CLINICAL TRIAL PROTOCOL

Protocol Title:	Benralizumab regulates atopic dermatitis through effects on eosinophils, basophils and innate lymphoid type 2 cells.
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Protocol Version:	8.1 – 27MAY2019
Short Title:	Effect of benralizumab in atopic dermatitis

The study will be performed in accordance with the ethical principles of the Declaration of Helsinki, and consistent with International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) and applicable regulatory requirements. Approval will be obtained from the local ethics committee, and all patients provided written and informed consent.

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Background

Atopic dermatitis (AD) is a common skin disease that is characterized by chronic, relapsing skin inflammation and eczematous, itchy lesions (Beiber, 2008). The prevalence of AD has increased over the past few decades, with 15-30% of children and 2-10% of adults being affected (Williams & Flohr, 2006). The pathogenesis of AD is as a result of a complex interaction between the epidermal barrier, the immune system and pruritus, with each factor contributing to disease initiation and progression (Kabashima, 2013). As the epidermal barrier breaks down in skin of AD patients, allergens are exposed to the underlying antigen presenting cells (APC's). Primed APC's migrate to local lymph nodes and activate naive T cells to Th2 cells. Th2 cells then traffic to the AD lesion and promote eosinophilic inflammation through release of Th2 cytokines (IL-4, IL-13, IL-25) and TSLP (Peng & Novak, 2015). Immune cells such as eosinophils, mast cells, basophils, innate lymphoid cells (IL-C2s) and dendritic cells infiltrate into the AD lesion, while circulating Th2 cells cause an increase in IgE and eosinophils in serum (Leung et al, 2004).

Interleukin-5 (IL-5) is a key cytokine involved in the differentiation and maturation of eosinophils from hematopoietic stem cells in the bone marrow, their mobilization and migration from the bone marrow to the blood, and their activation and survival in tissue (Blanchard and Rothenberg, 2009). The receptor for IL-5 (IL-5Rα) is expressed on eosinophils and basophils, but may also be expressed on other recently identified cells, such as innate lymphoid type 2 cells (ILC2).

There is evidence that in chronic allergic inflammatory diseases, eosinophilia may arise as a result of (i) the recruitment of mature cells from the periphery in response to locally elaborated chemoattractants such as eotaxin (Wardlaw et al., 1999) and/or (ii) the localized maturation of eosinophil lineage-committed progenitors, termed "*in situ* differentiation" in the presence of locally elaborated cytokines namely IL-5 (Sehmi et al., 2000; Sehmi et al., 2016). It is now apparent that type 2 innate lymphoid cells are an important source of IL-5 in mucosal tissue promoting eosinophilic inflammation including the skin in atopic dermatitis (Kim et al., 2013; Bonefeld et al., 2016). The aim of the current study is to target the local eosinophilopoietic process by interfering with the IL-5/IL-5Rα signaling axis. Our previous and ongoing studies show that eosinophils, basophils, eosinophil progenitor cells and innate lymphoid type 2 cells

express IL-5Rα and may be a target population for benralizumab, a humanized afucosylated antibody directed against IL-5Rα subunit.

Hypothesis

In atopic dermatitis, local IL-5 driven eosinophilopoietic processes contribute to development of tissue eosinophilia. Targeting the IL-5/ IL-5Rα axis will attenuate eosinophilic inflammation in the skin.

Study design

This randomized, double-blind, parallel group, placebo-controlled study will evaluate the effect of 3 doses of a fixed 30 mg dose of benralizumab administered subcutaneously (SC) every 4 weeks to patients with moderate-to-severe atopic dermatitis, on the severity of atopic dermatitis, and the cellular inflammation of skin lesions in these patients.

Screening and Baseline

During a screening period (Days -1 to 0), patients with moderate-to-severe atopic dermatitis who develop late phase cutaneous response following intradermal allergen challenge, will be recruited for the study.

