Primary objective	15
2.2 Secondary objectives	15
2.3 Safety objectives	15
2.4 Exploratory objectives	15
3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL	15
3.1 Inclusion criteria	15
3.2 Exclusion criteria	16
3.3 Subject enrolment and randomization	17
3.4 Procedures for handling incorrectly enrolled or randomized subjects.....	18
3.5 Methods for assigning treatment groups.....	18
3.6 Methods for ensuring blinding.....	18
3.7 Methods for unblinding.....	18
3.8 Restrictions	18
3.9 Discontinuation of investigational product.....	18
3.9.1 Procedures for discontinuation of a subject from investigational product.....	19
3.10 Criteria for withdrawal.....	19
3.10.1 Screen failures.....	19
3.10.2 Withdrawal of the informed consent.....	19
3.11 Discontinuation of the study	19
4. STUDY PLAN AND TIMING OF PROCEDURES.....	19

4.1	Enrolment/screening period	22
4.2	Treatment period	22
4.3	Follow-up period.....	23
5.	STUDY ASSESSMENTS	23
5.1	Efficacy assessments.....	23
5.1.3.1	Blood Sample Collection (for anti-FCER1, anti-TPO antibody, ECP, and eotaxin levels)	23
5.1.3.2	Skin Biopsy (for RNA-sequencing and immunocytochemistry)	23
5.1.4	Sample Processing	24
5.1.4.1	RNA sequencing from the skin biopsy samples.	24
5.1.4.2	Skin biopsy immunofluorescence.	24
5.1.4.3	Comparison of results	24
5.2	Safety assessments	24
5.2.1	Laboratory safety assessments	24
5.2.2	25	
5.2.3	ECG.....	25
5.2.3.1	Resting 12-lead ECG	25
5.2.4	Vital signs	25
5.2.5	Other safety assessments.....	25
5.3	Other assessments	25
5.3.1	Patient reported outcomes.....	25
5.3.1.1	Name of PRO method or questionnaire	25
5.4	Pharmacokinetics	25
5.4.1	Collection of samples.....	25
5.4.2	Determination of drug concentration	25
5.4.3	Storage and destruction of pharmacokinetic samples	26
5.5	Pharmacodynamics	26
5.5.1	Collection of samples.....	26
5.5.2	Storage, re-use and destruction of pharmacodynamic samples	26
5.6	Pharmacogenetics.....	26
5.6.1	Collection of pharmacogenetic samples	26
5.6.2	Storage, re-use and destruction of pharmacogenetic samples.....	26
5.7	Biomarker analysis.....	26
5.7.1	Storage, re-use and destruction of biological samples.....	26
5.7.2	Labelling and shipment of biological samples.....	26
5.7.3	Chain of custody of biological samples	27
5.7.4	Withdrawal of Informed Consent for donated biological samples	27
6.	SAFETY REPORTING AND MEDICAL MANAGEMENT	27
6.1	Definition of adverse events	27
6.1.1	Definitions of serious adverse event	28

6.1.2	Causality assessment.....	28
6.2	Recording of adverse events	28
6.2.1	Time period for collection of adverse events.....	28
6.2.2	Follow-up of unresolved adverse events.....	28
6.2.3	Variables collected for each adverse event.....	29
6.3	Reporting of serious adverse events.....	30
6.4	Overdose	31
6.5	Pregnancy.....	31
6.5.1	Maternal exposure.....	31
6.5.2	Paternal exposure	31
6.6	Management of toxicities (Dose Reductions).....	31
6.7	Study governance and oversight	31
6.7.1	Steering Committee	31
6.7.2	Data Monitoring Committee	32
6.7.3	Scientific Advisory Committee.....	32
7.	INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS	32
7.1	Identity of investigational product(s).....	32
7.2	Dose and treatment regimens.....	32
7.3	Labelling	32
7.4	Storage	32
7.5	Compliance	32
7.6	Accountability.....	32
7.7	Concomitant and other treatments	33
7.7.1	Other concomitant treatment.....	33
7.8	Post Study Access to Study Treatment	33
8.	STATISTICAL ANALYSES	33
8.1	Statistical considerations.....	33
8.2	Sample size estimate	34
8.3	Definitions of analysis sets	35
8.3.1	Efficacy analysis set.....	35
8.3.2	Safety analysis set.....	35
8.3.3	PK analysis set	35
8.3.4	PRO analysis set	35
8.4	Outcome measures for analyses.....	35
8.5	Methods for statistical analyses	35
8.5.1	Analysis of the primary variable(s).....	35

8.5.2	Analysis of the secondary variable(s)	35
8.5.3	Subgroup analysis	36
8.5.4	Interim analysis.....	36
8.5.5	Sensitivity analysis.....	36
8.5.6	Exploratory analysis.....	36
9.	STUDY AND DATA MANAGEMENT.....	36
9.1	Training of study site personnel.....	36
9.2	Monitoring of the study	36
9.2.1	Source data	37
9.2.2	Study agreements	37
9.2.3	Archiving of study documents	37
9.3	Study timetable and end of study	38
	Data management.....	38
9.4	38	
9.4.1	Serious Adverse Event (SAE) Reconciliation	38
9.4.2	Data Management of genotype data.....	38
9.4.3	Data associated with human biological samples.....	38
9.4.4	Management of external data.....	38
10.	ETHICAL AND REGULATORY REQUIREMENTS	38
10.1	Ethical conduct of the study.....	38
10.2	Subject data protection.....	38
10.3	Ethics and regulatory review	39
10.4	Informed consent	39
10.5	Changes to the protocol and informed consent form	39
10.6	Audits and inspections	40
11.	LIST OF REFERENCES	40

LIST OF TABLES

No table of figures entries found.

LIST OF FIGURES

No table of figures entries found.

LIST OF APPENDICES

Appendix A	Signatures
Appendix B	Additional Safety Information
Appendix C	IATA 6.2 Guidance document

Clinical Study Protocol
Drug Substance
Study Code
Edition Number 1
Date 21 December 2016

<i>Appendix D</i>	Pharmacogenetics Research
<i>Appendix X</i>	<i>Other</i>

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
AE	Adverse event
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IP	Investigational Product
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LSLV	Last Subject Last Visit
OAE	Other Significant Adverse Event
PGx	Pharmacogenetic research
PI	Principal Investigator
SAE	Serious adverse event
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Chronic idiopathic urticaria (CU) has been defined as the occurrence of spontaneous wheals, with or without angioedema, for more than 6 weeks of unknown cause [1-3]. CU affects 1% of US adults and can be a significant cause of economic burden [3, 4]. The pathogenic mechanism of CU is not completely understood, but IgG antibody to FcER1 α subunit has been identified in approximately 30-40% of patients with CU, while anti-IgE antibody has been identified in another 5-10% [5]. In addition, an increased prevalence of autoimmune diseases has been observed in some CU patients with 12–29% showing significantly higher serum anti-Thyroid Peroxidase (TPO) antibody [6, 7]. However, the presence or absence of anti-FcER1/anti-IgE serum antibodies or other autoantibodies have not been found to have an effect on treatment response to either oral antihistamines, biologics (i.e. omalizumab) or other anti-inflammatory/immunosuppressants used to treat CU [8-11].

Histological analyses reveals significantly higher numbers of infiltrating eosinophils and lymphocytes in urticarial wheals compared to uninvolved skin and skin of normal healthy control subjects [12, 13]. Monocytes and T-lymphocyte are predominant cellular infiltrates in CU although the T-cell sub-population is highly variable between patients [14]. Activated eosinophils could play a mechanistic role in CU by triggering the tissue factor pathway of the coagulation cascade and by releasing vascular endothelial growth factor, thus contributing to development and severity of disease [2, 15]. CU skin often shows a significant increase of vascular endothelial growth factor (VEGF), which increases vaso-permeability through an increase in local nitric oxide production [16-18]. CU wheals also show higher expression of several cytokines (including IL-5, the key cytokine involved in eosinophil differentiation), chemokines, and adhesion molecules compared to uninvolved skin, and healthy control subjects [12, 19]. In addition, serum eotaxin concentration has been found to be significantly higher in CU patients (N=100; median 140.1 pg/ml, range 33.7-718.7 pg/ml) compared to healthy controls (N=45; median 108.9 pg/ml, range 45.5-409.4 pg/ml) ($p = 0.032$) [20]. Taken together, these data strongly suggest the involvement of eosinophils in the pathogenesis of CU[2].

Existing treatment options such as use of H1-receptor antagonists with or without a leukotriene receptor antagonist (LTRA), cyclosporine and other anti-inflammatory agents have been frequently demonstrated to be inadequate in managing CU. Therefore, there is a need for novel therapeutic agents to control non-histaminergic hives. Omalizumab has recently been approved for the treatment of idiopathic CU unresponsive to H1-antagonists administered at FDA recommended doses [21]. Clinical trials have shown that up to 44% of CU patient unresponsive to H1-antagonists have a complete response to omalizumab but after discontinuation of therapy the hives in the majority of cases returned to near baseline [22]. Currently it is unknown how omalizumab works mechanistically other than binding peripheral IgE and downregulating IgE receptors on mast cells and basophils. It is also unclear how long to treat with this agent as it does not appear to drive CU into remission. Thus other options that have more plausible mechanisms of action are needed to treat CU and induce remission [23-25].

1.1. Research hypothesis

Benralizumab decreases Urticaria Activity Score 7 and Urticaria Control Score in human subjects with chronic urticaria unresponsive to second generation H1-antihistamines.

1.2. Rationale for study design, doses and control groups

The purpose of this study is to determine the percentage of CU patients previously unresponsive to second generation H1-antihistamines, who respond to Benralizumab as determined by significant improvement in UAS7 and UCT scores post-Benralizumab vs baseline or post-placebo.

Benralizumab, a humanized anti-IL5 receptor α monoclonal antibody, has been demonstrated to be very effective in decreasing baseline blood eosinophil counts, serum ECP (eosinophil cationic protein) levels, and sputum and tissue eosinophils resulting in clinical improvement in eosinophilic asthma and chronic obstructive pulmonary disease (COPD) [26-32].

Interestingly, IL5R is also expressed on B cells and regulates genes involved in B-cell maturation and antibody class switching which could explain the increased frequency of autoantibodies seen in CU patients and thus be a potential mechanistic target for benralizumab in the treatment of CU [33-38]. Although it has been suggested that Benralizumab could be effective in a wide range of eosinophilic disorders, its efficacy in controlling CU has never been studied. Thus the primary objective of this study is to assess the efficacy of Benralizumab in managing CU and to address existing knowledge gaps. Subjects, between >18 and $\leq$ 70 years, having symptoms for at least six weeks and unresponsive to H1-antagonists will be recruited from our patient registry for this proof of concept study and the primary endpoint will be complete response, partial response or no response using the urticaria activity score (UAS7) and urticaria control scores.

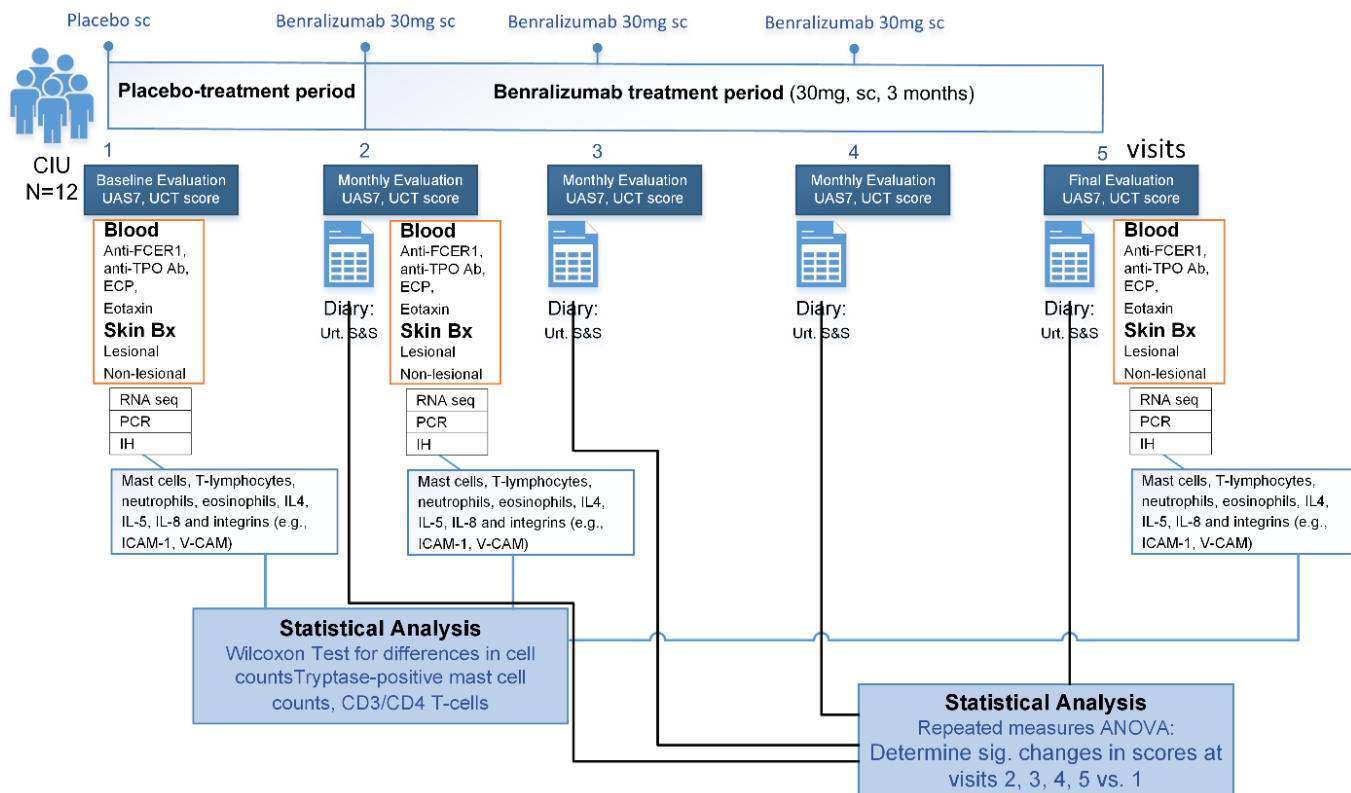
These results will determine on the optimal design of larger multicenter clinical trials for validating indications of Benralizumab in CU and FDA approval. Because of the small sample size and being conducted at a single site, a repeated measures study design is considered more appropriate.

Benefit/risk and ethical assessment

Benralizumab has already proven efficacious in decreasing asthma exacerbations in chronic moderate to severe asthma in patients not completely responsive to combination long-acting beta-agonists/corticosteroids. Based on its safety profile in human asthma subjects we believe that use of this treatment would not have harmful effects in CU patients and that the beneficial effects could be quite substantial in improving chronic urticaria control. Safety assessments will be performed throughout the study through clinical history, physical examination and blood examination as discussed in this protocol.

1.3. Study Design

Figure 1 Study Design



This is a single-blind, one-group, single center exploratory study to assess efficacy of Benralizumab 30mg in improving UAS7 and UCT scores and reducing inflammatory cell infiltration in skin in North American subjects with CU unresponsive to second generation H1-antihistamines. The design is explained in **Figure 1**.

