CHOOSING BETWEEN BIOLOGICAL AGENTS FOR SEVERE ALLERGIC EOSINOPHILIC ASTHMA

INTRODUCTION

Severe asthma is defined as asthma that requires treatment with high dose inhaled corticosteroids plus a second controller (and/or systemic corticosteroids) to prevent it from becoming 'uncontrolled' or which remains 'uncontrolled' despite this therapy [1]. Only a small number of people with asthma have severe disease, as the majority are well controlled on low dose inhaled corticosteroids. However, those who suffer with severe asthma have a highly disproportionate burden of illness, with increased clinical morbidity, high economic costs[2], and poor quality of life [3], making it an important chronic disease management problem for Australia and New Zealand.

With severe asthma defined on the basis of disease that is refractory to usual treatments that work well in those with milder asthma, it is not surprising that those with severe asthma demonstrate many differences to those with milder disease. Even within the group with severe asthma there is considerable heterogeneity; in terms of the natural history, pathology and expression of disease phenotypes, all of which have important implications for therapeutic control [4].

The advent of biological agents that target specific inflammatory pathways in asthma has been the most important recent therapeutic advance in targeting those with severe asthma. Asthma therapies such as bronchodilators and corticosteroids work well in those with mild asthma, but demonstrate limited effectiveness in severe disease. In contrast the new biologic agents, specifically target dysregulated mechanisms of active disease in severe asthma. This offers the hope of precision medicine, tailoring the right therapy to the right patient. However, in a disease that is acknowledged as heterogeneous the contrary is also true and unless the right patient is identified, the maximum benefits of these increasingly expensive therapies will not be realized[5].

 Severe asthma clinicians in Australia now have the choice to access treatment with two biologic agents; Omalizumab or Mepolizumab. Patients in NZ only have access to Omalizumab currently, and Mepolizumab is expected to be funded in the next few months. Omalizumab is a monoclonal antibody directed against IgE and approved for use in severe allergic asthma; Mepolizumab is a monoclonal antibody against interleukin (IL)-5, for use in severe eosinophilic asthma. These agents

do not work in all patients with severe asthma. However, they are excellent examples of progress toward precision medicine in airway diseases, whereby management is based on the pathogenesis of the active disease process with efficacy limited to specific asthma disease phenotypes. The challenge for clinicians in their prescription of these treatments are those cases where two severe asthma phenotypes (i.e. severe allergic asthma and severe eosinophilic asthma) have significant overlap in the one individual in terms of disease expression, and there is, as a consequence, a lack of precision in regard to which treatment should be applied.

While clinical trials have demonstrated the effectiveness of both agents against placebo, these trials were designed to maximise their treatment effect, against the broadest number of severe asthma patients. They have not directly compared these agents in terms of efficacy, nor have they provided sufficient clarity as to who may benefit more from one agent compared to the other. These are highly important clinical questions, and there have been calls for this research in international journals [6].

Hypothesis: in patients with the dual phenotypes of severe allergic and eosinophilic asthma, Mepolizumab is as effective as Omalizumab.

We also propose that key clinical biomarkers will clarify which patients will respond best to each of these interventions.

This study will be the first direct clinical comparison of these agents and will apply expert clinical characterisation, and incorporate cutting edge biotechnology to better inform treatment choices for severe asthma. There is an important and urgent management problem facing the Australian and New Zealand pharmaceutical schemes, where imprecision in prescribing will result in reduced clinical effectiveness as well as substantial and sustained economic cost.

RESEARCH PROPOSAL

Background

While only 3 to 10% of patients with asthma have severe disease [7], they suffer a high symptom burden and highly impaired health status [3,8]. Severe asthma in fact is responsible for the majority of health care expenditure for asthma with per patient costs for severe asthma ten times that for mild disease[9], making it a priority area for research in asthma, the most prevalent chronic respiratory condition affecting Australians.

Independently, a number of studies that have used unbiased population cluster analysis of asthma populations have demonstrated that severe asthma is a heterogeneous disease [10-12]. It is likely the result of genetic, epigenetic and multiple precipitating environmental factors that occur to varying degrees throughout life. This results in various clinical phenotypes of disease, many with overlapping features and many that remain poorly defined[4]. Early onset or childhood asthma tends be associated closely with allergy and expresses an inflammatory phenotype characterised by type 2 inflammation and airway eosinophilia and often is associated with other allergic diseases. In adults the relationship with allergy is less, and in those with late onset disease, asthma can be associated with airway eosinophilia and type 2 inflammation, refractory to inhaled corticosteroids as well as subgroups with minimal airway inflammation, or airway neutrophilia, obesity and fixed airflow obstruction [13]. This heterogeneity of disease means that a single therapeutic intervention is unlikely ever to be the answer to controlling severe asthma. While the occurrence each of these severe asthma clinical phenotypes are relatively small in number, the morbidity and cost of the disease justifies an approach that will carefully define what these clinical phenotypes are, the dysregulated disease mechanisms involved and research-based strategies to target therapies to correct or mitigate against the symptoms.

New monoclonal treatments for severe asthma

The emergence of monoclonal antibodies as treatments that target specific aspects of asthmatic inflammation offers considerable promise. In the past disease control in severe asthma was poor despite optimal inhaled therapies, leading to a reliance on oral corticosteroids with their limited efficacy and severe side effects. Conversely, the specificity of biologic agents means that their optimal performance will require careful characterization of severe asthmatic phenotypes.

