Title: Soluble FcεRI Targeting IgE Binding in Asthma Clinical Trial Proposal
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ABSTRACT

DISEASE
Allergic Asthma

Allergy induced asthma, affecting 25 million Americans a year, is a condition that can cause debilitating respiratory symptoms. Very often, an individual's airways become inflamed in response to allergen exposure and can lead to severe asthma that lower one's quality of life. Asthma symptoms are mediated by allergens causing a cascade with interleukins and T-helper cells, which eventually affects the Immunoglobulin E (IgE)-mast cell complex. IgE binds to FcεRI receptors on the surface of mast cells and our drug will affect this mechanism. Our drug is the soluble form of FcεRI, **soluble FcεRI (sFcεRI)**, which is found naturally in the human body and will interfere with the IgE-mast cell complex formation through competitive binding. It is a biologic that will reduce the activation of mast cells and basophils associated with airway inflammation, thereby alleviating allergic asthma.

TARGET
Immunoglobulin E

IgE are antibodies produced by the immune system that travel to cells to stimulate allergy symptoms like swelling, shortness of breath, and sneezing. Although IgE is typically the least abundant isotype, it is capable of triggering the most powerful inflammatory reactions. IgE in the plasma cause inflammatory responses by binding to FcεRI receptors located on the surfaces of mast cells and basophils to induce an inflammatory response cascade. The binding of an antigen to IgE (bound to FcεRI receptors) cross-links these receptors and results in the release of chemical mediators from the mast cells, which may lead to the development of a type I hypersensitivity reaction. Receptor-bound IgE can remain fixed to cells in tissues for weeks or months. IgE differs from other antibody isotypes because they predominantly act on tissues affecting asthma.

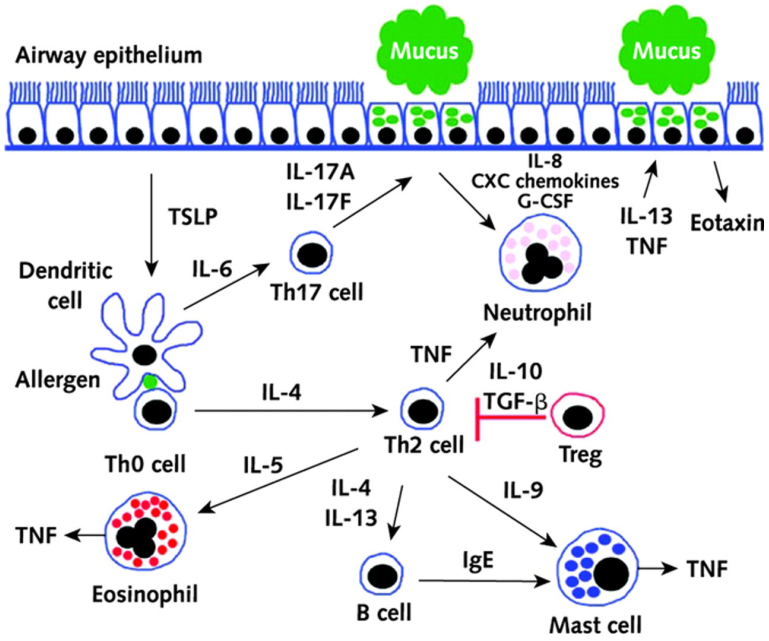

Figure 1. Type-2 Immune Response Pathway [1]

RELEVANCE TO DISEASE

There is currently no cure for asthma, but it can be managed with proper treatment. Asthma has been increasing since the early 1980s in all age, sex, and racial demographics, but is more common in adult women. It is the leading chronic disease in children and is more common in children (8.4%) than adults (7.6%). More than 11.5 million people with asthma reported having one or more asthma episodes or attacks in 2015.

Current treatment for asthma include corticosteroids which are given intravenously, typically only to patients who are vomiting or under respiratory failure. Bronchodilators include ipratropium (Atrovent) to treat severe asthma attacks. These are used when albuterol is not effective. Other types of treatments for severe asthma attacks range from omalizumab to intubation, mechanical ventilation and oxygen.