Effect of treatment on clinical disease

Skin lesions will be graded using validated scales before the start of treatment, and throughout the study to assess the effect of benralizumab on changes to the severity of disease. Biopsies from skin lesions and samples of peripheral blood will be examined for levels of cells that express IL-5Rα subunit including eosinophils, basophils, eosinophil progenitor cells, and innate lymphoid type 2 cells using flow cytometry. In addition, methylcellulose colony forming assay will be used to enumerate the IL-5 driven clonogenic potential of progenitor cell populations within the blood.

Effect of treatment on allergen-induced responses

An intradermal allergen challenge will be conducted 1 week after the last dose of study drug to determine the effect of benralizumab on allergen-induced responses in skin. The size of the resulting skin wheal will be measured at 24h post-challenge. Biopsies of the skin wheal will be obtained for measurements of eosinophils and basophils per mm^2 tissue using standard

histochemical and immuno-histochemical stains. Additional biopsies of the skin wheal will be collected to measure the frequency of eosinophils, basophils, eosinophil progenitor cells, and innate lymphoid type 2 cells by flow cytometry.

Follow-up
Patients will return for a safety follow-up visit on study Day 140 (week 20), which is 12 weeks after the last dose/11 weeks after the last study procedure.

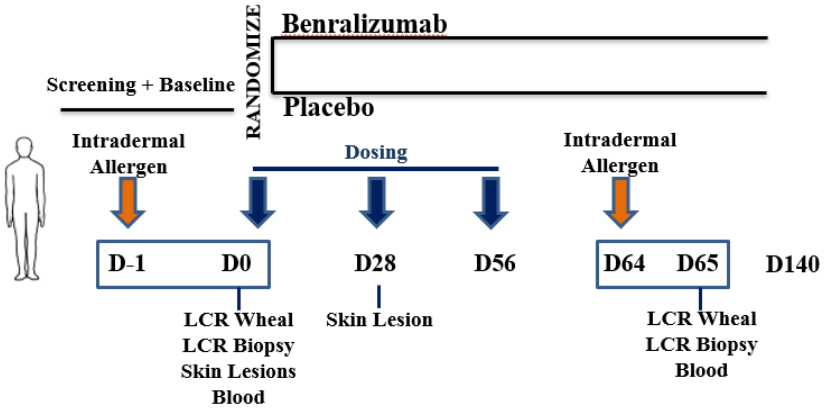

Figure 1 - Study flow chart.
Randomized double-blind,placebo-controlled, parallel-group prospective study. Patients with a history of atopic dermatitis will enter a screening period to assess responses to intradermal allergen challenge. Those developing a late cutaneous response 24 hours later will have the size of the wheal/flare measured and a punch biopsy obtained from the challenge site for measurement of eosinophils and basophils. Patients will then be randomized 1:1 to benralizumab (30 mg SC monthly) or placebo, with dosing at days 0, 28 and 56. Blood and a sample of a skin lesion will be collected on Day 0 before dosing. A skin lesion will be collected at Day 28 and blood will be collected again 65 days after the first dose, for assessments of eosinophils, basophils and other cells including eosinophil progenitors, ILC2s, hemopoietic progenitor cells and CD4+ T cells. On Day 64 an intradermal allergen challenge will be conducted and on Day 65 the size of the wheal/flare will be measured and a punch biopsy will be obtained from the challenge site for a measurement of eosinophils and basophils. Skin lesions will be assessed clinically before dosing and throughout the study using the EASI score. A safety follow up visit will take place at week 20, which is140 days after the first dose (12 weeks after the last dose). Each visit has a +/- 3 day window with the exception of Day 0 and Day 65 which must take place exactly 1 day after Day -1 and Day 64, respectively.

Duration of the study
The total length of a patient's participation in the trial is up to 25 weeks;

- up to 4 weeks screening period
- 8 weeks treatment period
- 1 week post-treatment period

- 12 weeks follow-up

Study period
Estimated date of first subject enrolled Q4 2017
Estimated date of last subject completed Q2 2019

Blinding
This study will be a double-blind study, using a placebo. The placebo will have an appearance identical to that of drug. The staff who administers the treatment, any study staff involved in subject evaluations, and the subjects, will remain blinded to treatment assignment for the duration of the study.