Mepolizumab

Mepolizumab provides an excellent example of this dilemma. Mepolizumab is a monoclonal antibody directed against IL-5 and prevents the recruitment of eosinophils to the airway, which is known to be associated often, but not always with, disease activity[14]. The initial phase three trial of mepolizumab recruited participants with symptomatic asthma that was not controlled despite inhaled corticosteroids and while a significant reduction in peripheral blood eosinophils was demonstrated, there was no effect on asthma symptoms, lung function or exacerbation frequency [15]. However, when investigators selected subjects with truly corticosteroid refractory asthma: symptomatic, with exacerbations and with persistent airway eosinophilia despite inhaled (ICS) or oral corticosteroids [16], Mepolizumab led to a reduction in exacerbations of approximately 50%, and improvements in quality of life. These observations prompted further larger scale phase three trials and selecting patients with refractory airway eosinophilia using peripheral blood eosinophils and severe asthma, investigators demonstrated a similar reduction in exacerbations [17].

Omalizumab

Omalizumab, is a monoclonal antibody directed against IgE, a molecule that plays a critical role in allergic inflammation, though not specifically asthma. In contrast to Mepolizumab, identifying those who will benefit most from Omalizumab is not so clear and a biomarker such as blood or airway eosinophilia remains elusive. Omalizumab exerts its benefits in those with moderate to severe allergic asthma, with the greatest benefit seen in exacerbation and hospitalisation reduction, though oral steroid reduction has not been documented [18]. People with persistent symptoms and evidence of type 2 inflammation; exhaled nitric oxide, blood eosinophilia and serum periostin despite ICS appear to derive the most benefits [19]while Omalizumab is of no demonstrable benefit in people who are non-atopic[20].

Better prescribing algorithms are needed.

A significant limitation of the clinical studies proving safety and efficacy of these targeted biologicals was that they were designed to satisfy international regulatory requirements principally by demonstrating superiority over placebo, thereby limiting the eligible pool of patients that could receive treatment, and further by focussing on relatively short-term outcomes that acted as a surrogate for long term effectiveness. Study design for both agents, was not concerned with identifying factors that predicted responsiveness. Subsequent investigator-initiated trials have

shown interesting effects of Omalizumab on immune regulation in difficult to control asthma. For example, investigators compared the treatment of Omalizumab in children with a history of asthma and found Omalizumab improved asthma control and substantially reduced acute exacerbations [21]. However, when the investigators compared Omalizumab to increasing the dose of ICS in the at-risk period, a benefit was only seen in those children who had a history of recent exacerbation and was most beneficial in those who were exacerbating with virus infections. In a subset of children they also demonstrated improved antiviral responses, following treatment with Omalizumab [22]. These findings suggest that there may be subsets of patients more likely to respond to treatment, via mechanisms not identified in the original regulatory trial designs.

Exacerbations are an important defining feature of severe asthma. One third of total annual asthma-related health care expenditure may be attributable to asthma-related hospitalisations[23,24]. Exacerbation risk is greatest in those with more severe disease, poor symptom control and persistent eosinophilic airway inflammation despite treatment[25,26]. The most common trigger for exacerbations in both children and adults are viral respiratory tract infections[27] which we and others have shown also to be linked to impaired antiviral interferon (IFN) responses[28-31]. The pathogenesis of virus-induced asthma exacerbations is complex and whilst only partially understood, it is evident there is an interaction between active type 2 airway inflammation in asthma and dysregulated antiviral responses. Type-2 biased immune responses are directed by a subset of TH2 effector lymphocytes that release signature cytokines IL-4, 5 and 13. These cells are not the exclusive source of these cytokines and a recently discovered subset of innate lymphoid cells (ILC-2) have been shown to play a crucial role in innate immune responses, with these cells releasing type 2 cytokines [32]. ILC-2 cells are directly activated by IL-25 and IL-33 released by airway epithelial cells and play a crucial role in perpetuating type-2 immune responses in asthma [33]. Viral infections however are usually associated with type I immune responses that should suppress allergic inflammation, but paradoxically the reverse appears to be the case in asthma. Allergic sensitisation and viral detection increase the odds of being admitted to the hospital with an asthma exacerbation [34] . Further, persistent eosinophilic airway inflammation has been associated with an increased intensity of symptoms following rhinovirus (RV) infection [35]. In the case of both Omalizumab and Mepolizumab, the greatest clinical benefit is their ability to reduce exacerbation frequency, yet relatively little is known about the mechanism(s) that leads to this outcome.

The literature currently fails to clearly differentiate between these two monoclonal therapies that target severe asthma (i.e. those with evidence of refractory type 2 airway inflammation) in terms of either efficacy or how to predict a favourable clinical response. Peripheral blood eosinophils do

appear to predict a response to Mepolizumab [36], and, whereas a similar single biomarker is not evident for Omalizumab, higher blood eosinophils at baseline are associated with a better response to Omalizumab[19].

We were able to study "real world experience" for the use of Omalizumab in Australia. Data from patients with poor asthma control despite optimised treatment, evidence of atopy and who had received Omalizumab prescribed by specialists, was entered into the Australian Xolair Registry (AXR). These data demonstrated that of 180 participants in the registry, after 6 months treatment, 150 (83%) were judged to have improved and continued Omalizumab treatment whereas 30 people discontinued due to either adverse events or treatment failure [37]. Treatment responders were shown to more likely have worse asthma symptoms with a higher asthma control questionnaire (ACQ) score at baseline and the analysis showed that a positive response to treatment with Omalizumab was not influenced by co-morbidities. Beyond these metrics no other factors predicted treatment success. Further interrogation of the AXR registry demonstrated that a large number of subjects would have been eligible for therapy with either Omalizumab or Mepolizumab based on Australian Pharmaceutical Benefits Scheme (PBS) criteria (Table 1).