IgE-mediated allergic asthma represents the condition affecting the majority of patients with asthma. These patients display Type- I hypersensitivity in which normally protective immune mechanisms cause detrimental reactions in the host. In the middle part of 20th century, asthma was identified as a disease or symptom complex that displayed paroxysmal dyspnea (attacks of severe shortness of breath with coughing) with reversible bronchoconstriction. Spasm of bronchial smooth muscle was thought to be the main mechanism of airway constriction and bronchodilators were used as treatment. In the 1960s, airway hyperreactivity was listed as an additional symptom. However, in the 1980s, researchers discovered that airway inflammation underpinned the disordered airway function. Eosinophils and mast cells were identified to contribute to changes in the airway pathway. Allergic asthma is caused by inflammation of the

air pathway and the build up of mucus in the airway in response to an allergen. When the airway swells and constricts, the individual will experience wheezing, coughing, and difficulty breathing. Allergic asthma is typically caused by airborne substances like dust mites, pollen, smoke, and spores.

Each IgE antibody has a specific receptor for each type of allergen. A wide variety of IgE antibodies indicates increased sensitivity to different foreign antigens. The immune response results from the activation of antibodies, mast cells, and basophils as well as the mediated release of histamine. By decreasing free IgE levels, fewer amounts of mast cells and basophils will be activated, thus, lowering the immune response and minimizing inflammation in the airway.

MODULATING THE TARGET

Soluble FcεRI (sFcεRI)

This high-affinity receptor is produced naturally in the body and is a circulating IgE receptor located in the human serum. IgE binds to sFcεRI receptors with equal affinity compared with FcεRI on mast cell surfaces. sFcεRI is present as both a free and IgE-bound protein. Through recombinant DNA techniques, researchers have linked the alpha chain of sFcεRI to be associated with the binding activity of IgE. The result of this complex formation is still under current investigation but data shows persistence of the IgE-sFcεRI complex once IgE is bounded to sFcεRI. Studies using ELISA also have shown that serum FcεRI levels correlate with serum IgE in patients and sFcεRI can outcompete the binding of IgE to FcεRI expressed at the cell surface. High levels of sFcεRI are not associated with increased susceptibility to allergic reactions.

Similar to another drug on the current market, sFcεRI and Omalizumab both prevent IgE-mediated activation of the immune system by clearing the serum of IgE. Omalizumab is a monoclonal antibody targeting serum IgE and down-regulates cell surface levels of FcεRI. It is the approved current treatment of severe allergic asthma. [2]

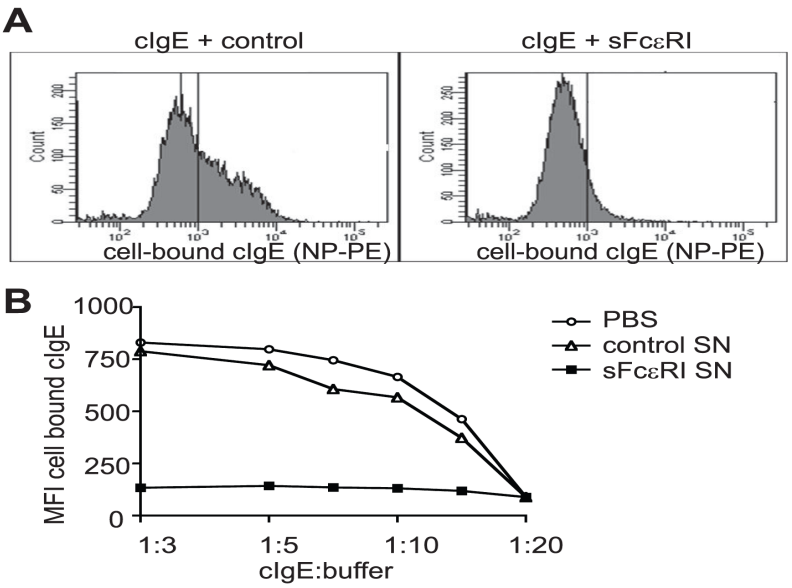

Figure 2.