Study Objectives

Primary Objective:
The primary objective is to evaluate the effect of benralizumab on the allergen-induced number of eosinophils in the skin assessed by histological examination on Day 65, at 24h post-intradermal allergen challenge, compared to placebo. Intradermal saline challenge will be used as a control.

Secondary Objectives:
The secondary objectives are to evaluate the effect of benralizumab on (i) the allergen-induced number of basophils in the skin, as assessed by immuno-histochemical staining of a biopsy of the skin wheal collected on Day 65, at 24h post-intradermal allergen challenge, compared to placebo, (ii) the allergen-induced late phase cutaneous response by measuring the skin wheal on Day 65, at 24h post-intradermal allergen challenge, compared to placebo, and (iii) on disease severity at 65 days post-treatment, by assessing skin lesions using the Eczema Area and Severity Index (EASI) score, compared to placebo.

Exploratory Objectives:
The exploratory objectives are to evaluate the effect of benralizumab on the number of eosinophils, basophils, eosinophil progenitor cells and innate lymphoid type 2 cells, CD4+ T cells and hemopoietic cells in skin lesions on Day 28 post-treatment, compared to placebo.

Comparisons of local versus systemic drug-induced changes will be made by making similar cellular assessments in the blood on Day 65. In addition, we will prepare methylcellulose cell cultures from samples of Day 65 peripheral blood to enumerate the out-growth potential of the circulating eosinophil/basophil progenitor cell population.

Selection of Study Population

Men and women, 18 to 65 years of age, with moderate-to-severe atopic dermatitis, whose disease cannot be adequately controlled with, and agree to withhold, topical/oral anti-inflammatory medications. The total enrollment for the study will be sufficient to result in 20 evaluable subjects. Any patients dropping out of the study prior to primary outcome assessment will be replaced.

Inclusion Criteria

The following inclusion criteria must be met for to enter the screening phase of the study:

1. Male and female patients 18 through 65 years of age.
2. Women of childbearing potential (WOCBP) must not be actively seeking pregnancy, and 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 levonorgestrel Intrauterine system, Depo-Provera™ injections, oral contraceptive, and Evra Patch™ or Nuvaring™. 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 IP. They must demonstrate a negative serum pregnancy test at screening and demonstrate a negative urine pregnancy test immediately before each dose of study drug or placebo. 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 randomization 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.

3. All male patients who are sexually active must agree to use an acceptable method of contraception (condom with or without spermicide, vasectomy) from the first dose of IP until 16 weeks after their last dose.
4. General good health
5. Moderate to severe atopic dermatitis
6. Able to understand and give written informed consent and has signed a written informed consent form approved by the investigator's REB

The following inclusion criteria must be met for entry into the dosing phase of the study:

1. Positive skin-prick test to common aeroallergens (including cat, dust mite, grass, pollen)
2. Positive late cutaneous response to intradermal allergen challenge

Exclusion Criteria
Patients should not enter the study if any of the following exclusion criteria are fulfilled:

1. History of anaphylaxis to any biologic therapy or vaccine
2. History of clinically significant hypotensive episodes or symptoms of fainting, dizziness, or light headedness, as judged by the investigator
3. Any history or symptoms of clinically significant cardiovascular disease, particularly coronary artery disease, arrhythmias, hypertension, or congestive heart failure
4. Any history or symptoms of clinically significant neurologic disease, including transient ischemic attack (TIA), stroke, seizure disorder, or behavioral disturbances
5. Any history or symptoms of clinically significant autoimmune disease