Table 1: Serum IgE and blood eosinophils in severe asthma patients included in the SAWD registry (measurements at baseline or within 3 years prior to baseline)

	Blood Eosinophils 0-150	Blood Eosinophils 150-300	Blood Eosinophils >300
IgE <30	13	8	5
IgE ≥ 30	65	58	71

<u>This raises the critical question of which drug should be chosen for individuals who present with both raised IgE and high blood eosinophils.</u>

There are no clinical studies that offer a head-to-head comparison between these agents to see if one is superior to the other in cases where either may be applicable. Indeed, it remains unclear if clinical or biological markers of disease can be used to identify responders, beyond the current prescribing recommendations that have been derived from the selection criteria used in regulatory clinical trials.

If the current circumstance remains it is likely that selection of treatment will default to personal physician preference, with the agent chosen being that which is easiest to administer or that which is easiest to apply for. This is clearly unsatisfactory for a disease such as severe asthma with these biological therapies being both long-term and expensive. Rather, the choice should be based on the agent that is best suited to control the disease process in an individual and one that will result in the greatest clinical efficacy. Failure to target an appropriate treatment may also have major clinical implications and could result in patients at risk of severe exacerbation and death from asthma not receiving efficacious treatment in a timely manner. Current restrictions of the Australian PBS also require a six month window between trials of monoclonal antibody therapies, further putting patients at risk if the more efficacious treatment is not chosen in the first place. The results of the study proposed here will be immediately translatable to the Australian and New Zealand health environments and provide clear direction for a change in policy for this important chronic condition.

HYPOTHESIS

We propose that:

"In patients with the dual phenotypes of severe allergic and eosinophilic asthma, Mepolizumab is as effective as Omalizumab".

In testing this hypothesis experimental analyses will be undertaken to identify key clinical biomarkers that may determine a clinical profile which responds best to each of these interventions if equivalence is not apparent (inferiority outcome).

AIMS

Aim 1: To determine if Mepolizumab is as effective as Omalizumab in adults with severe refractory asthma who exhibit a dual allergic/eosinophilic phenotype in terms of improvement in asthma control.

Aim 2: To determine those with severe refractory eosinophilic and allergic asthma if peripheral blood gene expression, immune cell subset analysis, exhaled volatile compounds and induced sputum provides novel information that will identify patients who are more likely to response to omalizumab or mepolizumab.

INVESTIGATIONAL PROCEDURES

CLINICAL

Aim 1: To determine if Mepolizumab is as effective as Omalizumab in adults with severe refractory asthma who exhibit a dual allergic/eosinophilic phenotype in terms of improvement in asthma control.

Participants

A non-inferiority un-blinded "pragmatic" randomised control trial (RCT) will be carried out. 190 participants will be recruited from patients who have been assessed by respiratory or immunology specialists in Australia and New Zealand and who dually qualify for treatment of severe asthma with either Mepolizumab or Omalizumab according to Australian PBS criteria or New Zealand Pharmac criteria (for Omalizumab). As Mepolizumab is currently unfunded in NZ, GlaxoSmithKline (GSK) has agreed to provide Mepolizumab on compassionate grounds to up to six patients recruited into this study. To qualify for PBS-subsidised provision of these drugs, the following criteria need to be satisfied and these criteria will be used to determine inclusion in this study.

INCLUSION CRITERIA

Treatment criteria

- Must be treated by a respiratory physician, clinical immunologist, allergist or general physician experienced in the management of patients with severe asthma.

AND

- Patient must be under the care of the same physician for at least 6 months; OR
  Patient must have been diagnosed by a multidisciplinary severe asthma clinic team,

AND

- Patient must have a diagnosis of asthma confirmed and documented by a respiratory physician, clinical immunologist, allergist or general physician experienced in the management of patients with severe asthma, defined by the following standard clinical features; (i) forced expiratory volume (FEV1) reversibility greater than or equal to 12% and greater than or equal to 200mL at baseline within 30 minutes after administration of salbutamol (200 to 400 micrograms) or (ii) airway hyper-responsiveness defined as a greater than 20% decline in FEV1 during a direct bronchial provocation test or greater than 15% decline during an indirect bronchial provocation test, (iii) peak expiratory flow (PEF) variability of greater than 15% between the two highest and two lowest peak expiratory flow rates during 14 days; OR

- Patient must have a diagnosis of asthma from at least two physicians experience in the management of patients with severe asthma,

AND

- Patient must have a duration of asthma of a least 1 year,

AND

- Patient must have past or current evidence of atopy, documented by skin prick testing or an in vitro measure of specific IgE, that is no more than 1 year old,

AND

- Patient must have total serum human immunoglobulin E greater than or equal to 30 IU/mL,

AND

- Patient must have blood eosinophil count greater than or equal to 300 cells per microliter in the last 12 months; OR
  Patient must have blood eosinophil count greater than or equal to 150 cells per micolitre while receiving treatment with oral corticosteroids in the last 12 months.

AND

- The treatment must not be used in combination with and within 4 weeks of another PBS-subsidised biological medicine prescribed for severe asthma.

AND

- Must be aged 12 years or older.