A. FcεRI-expressing MelJuso-αγ were loaded with cIgE and either a control serum or serum containing sFcεRI. Cell-bound IgE was visualized by flow cytometry. sFcεRI effectively blocked binding of cIgE to the FcεRI-expressing MelJuso-αγ cell surface.
B. cIgE was blocked in a dose-dependent manner as depicted in dilation curves compared to control solutions. [4]

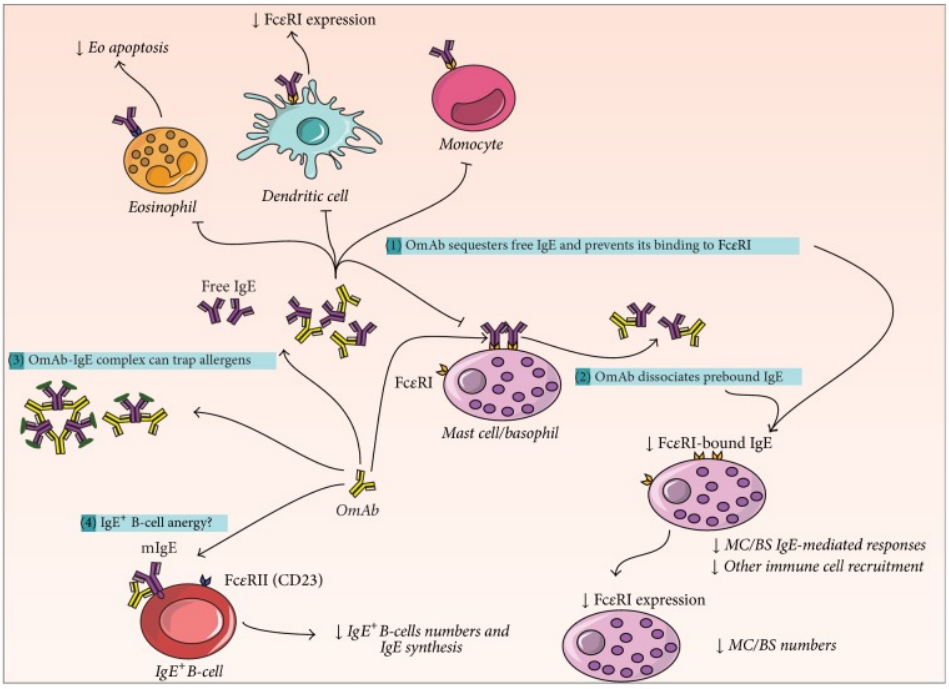

Figure 3. IgE Pathway. [3]

Drug Delivery Strategy

Administration of sFcεRI will be via intravenous injection. sFcεRI is a protein of ~40 kDa and contains an intact IgE-binding site. *In vitro* studies have demonstrated the ability of sFcεRI receptors to outcompete FcεRI receptors in binding serum IgE. With a high enough dosage, our drug will bind IgE with equally high affinity as demonstrated in the study.

Target Modulation

sFcεRI competitively binds and forms a complex with IgE, decreasing the concentration of IgE available to bind to mast cells and basophils. This binding blocks IgE from binding with other FcεRI receptors expressed on the cell surface. sFcεRI then helps reduce free IgE level. Reduced free IgE levels holds as a potential biomarker. In our quantitative assays, we will measure free IgE levels indirectly by measuring the level of binding between mAbs and FcεRI and sFcεRI receptors. If lower levels of mAbs bind to the soluble receptors then that means that higher levels of IgE successfully bound to them. With less free IgE available to bind to receptors on cells associated with the immune system, the allergic response activated by the formation of the IgE-FcεRI complex is minimized.