6. Any history of clinically significant haematologic abnormality, including coagulopathy or any history of chronic treatment with anticoagulants (e.g. warfarin, etc) or antiplatelet agent (e.g, aspirin, etc)
7. Clinically significant abnormalities in laboratory test results at enrolment and during the screening period (including complete blood count, coagulation, chemistry panel and urinalysis) unless judged not significant by the investigator.
8. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level ≥2.5 times the upper limit of normal (ULN) confirmed during screening period
9. Being pregnant or lactating or have positive serum pregnancy test at enrolment or positive urine pregnancy test during the study
10. Concomitant disease or condition which could interfere with the conduct of the study, or for which the treatment might interfere with the conduct of the study, or which would, in the opinion of the investigator, pose an unacceptable risk to the patient in this study, including, but not limited to, cancer, alcoholism, drug dependency or abuse, or psychiatric disease
11. Severe concomitant illness(es) that, in the investigator's judgment, would adversely affect the patient's participation in the study
12. Presence of skin comorbidities that may interfere with study assessments
13. History of cancer: Patients 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. Patients 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 of informed consent.
14. Patient who has a scheduled in-patient surgery or hospitalization during the study.
15. History of Guillain-Barré syndrome
16. 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

17. Positive hepatitis B surface antigen, or hepatitis C virus antibody serology, or a positive medical history for hepatitis B or C. Patients with a history of hepatitis B vaccination without history of hepatitis B are allowed to enrol
18. A history of known immunodeficiency disorder including a positive human immunodeficiency virus (HIV) test
19. Receipt of immunoglobulin or blood products within 30 days prior to the date informed consent is obtained
20. Receipt of any marketed (eg omalizumab) or investigational biologic within 4 months or 5 half-lives prior to randomization is obtained, whichever is longer
21. Treatment with an investigational drug within 8 weeks or within 5 half-lives (if known), whichever is longer, before the baseline visit
22. Any allergen immunotherapy within 4 months prior to or throughout the study.
23. Having used any of the following treatments within 4 weeks before the Day -2 baseline visit, or any condition that, in the opinion of the investigator, is likely to require such treatment(s) during study:
    a. Immunosuppressive/immunomodulating drugs (eg, systemic corticosteroids, cyclosporine, mycophenolate-mofetil, IFN-γ, Janus kinase inhibitors, azathioprine, methotrexate, etc.)
    b. Phototherapy for AD
24. Any cell-depleting agents including but not limited to rituximab: within 6 months before the baseline visit, or until lymphocyte count returns to normal, whichever is longer
25. Initiation of treatment of AD with prescription moisturizers or moisturizers containing additives such as ceramide, hyaluronic acid, urea, or filaggrin degradation products during the screening period (patients may continue using stable doses of such moisturizers if initiated before the screening visit)
26. Regular use (more than 2 visits per week) of a tanning booth/parlor within 4 weeks of the baseline visit
27. Active chronic or acute infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals within 2 weeks before the baseline visit, or superficial skin infections within 1 week before the baseline visit. NOTE: patients may be rescreened after infection resolves

28. Known or suspected history of immunosuppression, including history of invasive opportunistic infections (eg, histoplasmosis, listeriosis, coccidioidomycosis, pneumocystosis, aspergillosis) despite infection resolution: or unusually frequent, recurrent, or prolonged infections, per investigator judgment
29. Planned or anticipated major surgical procedure during the patient's participation in this study
30. Receipt of live attenuated vaccines 30 days prior to the date of randomization. Receipt of inactive/killed vaccinations (eg, inactive influenza) are allowed provided they are not administered within 1 week before/after any IP administration.
31. Patient is a member of the investigational team or his/her immediate family
32. Pregnant woman
33. Previously received benralizumab (MEDI-563)

Investigational product

Benralizumab 30 mg or placebo solution for injection will be administered SC every month, for 3 doses. Subjects will be randomized in a 1:1 ratio. The IP will be administered at the study centre on treatment visits on study days 0, 28 and 56 (± 3 days). The placebo will have an appearance identical to that of drug. Those administering treatment, any study staff involved in subject evaluations, and the subjects, will remain blinded to treatment assignment for the duration of the study.