Optimised asthma therapy:

(i) Adherence to maximal inhaled therapy, including high dose inhaled corticosteroid (ICS) plus long-acting beta-2 agonist (LABA) therapy for at least 12 months, unless contraindicated or not tolerated;

AND

(ii) treatment with oral corticosteroids, either daily oral corticosteroids for at least 6 weeks, OR a cumulative dose of oral corticosteroids of at least 500 mg prednisolone equivalent in the previous 12 months, unless contraindicated or not tolerated.

The following criteria indicate failure to achieve adequate control and must be demonstrated:

- an Asthma Control Questionnaire (ACQ-5) score of at least 2.0, as assessed in the previous month, AND

- while receiving optimised asthma therapy in the past 12 months, experienced at least 1 admission to hospital for a severe asthma exacerbation, OR 1 severe asthma exacerbation, requiring documented use of systemic corticosteroids (oral corticosteroids initiated or increased for at least 3 days, or parenteral corticosteroids) prescribed/supervised by a physician.

NZ specific criteria:

Participants recruited in New Zealand must meet the main inclusion criteria listed above. In addition they must be eligible for both Pharmac funded Omalizumab and Mepolizumab therapy in NZ. Participants in NZ will therefore represent a subset of all possible participants and will meet all of the following additional inclusion criteria:

1. Total serum IgE between 76 IU/mL and 1300 IU/ml at baseline
2. Proven adherence with optimal inhaled therapy including high dose inhaled corticosteroid (budesonide 1,600 mcg per day or fluticasone propionate 1,000 mcg per day or equivalent), plus long-acting beta-2 agonist therapy (at least salmeterol 50 mcg bd or eformoterol 12 mcg bd) for at least 12 months, unless contraindicated or not tolerated

3. Asthma Control Test (ACT) score of 10 or less
4. Systemic corticosteroids:
    a. Patient has received courses of systemic corticosteroids equivalent to at least 28 days treatment in the past 12 months, unless contraindicated or not tolerated OR
    b. Or patient has had at least 4 exacerbations needing systemic corticosteroids in the previous 12 months, where an exacerbation is defined as either documented use of oral corticosteroids for at least 3 days or parenteral steroids

Exclusion Criteria

1. Unwilling to provide consent
2. Other conditions likely to compromise participant safety or impact on the feasibility of results, at the discretion of the investigator (examples include pregnancy and malignancy).

RANDOMISATION AND DRUG PROVISION

Participants who are suitable for study inclusion and who have provided Informed Consent will be randomised (1:1) using the CReDITSS Online Randomisation Engine (CORE) at the HMRI to receive either open label Mepolizumab or Omalizumab for 6 months.

Site personnel will be unblinded to the randomisation process.

In Australia, Treatments will be sourced by the treating physician after successful application to the PBS authority.

In New Zealand, Omalizumab and Mepolizumab will be sourced by the treating physician after successful application to Pharmac.

CLINICAL ASSESSMENTS

Baseline data will be collected at Visit 0 by the local site at the time of randomisation and participant data forwarded electronically for collation by the central coordinating site.

Study-specific clinical data collected at baseline V0, by the site and sent electronically to the central site. This will include:

▪   Evidence of asthma (as defined in the inclusion criteria 4).

- Evidence of poor asthma control in the past 12 months (as defined inclusion criteria 8)
- Exhaled nitric oxide (FeNO), if available. The preferred device for use will be the Niox Vero, however an alternative device may be used by sites after review of its speciifcations.
- Post-bronchodilator spirometry
- Blood samples for measurement of:
  I. blood eosinophils,
  II. C reactive protein (CRP)
  III. total IgE
  IV. allergen specific IgE for mixed fungi, house dust mite, Australian grass mix, and animal dander (dog and cat) mix.
- Sputum induction (selected sites only)
- eNose
- Optional blood samples (included in visit blood sampling if consent provided)

Telephone contacts by the central coordinating site

The central site will telephone each randomised participant within 48hrs following the randomisation visit (Visit 0). The central site will take a standard asthma history. They will take a standard history of co-morbid conditions. They will determine current medications being used. They will assess asthma symptoms and calculate the ACQ5. This ACQ5 score will be used as the baseline metric to determine the primary outcome of the study.
The central site will then enter this data into the study database. They will generate a brief summary report for the referring site. They will generate the Australian PBS initial application form for the site and return this electronically to the site, together with the ACQ5 form.

The reffering clinican will need to sign the PBS initial application form and ACQ5 form acknowledging that both are correct. They will then submit these forms together with a prescription to the PBS.

Upon approval and receipt of the prescription the referring site will invite the patient to return to receive for their first dose (V1).

Those who elect to take part in the substudies defined in Aim 2, will have the following done:
- Blood taken for transcriptomics (x1 9ml EDTA tube)
- Blood taken for isolation of peripheral blood monocytes (1-2 9ml EDTA tube)
- Spirometry
- FeNO
- Measurement by the eNOSE

The central site will contact participants monthly by phone (P1, P2, P3, P4, P5 and P6 – see Time and Events table – Table 2) to determine if there has been change in clinical condition of asthma or otherwise or if any adverse event has occurred. In regard to asthma, this telephone call will involve the collection of:

- ACQ5
- ACQ6
- details of any exacerbations
- unplanned hospital or primary care visits
- use of prednisone
- adherence to asthma therapy

Those who elect to take part in the substudies defined in Aim 2, will have one or more of the following done, 4 weeks after starting treatment, 12 weeks and at the conclusion of treatment:
- Blood taken for transcriptomics (x1 9ml Paxgene tube)
- Blood taken for isolation of peripheral blood monocytes (1-2 9ml EDTA tube)
- Spirometry
- FeNO
- Measurement by the eNOSE