2. STUDY OBJECTIVES

2.1 Primary objective

Primary Objective:	Outcome Measure:
<i>To determine the clinical efficacy of Benralizumab when compared to placebo in patients with chronic urticaria who are refractory to treatment with H1-antihistamines.</i>	<i>Urticarial Activity Symptoms averaged over 7 days (UAS7) and Urticarial Control (UCT) scores 1</i>

2.2 Secondary objectives

Secondary Objective	Outcome Measure
<i>To investigate possible influence of Benralizumab on pathophysiology of CU in patients refractory to treatment with H1-antihistamines.</i>	<i>Blood anti-FCER1, anti-TPO antibody, ECP and eotaxin levels</i> <i>Gene expression profile using RNA-sequencing in skin biopsy samples (lesional vs. non-lesional skin); PCR validation for the top genes</i> <i>Inflammatory cell count and cytokine quantification in skin biopsy using immunocytochemistry</i>

2.3 Safety objectives

Safety Objective:	Outcome Measure :
<i>To evaluate the safety and tolerability of Benralizumab in CU patients in relation to H1-antihistamines</i>	<i>Adverse Events/Serious Adverse Events</i> <i>Vital signs (Pulse rate, Blood Pressure, Respiratory rate)</i> <i>Total count, Differential count, Hematocrit</i>

2.4 Exploratory objectives

Exploratory Objective:	Outcome Measure :
<i>To demonstrate an improvement in health-related quality of life in the study subjects in subjects treated with Benralizumab when compared to placebo.</i>	<i>Health Status as determined by Chronic Urticaria Quality of Life Questionnaire (CU-QoL)</i>

3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

Twelve (12) CU subjects and six (6) healthy control matches (total 18) who meet the inclusion and exclusion criteria will be enrolled.

3.1 Inclusion criteria

- Age >18 and ≤70 yrs;
- urticarial lesions for > 6 weeks of unknown cause;

- hives present most days of the week despite H1-antihistamines up to 4× the FDA approved dose;
- Pruritus severity ≥ 2
- Urticaria Activity Score (UAS, i.e., combined score of pruritus severity + # of hives + size of largest hive) ≥ 4
- Compliance $>70\%$ with completion of daily diaries for 2 weeks between visit 1 (informed consent) and visit 2 (placebo treatment)
- Informed Consent: Able to give written informed consent prior to participation in the study, which will include the ability to comply with the requirements and restrictions listed in the consent form. Subjects must be able to read, comprehend, and write at a level sufficient to complete study related materials.
- Female subjects: Women of childbearing potential (WOCBP) must use an effective form of birth control (confirmed by the Investigator). Effective forms of birth control include: true sexual abstinence, a vasectomized sexual partner, Implanon, female sterilization by tubal occlusion, any effective IUD intrauterine device/IUS levonogestrel Intrauterine system, Depo-Provera(tm) injections, oral contraceptive, and Evra Patch(tm) or Nuvaring(tm). WOCBP must agree to use effective method of birth control, as defined above, from enrolment, throughout the study duration and within 16 weeks after last dose of Benralizumab, and have negative serum pregnancy test result on Visit 0.
- Women not of childbearing potential are defined as women who are either permanently sterilized (hysterectomy, bilateral oophorectomy, or bilateral salpingectomy), or who are postmenopausal. Women will be considered postmenopausal if they have been amenorrheic for 12 months prior to the planned date of visit -1 without an alternative medical cause. The following age-specific requirements apply:
 - Women <50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatment and follicle stimulating hormone (FSH) levels in the postmenopausal range.
 - Women ≥ 50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatment.
- All male subjects who are sexually active must agree to use an acceptable method of contraception (condom with or without spermicide) from Visit 0 until 16 weeks after their last dose.

3.2 Exclusion criteria

- Physical urticarias (pressure, cold, exercise, dermatographism...)
- Urticarial vasculitis
- Secondary urticaria (underlying allergy to food/drugs or autoimmune disease, infection, malignancy, thyroid disease)
- Use of oral or topical corticosteroids, methotrexate, cyclosporine, other biologics (i.e., IVIG, omalizumab) and anti-inflammatory agents (hydroxychloroquine, sulfasalazine, dapsone or colchicine) within 4 weeks of enrolment.

- History of cancer: Subjects who have had basal cell carcinoma, localized squamous cell carcinoma of the skin, or in situ carcinoma of the cervix are eligible provided that the subject is in remission and curative therapy was completed at least 12 months prior to the date informed consent, and assent when applicable was obtained. Subjects who have had other malignancies are eligible provided that the subject is in remission and curative therapy was completed at least 5 years prior to the date informed consent, and assent when applicable, was obtained.
- A helminth parasitic infection diagnosed within 24 weeks prior to the date informed consent is obtained that has not been treated with, or has failed to respond to standard of care therapy.
- Pregnant or nursing
- If female and of child-bearing potential, must have negative pregnancy test and must adhere to acceptable method of contraception (with <1% failure rate) during the study and for four months after the study.
- Receipt of any investigational non biologic within 30 days or 5 half-lives prior to visit 0, whichever is longer.
- A history of known immunodeficiency disorder including a positive human immunodeficiency virus (HIV) test.
- Any other medical illness that precludes study involvement
- Positive hepatitis B surface antigen, or hepatitis C virus antibody serology, or a positive medical history for hepatitis B or C. Subjects with a history of hepatitis B vaccination without history of hepatitis B are allowed to be enrolled.
- Patients who are currently receiving or have previously received benralizumab or any other type of anti-interleukin therapy (i.e. mepolizumab, reslizumab, lebrikizumab etc.) within the last 4 months or 5 half-lives whichever is longer.
- History of anaphylaxis to any biologic therapy or vaccine.
- Receipt of immunoglobulin or blood products within 30 days prior to the date informed consent is obtained.

Healthy controls should have the same criteria except the urticarial lesions, hives, pruritus. These controls are not required to maintain daily diaries.

3.3 Subject enrolment and randomization

The Principal Investigator will:

- Obtain signed informed consent from the potential subject before any study specific procedures are performed.
- Assign potential subject a unique enrolment number.
- Determine subject eligibility. See Sections 3.1 and 3.2
- Because all subjects will receive the same intervention therefore randomization is not applicable.

3.4 Procedures for handling incorrectly enroled or randomized subjects

Subjects who fail to meet the inclusion criteria or who meet the exclusion criteria should not, under any circumstances, be enroled or randomized. There can be no exceptions to this rule.

Where subjects that do not meet the selection criteria are enroled in error or incorrectly started on treatment, or where subjects subsequently fail to meet the study criteria post initiation, the investigator will discontinue subject participation in the study. .

3.5 Methods for assigning treatment groups

In this single group study design all subjects with CU will receive placebo once and then Benralizumab 30mg every 4 weeks ×3 doses. Healthy controls will not be treated with the placebo or benralizumab but enroled for obtaining blood samples and one skin biopsy.

3.6 Methods for ensuring blinding

The study is single-blinded. The subjects only will be blinded to the intervention (placebo or Benralizumab) they will receive at the study site. Unblinded trained research staff will administer the placebo/Benralizumab at the study site. Close attention should however be paid to make the appearance, package and labels of the investigational product look identical to the subjects.

3.7 Methods for unblinding

Subjects only will be blinded to intervention throughout and after the study. Unblinding does not apply.

3.8 Restrictions

- There are no specific dietary or activity restrictions other than those typical for a patient with this disease. Patients should not donate blood or bone marrow at any time during the study period.
- Females of child-bearing potential (ie, females who are not chemically or surgically sterilised or females who are not post-menopause) must use 2 methods of reliable contraception, one of which must be a barrier method from the enrolment until 1 month after the last dose.
- Concomitant medications will exclude steroids, immunosuppressant and specific anti-inflammatory drugs such as colchicine.

3.9 Discontinuation of investigational product

- Subjects may be discontinued from investigational product (IP) in the following situations:
- Voluntary discontinuation by the patient who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment
- Safety reasons as judged by the investigator (e.g clinically significant ventricular pauses, syncope related to bradycardia, persistent increase in serum creatinine level of clinical relevance, persistent, unexplained anaemia (e.g. Hb <10 g/dL) or thrombocytopenia (e.g. platelet count <10 x10⁴/μL)

- Severe non-compliance to protocol as judged by the investigator
- Incorrect enrolment (ie, the patient does not meet the required inclusion/exclusion criteria) and the discontinuation is decided by the discussion (see Section 5.3)
- Pregnancy
- Development of a condition or an acute event (eg, MI or stroke) that necessitates treatment with one or more prohibited medications
- Once enrolled into the study all patients will be assessed until study closure unless informed consent is withdrawn for study participation.

Repeated attempts will be made to locate and determine the vital status and occurrence of any SAE for those patients who are considered initially lost to follow up. A patient will be classified as lost to follow up only if, he/she has failed to return for the required study visits and his/her vital status remains unknown despite multiple attempts to contact him/her via telephone, fax, email, and certified letter.

3.9.1 Procedures for discontinuation of a subject from investigational product

A subject that decides to discontinue investigational product will always be asked about the reason(s) and the presence of any AEs. If possible, the subject should be seen and assessed by the investigator(s) at discontinuation visit and follow up visit. AEs will be followed up (See Sections 7.2).

3.10 Criteria for withdrawal

3.10.1 Screen failures

Subjects not matching the inclusion and exclusion criteria will be deemed as screen failure and will not be enrolled.

3.10.2 Withdrawal of the informed consent

Subjects deciding to withdraw from the study after having consented initially will be withdrawn from the study. Samples and data from such subjects will be destroyed.

3.10.3 Need for concomitant medical treatment

A subject may be withdrawn from the study in the event of the subject needing administration of steroids and other biologics due to medical conditions unrelated to CU.

3.11 Discontinuation of the study

The investigator reserves the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the IRB. In terminating the study, the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

4. STUDY PLAN AND TIMING OF PROCEDURES

The study will be a single-blind (subject blinded), placebo-controlled repeated measures study at a single site. Twelve human subjects (aged >18—and ≤70 years, both men and women) with a physician-based diagnosis of CU and the other characteristics mentioned in the inclusion criteria while on H1 anti-histamines before enrolment will be included. After the initial

baseline assessment and sample collection at Visit 1, placebo for Benralizumab will be administered at the same visit (**Table 1**). Subsequently, at each of the Visits 2, 3 and 4 (four weeks apart), Benralizumab 30 mg subcutaneously will be administered. Samples (whole blood and skin biopsy) will be collected before placebo administration (Visit 1), 4 weeks after placebo (Visit 2) and 4 weeks after the last treatment with Benralizumab (Visit 5). Because of the small sample size, stratification based on disease severity will not be feasible. All subjects will be masked to treatment allocation. The primary endpoint will be extent of reduction in UAS7 and UCT scores post-Benralizumab versus post-placebo or versus baseline. Key secondary endpoints will be differential gene expression for pathway focussed genes and levels of anti-FCER1, anti-TPO antibody, ECP and eotaxin levels in blood, as well as the differences in inflammatory cell infiltrate and cytokines in skin biopsies post-Benralizumab vs. baseline /post-placebo. Efficacy analyses were by intention to treat (based on the full analysis set); safety analyses included patients according to study drug received.

Table 1 Study Plan detailing the procedures

Visit Visit window Days –28 to 0 for Visit 0 ±0 day for Visit 1 ±1 day for Visits 2, 3, 4 and 5 ±5 days for 60 day follow-up after Visit 5	0 Screening	1	2	3	4	5	60-day follow-up	Protocol Section
Week		4	8	12	16	20	28	Section
Written informed consent	×							10.4
Demographics	×							
Physical examination	×	×	×	×	×	×	×	5.2.2
Medical/surgical history	×							
Inclusion/exclusion criteria	×	×	×	×	×	×		3.1, 3.2
Vital signs	×	×	×	×	×	×	×	5.2.4
Assessment of daily diaries (UAS7, UCT)		×	×	×	×	×	×	5.1.1
Blood and Skin biopsy samples		×	×			×		5.1.3
Treatment dispensed to CU subjects only		×	×	×	×			7.1, 7.2
		(P)	(B, 1 st)	(B, 2 nd)	(B, 3 rd)			
Adverse event review (AEs and SAEs)	×	×	×	×	×	×	×	6.1, 6.2, 6.3
Blood samples for safety assessments		×	×	×	×	×	×	5.2.1

* before administration of placebo; P=Placebo; B= Benralizumab

4.1 Enrolment/screening period

Enrolment will begin in the second quarter of 2017 and will continue through the first quarter of 2018. A subject is expected to be in the study for seven months. Screening will be performed in the first month.

Methods of enrolment. The PI in this study has a steady pool of CU patients attending his clinic. Patients meeting the inclusion criteria will be informed about the study by conversations in person and / or using a recruitment material (advertisement). An outline of the advertisement is described below.

VOLUNTEERS NEEDED FOR BENRALIZUMAB CLINICAL RESEARCH TRIAL

Purpose- To investigate the usefulness of new medication named Benralizumab in the treatment of itchy skin rash and hives known as chronic urticaria, that has not responded adequately to standard treatment with H1-anti-histamine such as Allegra or others.

This investigational drug, **Benralizumab**, has been proven to be effective and safe in patients with asthma in other studies. Because of related immune mechanisms in asthma and chronic urticaria, this study will investigate if this drug is beneficial in treating chronic urticaria in patients who do not respond satisfactorily to standard treatments.

Duration of enrolment. Seven months

Inclusion/ Exclusion conditions. Participant aged >18 and ≤ 70 years must have itchy rash and hives on skin consistent with a diagnosis of chronic urticaria for six weeks or more that has not responded to standard drugs. They should not have taken immunosuppressant drugs such as topical or oral corticosteroids, cyclosporine or omalizumab or other anti-inflammatory drugs such as hydroxychloroquine, dapsone, sulfasalazine or colchicine within the last 4 weeks.

Activities. Upon meeting the inclusion and exclusion criteria as assessed during the screening visit, the volunteers will be required to report to the study site once a month for five office visits. Activities in these visits will include

- Physical examination (blood pressure, pulse rate) and skin examination
- Administration of investigational drug at the first four visits
- Blood sampling and skin biopsy of normal skin and hives three times during the study
- In addition, a diary for recording the symptoms will be provided. The participants should record their daily symptoms for the first five months and bring the diary to the study site at every visit.

Incentives. An incentive of \$250 per visit in check for time and travel to the study site is provided to all the participants.

Benefits. This study does not guarantee any treatment benefits resulting from the investigational drug.

Details on how to enrol. If interested, contact **Dr. Jonathan Bernstein at Phone number 513-235-7971. Or email jonathan.bernstein@uc.edu**

4.2 Treatment period

Each of the study Visits 0-5 will be scheduled 4 weeks apart. Placebo (for Benralizumab), subcutaneously, administered only once at visit 1 and Benralizumab 30 mg. subcutaneously during visits 2, 3 and 4.

4.3 Follow-up period

60 days ± 5 days after Visit 5. Subjects will be followed up at the clinic to assess for remission or relapse of urticaria and general well-being will be followed.

5. STUDY ASSESSMENTS

The study will assess the efficacy and safety of Benralizumab vs. placebo

5.1 Efficacy assessments

5.1.1 CU subjects only

UAS7 and UCT. Disease activity will be assessed by UAS7 and disease control by UCT. To obtain UAS7 values, patients will be asked to document every day for seven consecutive days the numbers of wheals and the intensity of pruritus they experienced over the last 24 h using a 0–3 point scale for wheals (0 for none, 1 for <20, 2 for 20–50, and 3 for >50) and pruritus (0 for none, 1 for mild: present but not annoying or troublesome, 2 for moderate: troublesome, but does not interfere with normal daily life activity or sleep, and 3 for intense: interferes with normal daily life or sleep). The UAS7 (minimum=0, maximum=42) is then calculated as the sum of the daily totals of the wheal and the itch scores.

Blood and Skin Biopsy. Whole blood sample (10 mL) and four 4sq.mm. skin biopsy (two from unaffected skin other two from lesional skin) will be collected at Visits 1 (baseline), 2 (post-placebo), and 5 (post-Benralizumab). If there are no lesion(s) at visit 5 then biopsies will be taken from skin adjacent to previous biopsy sites.

5.1.2 Healthy match subjects only

Blood and skin biopsy similar to CU subjects will be collected only once.

5.1.3 Methods and Procedures for Blood Sampling and Skin Biopsy Collection and Downstream Assays

5.1.3.1 Blood Sample Collection (for anti-FCER1, anti-TPO antibody, ECP, and eotaxin levels)

10 mL of venous blood will be obtained by venipuncture of cubital vein in the forearm and collected in a plain, red-top tube. Blood will be allowed to clot and separate within one hour. Serum will be sent to analysis lab frozen. Standard ELISA-based protocol will be followed for assessment of anti-FCER1, anti-TPO antibody, ECP, and eotaxin levels.

