PNEUMYXAB
The monoclonal antibody

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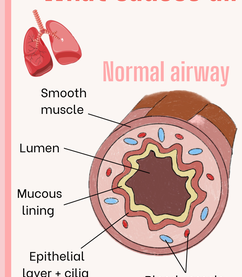
References

What is Hypersensitivity Pneumonitis?

Hypersensitivity Pneumonits (HP) is an immune system disorder in which an inflammatory immune response is triggered in the lungs by over **300 known inhaled allergens** such as various microorganisms, moulds or bird feathers. This inflammation causes **airway obstruction** leading to a shortness of breath and a lack of oxygen entering the blood which can cause dizziness and death. Severity ranges from short-term acute HP, to **chronic HP** where irreversible lung scarring and fibrosis is caused. In our proposal, we will be focussing on the chronic HP pathway.

What causes airway obstruction?



Inflammation is an immune response to tissue damage and infection. The mechanism involves mast cells secreting the cell signalling molecules histamine, which stimulates **vasodilation** and recruitment of other leukocytes. Increased blood flow through "leaky" capillaries causes **increased volumes of tissue fluid** and allows large quantities of leukocytes to enter the tissues. This leads to enflamed tissues in the airways.

As well as inflammation, **lung fibrosis** in chronic HP causes airway obstruction. Fibrosis is the **scarring and thickening of lung tissue** caused by the accumulation of collagen and extracellular matrix components. This results in the alveoli and bronchioles expanding less efficiently, leading to impaired respiratory function and gas exchange.

Existing treatments

Social

- Hypersensitivity Pneumonitis (HP)is caused by inhaling an allergen, so **identification and avoidance of the allergen** is an effective treatment and should always be the first step.
- **Avoiding vaping and second-hand vaping** is also effective as there have been cases where chronic vapers and even their partners have developed HP.
- There are **medical cessation programmes** in place to help people quit vaping and reduce the exposure to the allergen.

Clinical

- **Corticosteroids or immunosuppressants** are the most common current treatments and can be ingested orally by pills.
- These drugs prevent the immune system from responding to the antigen and reduce inflammation.
- The most common drug is **prednisone** which acts an anti-inflammatory glucocorticoid.
- It reverses capillary permeability and **inhibits pro-inflammatory cytokine production** by entering nucleus of T cells and altering gene expression.

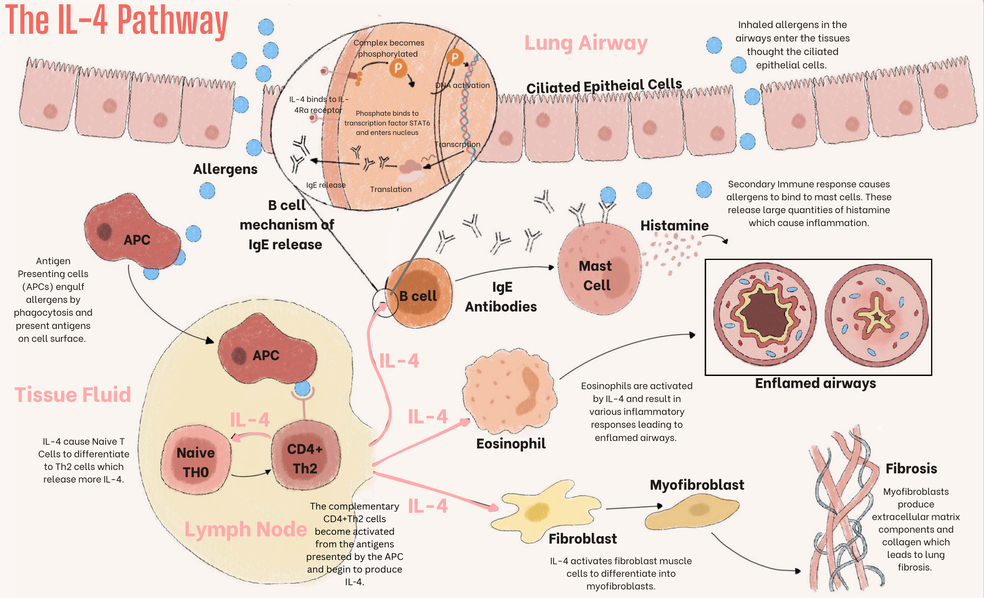
Issues with existing treatments

- It can be **hard to identify the allergen and avoid it** especially if the source of the allergen is your place of work such as a building site or working around animals.
- Corticosteroid treatments such as prednisone have **awful adverse side effects** such as acne, insomnia, headaches, irregular or absent menstrual periods, weak muscles or dizziness due to its hormonal nature.
- Other long term hormonal medication may be needed if prednisone is ineffective which can have further adverse side effects.