Investigational product administration

The IP will be administered by a study physician. For WOCBP urine pregnancy test will be done; IP will be administered only when the result of the test is negative. The site of injection of the IP is rotated such that the patient receives IP at a different anatomical site at each treatment visit. The injection site will be recorded at each treatment visit. After IP administration the subject should be observed for a minimum of 2 hours for the appearance of any acute drug reactions.

Efficacy Endpoints

Primary endpoint

1. The number of eosinophils per millimetre squared of skin, measured 24 hours post intradermal allergen challenge, 65 days after the first dose. The number of eosinophils in the skin will be assessed by histological examination of a punch skin biopsy obtained from the site of the intradermal allergen challenge.

Secondary endpoints

1. The number of basophils per millimetre squared of skin, measured 24 hours post intradermal allergen challenge, after 65 days of dosing. The number of basophils in the skin will be assessed by immunohistochemistry of a punch skin biopsy obtained from the site of the intradermal allergen challenge, using a commercial antibody specific for basophils.
2. The size of the late cutaneous response, measured 24 hours post intradermal allergen challenge, 65 days after the first dose. The late cutaneous response will be calculated using the length and width of the wheal and flare response to a standardized amount of allergen extract.
3. Eczema Area and Severity Index (EASI) score.

Exploratory endpoints

1. The number of eosinophils, basophils, eosinophil progenitors, ILC2s, CD4+ T cells and hemopoietic cells in skin lesions after 28 days of dosing. The number of cells in skin lesions will be assessed by flow cytometry following digestion of the biopsy and staining with a validated panel of antibodies.

Safety Endpoints

Blood and urine will be evaluated during screening for study eligibility, and at follow up to determine safety.

Safety Variables

Adverse Events

An AE is defined as any untoward medical occurrence in a clinical investigation subject who receives a pharmaceutical product at any dose, whether or not there is a causal relationship with the investigational treatment. An AE could therefore be any unfavorable or unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions. An unexpected AE is an AE not identified in nature, severity, or frequency in the current Investigator Drug Brochure.

The investigator will rate the intensity of AEs according to the following categories.

- Mild = symptoms or signs present; no limitation of activities; no medical intervention required.
- Moderate = some limitation of activities; no or minimal medical intervention required.
- Severe = marked limitation of activities; medical intervention required, possible including hospitalization.

Serious adverse events will be collected for the duration of the study. All AEs will be monitored until resolution or, if the AE is determined to be chronic, until stable. If an AE remains unresolved at the conclusion of the study, a clinical assessment will be made by the investigator and medical monitor to determine whether continued follow-up of the AE is warranted.

Serious Adverse Events

Serious AEs and AEs listed in the drug investigator brochure will be considered expected events. See the Investigator Drug Brochure for additional details of drug expected AEs.

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

- Not related
  The subject was not exposed to drug, or another cause is obvious.

- Probably not related
  The AE is more likely explained by another cause, and the time of occurrence of the AE is not reasonably related to drug administration.

- Possibly related
  The drug administration and the AE occurrence were reasonably related in time, and the AE is explained equally well by causes other than drug; or drug administration and the AE were not reasonably related in time, but the AE is not obviously a result of other causes.

- Probably related
  The drug administration and the AE occurrence were reasonably related in time, and the AE is more likely explained by drug exposure than by other mechanisms.

An SAE is defined as any untoward medical occurrence that:

- Results in death.

- Is life threatening (*i.e.*, the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred).

- Requires or prolongs hospitalization.

- Results in persistent or significant disability or incapacity (*i.e*., the event causes a substantial disruption of a person's ability to conduct normal life functions).

- Is a congenital anomaly or birth defect in the offspring of the treated subject.

- Is an important and significant medical event that, based upon appropriate medical judgment, may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes defining SAE.

Safety Reporting

The study drug, benralizumab, is an off-label indication for subjects recruited for this study. The Principal Investigator will therefore submit expedited reports, as indicated/necessary for reporting of serious adverse drug reactions, by fax, directly to Health Canada Biologics and Genetic Therapies Directorate.