5.1.3.2 Skin Biopsy (for RNA-sequencing and immunocytochemistry)

Skin biopsy performed using a minimally invasive disposable punch is a safe and painless procedure. A skin punch biopsy of a persistent hive can help diagnose underlying pathological changes by revealing the presence of inflammatory cell infiltration and deposition of immunoglobulins and complement. Using specific antibodies with immunofluorescence technique, it will be possible to investigate predominant cellular infiltrate in the lesional skin compared to normal skin in CU subjects normalized to samples from healthy controls.

Skin site in study subjects will be swabbed with isopropyl alcohol (70%) and anesthetized with local anesthetic (1 mL of 1% lidocaine + adrenaline). Using a punch biopsy needle a 4

sq.mm. full thickness skin biopsy from the lesional and normal will be obtained. The biopsies for immunocytochemistry will be preserved in Michel's medium or phosphate buffer saline and transported fresh to lab for further analysis. The biopsies for RNA-seq will be snap-frozen in liquid nitrogen and stored at -20C until analysis.

5.1.4 Sample Processing

5.1.4.1 RNA sequencing from the skin biopsy samples.

Total RNA will be isolated using the RNA isolation kit. The biopsy will be placed in a cryo-vial and snap-frozen in liquid nitrogen and stored at -20°C. During analysis ~75-100 mg Phase-Lock Gel Heavy (PLG-H) will be placed in a 1.5 ml tube and pelleted @ 12,000xg for 30s. The skin biopsies will be grinded to a fine powder in a pre-chilled mortar and pestle. Qiazol (Lysis Reagent) 300µL is added to a 1.5 ml tube. Powdered biopsy will be transferred to this tube and vortexed for 15s. The tubes will be shaken for 10 min and spun down for 15s. Chloroform 60 µL will be added and vortexed (15s) and spun down (15s) after 3 minutes. Partially separated mixture will be transferred to the PLG-H containing tube and mixed by inverting the tubes and then centrifuged @ 12,000xg for 15 min. The aqueous phase will be transferred to a new tube. One volume of 70% EtOH will be added to the aqueous phase and vortexed for 5s. Mixture will be transferred to a RNeasy MinElute spin column and RNA will be cleaned and concentrated using the vendor's protocol. Isolated RNA will be sent to sequencing core for library preparation, sequencing and preliminary data analysis (hierarchical clustering and pathway analysis).

5.1.4.2 Skin biopsy immunofluorescence.

In the lab, samples will be washed and then mounted and frozen in liquid nitrogen prior to sectioning. Using a cryostat, 5 micron sections will be cut and mounted onto charged glass slides. The tissue sections will be incubated with fluorescence tagged antibodies (fluorescein isothiocyanate, FITC) directed against mast cells, T-lymphocytes, neutrophils, eosinophils, IL4, IL-5, IL-8 and integrins. Each slide will be examined for fluorescence using a microscope with an ultraviolet light source and equipped with filters to detect the FITC signal. The location, pattern, and intensity of each immune reactant will be noted.

5.1.4.3 Comparison of results

Comparison of Gene expression and number of cell infiltrates or cytokine levels in CU subjects **post**-Benralizumab vs. **post**-placebo or baseline will identify the molecular mechanisms that are accountable for relapse in CU patients on H1-antihistamines prior to receiving Benralizumab. Results from healthy control samples will be used for normalization of results in CU samples.

5.2 Safety assessments

5.2.1 Laboratory safety assessments

The following laboratory variables (amend as appropriate) will be measured:

Table 2 Laboratory Safety Variables

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
B-Haemoglobin (Hb)	S/P-Albumin
B-Leukocyte count	S/P-Creatinine
B-Leukocyte differential count (absolute count)	S/P-Alanine transaminase (ALT)
B-Platelet count	

5.2.2

Physical examinations (PE) conducted throughout the study will be a focussed symptom-directed PE. The complete PE conducted at screening visits will also include the following assessments: medical history review, including history of allergies, and history of prior and current use of nicotine or nicotine-containing products, history of alcohol and illegal drug use, and history of prior (within 90 days) and current medication use.

5.2.3 ECG

not applicable

5.2.3.1 Resting 12-lead ECG

not applicable

5.2.4 Vital signs

Pulse rate, blood pressure, respiratory rate and surface body temperature will be assessed every visit.

5.2.5 Other safety assessments

Urine pregnancy tests will also be taken for all females at Visits 1-4, if applicable, before placebo/Benralizumab administration. If urine pregnancy test is positive, subjects will be deemed ineligible for further continuation in the study.

5.3 Other assessments

5.3.1 Patient reported outcomes

not applicable

5.3.1.1 Name of PRO method or questionnaire

not applicable

5.4 Pharmacokinetics

5.4.1 Collection of samples

not applicable

5.4.2 Determination of drug concentration

not applicable

5.4.3 Storage and destruction of pharmacokinetic samples

not applicable

5.5 Pharmacodynamics

not applicable

5.5.1 Collection of samples

not applicable

5.5.2 Storage, re-use and destruction of pharmacodynamic samples

not applicable

5.6 Pharmacogenetics

not applicable

5.6.1 Collection of pharmacogenetic samples

not applicable

5.6.2 Storage, re-use and destruction of pharmacogenetic samples

not applicable

5.7 Biomarker analysis

Significant differentially expressed genes in blood from CU subjects adjusted for healthy controls will be used to measure biomarkers important in CU and prediction of relevant immunological pathways or inflammation. Top genes will be validated by PCR assay.

Skin biopsies are well-tolerated and easily accessible. These biopsies characterise a system in which several intricate signaling networks are active and in which diverse drug targets may likely play a role directly or indirectly. Differences in types of cellular infiltrates or cytokines localized in skin biopsies will be ***pathological biomarkers*** for differentiating CU from healthy controls. Significant differences in cell infiltrates and cytokines post-treatment vs. placebo will determine ***pharmacodynamic biomarkers***.

Repeat samples will determine if Benralizumab modifies these discovered pathways or biomarker molecules. Correlation between biomarkers from blood and skin will also be analysed.

5.7.1 Storage, re-use and destruction of biological samples

. Samples left-over after the conclusion of the study will be disposed off using universal precautions.

5.7.2 Labelling and shipment of biological samples

The biological samples collected in this study are categorized as exempt human specimens and are assumed to be non-infectious. The label will be printed with the proper dimension of an Infectious Substance label (50 mm on a side, and the proper shipping name, "Biological Substance, Category B" in letters at least 6 mm high). Label will be affixed to the shipment

package by covering with clear plastic tape. Specimens will be shipped using the triple packaging system. The outer package will be marked with “Exempt human specimen”.

5.7.3 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle. The Principal Investigator will keep full traceability of collected biological samples from the subjects while in storage at the study site until shipment or disposal (where appropriate) and will keep documentation of receipt of arrival.

The sample receiver will keep full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps documentation of receipt of arrival.. Samples remaining at the end of the study will not be retained for further use but will be discarded.

5.7.4 Withdrawal of Informed Consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analysed, the investigator is not obliged to destroy the results of this research.

As collection of the biological samples is an integral part of the study, the subject will be withdrawn from further study participation.

The Principal Investigator:

- will ensure subjects’ withdrawal of informed consent to the use of donated samples is notified immediately to the Schulman IRB.
- will ensure that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- will ensure the lab holding the samples is informed about the withdrawn consent immediately and that samples are disposed/destroyed, the action documented and the signed document returned to the study site
- will ensure that the subject and the Schulman IRB are informed about the sample disposal

6. SAFETY REPORTING AND MEDICAL MANAGEMENT

Safety will be evaluated throughout the study. Evaluation for AEs, review of concomitant medications, and review of protocol restrictions will occur at the beginning of each visit.

6.1 Definition of adverse events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a Benralizumab or placebo, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, lab findings). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs. An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an AE and must be reported.
- Preexisting diseases, conditions, or laboratory abnormalities present or detected before the Visit 1 that do not worsen
- Situations where an untoward medical occurrence has not occurred (eg, hospitalization for elective surgery, social and/or convenience admissions)

Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be preexisting and should be documented on the medical history CRF.

6.1.1 Definitions of serious adverse event

- A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:
- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.
- The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

6.1.2 Causality assessment

The Investigator will assess causal relationship between Investigational Product (Benralizumab) and each Adverse Event, and answer ‘yes’ or ‘no’ to the question ‘Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?’

For SAEs a causal relationship will also be assessed for other concomitant medications, study procedures, and comparator study drugs. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as ‘yes’.

6.2 Recording of adverse events

6.2.1 Time period for collection of adverse events

AEs will be recorded after obtaining the written informed consent until the follow-up visit.

6.2.2 Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject’s last AE assessment in the study will be followed up by the investigator for as long as medically indicated, but without further recording in the CRF..

6.2.3 Variables collected for each adverse event

The following variables should be collected for each AE:

- AE (e.g., breathing difficulty, nasopharyngitis, upper respiratory infection, headache, bronchitis, sinusitis, influenza and pharyngitis)
- The date and time when the AE started and stopped
- Maximum intensity or changes in intensity
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) grade/changes in CTCAE grade (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study (yes or no)
- Outcome of AE(s).

In addition, the following variables should be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to ... (e.g., the subject being at risk of death at the time event, or has led to in-patient hospitalization, persistent or significant disability or incapacity or a medically important event or reaction that may not be immediately life-threatening)
- Date of hospitalisation
- Date of discharge
- **If death:**
 - Probable cause of death
 - Date of death
 - Autopsy performed
 - Causality assessment in relation to Study procedure(s)
 - Description of AE(s).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.1.11. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: 'Have you had any health problems since the previous visit/you were last asked?', or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report (CSR). Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in nonmandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Since the most common adverse effects previously noted for Benralizumab has been breathing difficulty, nasopharyngitis, upper respiratory infection, headache, bronchitis, sinusitis, influenza and pharyngitis, surveillance for these events will be undertaken. Any of these events that fulfil the criteria of an AE as judged by the investigator should be reported using the standard procedures for assessing severity, causality and seriousness.

6.3 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives within one day ie, immediately but no later than the end of the next business day of when he or she becomes aware of it. The designated AstraZeneca representative works with the investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site within one calendar day of initial receipt for fatal and life threatening events and within five calendar days of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day ie, immediately but no later than the end of the next business day of when he or she becomes aware of it.

The Investigator or other study site personnel reports a SAE to the appropriate AstraZeneca representative by telephone. The AstraZeneca representative will advise the Investigator/study site personnel how to proceed.

6.4 Overdose

Overdose is considered an adverse events and will be dealt with accordingly. An overdose is defined as any intake of study medication more than defined in the protocol. Because the placebo or drug will be administered in pre-filled syringes, the likelihood of an overdose is minimized. However, in the event of an overdose with Benralizumab, the time and extent of the overdose will be noted. Overdose with associated AE will be recorded as AE diagnosis/symptoms and overdose without AE will be recorded as Overdose only.

6.5 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca. Such cases should be reported to the AstraZeneca within 24 hours of becoming aware of the pregnancy.

6.5.1 Maternal exposure

If a subject becomes pregnant during the course of the study, the investigational product should be discontinued immediately. Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of any contraceptive medication.

Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The subject will be unblinded to the treatment and counseling may be offered based on whether the fetus has been exposed to the investigational drug or placebo.

6.5.2 Paternal exposure

There are no restrictions against fathering a child when treated with AZD6140. If paternal exposure pregnancy occurs in the course of the study, then investigators or other site personnel should inform appropriate AstraZeneca representatives within one day as described in the maternal exposure Section 13.3.1. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up and documented even if the subject was discontinued from the study.

6.6 Management of toxicities (Dose Reductions)

The study is designed to administer uniform dose of 30mg of Benralizumab. In the event of SAEs, the subject will be discontinued from the study and the event reported as mentioned above. Continued enrolment at reduced dosage is not applicable.

Dose Reductions: Not applicable.

6.7 Study governance and oversight

The Schulman IRB is the governing body who will review the research project in order to protect study participants. Every study is reviewed by an IRB before it begins. IRBs ensure that researchers and institutions abide by federal regulations and guidelines so that risk to human research participants is minimized.

6.7.1 Steering Committee

Not applicable

6.7.2 Data Monitoring Committee

We will select 3 individuals to review safety data throughout this study (Susan Pinney, PhD epidemiologist; Bill Ridgeway, MD; Vincent Martin, M.D.)

6.7.3 Scientific Advisory Committee

Not applicable

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Benralizumab	Subcutaneous, 30mg	AstraZeneca
Placebo for Benralizumab	Subcutaneous, 00mg of active drug	AstraZeneca

7.2 Dose and treatment regimens

Benralizumab 30mg every 4 weeks for 3 doses.

Placebo for Benralizumab administered once before treatment with Benralizumab

7.3 Labelling

Labelling of the investigational products will be carried out by AstraZeneca or a Contract Research Organization in accordance with current Good Manufacturing Practice (GMP). The labels will fulfil GMP Annex 13 requirements. All investigational products will be labelled.

7.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The investigational product label on the syringe container will specify the appropriate storage..

7.5 Compliance

Investigational products will not be used after the expiration date printed on the packaging, or if the individual pack is opened or appears damaged.

7.6 Accountability

Upon receipt of the clinical supplies, the Investigator or designee will conduct a complete inventory of all study products and assume responsibility for storage and dispensing. Dispensing and return of investigational study products must be appropriately documented. All used and unused supplies will be inventoried and disposed of by the Sponsor or its designee. In accordance with federal regulations, the Investigator must agree to keep all study products in a secure location with restricted access.

Investigational study products required for completion of this study (including a surplus to account for potential product failures) will be provided by the manufacturer. The recipient will acknowledge receipt of the investigational products indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all investigational study products dispensed will be maintained.

7.7 Concomitant and other treatments

Study does not involve administration of concomitant drug as part of the study. Benralizumab may have steroid sparing effect. A subject may be withdrawn from the study in the event of the subject needing administration of steroids and other biologics due to medical conditions unrelated to CU.

Restricted Medication/Class of drug	Usage
<i>Chronic corticosteroids</i>	<i>May administer two short courses (7-10 days) of prednisone 35mg during study for moderate to severe flares of hives</i>
Prohibited Medication/Class of drug	Usage
<i>Biologics</i>	<i>Omalizumab, IVIG</i>
<i>Anti-inflammatories</i>	<i>Hydroxychloroquine, Dapsone, Sulfasalazine, Colchicine</i>
Rescue/Supportive Medication/Class of drug:	Usage
<i>Hydroxyzine</i>	<i>Can use hydroxyzine 25mg up to 4 times a day as a rescue medication</i>

7.7.1 Other concomitant treatment

As mentioned in the exclusion criteria, only those participant who have not taken immunosuppressant or anti-inflammatory drug within 30 days of visit 1 will be considered for continuation in the study. Enrolment of subjects needing these forms of treatment due to any reason, within 4 weeks of enrolment or during the 6 months of study, will be discontinued.

7.8 Post Study Access to Study Treatment

Currently the study does not promises post study access to Benralizumab treatment even if it proves to be effective and safe because the results may not be generalizable. Larger studies will be needed to determine its indication in the treatment of CU.

8. STATISTICAL ANALYSES

8.1 Statistical considerations

Assessment of the effectiveness of treatments will be more sensitive in repeat-measures study designs in this pilot study. This design will make it possible to measure how the actual treatment vs. placebo affects each individual with CU. The variability between subjects will not mask the effects of actual vs. placebo. In this design each subject will serve as own control. The variability between subjects will be isolated, and analysis will highlight only on treatment effects.

In the context of a pilot study, the normality of data distribution and its effect on the p-values will be less important. The purposes of data analysis will be to get a hint of the data quality and biological significance rather than statistical significance and therefore to decide the need of further studies.