NON-CLINICAL PROGRAMS

First, we will examine the biological effect of sFcεRI. We will use human cell lines in an *in-vitro* cellular model to observe competitive binding of sFcεRI. We will then conduct further toxicity testing using cynomolgus monkeys. We have ruled out rodent models since rodent models will develop antibodies to the human-biologic protein thus rendering the protein ineffective. [10] Anaphylactic genicity, and IgE binding affinity to sFcεRI will be the primary pharmacodynamic studies. Repeat-dose toxicity studies will be conducted using cynomolgus monkeys after we have confirmed that these monkeys will not develop an immune response to our drug. These will be the non-clinical programs conducted to determine the rationale for first dose in humans to enable the first clinical trial.

Biological Effect

To demonstrate biological effect, we will use human cell lines in an *in-vitro* cellular model. Since our drug is a human recombinant protein, animals may form antibodies against the human recombinant protein and experience an autoimmune reaction that will cancel out the therapeutic effects of the drug. There is a chance patients may also develop a human anti-mouse antibody (HAMA) which is an antibody that reacts to immunoglobulins found in mice. Patients may react to the new antibodies as if they were a foreign substance, creating a new set of antibodies to combat these new mouse antibodies. Monoclonal antibodies generated using this method do not suffer from drawbacks related to the HAMA response. Using an *in-vitro* model allows us to observe competitive binding of sFcεRI and the FcεRI-mast cell complex to IgE.

(1) IgE Binding Affinity to sFcεRI in Human Serum

To investigate the IgE binding affinity for the soluble version of the IgE-binding alpha-chain of sFcεR in human serum. sFcεRI immunoprecipitates as a protein of ~40 kDa and contains an intact IgE-binding site. In human serum, sFcεRI is found as a soluble free IgE receptor as well as a complex with IgE. Using ELISA, we will determine whether serum sFcεRI correlate with serum IgE in human serum samples. [4]

Duration of bound IgE in human plasma will last from "weeks to months." Time for degradation of soluble receptor IgE is determined to be a half-life of 10 days. Half-life of free IgE in plasma is about 2-3 days. To manufacture the drug, the gene for sFcεRI will be isolated and then made into cDNA. It will be put into a bacterial plasmid and used to transform bacteria or yeast. After the culture grows to 10^10, expression of the gene will be induced and the protein will be purified. [6]

(2) Anaphylactic Genecity

To test for anaphylactic genecity, human IgE variants with amino acid residue substitutions will be generated, and amino acid residues within the IgE epitope required for binding will be investigated by identifying IgE binding to the human high-affinity IgE receptor (FcεRI) and sFcεRI. We will investigate the recognition site for human FcεRI and sFcεRI. The drug will be considered non-anaphylactogenic because it will not be able to bind to IgE currently bound to FcεRI. This would result in inability to cross-link IgE currently bond to FcεRI.

(3) Repeat-dose Toxicity Studies

Further testing will be done on cynomolgus monkeys (*Macaca fascicularis*), a non-human primate that shares the same epitopes as humans. Cynomolgus monkeys are typically used in biomedical research since they share many immunological and physiological features with humans. [5] About 90-93% of their genetic makeup is the same. These monkeys can also be sensitized to common human allergens like pollen and dust mites. [15] To examine biological effect, we will take baseline blood samples to examine free IgE and IgE-sFcεRI levels in the plasma. We will then induce an allergic response using dust mites in cynomolgus monkeys and quantify the duration and severity of the animal's reaction by measuring AhR activation, airway inflammation, goblet cell hyperplasia, and epithelial hypertrophy. Following baseline sampling, we will administer sFcεRI, test free and IgE-sFcεRI plasma levels, induce allergic responses, and quantify the reactions.

FIRST-DOSE RATIONALIZATION

Doses and dosing frequency are determined by serum total IgE level, measured before the start of treatment, and body weight. Rationale for dosage is determined by considering the 7 following criteria: (1) No Observed Adverse Effect Level (NOAEL) Determination, (2) Human Equivalent Dose (HED) Calculation, (3) Most Appropriate Species Selection, (4) Application of Safety Factor, (5) Considering of the Pharmacologically Active Dose (PAD).