End of treatment trial visit (Visit 2)

The subject will be contacted by the central site 2 weeks before Visit 2 (P6). Data required for possible reapplication for their monoclonal therapy will be obtained. A report including this data will be sent to the referring local clinician. This report will included ACQ5 results, prednisone dose, number of asthma exacerbations in the last 6 months and an assessment indicating if the subject meets PBS criteria for continuation, together with a completed PBS continuation form and ACQ5 form. At Visit 2 the referring clinician will determine if the subject has experienced clinical success and should reapply for the treatment. The subject will be determined as a treatment success or failure. Subjects may also at this time elect to discontinue treatment or may elect to trial the alternative monoclonal antibody therapy.

Clinical assessments and blood samples will be collected from all participants at the end of the 6 month treatment trial and study-specific data recorded by the local site. As described for Visit 0, a phone assessment by the central site will be made within 48 hours of the 6 month visit taking place.

Extra study visits for sub-study participants (optional)

Extra study visits can take place 4 and/or 12 weeks after treatment initiation (see Table 2). These study visits will be for those subjects who provide consent to participate in the sub-studies. If these visits are completed a phone assessment will be made within one week of each extra visit by the central site.

Treatment failure and treatment with the alternate treatment

If a randomised participant is deemed a treatment failure i.e. no change in ACQ5 of at least -0.5 from baseline or an inability to reduce either regular prednisone dose or intermittent prednisone dose usage by at least 15%, they will undergo an 8 week washout and then be reassessed at the study site as for Visit 0 of the first treatment period. If it is judged to be clinically appropriate by

their treating physician the participant will be asked if they are willing to continue in the study. Those subjects that agree to continue their involvement in the study will treated with the alternate monoclonal antibody for a further period of 6 months. All study assessments described for the initial randomised treatment period will be repeated for this second treatment period.

Participants who decline to continue in the study after initial treatment failure will be managed according to standard clinical practice at their clinical centre which may include trial of the alternate monoclonal antibody treatment.

Table 2: Schedule of Time and Events

	V0	☎1	V1 Treatment start	Optional visits Weeks 4 & 12[d]	☎2	☎3	☎4	☎5	☎6	V2
Week	-4 to -2	≤48 hours post-V0	0	4	4	8	12	16	22	24
Asthma History	X									
Comorbidities	X									
ACQ5 & ACQ6	X	X	X	X	X	X	X	X	X	
Exacerbation occurrence	X	X	X	X	X	X	X	X	X	
Asthma meds	X	X	X	X	X	X	X	X	X	
OCS dose	X	X	X	X	X	X	X	X	X	
Spirometry[a,]	X		X	X						X
FeNO [b]	X		X	X						X
Paxgene [b]			X	X						X
FBC	X			X						X
EDTA blood samples			X	X						X
CRP	X			X						X
IgE & RAST	X									X[e]
Sputum induction [b]			X	X						X
eNOSE [b]	X		X	X						X
mAb treatment randomisation	X									

a. Post-bronchodilator

b. will be done by selected sites only

d. Sub-study sample collection visit

e. IgE only

STUDY ENDPOINTS

Primary Outcome

- Change in ACQ5 after 6 months of treatment, adjusted for baseline ACQ5. Mean ACQ5 in the Mepolizumab group will be compared to that in the Omalizumab group using a pre-specified non-inferiority margin (Δ) of 0.35.

Secondary outcomes

- Number of Exacerbations, requiring change in oral corticosteroids, with either a course of prednisone for at least 3 days or, for those subjects on maintenance OCS, an increase in dose of at least 50% for at least 3 days.
- Time to first exacerbation
- Number of admissions to hospital and /or ED presentations
- Reduction in dose of regular OCS
- Reduction in total OCS use during the 6 month treatment period
- Changes in spirometry (FEV1 or FVC)
- Change in blood eosinophil count
- Proportion continuing on randomisation assigned medication after 6 months (successful treatment)
- Adverse events

Treatment Failure

After 6 months of treatment subjects will be clinically assessed to determine if the treatment has succeeded or failed. The following criteria will be used be regarded as the treatment having failed:

1. No improvement in ACQ5 of at least 0.5 (minimum clinically important difference) from baseline, and

2. No reduction in regular prednisone dose or intermittent prednisone usage by at least 15% and

3. An intolerance to the agent or the emergence of clinically significant side-effects.

In New Zealand participants will be considered to have treatment failure if they do not meet the following additional criteria:

1. An increase in the Asthma Control Test (ACT) score of at least 5 from baseline AND
2. A reduction in the maintenance oral corticosteroid dose or number of exacerbations of at least 50% from baseline.

Alternate treatment challenge

Those participants who are deemed to have failed initial therapy will then undergo a 4 week washout period. At the end of this time they will then be offered therapy with the alternative monoclonal antibody for 6 months where the same visit schedule will be followed as was done for the initial randomised treatment period.

In Australia, the supply of the alternate medication will be provided on compassionate grounds from either GSK (mepolizumab) or Novartis (omalizumab) for 6 months. In New Zealand, use of omalizumab as an alternate medication will be funded by Pharmac whereas mepolizumab will be provided by GSK under compassionate access. If Pharmac fund Mepolizumab during the study, participants randomised after this point will be placed on Pharmac funded mepolizumab if they meet the Pharmac criteria in place at that time.