8.2 Sample size estimate

Sample size calculation is shown below in Figures 2 and 3

Sample size determination based significant differences clinical measures:

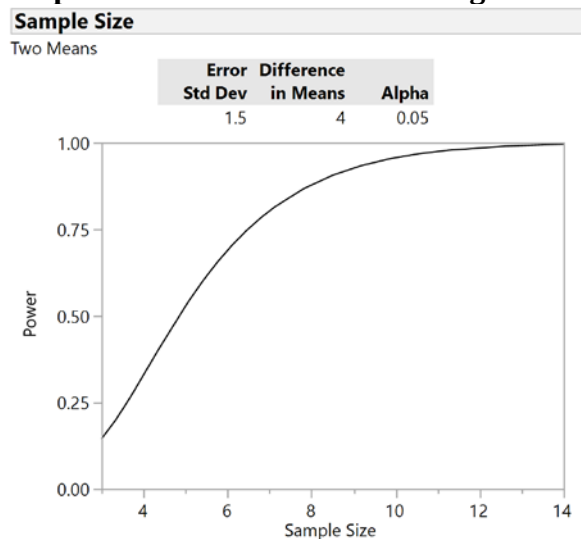


Figure 2. A sample size of 12 cases and 6 controls will determine statistical significance of a difference in UAS $\geq 4 \pm 1.5$ from baseline at alpha = 0.05 and power >0.95.

Sample size determination based on gene expression assays: Fold-change in gene-expression for the relevant genes compared to house-keeping genes at the end of placebo-treatment period and Benralizumab treatment period.

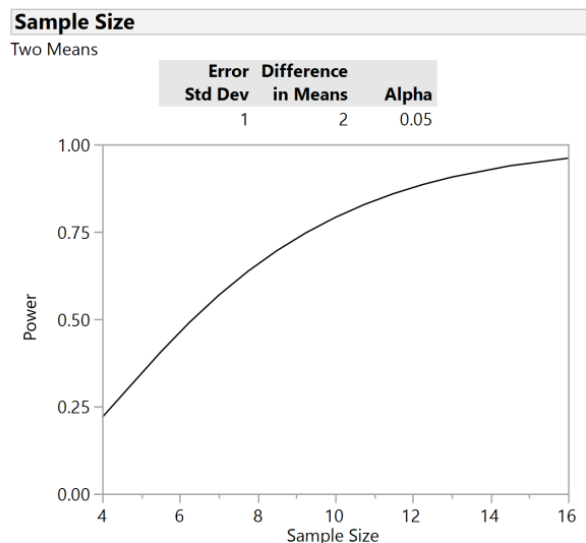


Figure 3. Sample size determination for Aim 2. A $2(\pm 0.5)$ fold-difference in gene expression of between samples collected after placebo period vs samples collected after Benralizumab treatment will be statistically significant within 95% CI and power of 0.8

8.3 Definitions of analysis sets

8.3.1 Efficacy analysis set

Clinical

- UAS7 score
- UCT score
- Urticaria QOL

Pathological

- **RNA-sequencing:** Differential gene expression in post-treatment with Benralizumab vs. placebo/baseline.
- **Immunofluorescence:** Cell infiltrate, cytokine profile in skin biopsy from normal vs. lesional skin post-treatment with Benralizumab vs. placebo/baseline.

8.3.2 Safety analysis set

Clinical

- **Vitals:** Pulse, BP, respiratory rate, body surface temperature
- **Hematology:** Complete blood count with differential Including number of eosinophils/ μ L, %Eosinophils, platelet count
- **Clinical chemistry:** Serum Creatinine, Albumin, Alkaline phosphatase.

8.3.3 PK analysis set

Will not be performed.

8.3.4 PRO analysis set

Optional. It will include patient's perception of quality of life and performance improvement post-treatment vs. baseline.

8.4 Outcome measures for analyses

Decrease in UAS7 and UCT score and pruritus severity at Visit 5 (post-Benralizumab) compared to baseline (Visit 1) and post-placebo (Visit 2).

8.5 Methods for statistical analyses

Repeated measure ANOVA of the efficacy variables in CU subjects normalized with data from healthy controls will be performed. Analysis will be adjusted for stratified age groups, gender and duration of disease.

8.5.1 Analysis of the primary variable(s)

UAS7 and UCT scores at baseline or post-placebo will be compared to post-treatment scores. Statistical significance will be determined by a significant effect size. P-values for statistical significance may not be very useful because of small sample size in this study.

8.5.2 Analysis of the secondary variable(s)

Effect of treatment with Benralizumab vs. placebo will be determined by log-2 fold-change in gene expression in targeted sequencing.

8.5.3 Subgroup analysis

Because of a small sample size, further sub-group analysis may not be feasible. Cluster analysis, however, will be explored if possible to determine if the duration of the disease, gender or age-group influences the response to Benralizumab in CU.

8.5.4 Interim analysis

UAS7 and UCT scores will be compared at every visit from the Visits 3-5 to determine the trends in the response to Benralizumab vs. baseline and post-placebo scores.

8.5.5 Sensitivity analysis

Principal components analysis will be applied to the gene expression data from RNA sequencing that can determine general patterns of expression alteration post-Benralizumab vs. post-placebo. Such analyses may provide reasonable power to distinguish moderate-sized alterations in clinically relevant genes.

8.5.6 Exploratory analysis

Improvement in health-related quality of life in the CU subjects post-Benralizumab vs. baseline or post-placebo will be compared by Chronic Urticaria Quality of Life Questionnaire (CU-QoL).

9. STUDY AND DATA MANAGEMENT

9.1 Training of study site personnel

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

Bench scientists who will perform the blood and skin biopsy processing have training in the methods needed in this study.

9.2 Monitoring of the study

The investigator will be responsible for all monitoring and quality controls in the study. The investigator will ensure

- that the investigational team is adhering to the protocol
- that data are being accurately and timely recorded in the CRFs,
- that biological samples are handled in accordance with the Laboratory Manual and
- that study drug accountability checks are being performed
- withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

9.2.1 Source data

Source data are any data generated as a result of the subject's inclusion in the study (including run-in and/or follow up related to the study) and includes all related medical examinations and other records. Original data will be recorded on the CRFs and regarded as source data.

9.2.2 Study agreements

The Principal Investigator should comply with all the terms, conditions, and obligations of the study agreement for this study. In the event of any inconsistency between this study protocol and the study agreement, the terms of study protocol shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the study agreement shall prevail.

9.2.3 Archiving of study documents

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IRB approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data should include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender);
- Documentation that subject meets eligibility criteria, i.e., history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);
- Documentation of the reason(s) a consented subject is not enrolled
- Participation in study (including study number);
- Study discussed and date of informed consent;
- Dates of all visits;
- Documentation that protocol specific procedures were performed;
- Results of efficacy parameters, as required by the protocol;
- Start and end date of study procedure
- Record of all AEs and other safety parameters (start and end date, and including causality and severity);
- Concomitant medication (including start and end date, dose if relevant; dose changes);
- Date of study completion and reason for early discontinuation, if it occurs.

All clinical study documents must be retained by the investigator until at least 2 years or until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if specified by regulatory requirements, by local regulations, or by an agreement with AstraZeneca.

9.3 Study timetable and end of study

The study will start enrolling subjects beginning 2nd quarter of 2017 and is expected to be completed in about a year. The enrolment duration per subject is 6 months of active enrolment followed by 2 months of follow-up period.

9.4 Data management

9.4.1 Serious Adverse Event (SAE) Reconciliation

SAE reporting and reconciliation will follow Good Clinical Principles and NIH guidelines. The event onset date will be entered as the date the outcome of the event fulfilled one of the serious criteria or the investigator considers the event to meet one of the serious categories.

9.4.2 Data Management of genotype data

De-identified code using initials and 4 digit numeric letters will be used to code the RNA-extracts to the genomics core. Data analysis and secured data storage will be performed on the deidentified data.

9.4.3 Data associated with human biological samples

The investigator will take necessary steps to maintain confidentiality of data. Personal identifiers, such as subject names, their address, SSN, Health insurance provider, etc. will not be disclosed on study samples or data. The analysis data will be encrypted using third party vendor software and shared with the study team only.

9.4.4 Management of external data

The data generated in this study will not be linked to any external data such as EHR data.

10. ETHICAL AND REGULATORY REQUIREMENTS

10.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) /Good Clinical Practice (GCP), applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

10.2 Subject data protection

The investigator will assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, another unique identifier and an identification code will be recorded on any form or biological sample submitted to the , IRB, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject in accordance with local regulations. The investigator will keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial. Subject data will be processed in accordance with all applicable regulations.

The investigator agrees that all information received from AstraZeneca, including but not limited to the investigator brochure, this protocol, CRF, and any other study information, remain the sole and exclusive property of AstraZeneca during the conduct of the study and

thereafter. Information in this protocol, consent forms and any other study information remain the shared property of the investigator and AstraZeneca during the study and thereafter. The investigator is free to publish the deidentified data, use in a grant application, and/or refine a hypothesis. . The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

10.3 Ethics and regulatory review

The Schulman Institutional Review Board (Schulman-IRB) should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable Schulman-IRB, and to the study site staff.

The opinion of the Schulman-IRB should be given in writing. The investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study. The Schulman-IRB should approve all advertising used to recruit subjects for the study. Schulman-IRB should approve any modifications to the Informed Consent Form that are needed to meet local requirements. The protocol should be re-approved by the Schulman-IRB annually.

Before enrolment of any subject into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations. AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities. AstraZeneca will provide Regulatory Authorities, Ethics Committees and Principal Investigators with safety updates/reports according to local requirements, including SUSARs (Suspected Unexpected Serious Adverse Reactions), where relevant.

10.4 Informed consent

The Principal Investigator(s) at each centre will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

10.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal

Investigator and AstraZeneca. If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Revised Clinical Study Protocol).

The amendment is to be approved by the relevant IRB and if applicable, also the national regulatory authority approval, before implementation. Local requirements are to be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to the Principal Investigator.

If a protocol amendment requires a change to a study site's Informed Consent Form, AstraZeneca and the study site IRB will approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the IRB.

10.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, GCP, guidelines of the ICH, and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

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FINAL PROTOCOL

includes **Statistical Analysis Plan** (section 8, page 33)

Summary of Changes in Final Protocol

- Removal of 'Urticaria Control Score' as an outcome measure for the primary objective.

Summary of Changes in Statistical Analysis Plan in Final Protocol

Removal of UCT score

- as one of the parameters in the Efficacy analysis set (Clinical),
- as an outcome measure for (statistical) analyses (Section 8.4, page 35), and
- as one of the measures for analysis of the primary variable(s) or outcomes (Section 8.5.1, page 35).

Clinical Study Protocol

Drug Substance

Study Code

Edition Number 1

Date 21 December 2016

Treatment of chronic urticaria unresponsive to H1-antihistamines with an anti-IL5Ralpha monoclonal antibody

Sponsor:

The following Amendment(s) and Administrative Changes have been made to this protocol since the date of preparation:

Amendment No.	Date of Amendment	Local Amendment No:	Date of Local Amendment
Administrative Change No.	Date of Administrative Change	Local Administrative Change No.	Date of Local Administrative Change

PROTOCOL SYNOPSIS

Treatment of chronic urticaria unresponsive to H1-antihistamines with an anti-IL5Ralpha monoclonal antibody

Principal Investigator

Jonathan A. Bernstein

Study site(s)

Bernstein Clinical Research Center (office visits and sample collection) **Number of subjects planned**

18 human subjects (12 chronic urticaria cases, 6 normal controls)

Study period		Phase of development
Estimated date of first subject enrolled	2 nd Quarter, 2017	Phase 0
Estimated date of last subject completed	1 st Quarter, 2018	

Study design

This exploratory study will assess the efficacy of Benralizumab, anti-IL5 receptor α monoclonal antibody, in the treatment of chronic urticaria (CU) refractory to H1-antihistamines. This is a single center, single-blind, case-controlled, uniform dose multi-treatment (Benralizumab 30mg $\times$ 3 treatments), repeated measures study. The study will enrol up to 18 subjects (12 CU cases, 6 normal controls). The study duration is about one year.

Eligible subjects will be male or non-pregnant/non-lactating female subjects, age >18 and ≤ 70 years, with CU unresponsive to up to 4 $\times$ the FDA approved doses of H1-antihistamines and age, gender-matched non-CU healthy controls.

There will be 7 office visits. During screening visit 0, after signing an informed consent, CU subjects meeting inclusion/exclusion criteria with a Urticaria Activity Score 7 (UAS7) ≥ 4 , will be enrolled and asked to complete daily diaries for 2 weeks prior to visit 1 to ensure $>70\%$ compliance with diaries. During visit 1, subjects will be administered a placebo subcutaneously. All subjects will return after 4 weeks and those subjects still with a UAS7 ≥ 4 at visit 2 will be switched to Benralizumab 30mg subcutaneously and continue to receive Benralizumab at visits 3 and 4 at one month intervals. Subjects will be assessed after completing the medication at visit 5 and after being off medication for 60 days at visit 6.

Clinical outcomes, i.e. UAS7 ~~and urticarial control test (UCT) score~~ and the urticaria quality of life questionnaire, will be assessed at every (screening visit and visits 1-6) visit. Blood samples and skin biopsies for efficacy assessment will be collected at baseline, 4-weeks post-placebo and 4-weeks after the third dose of Benralizumab. Blood samples in such assessments

will be used for quantification of anti-FCER1, anti-TPO and skin biopsies for determining fold-changes in gene expression and immunohistochemistry.

Repeated measures statistical analysis will be performed on the outcomes to determine the clinical efficacy of Benralizumab in CU that can be interpreted in view of the molecular pathways predicted by gene expression assays and expression of cellular proteins before and after treatment with Benralizumab and placebo. Safety of the drug in the treatment of CU will be measured by hematological tests (total and differential count) and assessment of vital sign parameters at each clinical visit including visit 5 which is one month after receiving the last dose of Benralizumab.

Clinical Assessment

- For subjects meeting inclusion criteria, chronic medications not permitted in the study will be discontinued at the screening visit; in these circumstances visit 1 will take place at least 30 days after the screening visit.
- Placebo will be administered at week 4 and subjects will enter a 4 week run in period followed by treatment with Benralizumab 30mg subcutaneously monthly for 3 months (a total of 3 active doses).
- All patients will be blinded to treatment.
- Daily diaries of urticarial signs and symptoms will be maintained.
- Mean UAS7 ~~and UCT scores~~ will be calculated at monthly visits; urticaria QOL will also be assessed at each visit
- Therapeutic response will be determined at each montlymonthly. Response will be defined as well-controlled urticaria ($UAS7 \leq 6$) or complete response ($UAS7 = 0$)
- Rescue medications include; hydroxyzine 25mg or less up to four times daily and up to two short courses of oral corticosteroids (5-10 days) for severe symptoms during the study.

Objectives

Primary Objective	Outcome Measure
<i>To determine the clinical efficacy of Benralizumab when compared to placebo in patients with chronic urticaria who are refractory to treatment with H1-antihistamines.</i>	1. Urticarial Activity Score averaged over 7 days (UAS7) 2. ~~Urticaria control (UCT) scores~~
Secondary Objective	Outcome Measure
<i>To investigate possible influence of Benralizumab on pathophysiology of CU in patients refractory to treatment with H1-antihistamines.</i>	1. Blood anti-FCER1, anti-TPO antibody, ECP and eotaxin levels 2. Gene expression profile using RNA-sequencing in skin biopsy samples (lesional vs. non-lesional skin); PCR validation for the top genes 3. Inflammatory cell count and cytokine quantification in skin biopsy using immunocytochemistry
Safety Objective	Outcome Measure
<i>To evaluate the safety and tolerability of Benralizumab in CU patients in relation to H1-antihistamines</i>	1. Adverse Events/Serious Adverse Events 2. Vital signs 3. Collection of clinical chemistry/hematology parameters
Exploratory Objective:	Outcome Measure :
<i>To demonstrate an improvement in health-related quality of life in the study subjects in subjects treated with Benralizumab when compared to placebo.</i>	<i>Health Status as determined by Chronic Urticaria Quality of Life Questionnaire (CU-QoL)</i>

Target subject population

Human subjects aged >18-70 yrs, having urticarial lesions for >6 weeks of unknown cause and hives present most days of the week despite treatment with 4× the FDA approved H1-antihistamines.