Adverse drug reactions that are *both serious and unexpected* are subject to expedited reporting to Health Canada. Expedited reporting of reactions which are serious but expected is not required. Expedited reporting is not required for serious events from clinical investigations that are considered unrelated to the study product, whether or not the event is expected.

In accordance with Health Canada requirements, an Adverse Drug Reaction Report will be filed in the cases

- where the adverse drug reaction is neither fatal nor life-threatening, within 15 days after becoming aware of the information;
- where adverse drug reactions is fatal or life-threatening, immediately where possible and, in any event, within 7 days after becoming aware of the information;
- within 8 days after having informed Health Canada of the adverse drug reactions, submit as complete as possible, a report which includes an assessment of the importance and implication of any findings

All safety reports submitted to Health Canada will also be submitted to AstraZeneca at the following address: AEMailboxClinicalTrialTCS@astrazeneca.com.

Procedures

Skin Prick Test
A skin prick test will be used to determine the allergen(s) to which each subject is sensitized. Standard allergen extracts will include ragweed, trees, grass, dog, cat, horse, dust mites (Dermatophagoides *farinae* and D. pteronyssinus), Alternaria, and Aspergillus. Extracts will be applied to the back then pricking the skin to allow exposure, and then evaluating the local reaction in the skin. A positive control (1 mg/mL histamine) and a negative control (diluent) are applied to the skin. If an allergen provokes an allergic reaction, a raised itchy bump (wheal)

develops. The size of the wheal (the raised area, not the redness) will be measured and recorded with a ruler in millimeters in the horizontal and vertical directions, perpendicular to each other after approximately 15 minutes. The size of the wheal for each antigen will be recorded on the Skin Test Form, along with any observed adverse reaction or event and any actions taken.
A reaction greater than 2 × 2 mm will be regarded as positive, provided that the positive and negative controls are appropriately positive (histamine) and negative (diluent), respectively. The investigator is to choose an allergen for injection on the basis of the skin response. The investigator may choose to perform a RAST test to assist with selection of an appropriate allergen. This procedure will be completed in accordance with the allergen skin testing using the epicutaneous method SOP at McMaster University (Hamilton, Ontario, Canada).

Allergen extracts manufactured following GMP guidelines, will be selected, prepared by staff and administered to the subjects by injection in accordance with procedures approved by Health Canada.

Intradermal Allergen Challenge
The allergen extract selected for intradermal testing will be determined by the results of the skin prick test, being the allergen that gives the largest skin wheal. The selected allergen will be injected in a volume of 100 µL intradermally in two adjacent and standardized locations on the subject's back. A saline control will also be injected in a volume of 100 µL intradermally in one location of the subject's back. At 24 hours after challenge, the size of the wheal and flare will be measured, and a punch biopsy will be taken from the centre of the sites (2 allergen, 1 diluent) plus an adjacent unchallenged/unaffected site.

Skin Sampling
Punch biopsies will be obtained from the site of the intradermal allergen (A) and saline (S) challenges and an unaffected site (U) adjacent to intradermal challenges using a sterile 4 mm skin punch by applying and twisting until the blade of the skin punch has pierced the skin. The biopsy will be removed using sterile forceps and a scalpel. These samples will be processed for histologic examination of inflammatory cells, including eosinophils, mast cells and basophils.

A skin lesion (L) will be sampled by excising not more than 1 cm x 3 cm using a sterile scalpel. These skin biopsies will be analyzed for inflammatory cells including eosinophils, eosinophil progenitors, basophils and mast cells. If the lesion is too small to obtain sufficient cell numbers

for flow cytometry, only a punch biopsy will be obtained for microscopy. A second skin lesion on the contralateral side will be identified for observation throughout the study (measure size, photograph, conduct EASI assessment at each visit).