If the alternate treatment is successful, an application will be made for continuation of the treatment from the PBS, for Australian participants, or Pharmac, for New Zealand participants. If mepolizumab is not funded by Pharmac at that time GSK will supply ongoing mepolizumab on a compassionate basis up to a maximum of 6 participants.

Based on the experience gained from the Australian Xolair data registry, a 16% failure rate with the first agent is anticipated. This expectation suggests treatment with either Mepolizumab or Omalizumab as an alternate may be required for approximately 30 participants of the initial cohort of 190 randomised subjects.   `

DATA COLLECTION

Data will be collected by the sites recruiting participants on a clinical record form, with data forwarded to the central site for data entry. The data will be entered into the secure Severe Asthma Network database, 'SAWD' that works on a Research Electronic Data Capture (REDCap) platform.

The central site will conduct all phone assessments and will enter this data directly into the database.

All data will stored in password protected secure electronic format, with access limited to those with database administrator rights. Access to the database is managed by the Data Custodian and individual user rights within the database are controlled according to the specific role/access permission of the user. All users require individual log-in details which remain confidential.

The SAWD REDCap database is stored and run from the HMRI data centre with all data both physically and virtually secured. The application security provides role based access and uses 256 bit grade encryption to protect the authentication details and all communication between clients and the servers will utilise HTTPS via SSL. The HMRI data centre offers cold disaster recovery – if the infrastructure housing the application fails, HMRI provide an offsite fully operational replica of the system within the University of Newcastle Callaghan Campus data centre that can be turned on within moments. Backups are taken at 30 minute increments, stored to disk and tape and are housed onsite and offsite. Access to the server physically and virtually is limited to HMRI IT Services staff only.

STATISTICAL PLAN FOR AIM 1

The hypothesis of this study aim is that Mepolizumab is as effective as Omalizumab in improving asthma symptom control.

Web-based randomisation
The Clinical Research Design, IT and Statistical Support (CReDITSS) unit at the Hunter Medical Research Institute is a consulting unit that provides statistical services to researchers. Randomisation will be performed using the CReDITSS Online Randomisation Engine (CORE): a web-based randomisation and data entry system deployed as a standalone application for individual research projects. We will use permuted block randomisation, with block sizes of 4 or 6, stratified by baseline eosinophil count (using a median split).

Blinding
Owing to the pragmatic nature of the trial, neither participants, treating clinicians nor site staff will be blinded. However, research staff recording outcomes by phone will be blinded. In addition, data managers and statisticians will be blinded.

Sample Labelling

All subjects, questionnaires and phone interview records and laboratory samples will be labelled using a code in the format CAM – site number – subject number: CAM-XXX-XXX.

A list will be maintained at each site by the study coordinator and a master identification list maintained at the central site. This coding is to allow the blinding of data managers and statisticians.

Sample size/power

The sample size has been selected to provide 80% power to test the one-sided null hypothesis H_0: $\mu_M - \mu_O \geq \Delta$ where μ_M is the mean 6 month ACQ5 in the Mepolizumab group, μ_O is the mean 6 month ACQ5 in the Omalizumab group and Δ is the non-inferiority margin, which we have specified as 0.35. The corresponding alternative hypothesis is H_A: $\mu_M - \mu_O < \Delta$.

The non-inferiority margin has been selected as a proportion (slightly less than half) of the effect size observed in two of the largest superiority trials comparing Omalizumab to placebo [39]. Using pilot data, we estimated a standard deviation (SD) of 1.05 for the mean ACQ5 and a correlation of 0.4 between baseline and 6 month ACQ5 for patients treated with Omalizumab and Mepolizumab. To test the non-inferiority of Mepolizumab compared to Omalizumab will require 95 participants per group (1:1 allocation ratio). This will provide 80% power to estimate a one-sided 95% confidence interval (alpha=0.05) for the baseline-adjusted group difference in mean ACQ that excludes the 0.35-point non-inferiority margin, assuming a SD of 0.962 for the baseline-adjusted 6 month mean ACQ5 (based on the ACQ5 SD of 1.05 and a correlation of 0.4 between baseline and follow-up ACQ5). Allowing for a 5% loss to follow-up at the 6 month visit, the study will recruit 200 subjects.

Statistical analysis

<u>Efficacy of the intervention</u>: All analyses will be conducted blind to group allocation. The primary endpoint will be analysed for the as treated population to minimise type I error inflation by treatment failure and subsequent crossover. A sensitivity analysis will be performed for the intention-to-treat (ITT) population.

Treatment group differences in six-month ACQ5 will be estimated using a Generalised Linear Model (GLM) with a normal response distribution and identity link function. The model will be

fitted in an ANCOVA framework (i.e. adjusted for baseline). The model will include fixed effects for group and baseline eosinophil count. The treatment effects will be estimated as baseline-adjusted, least-square mean differences between the two treatment groups at 6 month follow-up. Secondary analyses will be assessed using the same GLM approach, with response link function as appropriate to the response distribution.

Attrition: The GLM provides unbiased estimates of treatment effect under the assumption that data are missing completely at random. Based on previous experience with this patient population, attrition is anticipated to be very low. However, sensitivity analyses such as multiple imputation and pattern mixture modelling will be used to investigate the robustness of conclusions to different missing data mechanisms.

EXPERIMENTAL APPROACH

LABORATORY-BASED PROCESSES

Aim 2: To determine those with severe refractory eosinophilic and allergic asthma if peripheral blood gene expression, immune cell subset analysis, exhaled volatile compounds and induced sputum provides novel information that will identify patients who are more likely to response to omalizumab or mepolizumab.