Duration of treatment

For completion of the study protocols the subjects will need to be enrolled in the study for up to 7 months.

Investigational product, dosage and mode of administration

- Benralizumab 30mg every 4 weeks ×3 months subcutaneously
- Placebo for Benralizumab ×one month subcutaneously

Statistical methods

Efficacy Variables in Clinical Assessments:

1. **Primary Variable** – Overall change from baseline mean UAS i.e., averaged 4-week score during placebo treatment – averaged final 4-week score during Benralizumab treatment.
2. **Secondary Variable**

- a. Percentage of responders (percentage of subjects demonstrating a change from baseline/post-placebo in mean UAS of $\geq 50\%$).
- b. Log2-fold change in gene expression profile post-Benralizumab vs. baseline/post-placebo
- c. Differences in counts of cell infiltrates/cytokine levels in skin biopsies post-Benralizumab vs. baseline/post-placebo.

ETABLE OF CONTENTS

PAGE

TITLE PAGE	1
PROTOCOL SYNOPSIS.....	2
ETABLE OF CONTENTS	6
LIST OF ABBREVIATIONS AND DEFINITION OF TERMS	11
1. INTRODUCTION	12
1.1 Background and rationale for conducting this study	12
1.1. Research hypothesis.....	13
1.2. Rationale for study design, doses and control groups.....	13
1.3. Study Design.....	14
2. STUDY OBJECTIVES.....	15
2.1 Primary objective	15
2.2 Secondary objectives	15
2.3 Safety objectives	15
2.4 Exploratory objectives	15
3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL	15
3.1 Inclusion criteria	15
3.2 Exclusion criteria	16
3.3 Subject enrolment and randomization	17
3.4 Procedures for handling incorrectly enroled or randomized subjects.....	18
3.5 Methods for assigning treatment groups.....	18
3.6 Methods for ensuring blinding.....	18
3.7 Methods for unblinding.....	18
3.8 Restrictions	18
3.9 Discontinuation of investigational product.....	18
3.9.1 Procedures for discontinuation of a subject from investigational product.....	19
3.10 Criteria for withdrawal.....	19
3.10.1 Screen failures.....	19
3.10.2 Withdrawal of the informed consent.....	19
3.11 Discontinuation of the study	19
4. STUDY PLAN AND TIMING OF PROCEDURES.....	19

4.1	Enrolment/screening period	22
4.2	Treatment period	22
4.3	Follow-up period.....	23
5.	STUDY ASSESSMENTS	23
5.1	Efficacy assessments.....	23
5.1.3.1	Blood Sample Collection (for anti-FCER1, anti-TPO antibody, ECP, and eotaxin levels)	23
5.1.3.2	Skin Biopsy (for RNA-sequencing and immunocytochemistry)	23
5.1.4	Sample Processing	24
5.1.4.1	RNA sequencing from the skin biopsy samples.	24
5.1.4.2	Skin biopsy immunofluorescence.	24
5.1.4.3	Comparison of results	24
5.2	Safety assessments	24
5.2.1	Laboratory safety assessments	24
5.2.2	25	
5.2.3	ECG.....	25
5.2.3.1	Resting 12-lead ECG	25
5.2.4	Vital signs	25
5.2.5	Other safety assessments.....	25
5.3	Other assessments	25
5.3.1	Patient reported outcomes.....	25
5.3.1.1	Name of PRO method or questionnaire	25
5.4	Pharmacokinetics	25
5.4.1	Collection of samples.....	25
5.4.2	Determination of drug concentration	25
5.4.3	Storage and destruction of pharmacokinetic samples	26
5.5	Pharmacodynamics	26
5.5.1	Collection of samples.....	26
5.5.2	Storage, re-use and destruction of pharmacodynamic samples	26
5.6	Pharmacogenetics.....	26
5.6.1	Collection of pharmacogenetic samples	26
5.6.2	Storage, re-use and destruction of pharmacogenetic samples.....	26
5.7	Biomarker analysis.....	26
5.7.1	Storage, re-use and destruction of biological samples	26
5.7.2	Labelling and shipment of biological samples.....	26
5.7.3	Chain of custody of biological samples	27
5.7.4	Withdrawal of Informed Consent for donated biological samples	27
6.	SAFETY REPORTING AND MEDICAL MANAGEMENT	27
6.1	Definition of adverse events	27
6.1.1	Definitions of serious adverse event	28

6.1.2	Causality assessment.....	28
6.2	Recording of adverse events	28
6.2.1	Time period for collection of adverse events.....	28
6.2.2	Follow-up of unresolved adverse events.....	28
6.2.3	Variables collected for each adverse event.....	29
6.3	Reporting of serious adverse events.....	30
6.4	Overdose	31
6.5	Pregnancy.....	31
6.5.1	Maternal exposure.....	31
6.5.2	Paternal exposure	31
6.6	Management of toxicities (Dose Reductions).....	31
6.7	Study governance and oversight	31
6.7.1	Steering Committee	31
6.7.2	Data Monitoring Committee	32
6.7.3	Scientific Advisory Committee.....	32
7.	INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS	32
7.1	Identity of investigational product(s).....	32
7.2	Dose and treatment regimens.....	32
7.3	Labelling	32
7.4	Storage	32
7.5	Compliance	32
7.6	Accountability.....	32
7.7	Concomitant and other treatments	33
7.7.1	Other concomitant treatment.....	33
7.8	Post Study Access to Study Treatment	33
8.	STATISTICAL ANALYSES	33
8.1	Statistical considerations.....	33
8.2	Sample size estimate	34
8.3	Definitions of analysis sets	35
8.3.1	Efficacy analysis set.....	35
8.3.2	Safety analysis set	35
8.3.3	PK analysis set	35
8.3.4	PRO analysis set	35
8.4	Outcome measures for analyses.....	35
8.5	Methods for statistical analyses	35
8.5.1	Analysis of the primary variable(s).....	35

8.5.2	Analysis of the secondary variable(s)	35
8.5.3	Subgroup analysis	36
8.5.4	Interim analysis.....	36
8.5.5	Sensitivity analysis.....	36
8.5.6	Exploratory analysis.....	36
9.	STUDY AND DATA MANAGEMENT.....	36
9.1	Training of study site personnel.....	36
9.2	Monitoring of the study	36
9.2.1	Source data	37
9.2.2	Study agreements	37
9.2.3	Archiving of study documents	37
9.3	Study timetable and end of study	38
	Data management.....	38
9.4	38	
9.4.1	Serious Adverse Event (SAE) Reconciliation	38
9.4.2	Data Management of genotype data.....	38
9.4.3	Data associated with human biological samples.....	38
9.4.4	Management of external data.....	38
10.	ETHICAL AND REGULATORY REQUIREMENTS	38
10.1	Ethical conduct of the study.....	38
10.2	Subject data protection.....	38
10.3	Ethics and regulatory review	39
10.4	Informed consent	39
10.5	Changes to the protocol and informed consent form	39
10.6	Audits and inspections	40
11.	LIST OF REFERENCES	40

LIST OF TABLES

No table of figures entries found.

LIST OF FIGURES

No table of figures entries found.

LIST OF APPENDICES

Appendix A	Signatures
Appendix B	Additional Safety Information
Appendix C	IATA 6.2 Guidance document

Clinical Study Protocol
Drug Substance
Study Code
Edition Number 1
Date 21 December 2016

<i>Appendix D</i>	Pharmacogenetics Research
<i>Appendix X</i>	<i>Other</i>

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation or special term	Explanation
AE	Adverse event
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IP	Investigational Product
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LSLV	Last Subject Last Visit
OAE	Other Significant Adverse Event
PGx	Pharmacogenetic research
PI	Principal Investigator
SAE	Serious adverse event
WBDC	Web Based Data Capture

1. INTRODUCTION

1.1 Background and rationale for conducting this study

Chronic idiopathic urticaria (CU) has been defined as the occurrence of spontaneous wheals, with or without angioedema, for more than 6 weeks of unknown cause [1-3]. CU affects 1% of US adults and can be a significant cause of economic burden [3, 4]. The pathogenic mechanism of CU is not completely understood, but IgG antibody to FcER1 α subunit has been identified in approximately 30-40% of patients with CU, while anti-IgE antibody has been identified in another 5-10% [5]. In addition, an increased prevalence of autoimmune diseases has been observed in some CU patients with 12–29% showing significantly higher serum anti-Thyroid Peroxidase (TPO) antibody [6, 7]. However, the presence or absence of anti-FcER1/anti-IgE serum antibodies or other autoantibodies have not been found to have an effect on treatment response to either oral antihistamines, biologics (i.e. omalizumab) or other anti-inflammatory/immunosuppressants used to treat CU [8-11].

Histological analyses reveals significantly higher numbers of infiltrating eosinophils and lymphocytes in urticarial wheals compared to uninvolved skin and skin of normal healthy control subjects [12, 13]. Monocytes and T-lymphocyte are predominant cellular infiltrates in CU although the T-cell sub-population is highly variable between patients [14]. Activated eosinophils could play a mechanistic role in CU by triggering the tissue factor pathway of the coagulation cascade and by releasing vascular endothelial growth factor, thus contributing to development and severity of disease [2, 15]. CU skin often shows a significant increase of vascular endothelial growth factor (VEGF), which increases vaso-permeability through an increase in local nitric oxide production [16-18]. CU wheals also show higher expression of several cytokines (including IL-5, the key cytokine involved in eosinophil differentiation), chemokines, and adhesion molecules compared to uninvolved skin, and healthy control subjects [12, 19]. In addition, serum eotaxin concentration has been found to be significantly higher in CU patients (N=100; median 140.1 pg/ml, range 33.7-718.7 pg/ml) compared to healthy controls (N=45; median 108.9 pg/ml, range 45.5-409.4 pg/ml) ($p = 0.032$) [20]. Taken together, these data strongly suggest the involvement of eosinophils in the pathogenesis of CU[2].

Existing treatment options such as use of H1-receptor antagonists with or without a leukotriene receptor antagonist (LTRA), cyclosporine and other anti-inflammatory agents have been frequently demonstrated to be inadequate in managing CU. Therefore, there is a need for novel therapeutic agents to control non-histaminergic hives. Omalizumab has recently been approved for the treatment of idiopathic CU unresponsive to H1-antagonists administered at FDA recommended doses [21]. Clinical trials have shown that up to 44% of CU patient unresponsive to H1-antagonists have a complete response to omalizumab but after discontinuation of therapy the hives in the majority of cases returned to near baseline [22]. Currently it is unknown how omalizumab works mechanistically other than binding peripheral IgE and downregulating IgE receptors on mast cells and basophils. It is also unclear how long to treat with this agent as it does not appear to drive CU into remission. Thus other options that have more plausible mechanisms of action are needed to treat CU and induce remission [23-25].

1.1. Research hypothesis

Benralizumab decreases Urticaria Activity Score 7 ~~and Urticaria Control Score~~ in human subjects with chronic urticaria unresponsive to second generation H1-antihistamines.

1.2. Rationale for study design, doses and control groups

The purpose of this study is to determine the percentage of CU patients previously unresponsive to second generation H1-antihistamines, who respond to Benralizumab as determined by significant improvement in UAS7 ~~and UCT-scores~~ post-Benralizumab vs baseline or post-placebo.

Benralizumab, a humanized anti-IL5 receptor α monoclonal antibody, has been demonstrated to be very effective in decreasing baseline blood eosinophil counts, serum ECP (eosinophil cationic protein) levels, and sputum and tissue eosinophils resulting in clinical improvement in eosinophilic asthma and chronic obstructive pulmonary disease (COPD) [26-32].

Interestingly, IL5R is also expressed on B cells and regulates genes involved in B-cell maturation and antibody class switching which could explain the increased frequency of autoantibodies seen in CU patients and thus be a potential mechanistic target for benralizumab in the treatment of CU [33-38]. Although it has been suggested that Bernalizumab could be effective in a wide range of eosinophilic disorders, its efficacy in controlling CU has never been studied. Thus the primary objective of this study is to assess the efficacy of Benralizumab in managing CU and to address existing knowledge gaps. Subjects, between >18 and $\leq$ 70 years, having symptoms for at least six weeks and unresponsive to H1-antagonists will be recruited from our patient registry for this proof of concept study and the primary endpoint will be complete response, partial response or no response using the urticaria activity score (UAS7) ~~and urticaria control scores~~.

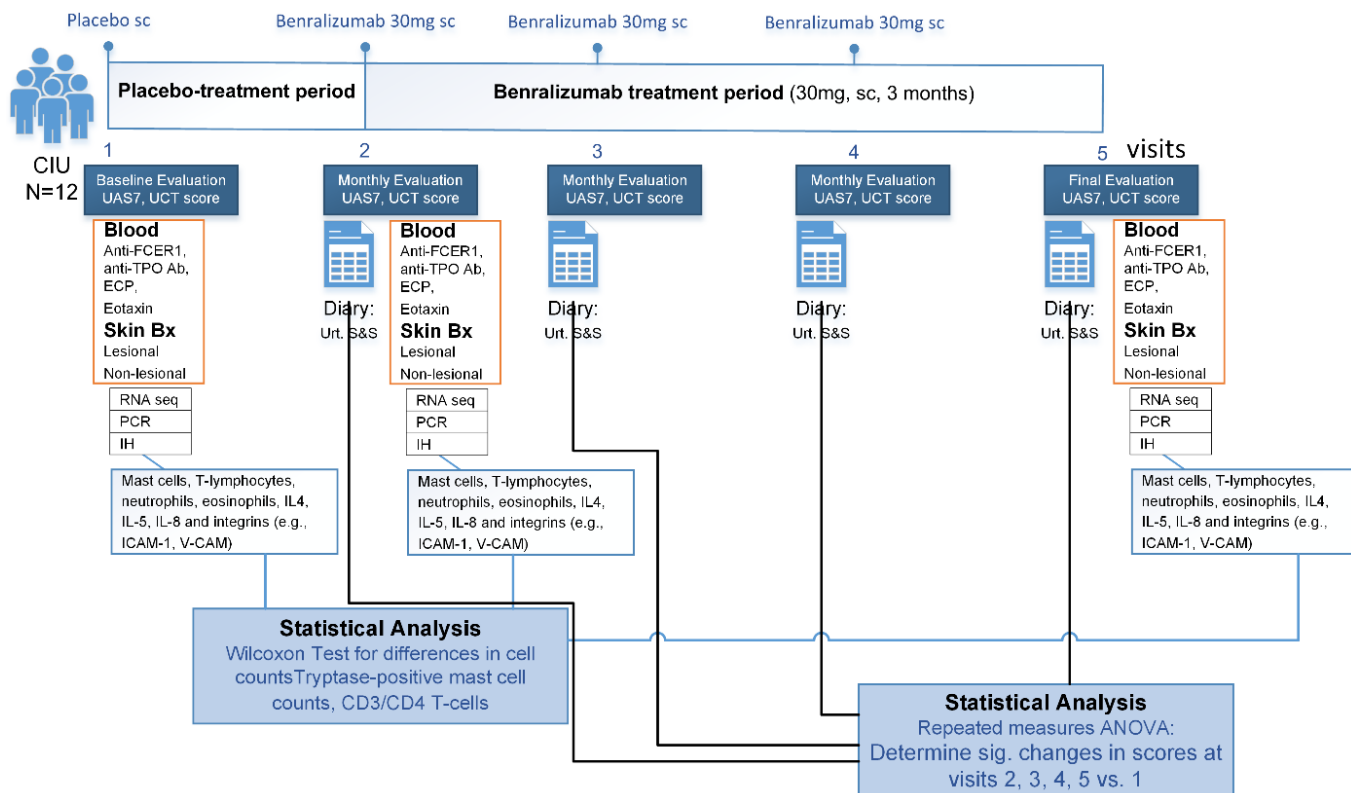
These results will determine on the optimal design of larger multicenter clinical trials for validating indications of Benralizumab in CU and FDA approval. Because of the small sample size and being conducted at a single site, a repeated measures study design is considered more appropriate.