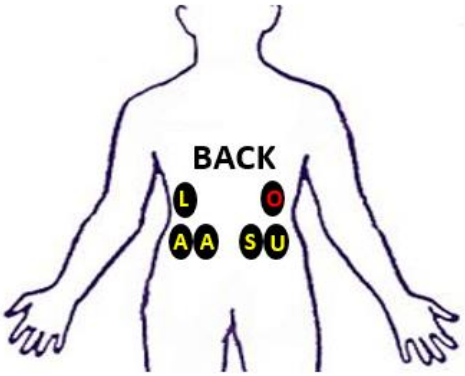

Figure 2 – Site of skin biopsies

Before a biopsy is taken, the skin is thoroughly cleaned and local anesthetic (2% lidocaine) is injected to numb the skin. The site of skin biopsies and excision will be sutured and covered with a sterile bandage. Subjects will be instructed how to keep the site clean, and sutures will be removed at the next study visit.

Blood Sampling

At visit 1 (D-1, screening) and visit 7 (D140) blood will be collected for safety labs including complete blood count, coagulation, and chemistry including alanine aminotransferase and aspartate aminotransferase. At visit 1 blood will also be collected for virology (hepatitis B and C, and HIV). Incidental findings would be given the appropriate medical follow up. Women will have blood drawn for pregnancy testing or FSH to confirm a woman is post-menopausal. At visits 2 (D0) and 6 (D65), 30 mL of blood will be drawn from a vein in the arm using aseptic techniques. The blood will be used to measure circulating levels of inflammatory cells, including eosinophils, basophils and eosinophil/basophil progenitors. Those performing patient and laboratory assessments will remain blinded to the results of the blood cell counts.

Urine Sampling

At visit 1 (screening) and visit 8 (follow up) urine will be collected for safety urinalysis. After enrolment, urine will be collected from women at visits 2, 3 and 4 before dosing for urine pregnancy testing.

Eczema Area and Severity Index
The EASI is a validated measure used in clinical practice and clinical trials to assess the severity and extent of atopic dermatitis. It is a composite index with scores ranging from 0 to 72. Four atopic dermatitis disease characteristics (erythema, thickness [induration, papulation, edema], scratching [excoriation], and lichenification) will each be assessed for severity by the investigator or designee on a scale of "0" (absent) through "3" (severe). In addition, the area of AD involvement will be assessed as a percentage by body area of head, trunk, upper limbs, and lower limbs, and converted to a score of 0 to 6. In each body region, the area is expressed as 0, 1 (1% to 9%), 2 (10% to 29%), 3 (30% to 49%), 4 (50% to 69%), 5 (70% to 89%), or 6 (90% to 100%).

Statistical methods
Using data from a study with the same model (intradermal allergen challenge) in the same patient population (moderate-to-severe atopic dermatitis), the sample size requirement was determined using data collected from 7 subjects undergoing identical challenge protocols in our laboratory in an ongoing study of the protective effects of prednisone treatment. In the 4 prednisone-treated subjects the mean increase in skin eosinophils during the screening portion of the study was 59.8 (42.9) [cells/mm^2;M(SD)]. This was reduced to 16.25 (20.6) during the prednisone treatment period. The mean % reduction in the delta between the screening and prednisone challenges was 80.4 (41.5). To calculate the sample size for the current study, we have assumed that treatment with benralizumab will yield a similar protective effect as prednisone, and that there will be a similar between subject variability in this protective effect. We also assumed that there will be no protective effect in the placebo arm, but that the variability in that arm will be similar to that seen in the treatment arm. Thus, using projected mean % reduction in the delta eosinophils of 80 in the prednisone arm, 0 in the placebo arm with a SD of 40 in both of those arms, a sample size of 6 would be needed to demonstrate statistical difference

using an independent Student t-test. We have chosen to put 10 subjects into each arm to allow for subject drop out and also to give increased power for secondary mechanism based investigations.