The registration trials for both Mepolizumab and Omalizumab, identified a clinical phenotype, i.e. dual atopy and persistent eosinophilia that may potentially respond to both agents. This description however may be a superficial means of describing this heterogenous group of patients. A more detailed analysis of the pathology present, before and after treatment, would better define whether participants will respond to one agent better than the other and may also provide invaluable data for future clinical interventions.

From two subsets of participants we will seek to determine the influence of treatment on immune responses in asthma.

Participants will be asked to provide consent for the additional blood samples required for this aspect of the research study.

Aim 2a: Characterisation of peripheral blood molecular signature to predict response to treatment.

We will collect blood samples (i.e. 3 x 9ml EDTA tubes & 1 x 2.5 ml PaxGene tube) for storage and measurement of biomarkers and gene associations using RNA-Seq to predict response from 80 participants, 40 assigned to each treatment arm.

Blood samples will be collected at randomisation (V0) and again after 6 months of treatment (V2). In addition, blood samples will be collected from participants who have been deemed treatment failures as described above.

Samples for RNA-Seq will be collected in Paxgene tubes and stored, batched and sent to the central lab (HMRI) for processing. The RNA-Seq will be used to perform determine responder gene(s) profiles in severe asthma patients to be characterised before and after treatment. This transcriptomic profile will be compared to that of our collaborators in the large U-BIOPRED population that assessed severe asthma at baseline [40].

A subgroup of individuals will have systemic and airway immune profiles characterised before and after treatment with Mepolizumab.

Forty (20) participants recruited from the John Hunter Hospital, Newcastle and Princess Alexandra Hospital, Brisbane will have blood (1 x 9ml EDTA tubes) taken for isolation of peripheral blood monocytes (PBMCs) and also be asked to undergo a sputum induction.

PBMCs will be characterised by flow cytometry to determine and quantify the presence lymphocyte populations; CD3+ CD4+ T helper (TH-1/2/17), T Regulatory cells; CD3+, CD4+, CD25+, CD8+ T cells, Plasmacytoid dendritic cells (pDCs), NKT cells; CD3+CD56+, NK cells CD3- CD56+, monocyte derived dendritic cells; CD14+, CD11c+ and plasmacytoid dendritic cells; CD3-CD304+. Innate lymphoid 2 cells. Total ILCs will be identified as CD45+ cells, negative for lineage markers and CD127+; ILC2 are positive for the prostaglandin D2 receptor (CRTH2) or IL-33 receptor (ST2).

- A subset of PBMCs will be stimulated to assess immune response to important asthma triggers. PBMCs will be cultured with Influenza, Rhinovirus, House dust mite extract and LPS. Responses will be determined in regard to release of; IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-10, IL-5, IL-4, IL-13. Responses will be assessed at 24hrs and after 7 days, by ELISA or flow cytometry bead array.

- Induced sputum will be collected and processed. Cell count and differential performed on selected mucus plugs. Cell count will be determined using microscopy followed by flow cytometry to quantify monocyte and lymphocyte populations.

Aim 2b: Characterisation of exhaled molecular signature using an "electronic nose" to predict response to treatment.

The "SpiroNose" is an electronic nose especially designed for medical purposes that can be used as add-on to routine lung function testing[41]. This technology will be supplied through our collaboration with AI-Sterk. The "SpiroNose" incorporates e-nose assessment in routine daily practice. The SpiroNose consists of 8 separate sensor arrays, each comprising 4 metal oxide semiconductor sensors. Four of the sensor arrays are used as reference arrays to detect the ambient volatile organic compounds (VOCs) and 4 sensor arrays are used to monitor the VOCs in exhaled breath.

Deidentified data (changes in electrical voltage) are transmitted in real-time and stored in the online server of "BreathCloud". "BreathCloud" is a reference database of exhaled biomarker profiles linked to a computer programme by a corresponding application to enable point-of-care personalized medicine.

This database provides immediate diagnostic answers for the individual patient. For each patient, BreathCloud automatically selects the most optimal model, based on patient characteristics and differential diagnosis, in order to create a final report.

The "SpiroNose" will be performed on those participants (n=80) who consent to participate in Aim 2a. This will provide the unique opportunity to combine data from clinical characterisation and a systemic molecular signature of disease and relate these data directly to treatment efficacy.

FINAL OUTCOME

At the conclusion of this study the information gained may provide the opportunity to determine a precise clinical profile that can be immediately applied in real world severe asthma clinics. It is hoped that these data will enable an accurate predictor of success for treatment with both Omalizumab and Mepolizumab in people with severe allergic/eosinophilic asthma. If this outcome

is demonstrated it will be a significant improvement on the present situation where imprecision may lead to suboptimal selection of treatment.

REFERENCES

1.   Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343-73.

2.   Kerkhof M, Tran TN, Soriano JB, et al. Healthcare resource use and costs of severe, uncontrolled eosinophilic asthma in the UK general population. Thorax 2017.

3.   Foster JM, McDonald VM, Guo M, Reddel HK. "I have lost in every facet of my life": the hidden burden of severe asthma. Eur Respir J 2017;50.

4.   Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med 2012;18:716-25.

5.   McDonald VM, Maltby S, Reddel HK, et al. Severe asthma: Current management, targeted therapies and future directions-A roundtable report. Respirology 2017;22:53-60.