Benefit/risk and ethical assessment

Benralizumab has already proven efficacious in decreasing asthma exacerbations in chronic moderate to severe asthma in patients not completely responsive to combination long-acting beta-agonists/corticosteroids. Based on its safety profile in human asthma subjects we believe that use of this treatment would not have harmful effects in CU patients and that the beneficial effects could be quite substantial in improving chronic urticaria control. Safety assessments will be performed throughout the study through clinical history, physical examination and blood examination as discussed in this protocol.

1.3. Study Design

Figure 1 Study Design



This is a single-blind, one-group, single center exploratory study to assess efficacy of Benralizumab 30mg in improving UAS7 ~~and UCT scores~~ and reducing inflammatory cell infiltration in skin in North American subjects with CU unresponsive to second generation H1-antihistamines. The design is explained in **Figure 1**.

2. STUDY OBJECTIVES

2.1 Primary objective

Primary Objective:	Outcome Measure:
<i>To determine the clinical efficacy of Benralizumab when compared to placebo in patients with chronic urticaria who are refractory to treatment with H1-antihistamines.</i>	<i>Urticarial Activity Symptoms averaged over 7 days (UAS7) ~~and Urticarial Control (UCT) scores 1~~</i>

2.2 Secondary objectives

Secondary Objective	Outcome Measure
<i>To investigate possible influence of Benralizumab on pathophysiology of CU in patients refractory to treatment with H1-antihistamines.</i>	<i>Blood anti-FCER1, anti-TPO antibody, ECP and eotaxin levels</i> <i>Gene expression profile using RNA-sequencing in skin biopsy samples (lesional vs. non-lesional skin); PCR validation for the top genes</i> <i>Inflammatory cell count and cytokine quantification in skin biopsy using immunocytochemistry</i>

2.3 Safety objectives

Safety Objective:	Outcome Measure :
<i>To evaluate the safety and tolerability of Benralizumab in CU patients in relation to H1-antihistamines</i>	<i>Adverse Events/Serious Adverse Events</i> <i>Vital signs (Pulse rate, Blood Pressure, Respiratory rate)</i> <i>Total count, Differential count, Hematocrit</i>

2.4 Exploratory objectives

Exploratory Objective:	Outcome Measure :
<i>To demonstrate an improvement in health-related quality of life in the study subjects in subjects treated with Benralizumab when compared to placebo.</i>	<i>Health Status as determined by Chronic Urticaria Quality of Life Questionnaire (CU-QoL)</i>

3. SUBJECT SELECTION, ENROLMENT, RANDOMISATION, RESTRICTIONS, DISCONTINUATION AND WITHDRAWAL

Twelve (12) CU subjects and six (6) healthy control matches (total 18) who meet the inclusion and exclusion criteria will be enrolled.

3.1 Inclusion criteria

- Age >18 and ≤70 yrs;
- urticarial lesions for > 6 weeks of unknown cause;

- hives present most days of the week despite H1-antihistamines up to 4× the FDA approved dose;
- Pruritus severity ≥ 2
- Urticaria Activity Score (UAS, i.e., combined score of pruritus severity + # of hives + size of largest hive) ≥ 4
- Compliance $>70\%$ with completion of daily diaries for 2 weeks between visit 1 (informed consent) and visit 2 (placebo treatment)
- Informed Consent: Able to give written informed consent prior to participation in the study, which will include the ability to comply with the requirements and restrictions listed in the consent form. Subjects must be able to read, comprehend, and write at a level sufficient to complete study related materials.
- Female subjects: Women of childbearing potential (WOCBP) must use an effective form of birth control (confirmed by the Investigator). Effective forms of birth control include: true sexual abstinence, a vasectomized sexual partner, Implanon, female sterilization by tubal occlusion, any effective IUD intrauterine device/IUS levonogestrel Intrauterine system, Depo-Provera(tm) injections, oral contraceptive, and Evra Patch(tm) or Nuvaring(tm). WOCBP must agree to use effective method of birth control, as defined above, from enrolment, throughout the study duration and within 16 weeks after last dose of Benralizumab, and have negative serum pregnancy test result on Visit 0.
- Women not of childbearing potential are defined as women who are either permanently sterilized (hysterectomy, bilateral oophorectomy, or bilateral salpingectomy), or who are postmenopausal. Women will be considered postmenopausal if they have been amenorrheic for 12 months prior to the planned date of visit -1 without an alternative medical cause. The following age-specific requirements apply:
 - Women <50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatment and follicle stimulating hormone (FSH) levels in the postmenopausal range.
 - Women ≥ 50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatment.
- All male subjects who are sexually active must agree to use an acceptable method of contraception (condom with or without spermicide) from Visit 0 until 16 weeks after their last dose.

3.2 Exclusion criteria

- Physical urticarias (pressure, cold, exercise, dermatographism...)
- Urticarial vasculitis
- Secondary urticaria (underlying allergy to food/drugs or autoimmune disease, infection, malignancy, thyroid disease)
- Use of oral or topical corticosteroids, methotrexate, cyclosporine, other biologics (i.e., IVIG, omalizumab) and anti-inflammatory agents (hydroxychloroquine, sulfasalazine, dapsone or colchicine) within 4 weeks of enrolment.

- History of cancer: Subjects who have had basal cell carcinoma, localized squamous cell carcinoma of the skin, or in situ carcinoma of the cervix are eligible provided that the subject is in remission and curative therapy was completed at least 12 months prior to the date informed consent, and assent when applicable was obtained. Subjects who have had other malignancies are eligible provided that the subject is in remission and curative therapy was completed at least 5 years prior to the date informed consent, and assent when applicable, was obtained.
- A helminth parasitic infection diagnosed within 24 weeks prior to the date informed consent is obtained that has not been treated with, or has failed to respond to standard of care therapy.
- Pregnant or nursing
- If female and of child-bearing potential, must have negative pregnancy test and must adhere to acceptable method of contraception (with <1% failure rate) during the study and for four months after the study.
- Receipt of any investigational non biologic within 30 days or 5 half-lives prior to visit 0, whichever is longer.
- A history of known immunodeficiency disorder including a positive human immunodeficiency virus (HIV) test.
- Any other medical illness that precludes study involvement
- Positive hepatitis B surface antigen, or hepatitis C virus antibody serology, or a positive medical history for hepatitis B or C. Subjects with a history of hepatitis B vaccination without history of hepatitis B are allowed to be enrolled.
- Patients who are currently receiving or have previously received benralizumab or any other type of anti-interleukin therapy (i.e. mepolizumab, reslizumab, lebrikizumab etc.) within the last 4 months or 5 half-lives whichever is longer.
- History of anaphylaxis to any biologic therapy or vaccine.
- Receipt of immunoglobulin or blood products within 30 days prior to the date informed consent is obtained.

Healthy controls should have the same criteria except the urticarial lesions, hives, pruritus. These controls are not required to maintain daily diaries.

3.3 Subject enrolment and randomization

The Principal Investigator will:

- Obtain signed informed consent from the potential subject before any study specific procedures are performed.
- Assign potential subject a unique enrolment number.
- Determine subject eligibility. See Sections 3.1 and 3.2
- Because all subjects will receive the same intervention therefore randomization is not applicable.

3.4 Procedures for handling incorrectly enroled or randomized subjects

Subjects who fail to meet the inclusion criteria or who meet the exclusion criteria should not, under any circumstances, be enroled or randomized. There can be no exceptions to this rule.

Where subjects that do not meet the selection criteria are enroled in error or incorrectly started on treatment, or where subjects subsequently fail to meet the study criteria post initiation, the investigator will discontinue subject participation in the study. .

3.5 Methods for assigning treatment groups

In this single group study design all subjects with CU will receive placebo once and then Benralizumab 30mg every 4 weeks ×3 doses. Healthy controls will not be treated with the placebo or benralizumab but enroled for obtaining blood samples and one skin biopsy.

3.6 Methods for ensuring blinding

The study is single-blinded. The subjects only will be blinded to the intervention (placebo or Benralizumab) they will receive at the study site. Unblinded trained research staff will administer the placebo/Benralizumab at the study site. Close attention should however be paid to make the appearance, package and labels of the investigational product look identical to the subjects.

3.7 Methods for unblinding

Subjects only will be blinded to intervention throughout and after the study. Unblinding does not apply.

3.8 Restrictions

- There are no specific dietary or activity restrictions other than those typical for a patient with this disease. Patients should not donate blood or bone marrow at any time during the study period.
- Females of child-bearing potential (ie, females who are not chemically or surgically sterilised or females who are not post-menopause) must use 2 methods of reliable contraception, one of which must be a barrier method from the enrolment until 1 month after the last dose.
- Concomitant medications will exclude steroids, immunosuppressant and specific anti-inflammatory drugs such as colchicine.

3.9 Discontinuation of investigational product

- Subjects may be discontinued from investigational product (IP) in the following situations:
- Voluntary discontinuation by the patient who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment
- Safety reasons as judged by the investigator (e.g clinically significant ventricular pauses, syncope related to bradycardia, persistent increase in serum creatinine level of clinical relevance, persistent, unexplained anaemia (e.g. Hb <10 g/dL) or thrombocytopenia (e.g. platelet count <10 x10⁴/μL)

- Severe non-compliance to protocol as judged by the investigator
- Incorrect enrolment (ie, the patient does not meet the required inclusion/exclusion criteria) and the discontinuation is decided by the discussion (see Section 5.3)
- Pregnancy
- Development of a condition or an acute event (eg, MI or stroke) that necessitates treatment with one or more prohibited medications
- Once enrolled into the study all patients will be assessed until study closure unless informed consent is withdrawn for study participation.

Repeated attempts will be made to locate and determine the vital status and occurrence of any SAE for those patients who are considered initially lost to follow up. A patient will be classified as lost to follow up only if, he/she has failed to return for the required study visits and his/her vital status remains unknown despite multiple attempts to contact him/her via telephone, fax, email, and certified letter.

3.9.1 Procedures for discontinuation of a subject from investigational product

A subject that decides to discontinue investigational product will always be asked about the reason(s) and the presence of any AEs. If possible, the subject should be seen and assessed by the investigator(s) at discontinuation visit and follow up visit. AEs will be followed up (See Sections 7.2).

3.10 Criteria for withdrawal

3.10.1 Screen failures

Subjects not matching the inclusion and exclusion criteria will be deemed as screen failure and will not be enrolled.

3.10.2 Withdrawal of the informed consent

Subjects deciding to withdraw from the study after having consented initially will be withdrawn from the study. Samples and data from such subjects will be destroyed.

3.10.3 Need for concomitant medical treatment

A subject may be withdrawn from the study in the event of the subject needing administration of steroids and other biologics due to medical conditions unrelated to CU.

3.11 Discontinuation of the study

The investigator reserves the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the IRB. In terminating the study, the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

4. STUDY PLAN AND TIMING OF PROCEDURES

The study will be a single-blind (subject blinded), placebo-controlled repeated measures study at a single site. Twelve human subjects (aged >18—and ≤70 years, both men and women) with a physician-based diagnosis of CU and the other characteristics mentioned in the inclusion criteria while on H1 anti-histamines before enrolment will be included. After the initial

baseline assessment and sample collection at Visit 1, placebo for Benralizumab will be administered at the same visit (**Table 1**). Subsequently, at each of the Visits 2, 3 and 4 (four weeks apart), Benralizumab 30 mg subcutaneously will be administered. Samples (whole blood and skin biopsy) will be collected before placebo administration (Visit 1), 4 weeks after placebo (Visit 2) and 4 weeks after the last treatment with Benralizumab (Visit 5). Because of the small sample size, stratification based on disease severity will not be feasible. All subjects will be masked to treatment allocation. The primary endpoint will be extent of reduction in UAS7 ~~and UCT scores~~ post-Benralizumab versus post-placebo or versus baseline. Key secondary endpoints will be differential gene expression for pathway focussed genes and levels of anti-FCER1, anti-TPO antibody, ECP and eotaxin levels in blood, as well as the differences in inflammatory cell infiltrate and cytokines in skin biopsies post-Benralizumab vs. baseline /post-placebo. Efficacy analyses were by intention to treat (based on the full analysis set); safety analyses included patients according to study drug received.

Table 1 Study Plan detailing the procedures

Visit Visit window Days –28 to 0 for Visit 0 ±0 day for Visit 1 ±1 day for Visits 2, 3, 4 and 5 ±5 days for 60 day follow-up after Visit 5	0 Screening	1	2	3	4	5	60-day follow-up	Protocol Section
Week		4	8	12	16	20	28	Section
Written informed consent	×							10.4
Demographics	×							
Physical examination	×	×	×	×	×	×	×	5.2.2
Medical/surgical history	×							
Inclusion/exclusion criteria	×	×	×	×	×	×		3.1, 3.2
Vital signs	×	×	×	×	×	×	×	5.2.4
Assessment of daily diaries (UAS7, ~~UCT~~)		×	×	×	×	×	×	5.1.1
Blood and Skin biopsy samples		×	×			×		5.1.3
Treatment dispensed to CU subjects only		×	×	×	×			7.1, 7.2
		(P)	(B, 1 st)	(B, 2 nd)	(B, 3 rd)			
Adverse event review (AEs and SAEs)	×	×	×	×	×	×	×	6.1, 6.2, 6.3
Blood samples for safety assessments		×	×	×	×	×	×	5.2.1

* before administration of placebo; P=Placebo; B= Benralizumab

4.1 Enrolment/screening period

Enrolment will begin in the second quarter of 2017 and will continue through the first quarter of 2018. A subject is expected to be in the study for seven months. Screening will be performed in the first month.

Methods of enrolment. The PI in this study has a steady pool of CU patients attending his clinic. Patients meeting the inclusion criteria will be informed about the study by conversations in person and / or using a recruitment material (advertisement). An outline of the advertisement is described below.

VOLUNTEERS NEEDED FOR BENRALIZUMAB CLINICAL RESEARCH TRIAL

Purpose- To investigate the usefulness of new medication named Benralizumab in the treatment of itchy skin rash and hives known as chronic urticaria, that has not responded adequately to standard treatment with H1-anti-histamine such as Allegra or others.

This investigational drug, **Benralizumab**, has been proven to be effective and safe in patients with asthma in other studies. Because of related immune mechanisms in asthma and chronic urticaria, this study will investigate if this drug is beneficial in treating chronic urticaria in patients who do not respond satisfactorily to standard treatments.

Duration of enrolment. Seven months

Inclusion/ Exclusion conditions. Participant aged >18 and ≤ 70 years must have itchy rash and hives on skin consistent with a diagnosis of chronic urticaria for six weeks or more that has not responded to standard drugs. They should not have taken immunosuppressant drugs such as topical or oral corticosteroids, cyclosporine or omalizumab or other anti-inflammatory drugs such as hydroxychloroquine, dapsone, sulfasalazine or colchicine within the last 4 weeks.

Activities. Upon meeting the inclusion and exclusion criteria as assessed during the screening visit, the volunteers will be required to report to the study site once a month for five office visits. Activities in these visits will include

- Physical examination (blood pressure, pulse rate) and skin examination
- Administration of investigational drug at the first four visits
- Blood sampling and skin biopsy of normal skin and hives three times during the study
- In addition, a diary for recording the symptoms will be provided. The participants should record their daily symptoms for the first five months and bring the diary to the study site at every visit.

Incentives. An incentive of \$250 per visit in check for time and travel to the study site is provided to all the participants.

Benefits. This study does not guarantee any treatment benefits resulting from the investigational drug.

Details on how to enrol. If interested, contact **Dr. Jonathan Bernstein at Phone number 513-235-7971. Or email jonathan.bernstein@uc.edu**

4.2 Treatment period

Each of the study Visits 0-5 will be scheduled 4 weeks apart. Placebo (for Benralizumab), subcutaneously, administered only once at visit 1 and Benralizumab 30 mg. subcutaneously during visits 2, 3 and 4.

4.3 Follow-up period

60 days ± 5 days after Visit 5. Subjects will be followed up at the clinic to assess for remission or relapse of urticaria and general well-being will be followed.