The primary analysis will be on the % reduction of the allergen induced increase in tissue eosinophils. The allergen induced increase in tissue eosinophils will be calculated by subtracting the cell count made with saline from the count made with allergen 24h post challenge. This will be performed during the screening phase (Day 0) and the treatment phase (Day 65) of the study. The percent reduction will be calculated by expressing this increase in the treatment phase as a percentage of that in the screening phase. The percent increases during the placebo and active treatment arms of the study will be compared using an independent Student t-test.

Statistical significance will be set at $p<0.05$.

Ethics

Research Ethics Board

The Principal Investigator (PI) will provide the Research Ethics Board (REB) with all appropriate material, including the informed consent document. The study will not be started until appropriate REB approval of the protocol and the informed consent document is obtained. Appropriate reports on the progress of the study will be made to the REB by the Principal Investigator.

Ethical Conduct of the Study

This study will be conducted in accordance with the protocol, Good Clinical Practice (GCP) according to International Conference on Harmonization (ICH) guidelines, and regulatory requirements for the participating institution. The study will be conducted by scientifically qualified persons and all medical procedures will be performed by a qualified medically trained physician.

Subject Information and Consent

All subjects will provide informed signed consent. Before entering subjects into the study, a copy of the REB-approved IC will be reviewed with the potential participant, and signed and

dated. The investigator will provide a copy of the signed informed consent form to each subject and will maintain the original document in the subject's study file.

Study Feasibility

Clinical trial experience
The laboratory at McMaster University has a dedicated research staff with experience conducting phase II clinical trials under the supervision of Dr. Gauvreau. Furthermore, Dr. Gauvreau is experienced with Health Canada submissions and reporting, and has published widely on the effect of investigational medications in a model of allergy (Gauvreau 2014, 2015, 2016).

Patient recruitment
The atopic dermatitis patient population is in need of an effective therapeutic for management of their disease, and these patients are readily accessible from Dr. Lima's outpatient dermatology clinic. Dr. Lima manages over 100 patients with atopic dermatitis who are eager to test a new medication for control of their disease. Based on routine clinical patch testing, we anticipate the majority of patients with moderate to severe atopic dermatitis will develop late cutaneous responses to intradermal allergen challenge, and will develop elevated levels of eosinophils and basophils following intradermal allergen challenge (Macfarlane 1999). We do not anticipate any issues with recruitment or eligibility.

Outcome measurements
Dr. Sehmi and Dr. Gauvreau have published widely on mechanisms of allergic inflammation, using allergen-challenge models to evaluate drug efficacy and mechanisms of disease in patients with allergic asthma (Gauvreau 2014, 2015, 2016, Salter 2016, Smith 2015, 2016). Together with Medimmune, Dr. Sehmi and Gauvreau have validated a flow cytometry panel for measuring basophils, ILC2s, CD4+ T cells and eosinophil progenitor cells to examine the effects benralizumab in subjects with allergic asthma in our laboratory (D3250C00040 - ARIA study). This same staining panel has been optimized for measurement of these cells in skin of patients with atopic dermatitis and pilot data shows positive detection of all cell types of interest. We have recruited a research pathologist to conduct all tissue staining and assessments for the proposed study (and ARIA).

Study Schematic

Study Procedures	**Visit 1 Day -1**	**Visit 2 Day 0**	**Visit 2b Day 14**	**Visit 3 Day 28**	**Visit 4 Day 56**	**Visit 5 Day 64**	**Visit 6 Day 65**	**Visit 7 Day 140**
Physical Exam/Medical History	x							
Vital Signs	x	x		x	x	x	x	x
Examination of Skin Examination of contralateral lesion	x	x	x	x	x		x	
Skin Prick Test	x					x		
Patient Questionnaires: EASI, IGA, SCORAD, DLQI, POEM, PGAD		x	x	x	x		x	
Blood Sample Safety Efficacy	x	x					x	x
Urine Sample Safety Pregnancy	x	x*		x*	x*			x
Intradermal challenges	x					x		
Skin biopsies From intradermal challenges From lesion		x x		x			x	
Drug administration		x		x	x			

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