Our Proposal

We aim to treat chronic Hypersensitivity Pneumonitis by **inhibiting the inflammatory and fibrotic pathway** in the airways of the lungs through the use of a new **monoclonal antibody Pneumixab**. Our innovation involves **Pneumixab** binding to the cell signalling interleukin, **IL-4,** changing its shape to ensure it cannot bind to its IL-4Ra receptors, thereby **reducing inflammation and fibrosis** in the lungs. With easy, at home administration by **self-injections**, our drug will be accessible to all. Unlike existing treatments, our **Pneumixab** treatment has **very few side effects** due to its specific targeting of a singular cell signalling molecule. Our solution combines the established benefits of interleukin inhibition with the revolutionary development of monoclonal antibody therapy.

The IL-4 Pathway



The Dangers of HP

Chronic Hypersensitivity Pneumonitis (HP) is a dangerous disease with an average life expectancy of **3-5 years** after diagnosis due to the **irreversibility of pulmonary fibrosis** (lung scarring). It causes shortness of breath, pulmonary hypertension (high blood pressure between heart and lungs), lung failure and death. The main risk factors of HP include bird handling, farming, and mould as these are the main sources of the inhaled allergens. This means that those working in the **primary economic sector** develop chronic HP far more frequently than others. People in these professions also typically have a **lower income** so have less money to spend on healthcare, which is why we must develop a **cheap and effective treatment** for this disease.

How does Pneumixab work?

Our monoclonal antibody **Pneumixab** will have a **specific complementary shape** to the IL-4 cytokine. It will bind to IL-4 and change it's shape so that it can no longer bind to the **IL-4Ra receptor** on the surface of the B-cells, eosinophils, fibroblasts and Th0 cells. This means that the ongoing **pathway is inhibited** and the sequential inflammatory and fibrotic responses are prevented. This reduces airway obstruction and the irreversible scarring of the lungs.

What IL-4 does

IL-4 is produced and released by activated **CD4+ T-helper lymphocytes (Th2)** and causes 4 main immune responses.

1. IL-4 causes the **proliferation of B-cells** that produce **immunoglobulin E (IgE)** antibodies. These bind to high affinity receptors on **mast cells** which then release **histamine** leading to inflammation.
2. IL-4 stimulates the **activation of eosinophils** which are involved in numerous inflammatory responses such as vasodilation and bronchoconstriction.
3. IL-4 causes the **differentiation of more naive Th0 cells to Th2 cells** which then produce **more IL-4** establishing a chain-reaction-like loop.
4. IL-4 stimulates the **activation of fibroblasts to myofibroblasts** which produce collagen and various extra cellular matrix components. These cause the **stiffening and thickening** of muscle and connective tissue walls. This leads to **lung scarring** and reduces the eslaticity of the airways and alveoli.

Costs

- **Preclinical studies**: £5 million - staff and lab equipment.
- **Clinical trials**: £100 million - staff, recruitment of volunteers, equipment and manufacture of the drug.
- **Production**: Initially, £664 000 per kg produced therefore £199 per dose. However, after mass production and technical advances, it is possible to halve this price.
- **Distribution**: Estimated £21 per dose - including packaging, transportation and storage.
- Allergy related conditions currently cost the NHS **£1 billion a year** so our new treatment could save the NHS millions.
- Monoclonal Antibody treatment is becoming cheaper. The rabies treatment proposed by the Serum Institute of India currently costs only **£20 a dose**.
- **Overall**: This is an expensive drug, with development costing over **£100 million** and each dose costing around **£220**, which is a yearly cost of **£5720**. However, this is a vast decrease on current treatment costs and with economies of scale and improved technology, this figure could be greatly reduced still.