(1) No Observed Adverse Effect Level (NOAEL)

The FDA guidance focuses on the use of the NOAEL to establish the maximum recommended starting dose. In general, NOAEL is determined in various toxicity tests performed in the most sensitive and relevant animal species. NOAEL is calculated using the following equation: **NOAEL (No Observed Effect Level) = (LD50 * Avg Wt. of a human (60 kg)) / 2000.** To calculate NOAEL, we will use the cynomolgus monkey as the most relevant animal species by administering daily doses of soluble sFcεRI to determine whether one or more organ or system is adversely affected following exposures of 6 month duration. The NOAEL in previous IgE drug Xolair, was determined to be 50 mg/kg in cynomolgus monkeys. The LD50 was determined to be 166 mg/kg in cynomolgus monkeys and 500 μg/kg in mice.

(2) Human Equivalent Dose (HED)

The Human Equivalent Dose (HED) was determined using the following table (Table 1) by calculating the conversion of animal doses to HED based on body surface area. To convert animal dose in mg/kg to HED, we will multiply the animal dose of monkeys, 50 mg/kg by 0.32. The HED is 16 mg/kg.

Table 1: Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area

Species	To Convert Animal Dose in mg/kg to Dose in mg/m², Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys[c]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

[a] Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.

[b] This k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

[c] For example, cynomolgus, rhesus, and stumptail.

Table 1. Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area. [13]

(3) Most Appropriate Species Selection

Both rodent and non- rodent models are used for preclinical safety assessments, but it has been demonstrated that non- rodent models may be better at predicting MTD in humans. [14]

Traditionally one tenth of the lethal dose for mice (LD10), or one sixth the highest non- severely toxic dose (HNSTD) in a more sensitive species (e.g. monkey). However, we have ruled out rodent models since rodent models will develop antibodies to the human-biologic protein thus rendering the protein ineffective. We have selected cynomolgus monkeys, a non-human primate that shares the same epitopes as humans. Cynomolgus monkeys are typically used for asthma models because their immunological and physiological parameters for allergic responses are similar to those of humans.

(4) Application of Safety Factor

The application of safety factor is the HED divided to introduce a margin of safety between the HED and MRSD. The default application of safety factor is 10. The application of safety factor for this study will also be 10. Increasing the safety factor is not necessary because there is no steep dose response curve, severe toxicities, unexplained mortality in animals, and animal models do not have limited utility. There are also no novel therapeutic targets since the current primary drug used to treat severe asthma, Omalizumab, is also targeting IgE. We will not decrease the safety factor since the NOAEL was not determined based on toxicity studies of longer duration.

(5) Pharmacologically Active Dose (PAD)

Selection of the pharmacologically active dose (PAD) depends on many factors and differs among pharmacological drug classes. *In-vivo* studies can derive the HED from a PAD estimate using the body surface area conversion (Table 1).

The Maximum Recommended Starting Dose (MRSD) can be calculated using the following equation: **MSRD = HED / 10.** Using the HED calculated earlier, 16 mg/kg, dividing the HED by 10 will result in the MSRD to be 1.6 mg/kg. The average human weighs between 60-70 kg. Multiplying the average human weight of 60-70 kg by 1.6 mg/kg results in the clinical starting dose to be 96-112 mg. However, doses are also determined by serum total IgE level. If the pretreatment serum total IgE level is above 100 IU/mL, the MSRD will be multiplied by 2. The clinical starting dose will be 192-224 mg. Clinical starting doses for serum total IgE level 30-10 IU/mL will be 96-112 mg. These doses will be intravenous doses administered every 4 weeks.