5. STUDY ASSESSMENTS

The study will assess the efficacy and safety of Benralizumab vs. placebo

5.1 Efficacy assessments

5.1.1 CU subjects only

UAS7 ~~and~~ UCT. Disease activity will be assessed by UAS7 ~~and disease control by UCT~~. To obtain UAS7 values, patients will be asked to document every day for seven consecutive days the numbers of wheals and the intensity of pruritus they experienced over the last 24 h using a 0–3 point scale for wheals (0 for none, 1 for <20, 2 for 20–50, and 3 for >50) and pruritus (0 for none, 1 for mild: present but not annoying or troublesome, 2 for moderate: troublesome, but does not interfere with normal daily life activity or sleep, and 3 for intense: interferes with normal daily life or sleep). The UAS7 (minimum=0, maximum=42) is then calculated as the sum of the daily totals of the wheal and the itch scores.

Blood and Skin Biopsy. Whole blood sample (10 mL) and four 4sq.mm. skin biopsy (two from unaffected skin other two from lesional skin) will be collected at Visits 1 (baseline), 2 (post-placebo), and 5 (post-Benralizumab). If there are no lesion(s) at visit 5 then biopsies will be taken from skin adjacent to previous biopsy sites.

5.1.2 Healthy match subjects only

Blood and skin biopsy similar to CU subjects will be collected only once.

5.1.3 Methods and Procedures for Blood Sampling and Skin Biopsy Collection and Downstream Assays

5.1.3.1 Blood Sample Collection (for anti-FCER1, anti-TPO antibody, ECP, and eotaxin levels)

10 mL of venous blood will be obtained by venipuncture of cubital vein in the forearm and collected in a plain, red-top tube. Blood will be allowed to clot and separate within one hour. Serum will be sent to analysis lab frozen. Standard ELISA-based protocol will be followed for assessment of anti-FCER1, anti-TPO antibody, ECP, and eotaxin levels.

5.1.3.2 Skin Biopsy (for RNA-sequencing and immunocytochemistry)

Skin biopsy performed using a minimally invasive disposable punch is a safe and painless procedure. A skin punch biopsy of a persistent hive can help diagnose underlying pathological changes by revealing the presence of inflammatory cell infiltration and deposition of immunoglobulins and complement. Using specific antibodies with immunofluorescence technique, it will be possible to investigate predominant cellular infiltrate in the lesional skin compared to normal skin in CU subjects normalized to samples from healthy controls.

Skin site in study subjects will be swabbed with isopropyl alcohol (70%) and anesthetized with local anesthetic (1 mL of 1% lidocaine + adrenaline). Using a punch biopsy needle a 4

sq.mm. full thickness skin biopsy from the lesional and normal will be obtained. The biopsies for immunocytochemistry will be preserved in Michel's medium or phosphate buffer saline and transported fresh to lab for further analysis. The biopsies for RNA-seq will be snap-frozen in liquid nitrogen and stored at -20C until analysis.

5.1.4 Sample Processing

5.1.4.1 RNA sequencing from the skin biopsy samples.

Total RNA will be isolated using the RNA isolation kit. The biopsy will be placed in a cryo-vial and snap-frozen in liquid nitrogen and stored at -20°C. During analysis ~75-100 mg Phase-Lock Gel Heavy (PLG-H) will be placed in a 1.5 ml tube and pelleted @ 12,000xg for 30s. The skin biopsies will be grinded to a fine powder in a pre-chilled mortar and pestle. Qiazol (Lysis Reagent) 300µL is added to a 1.5 ml tube. Powdered biopsy will be transferred to this tube and vortexed for 15s. The tubes will be shaken for 10 min and spun down for 15s. Chloroform 60 µL will be added and vortexed (15s) and spun down (15s) after 3 minutes. Partially separated mixture will be transferred to the PLG-H containing tube and mixed by inverting the tubes and then centrifuged @ 12,000xg for 15 min. The aqueous phase will be transferred to a new tube. One volume of 70% EtOH will be added to the aqueous phase and vortexed for 5s. Mixture will be transferred to a RNeasy MinElute spin column and RNA will be cleaned and concentrated using the vendor's protocol. Isolated RNA will be sent to sequencing core for library preparation, sequencing and preliminary data analysis (hierarchical clustering and pathway analysis).

5.1.4.2 Skin biopsy immunofluorescence.

In the lab, samples will be washed and then mounted and frozen in liquid nitrogen prior to sectioning. Using a cryostat, 5 micron sections will be cut and mounted onto charged glass slides. The tissue sections will be incubated with fluorescence tagged antibodies (fluorescein isothiocyanate, FITC) directed against mast cells, T-lymphocytes, neutrophils, eosinophils, IL4, IL-5, IL-8 and integrins. Each slide will be examined for fluorescence using a microscope with an ultraviolet light source and equipped with filters to detect the FITC signal. The location, pattern, and intensity of each immune reactant will be noted.

5.1.4.3 Comparison of results

Comparison of Gene expression and number of cell infiltrates or cytokine levels in CU subjects **post**-Benralizumab vs. **post**-placebo or baseline will identify the molecular mechanisms that are accountable for relapse in CU patients on H1-antihistamines prior to receiving Benralizumab. Results from healthy control samples will be used for normalization of results in CU samples.

5.2 Safety assessments

5.2.1 Laboratory safety assessments

The following laboratory variables (amend as appropriate) will be measured:

Table 2 Laboratory Safety Variables

Haematology/Haemostasis (whole blood)	Clinical Chemistry (serum or plasma)
B-Haemoglobin (Hb)	S/P-Albumin
B-Leukocyte count	S/P-Creatinine
B-Leukocyte differential count (absolute count)	S/P-Alanine transaminase (ALT)
B-Platelet count	

5.2.2

Physical examinations (PE) conducted throughout the study will be a focussed symptom-directed PE. The complete PE conducted at screening visits will also include the following assessments: medical history review, including history of allergies, and history of prior and current use of nicotine or nicotine-containing products, history of alcohol and illegal drug use, and history of prior (within 90 days) and current medication use.

5.2.3 ECG

not applicable

5.2.3.1 Resting 12-lead ECG

not applicable

5.2.4 Vital signs

Pulse rate, blood pressure, respiratory rate and surface body temperature will be assessed every visit.

5.2.5 Other safety assessments

Urine pregnancy tests will also be taken for all females at Visits 1-4, if applicable, before placebo/Benralizumab administration. If urine pregnancy test is positive, subjects will be deemed ineligible for further continuation in the study.

5.3 Other assessments

5.3.1 Patient reported outcomes

not applicable

5.3.1.1 Name of PRO method or questionnaire

not applicable

5.4 Pharmacokinetics

5.4.1 Collection of samples

not applicable

5.4.2 Determination of drug concentration

not applicable

5.4.3 Storage and destruction of pharmacokinetic samples

not applicable

5.5 Pharmacodynamics

not applicable

5.5.1 Collection of samples

not applicable

5.5.2 Storage, re-use and destruction of pharmacodynamic samples

not applicable

5.6 Pharmacogenetics

not applicable

5.6.1 Collection of pharmacogenetic samples

not applicable

5.6.2 Storage, re-use and destruction of pharmacogenetic samples

not applicable

5.7 Biomarker analysis

Significant differentially expressed genes in blood from CU subjects adjusted for healthy controls will be used to measure biomarkers important in CU and prediction of relevant immunological pathways or inflammation. Top genes will be validated by PCR assay.

Skin biopsies are well-tolerated and easily accessible. These biopsies characterise a system in which several intricate signaling networks are active and in which diverse drug targets may likely play a role directly or indirectly. Differences in types of cellular infiltrates or cytokines localized in skin biopsies will be ***pathological biomarkers*** for differentiating CU from healthy controls. Significant differences in cell infiltrates and cytokines post-treatment vs. placebo will determine ***pharmacodynamic biomarkers***.

Repeat samples will determine if Benralizumab modifies these discovered pathways or biomarker molecules. Correlation between biomarkers from blood and skin will also be analysed.

5.7.1 Storage, re-use and destruction of biological samples

Samples left-over after the conclusion of the study will be disposed off using universal precautions.

5.7.2 Labelling and shipment of biological samples

The biological samples collected in this study are categorized as exempt human specimens and are assumed to be non-infectious. The label will be printed with the proper dimension of an Infectious Substance label (50 mm on a side, and the proper shipping name, "Biological Substance, Category B" in letters at least 6 mm high). Label will be affixed to the shipment

package by covering with clear plastic tape. Specimens will be shipped using the triple packaging system. The outer package will be marked with “Exempt human specimen”.

5.7.3 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle. The Principal Investigator will keep full traceability of collected biological samples from the subjects while in storage at the study site until shipment or disposal (where appropriate) and will keep documentation of receipt of arrival.

The sample receiver will keep full traceability of the samples while in storage and during use until used or disposed of or until further shipment and keeps documentation of receipt of arrival.. Samples remaining at the end of the study will not be retained for further use but will be discarded.

5.7.4 Withdrawal of Informed Consent for donated biological samples

If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analysed, the investigator is not obliged to destroy the results of this research.

As collection of the biological samples is an integral part of the study, the subject will be withdrawn from further study participation.

The Principal Investigator:

- will ensure subjects’ withdrawal of informed consent to the use of donated samples is notified immediately to the Schulman IRB.
- will ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- will ensure the lab holding the samples is informed about the withdrawn consent immediately and that samples are disposed/destroyed, the action documented and the signed document returned to the study site
- will ensure that the subject and the Schulman IRB are informed about the sample disposal

6. SAFETY REPORTING AND MEDICAL MANAGEMENT

Safety will be evaluated throughout the study. Evaluation for AEs, review of concomitant medications, and review of protocol restrictions will occur at the beginning of each visit.

6.1 Definition of adverse events

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a Benralizumab or placebo, whether or not considered causally related to the product. An undesirable medical condition can be symptoms (eg, nausea, chest pain), signs (eg, tachycardia, enlarged liver) or the abnormal results of an investigation (eg, lab findings). In clinical studies, an AE can include an undesirable medical condition occurring at any time, including run-in or washout periods, even if no study treatment has been administered.

The term AE is used to include both serious and non-serious AEs. An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an AE and must be reported.
- Preexisting diseases, conditions, or laboratory abnormalities present or detected before the Visit 1 that do not worsen
- Situations where an untoward medical occurrence has not occurred (eg, hospitalization for elective surgery, social and/or convenience admissions)

Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be preexisting and should be documented on the medical history CRF.

6.1.1 Definitions of serious adverse event

- A serious adverse event is an AE occurring during any study phase (ie, run-in, treatment, washout, follow-up), that fulfils one or more of the following criteria:
- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.
- The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to AstraZeneca.

6.1.2 Causality assessment

The Investigator will assess causal relationship between Investigational Product (Benralizumab) and each Adverse Event, and answer 'yes' or 'no' to the question 'Do you consider that there is a reasonable possibility that the event may have been caused by the investigational product?'

For SAEs a causal relationship will also be assessed for other concomitant medications, study procedures, and comparator study drugs. Note that for SAEs that could be associated with any study procedure the causal relationship is implied as 'yes'.

6.2 Recording of adverse events

6.2.1 Time period for collection of adverse events

AEs will be recorded after obtaining the written informed consent until the follow-up visit.

6.2.2 Follow-up of unresolved adverse events

Any AEs that are unresolved at the subject's last AE assessment in the study will be followed up by the investigator for as long as medically indicated, but without further recording in the CRF..

6.2.3 Variables collected for each adverse event

The following variables should be collected for each AE:

- AE (e.g., breathing difficulty, nasopharyngitis, upper respiratory infection, headache, bronchitis, sinusitis, influenza and pharyngitis)
- The date and time when the AE started and stopped
- Maximum intensity or changes in intensity
- Common Terminology Criteria for Adverse Events v4.0 (CTCAE) grade/changes in CTCAE grade (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)
- Whether the AE is serious or not
- Investigator causality rating against the Investigational Product (yes or no)
- Action taken with regard to investigational product
- AE caused subject's withdrawal from study (yes or no)
- Outcome of AE(s).

In addition, the following variables should be collected for SAEs:

- Date AE met criteria for serious AE
- Date Investigator became aware of serious AE
- AE is serious due to ... (e.g., the subject being at risk of death at the time event, or has led to in-patient hospitalization, persistent or significant disability or incapacity or a medically important event or reaction that may not be immediately life-threatening)
- Date of hospitalisation
- Date of discharge
- **If death:**
 - Probable cause of death
 - Date of death
 - Autopsy performed
 - Causality assessment in relation to Study procedure(s)
 - Description of AE(s).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 6.1.11. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not a SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke but would be a SAE.

Adverse Events based on signs and symptoms

All AEs spontaneously reported by the subject or reported in response to the open question from the study personnel: 'Have you had any health problems since the previous visit/you were last asked?', or revealed by observation will be collected and recorded in the CRF. When collecting AEs, the recording of diagnoses is preferred (when possible) to recording a list of signs and symptoms. However, if a diagnosis is known and there are other signs or symptoms that are not generally part of the diagnosis, the diagnosis and each sign or symptom will be recorded separately.

Adverse Events based on examinations and tests

The results from protocol mandated laboratory tests and vital signs will be summarised in the clinical study report (CSR). Deterioration as compared to baseline in protocol-mandated laboratory values and vital signs should therefore only be reported as AEs if they fulfil any of the SAE criteria or are the reason for discontinuation of treatment with the investigational product.

If deterioration in a laboratory value/vital sign is associated with clinical signs and symptoms, the sign or symptom will be reported as an AE and the associated laboratory result/vital sign will be considered as additional information. Wherever possible the reporting investigator uses the clinical, rather than the laboratory term (eg, anaemia versus low haemoglobin value). In the absence of clinical signs or symptoms, clinically relevant deteriorations in nonmandated parameters should be reported as AE(s).

Any new or aggravated clinically relevant abnormal medical finding at a physical examination as compared with the baseline assessment will be reported as an AE.

Since the most common adverse effects previously noted for Benralizumab has been breathing difficulty, nasopharyngitis, upper respiratory infection, headache, bronchitis, sinusitis, influenza and pharyngitis, surveillance for these events will be undertaken. Any of these events that fulfil the criteria of an AE as judged by the investigator should be reported using the standard procedures for assessing severity, causality and seriousness.

6.3 Reporting of serious adverse events

All SAEs have to be reported, whether or not considered causally related to the investigational product, or to the study procedure(s). All SAEs will be recorded in the CRF.

If any SAE occurs in the course of the study, then investigators or other site personnel inform appropriate AstraZeneca representatives within one day ie, immediately but no later than the end of the next business day of when he or she becomes aware of it. The designated AstraZeneca representative works with the investigator to ensure that all the necessary information is provided to the AstraZeneca Patient Safety data entry site within one calendar day of initial receipt for fatal and life threatening events and within five calendar days of initial receipt for all other SAEs.

For fatal or life-threatening AEs where important or relevant information is missing, active follow-up is undertaken immediately. Investigators or other site personnel inform AstraZeneca representatives of any follow-up information on a previously reported SAE within one calendar day ie, immediately but no later than the end of the next business day of when he or she becomes aware of it.

The Investigator or other study site personnel reports a SAE to the appropriate AstraZeneca representative by telephone. The AstraZeneca representative will advise the Investigator/study site personnel how to proceed.

6.4 Overdose

Overdose is considered an adverse events and will be dealt with accordingly. An overdose is defined as any intake of study medication more than defined in the protocol. Because the placebo or drug will be administered in pre-filled syringes, the likelihood of an overdose is minimized. However, in the event of an overdose with Benralizumab, the time and extent of the overdose will be noted. Overdose with associated AE will be recorded as AE diagnosis/symptoms and overdose without AE will be recorded as Overdose only.

6.5 Pregnancy

All outcomes of pregnancy should be reported to AstraZeneca. Such cases should be reported to the AstraZeneca within 24 hours of becoming aware of the pregnancy.