Feasibility

Subcutaneous injections - beneath a layer of fat eg abdomen, around the navel, front/outer thigh, upper arm, or upper/outer quadrant of the buttocks - allow for **self-administration**, increasing accessibility for those in developing countries - where HP is most common - without access to infusion centres.

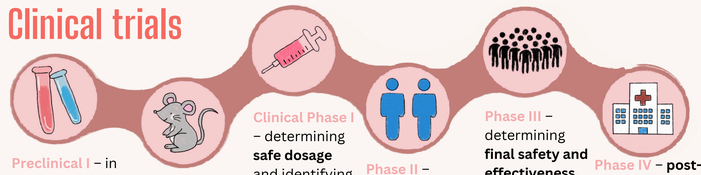
In clinical trials since 2003, monoclonal antibodies (mAb) being tested to treat both asthma and malignant tumours have an **incidence of anaphylaxis** an estimated of 0.1-0.2%. Despite the dangers, **6 mAb treatments** have successfully passed clinical trials which shows the **feasibility** of our mAb drug.

Monoclonal antibodies which focus on IL-4 inhibition have passed clinical trials previously. For example **Dupilumab** binds to the alpha subunit of the IL-4Ra receptor to prevent IL-4 from binding. It has been **approved for use in over 60 countries** to mainly treat the condition Atopic Dermatitis. This shows that our similar treatment is **definitely feasible**.

Monoclonal antibodies must be kept long term at low temperatures, ranging from **-20°C to -80°C**. Short term, they can be kept in temperatures up to **4°C**. This means that the drug can be **less accessible in rural areas** developing countries, who dont have access to refrigeration.

The second phase of **preclinical testing on mice** may have **ethical limitations** as the mice may suffer from side effects of the treatment. However there is **yet to be an alternative** introduced which does not involve preclinical animal trials.

Clinical trials



Preclinical I – in vitro **test tube** or cell culture tests. For observing **metabolism and mechanism of drug** following purification and formulation.

Preclinical II – in vivo trials in animals **typically mice** for immunological drugs. For monitoring **toxicity and drug safety.**

Clinical Phase I – determining **safe dosage** and identifying any **side effects.** Occurs over a few weeks/month with a small group of **20-80 healthy volunteers.**

Phase II – experimental drug testing on to determine **efficacy** and further evaluate **safety**. With a larger group of **100-300 individuals with chronic HP** and last for months.

Phase III – determining **final safety and effectiveness**, complex examination of the risk/benefit profile of the drug. **Compare efficacy to existing drugs** with around **1000-3000 subjects** over 6 months.

Phase IV – **post-marketing surveillance (PMS)** studies to provide evidence regarding benefits, **optimal use of drug** and long-term adverse effects on a **large patient population.**

How are the monoclonal antibodies developed?

Step 1: Identify the gene coding for the cytokine (gene IL4 **ENSG00000113520 for IL-4**). Isolate and insert it into an **expression vector** (eg. Bacterium or yeast cell).

Step 2: **Inject mice with these vectors**. This causes the mice to produce **B cells** through a primary immune response which release the **complementary antibodies** for the cytokine.

Step 3: **Extract these B cells from the spleen** of the mouse and fuse with **myeloma** (fast-proliferating tumour) cells to produce a **hybridoma**.

Step 4: **Culture hybridomas** in a selective medium (**HAT medium**) that causes only the fused cells to grow and the unfused myeloma and B cells to die.

Step 5: **Screen hybridomas** for correct production of the antibody, usually done using an **assay such as ELISA.**

Step 6: **Clone the hydridomas** through the process of **limiting dilution**, to ensure that each hybridoma cell line produces **identical antibodies.**

Step 7: **Culture the hybridomas in bioreactors** to produce large quantities of the monoclonal antibodies and collect the **culture supernatant**.

Step 8: Carry out **initial clarification,** where cells and debris are removed by **centrifugation or filtration.** Then, use **chromatography** to purify the antibodies.

Step 9: **Polish to remove aggregates** and any remaining impurities then formulate the monoclonal antibodies into **solutions for therapeutic use** by adding adjusting buffers and stabilisers.