PATIENT DESIGN

Clinical trials for drugs dealing with non life threatening conditions are often divided into two parts with the first involving healthy normal volunteers to test for safety and the second involving patients. However, we will bypass this first phase with normal volunteers due to concerns associated with depressed immune responses that may arise from below average IgE levels in healthy individuals. The first clinical trial will involve 150 participants who are between 18-25 years in age with severe allergic asthma. In order to be eligible, the asthmatic patients must be non-smokers and cannot have other autoimmune conditions or lung disorders. In the month prior to the first injection, participants will be asked to record the frequency and severity

of asthma attacks they experience. They will continue updating this log throughout the experiment. A third of the participants will make up the control group and will be administered saline instead of the drug. Initially, each individual will receive 1 injection every 3 weeks over the course of 6 months. The dosage of the drug will be based on the severity of the participant's condition. This will be determined from their asthma attack log as well as their baseline free-IgE serum levels. Before subsequent injections, blood samples will be drawn to measure free IgE levels, free sFcεRI levels, and IgE-sFcεRI levels. Also, specific monoclonal antibodies will be administered in vitro to quantify IgE binding to both the sFcεRI and FcεRI. After samples have been collected, subjects will receive an injection based on their treatment group. Treatment frequency will be altered based on receptor occupancy relative to baseline measurements. Asthma patients have elevated levels of free IgE (554 +/- 447 IU/mL), and we want to adjust the injection frequency to maintain free IgE levels lower than 50% of their baseline values without having the value fall below the normal range of IgE (69 +/- IU/mL) in healthy people. [1]

There is insufficient evidence regarding biological effects of sFcεRI outside of IgE binding. Therefore, we cannot predict side effects arising from sFcεRI. However, other asthma-related drugs targeting IgE share common side effects: increased risk of parasitic infections, rashes, fever, and muscle pains due to decreased IgE levels. There may also be inflammation at the injection site, headaches, and sore throats.

PROOF-OF-CONCEPT

To determine whether our target, sFcεRI, localizes in specific tissues, we will use different human cell lines for immunohistochemistry (IHC). IHC functions by using washing cells with PBS and inhibiting peroxidases with peroxide solution. Next, we will use 100% ethanol to fix cells and permeabilize cells to allow for our mAbs to enter the cells. Then, a blocking buffer will be added to block non-specific sites. Finally we will add our substrates needed to induce fluorescence. We will also use 6-12 months to create two monoclonal Abs specific to sFcεRI and FcεRI receptors. Our mAb specific to FcεRI will bind to the gamma or beta chain that sFcεRI lacks because the latter only has the alpha chain. Furthermore, we want to first identify the tissue location of FcεRI receptors by adding the mAbs specific to FcεRI and then determining the level and location of fluorescence with fluorescence microscopy. Afterwards, we want to identify the tissue location of sFcεRI receptors by adding mAbs specific to sFcεRI and then determining the level of fluorescence in the tissue cells.

After we have identified the tissue site where sFcεRI localizes, we want to prove that IgE is actually binding to sFcεRI. We will run assays on blood samples from human patients to measure the expression of IgE bound to our drug. Additionally, we also want to quantify the level of binding between IgE-FcεRI in comparison with IgE-sFcεRI. In order to quantify the binding of our drug to IgE, we will use the two specific mAbs used previously for our respective receptors, sFcεRI and the FcεRI receptors on mast cells. These mAbs bind to their receptors with

high specificity and affinity. If the mAbs are able to bind to their targets, then it means that IgE did not bind to the receptors. To allow for quantitative comparison, we will determine the saturation levels of IgE when bound to our soluble FcεRI receptors compared to saturation levels of IgE when bound to FcεRI receptors on mast cell surfaces. We would then isolate the plasma from our blood samples, centrifuge to concentrate these receptors and perform fluorescence-activated cell sorting in order to determine the level of binding. FACS is used to indicate the leveling of binding between IgE and our two receptors, our drug and the native FcεRI receptor. We can also show whether or not our drug is outcompeting FcεRI receptors by comparing the level of mAbs that were able to bind to their targets and our baseline percentages.

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