6.5.1 Maternal exposure

If a subject becomes pregnant during the course of the study, the investigational product should be discontinued immediately. Pregnancy itself is not regarded as an AE unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of any contraceptive medication.

Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The subject will be unblinded to the treatment and counseling may be offered based on whether the fetus has been exposed to the investigational drug or placebo.

6.5.2 Paternal exposure

There are no restrictions against fathering a child when treated with AZD6140. If paternal exposure pregnancy occurs in the course of the study, then investigators or other site personnel should inform appropriate AstraZeneca representatives within one day as described in the maternal exposure Section 13.3.1. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up and documented even if the subject was discontinued from the study.

6.6 Management of toxicities (Dose Reductions)

The study is designed to administer uniform dose of 30mg of Benralizumab. In the event of SAEs, the subject will be discontinued from the study and the event reported as mentioned above. Continued enrolment at reduced dosage is not applicable.

Dose Reductions: Not applicable.

6.7 Study governance and oversight

The Schulman IRB is the governing body who will review the research project in order to protect study participants. Every study is reviewed by an IRB before it begins. IRBs ensure that researchers and institutions abide by federal regulations and guidelines so that risk to human research participants is minimized.

6.7.1 Steering Committee

Not applicable

6.7.2 Data Monitoring Committee

We will select 3 individuals to review safety data throughout this study (Susan Pinney, PhD epidemiologist; Bill Ridgeway, MD; Vincent Martin, M.D.)

6.7.3 Scientific Advisory Committee

Not applicable

7. INVESTIGATIONAL PRODUCT AND OTHER TREATMENTS

7.1 Identity of investigational product(s)

Investigational product	Dosage form and strength	Manufacturer
Benralizumab	Subcutaneous, 30mg	AstraZeneca
Placebo for Benralizumab	Subcutaneous, 00mg of active drug	AstraZeneca

7.2 Dose and treatment regimens

Benralizumab 30mg every 4 weeks for 3 doses.

Placebo for Benralizumab administered once before treatment with Benralizumab

7.3 Labelling

Labelling of the investigational products will be carried out by AstraZeneca or a Contract Research Organization in accordance with current Good Manufacturing Practice (GMP). The labels will fulfil GMP Annex 13 requirements. All investigational products will be labelled.

7.4 Storage

All study drugs should be kept in a secure place under appropriate storage conditions. The investigational product label on the syringe container will specify the appropriate storage..

7.5 Compliance

Investigational products will not be used after the expiration date printed on the packaging, or if the individual pack is opened or appears damaged.

7.6 Accountability

Upon receipt of the clinical supplies, the Investigator or designee will conduct a complete inventory of all study products and assume responsibility for storage and dispensing. Dispensing and return of investigational study products must be appropriately documented. All used and unused supplies will be inventoried and disposed of by the Sponsor or its designee. In accordance with federal regulations, the Investigator must agree to keep all study products in a secure location with restricted access.

Investigational study products required for completion of this study (including a surplus to account for potential product failures) will be provided by the manufacturer. The recipient will acknowledge receipt of the investigational products indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all investigational study products dispensed will be maintained.

7.7 Concomitant and other treatments

Study does not involve administration of concomitant drug as part of the study. Benralizumab may have steroid sparing effect. A subject may be withdrawn from the study in the event of the subject needing administration of steroids and other biologics due to medical conditions unrelated to CU.

Restricted Medication/Class of drug	Usage
<i>Chronic corticosteroids</i>	<i>May administer two short courses (7-10 days) of prednisone 35mg during study for moderate to severe flares of hives</i>
Prohibited Medication/Class of drug	Usage
<i>Biologics</i>	<i>Omalizumab, IVIG</i>
<i>Anti-inflammatories</i>	<i>Hydroxychloroquine, Dapsone, Sulfasalazine, Colchicine</i>
Rescue/Supportive Medication/Class of drug:	Usage
<i>Hydroxyzine</i>	<i>Can use hydroxyzine 25mg up to 4 times a day as a rescue medication</i>

7.7.1 Other concomitant treatment

As mentioned in the exclusion criteria, only those participant who have not taken immunosuppressant or anti-inflammatory drug within 30 days of visit 1 will be considered for continuation in the study. Enrolment of subjects needing these forms of treatment due to any reason, within 4 weeks of enrolment or during the 6 months of study, will be discontinued.

7.8 Post Study Access to Study Treatment

Currently the study does not promises post study access to Benralizumab treatment even if it proves to be effective and safe because the results may not be generalizable. Larger studies will be needed to determine its indication in the treatment of CU.

8. STATISTICAL ANALYSES

8.1 Statistical considerations

Assessment of the effectiveness of treatments will be more sensitive in repeat-measures study designs in this pilot study. This design will make it possible to measure how the actual treatment vs. placebo affects each individual with CU. The variability between subjects will not mask the effects of actual vs. placebo. In this design each subject will serve as own control. The variability between subjects will be isolated, and analysis will highlight only on treatment effects.

In the context of a pilot study, the normality of data distribution and its effect on the p-values will be less important. The purposes of data analysis will be to get a hint of the data quality and biological significance rather than statistical significance and therefore to decide the need of further studies.

8.2 Sample size estimate

Sample size calculation is shown below in Figures 2 and 3

Sample size determination based significant differences clinical measures:

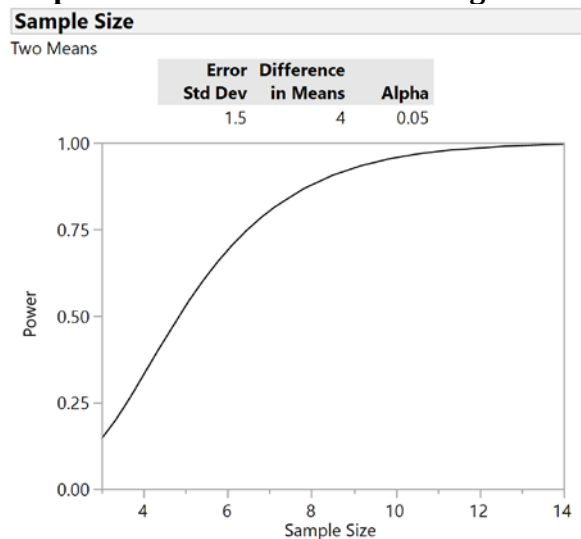


Figure 2. A sample size of 12 cases and 6 controls will determine statistical significance of a difference in UAS $\geq 4 \pm 1.5$ from baseline at alpha = 0.05 and power >0.95.

Sample size determination based on gene expression assays: Fold-change in gene-expression for the relevant genes compared to house-keeping genes at the end of placebo-treatment period and Benralizumab treatment period.

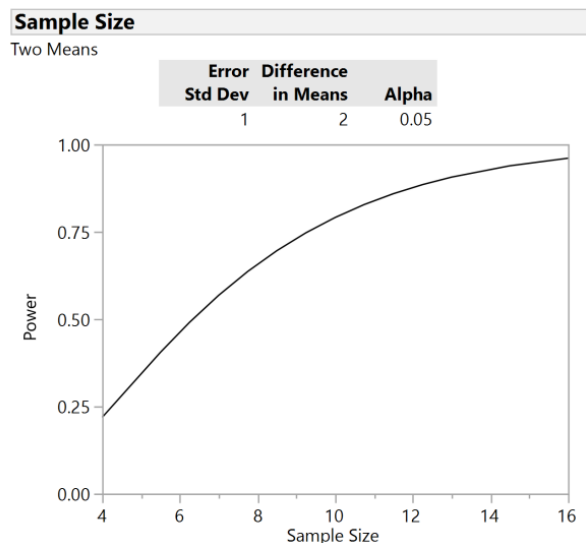


Figure 3. Sample size determination for Aim 2. A $2(\pm 0.5)$ fold-difference in gene expression of between samples collected after placebo period vs samples collected after Benralizumab treatment will be statistically significant within 95% CI and power of 0.8

8.3 Definitions of analysis sets

8.3.1 Efficacy analysis set

Clinical

- UAS7 score
- ~~UCT score~~
- Urticaria QOL

Pathological

- **RNA-sequencing:** Differential gene expression in post-treatment with Benralizumab vs. placebo/baseline.
- **Immunofluorescence:** Cell infiltrate, cytokine profile in skin biopsy from normal vs. lesional skin post-treatment with Benralizumab vs. placebo/baseline.

8.3.2 Safety analysis set

Clinical

- **Vitals:** Pulse, BP, respiratory rate, body surface temperature
- **Hematology:** Complete blood count with differential Including number of eosinophils/ μ L, %Eosinophils, platelet count
- **Clinical chemistry:** Serum Creatinine, Albumin, Alkaline phosphatase.

8.3.3 PK analysis set

Will not be performed.

8.3.4 PRO analysis set

Optional. It will include patient's perception of quality of life and performance improvement post-treatment vs. baseline.

8.4 Outcome measures for analyses

Decrease in UAS7 ~~and UCT score~~ and pruritus severity at Visit 5 (post-Benralizumab) compared to baseline (Visit 1) and post-placebo (Visit 2).

8.5 Methods for statistical analyses

Repeated measure ANOVA of the efficacy variables in CU subjects normalized with data from healthy controls will be performed. Analysis will be adjusted for stratified age groups, gender and duration of disease.

8.5.1 Analysis of the primary variable(s)

UAS7 ~~and UCT scores~~ at baseline or post-placebo will be compared to post-treatment scores. Statistical significance will be determined by a significant effect size. P-values for statistical significance may not be very useful because of small sample size in this study.

8.5.2 Analysis of the secondary variable(s)

Effect of treatment with Benralizumab vs. placebo will be determined by log-2 fold-change in gene expression in targeted sequencing.

8.5.3 Subgroup analysis

Because of a small sample size, further sub-group analysis may not be feasible. Cluster analysis, however, will be explored if possible to determine if the duration of the disease, gender or age-group influences the response to Benralizumab in CU.

8.5.4 Interim analysis

UAS7 ~~and UCT-scores~~ will be compared at every visit from the Visits 3-5 to determine the trends in the response to Benralizumab vs. baseline and post-placebo scores.

8.5.5 Sensitivity analysis

Principal components analysis will be applied to the gene expression data from RNA sequencing that can determine general patterns of expression alteration post-Benralizumab vs. post-placebo. Such analyses may provide reasonable power to distinguish moderate-sized alterations in clinically relevant genes.

8.5.6 Exploratory analysis

Improvement in health-related quality of life in the CU subjects post-Benralizumab vs. baseline or post-placebo will be compared by Chronic Urticaria Quality of Life Questionnaire (CU-QoL).

9. STUDY AND DATA MANAGEMENT

9.1 Training of study site personnel

The Principal Investigator will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The Principal Investigator will maintain a record of all individuals involved in the study (medical, nursing and other staff).

Bench scientists who will perform the blood and skin biopsy processing have training in the methods needed in this study.

9.2 Monitoring of the study

The investigator will be responsible for all monitoring and quality controls in the study. The investigator will ensure

- that the investigational team is adhering to the protocol
- that data are being accurately and timely recorded in the CRFs,
- that biological samples are handled in accordance with the Laboratory Manual and
- that study drug accountability checks are being performed
- withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

9.2.1 Source data

Source data are any data generated as a result of the subject's inclusion in the study (including run-in and/or follow up related to the study) and includes all related medical examinations and other records. Original data will be recorded on the CRFs and regarded as source data.

9.2.2 Study agreements

The Principal Investigator should comply with all the terms, conditions, and obligations of the study agreement for this study. In the event of any inconsistency between this study protocol and the study agreement, the terms of study protocol shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the study agreement shall prevail.

9.2.3 Archiving of study documents

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IRB approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data should include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender);
- Documentation that subject meets eligibility criteria, i.e., history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);
- Documentation of the reason(s) a consented subject is not enrolled
- Participation in study (including study number);
- Study discussed and date of informed consent;
- Dates of all visits;
- Documentation that protocol specific procedures were performed;
- Results of efficacy parameters, as required by the protocol;
- Start and end date of study procedure
- Record of all AEs and other safety parameters (start and end date, and including causality and severity);
- Concomitant medication (including start and end date, dose if relevant; dose changes);
- Date of study completion and reason for early discontinuation, if it occurs.

All clinical study documents must be retained by the investigator until at least 2 years or until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if specified by regulatory requirements, by local regulations, or by an agreement with AstraZeneca.

9.3 Study timetable and end of study

The study will start enrolling subjects beginning 2nd quarter of 2017 and is expected to be completed in about a year. The enrolment duration per subject is 6 months of active enrolment followed by 2 months of follow-up period.

9.4 Data management

9.4.1 Serious Adverse Event (SAE) Reconciliation

SAE reporting and reconciliation will follow Good Clinical Principles and NIH guidelines. The event onset date will be entered as the date the outcome of the event fulfilled one of the serious criteria or the investigator considers the event to meet one of the serious categories.

9.4.2 Data Management of genotype data

De-identified code using initials and 4 digit numeric letters will be used to code the RNA-extracts to the genomics core. Data analysis and secured data storage will be performed on the deidentified data.

9.4.3 Data associated with human biological samples

The investigator will take necessary steps to maintain confidentiality of data. Personal identifiers, such as subject names, their address, SSN, Health insurance provider, etc. will not be disclosed on study samples or data. The analysis data will be encrypted using third party vendor software and shared with the study team only.

9.4.4 Management of external data

The data generated in this study will not be linked to any external data such as EHR data.

10. ETHICAL AND REGULATORY REQUIREMENTS

10.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) /Good Clinical Practice (GCP), applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

10.2 Subject data protection

The investigator will assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, another unique identifier and an identification code will be recorded on any form or biological sample submitted to the , IRB, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject in accordance with local regulations. The investigator will keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial. Subject data will be processed in accordance with all applicable regulations.

The investigator agrees that all information received from AstraZeneca, including but not limited to the investigator brochure, this protocol, CRF, and any other study information, remain the sole and exclusive property of AstraZeneca during the conduct of the study and

thereafter. Information in this protocol, consent forms and any other study information remain the shared property of the investigator and AstraZeneca during the study and thereafter. The investigator is free to publish the deidentified data, use in a grant application, and/or refine a hypothesis. . The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

10.3 Ethics and regulatory review

The Schulman Institutional Review Board (Schulman-IRB) should approve the final study protocol, including the final version of the Informed Consent Form and any other written information and/or materials to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable Schulman-IRB, and to the study site staff.

The opinion of the Schulman-IRB should be given in writing. The investigator should submit the written approval to AstraZeneca before enrolment of any subject into the study. The Schulman-IRB should approve all advertising used to recruit subjects for the study. Schulman-IRB should approve any modifications to the Informed Consent Form that are needed to meet local requirements. The protocol should be re-approved by the Schulman-IRB annually.

Before enrolment of any subject into the study, the final study protocol, including the final version of the Informed Consent Form, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations. AstraZeneca will handle the distribution of any of these documents to the national regulatory authorities. AstraZeneca will provide Regulatory Authorities, Ethics Committees and Principal Investigators with safety updates/reports according to local requirements, including SUSARs (Suspected Unexpected Serious Adverse Reactions), where relevant.

10.4 Informed consent

The Principal Investigator(s) at each centre will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an Ethics Committee.

10.5 Changes to the protocol and informed consent form

Study procedures will not be changed without the mutual agreement of the Principal

Investigator and AstraZeneca. If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol (Revised Clinical Study Protocol).

The amendment is to be approved by the relevant IRB and if applicable, also the national regulatory authority approval, before implementation. Local requirements are to be followed for revised protocols.

AstraZeneca will distribute any subsequent amendments and new versions of the protocol to the Principal Investigator.

If a protocol amendment requires a change to a study site's Informed Consent Form, AstraZeneca and the study site IRB will approve the revised Informed Consent Form before the revised form is used.

If local regulations require, any administrative change will be communicated to or approved by the IRB.

10.6 Audits and inspections

Authorised representatives of AstraZeneca, a regulatory authority, or an Ethics Committee may perform audits or inspections at the centre, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, GCP, guidelines of the ICH, and any applicable regulatory requirements. The investigator will contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at the centre.

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