6.   Israel E, Panettieri RA, Jr. Anti-IgE or Anti-IL5: That Is the Question. J Allergy Clin Immunol Pract 2017.

7.   Chung KF, Bel EH, Wenzel SE. Difficult-to-Treat Severe Asthma. European Respiratory Society Monograph 2011;15:51.

8.   Shaw DE, Sousa AR, Fowler SJ, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. Eur Respir J 2015;46:1308-21.

9.   Sadatsafavi M, Lynd L, Marra C, et al. Direct health care costs associated with asthma in British Columbia. Can Respir J 2010;17:74-80.

10.   Moore WC BE, Curran-Everett D, et al. . Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. journal of allergy and clinical immunology 2007;119.

11.   Moore WC MD, Wenzel Se, et al. Identification of Asthma Phenotypes Using Cluster Analysis in the Severe Asthma Research Program. American Journal of Respiratory Critical Care Medicine 2010;181.

12.   Hastie AT MW, Meyers DA, et al. . Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. Journal of allergy and clinical immunology 2010;125.

13.   Moore WC, Meyers DA, Wenzel SE, et al. Identification of Asthma Phenotypes Using Cluster Analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010;181:315-23.

14.   Abonia JP, Putnam PE. Mepolizumab in eosinophilic disorders. Expert review of clinical immunology 2011;7:411-7.

15.   Flood-Page P, Swenson C, Faiferman I, et al. A Study to Evaluate Safety and Efficacy of Mepolizumab in Patients with Moderate Persistent Asthma. Am J Respir Crit Care Med 2007;176:1062-71.

16.   Nair P, Pizzichini MMM, Kjarsgaard M, et al. Mepolizumab for Prednisone-Dependent Asthma with Sputum Eosinophilia. N Engl J Med 2009;360:985-93.

17.   Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.

18.   Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. Cochrane Database Syst Rev 2014:CD003559.

19.   Hanania NA, Wenzel S, Rosen K, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. Am J Respir Crit Care Med 2013;187:804-11.

20.   Garcia G, Magnan A, Chiron R, et al. A proof-of-concept, randomized, controlled trial of omalizumab in patients with severe, difficult-to-control, nonatopic asthma. Chest 2013;144:411-9.

21.   Busse WW, Morgan WJ, Gergen PJ, et al. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. N Engl J Med 2011;364:1005-15.

22.   Teach SJ, Gill MA, Togias A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. J Allergy Clin Immunol 2015;136:1476-85.

23.   Available at: http://www.european-lung-foundation.org/16385-lung-health-in-europe.htm. Accessed July 12.

24.   American Lung Association. Trends in asthma morbidity and mortality. Available at: http://www.lungusa.org/site/pp.asp?c=dvLUK9O0E&b=33347. Accessed July 12.

25.   JATAKANON A, LIM S, BARNES PJ. Changes in Sputum Eosinophils Predict Loss of Asthma Control. American Journal of Respiratory and Critical Care Medicine 2000;161:64-72.

26.   Bateman ED, Reddel HK, Eriksson G, et al. Overall asthma control: the relationship between current control and future risk. J Allergy Clin Immunol 2010;125:600-8, 8 e1-8 e6.

27.   Sears MR. Epidemiology of asthma exacerbations. J Allergy Clin Immunol 2008;122:662-8; quiz 9-70.

28.   Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. Nature Medicine 2006;12:1023-6.

29.   Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. J Exp Med 2005;201:937-47.

30. Forbes RL, Gibson PG, Murphy VE, Wark PA. Impaired type I and III interferon response to rhinovirus infection during pregnancy and asthma. Thorax 2011.

31. Simpson JL, Carroll M, Yang IA, et al. Reduced Antiviral Interferon Production in Poorly Controlled Asthma Is Associated With Neutrophilic Inflammation and High-Dose Inhaled Corticosteroids. Chest 2016;149:704-13.

32. Spits H, Artis D, Colonna M, et al. Innate lymphoid cells--a proposal for uniform nomenclature. Nat Rev Immunol 2013;13:145-9.

33. Bernink J, Mjosberg J, Spits H. Th1- and Th2-like subsets of innate lymphoid cells. Immunological reviews 2013;252:133-8.

34. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. Bmj 2002;324:763.

35. DeMore JP, Weisshaar EH, Vrtis RF, et al. Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16. The Journal of allergy and clinical immunology 2009;124:245-52, 52 e1-3.

36. Ortega H, Katz L, Gunsoy N, Keene O, Yancey S. Blood eosinophil counts predict treatment response in patients with severe eosinophilic asthma. J Allergy Clin Immunol 2015;136:825-6.

37. Gibson PG, Reddel H, McDonald VM, et al. Effectiveness and response predictors of omalizumab in a severe allergic asthma population with a high prevalence of comorbidities: the Australian Xolair Registry. Internal medicine journal 2016;46:1054-62.

38. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999;14:902-7.

39. Hanania NA, Alpan O, Hamilos DL, et al. Omalizumab in severe allergic asthma inadequately controlled with standard therapy: a randomized trial. Ann Intern Med 2011;154:573-82.

40. Bigler J, Boedigheimer M, Schofield JPR, et al. A Severe Asthma Disease Signature from Gene Expression Profiling of Peripheral Blood from U-BIOPRED Cohorts. Am J Respir Crit Care Med 2017;195:1311-20.

41. de Vries R, Brinkman P, van der Schee MP, et al. Integration of electronic nose technology with spirometry: validation of a new approach for exhaled breath analysis. J Breath Res 2015;9:046001.