Lipocalin mutein dry powder formulation for treatment of asthma

Field of the Invention

The present invention relates to the treatment of asthma in a human subject by administering by oral inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, to said subject. The invention also relates to the dry powder formulation comprising the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof.

Background

Asthma is a chronic, complex and heterogeneous respiratory disease characterised by a range of pathogenic features including pulmonary inflammation, mucus hypersecretion, variable airway obstruction and airway remodelling. It is defined by a history of respiratory symptoms that include wheezing, shortness of breath and cough which vary over time and in severity. Both symptoms and airway obstruction can be triggered by a range of factors including exercise, exposure to inhaled irritants or allergens or respiratory infections. Patients are at risk of worsening of their asthma (exacerbations). These exacerbations of asthma can be life threatening and can significantly impact the patient's quality of life. The treatment for most asthma patients, consists of a treatment regime of a controller and bronchodilator therapy. Inhaled corticosteroids (ICS) are considered the "gold standard" in controlling asthma symptoms and long acting beta-agonists (LABA) are the most effective bronchodilators currently available. Oral corticosteroids remain standard of care in severe asthma but are associated with significant side-effects, whilst omalizumab, an anti-IgE monoclonal antibody; benralizumab, mepolizumab and reslizumab, anti-IL-5 antibodies, and dupilumab (US) a monoclonal antibody blocker of IL-4Rα and IL-13 offer a limited number of options for the severe patients. Additionally, patients frequently remain uncontrolled on ICS/LABA and even the limited number of alternative therapies, highlighting an important unmet need (Ray, A., *et al.*, 2016, Current concepts of severe asthma. J. Clin. Invest. 126, 2394–2403).

Interleukin-4, interleukin-13, interleukin-4-receptor alpha and the signal transducer and activator of transcription factor-6 are key components in the development of airway inflammation, mucus production, and airway hyper-responsiveness in asthma.

Lipocalins are proteinaceous binding molecules, which have naturally evolved to bind ligands. Lipocalins occur in many organisms, including vertebrates, insects, plants and bacteria. The members of the lipocalin protein family (Pervaiz, S., & Brew, K. (1987) *FASEB J.* 1, 209-214) are typically small, secreted proteins and have a single polypeptide chain. They are

characterized by a range of different molecular-recognition properties: their ability to bind various, principally hydrophobic molecules (such as retinoids, fatty acids, cholesterols, prostaglandins, biliverdins, pheromones, tastants, and odorants), their binding to specific cell-surface receptors and their formation of macromolecular complexes. Although they have, in the past, been classified primarily as transport proteins, it is now clear that the lipocalins fulfil a variety of physiological functions. These include roles in retinol transport, olfaction, pheromone signalling, and the synthesis of prostaglandins. The lipocalins have also been implicated in the regulation of the immune response and the mediation of cell homoeostasis (reviewed, for example, in Flower, D.R. (1996) *Biochem. J.* 318, 1-14 and Flower, D.R. et al. (2000) *Biochim. Biophys. Acta* 1482, 9-24).

The lipocalins share unusually low levels of overall sequence conservation, often with sequence identities of less than 20%. In strong contrast, their overall folding pattern is highly conserved. The central part of the lipocalin structure consists of a single eight-stranded anti-parallel β - sheet closed back on itself to form a continuously hydrogen-bonded β-barrel. This β-barrel forms a central cavity. One end of the barrel is sterically blocked by the N-terminal peptide segment that runs across its bottom as well as three peptide loops connecting the β-strands. The other end of the β-barrel is open to solvent and encompasses a target-binding site, which is formed by four flexible peptide loops. It is this diversity of the loops in the otherwise rigid lipocalin scaffold that gives rise to a variety of different binding modes each capable of accommodating targets of different size, shape, and chemical character (reviewed, e.g., in Flower, D.R. (1996), *supra*; Flower, D.R. et al. (2000), *supra*, or Skerra, A. (2000) *Biochim. Biophys. Acta* 1482, 337-350).

Human tear lipocalin (TLPC or Tlc), also termed lipocalin-1, tear pre-albumin or von Ebner gland protein, was originally described as a major protein of human tear fluid (approximately one third of the total protein content), but has also been identified in several other secretory tissues including prostate, adrenal gland, thymus, mammary gland, testis, nasal mucosa and tracheal mucosa as well as corticotrophs of the pituitary gland. Homologous proteins have been found in rhesus monkey, chimpanzee, rat, mouse, pig, hamster, cow, dog and horse. Tear lipocalin is an unusual lipocalin member in that it exhibits an unusually broad ligand specificity, when compared to other lipocalins, and in its high promiscuity for relative insoluble lipids (see Redl, B. (2000) *Biochim. Biophys. Acta* 1482; 241-248). This feature of tear lipocalin has been attributed to the protein's function in inhibiting bacterial and fungal growth at the cornea. A remarkable number of lipophilic compounds of different chemical classes such as fatty acids, fatty alcohols, phospholipids, glycolipids and cholesterol are endogenous ligands of this protein. Interestingly, in contrast to other lipocalins, the strength of ligand (target) binding to tear lipocalin correlates with the length of the hydrocarbon tail for both alkyl amides and fatty acids. Thus, tear lipocalin binds most strongly to the least soluble lipids (Glasgow, B.J. et al. (1995)

Curr. Eye Res. 14, 363-372; Gasymov, O.K. et al. (1999) *Biochim. Biophys. Acta* 1433, 307-320). The 1.8-A crystal structure of tear lipocalin revealed an unusually large cavity inside its β-barrel (Breustedt, D.A. et al. (2005) *J. Biol. Chem.* 280, 1, 484-493).

International patent application WO 2005/19256 discloses muteins of tear lipocalin with at least one binding site for different or the same target ligand and provides a method for the generation of such muteins of human tear lipocalin. According to this PCT application, certain amino acid stretches within the primary sequence of tear lipocalin, in particular the loop regions that include amino acids 7-14, 24-36, 41-49, 53-66, 69-77, 79-84, 87-98, and 103-110 of mature human tear lipocalin, are subjected to mutagenesis in order to generate muteins with binding affinities. The resulting muteins have binding affinities for the selected ligand (K_D) in the nanomolar range, in most cases >100 nM. International patent application WO 2008/015239 discloses muteins of tear lipocalin binding a given non-natural ligand, including the IL-4 receptor alpha. Binding affinities are in the nanomolar range. International patent application WO 2011/154420 describes high affinity muteins of human tear lipocalin that bind to human IL-4 receptor alpha in the nanomolar range and methods for producing such high affinity muteins. International patent application WO 2013/087660 describes the use of muteins of human tear lipocalin to treat disorders in which the IL-4/IL-13 pathway contributes to disease pathogenesis, including asthma.

International patent application WO 2020/200960 describes the use of muteins of human tear lipocalin to treat asthma by inhalation, e.g. by nebulisation, wherein the delivered dose of the lipocalin mutein is from about 0.1mg to about 160mg.

The advantages of pulmonary delivery of active agents, such as lipocalin muteins, include the convenience of patient self-administration, the potential for reduced drug side-effects, ease of delivery by inhalation, the elimination of needles, and the like. Many clinical studies with inhaled proteins, peptides, DNA and small molecules have demonstrated that efficacy can be achieved both within the lungs and systemically. However, many molecules which require high payload for delivery, and in particular biological molecules, present problems for the development of inhalable formulations. Formulations must provide stability to the biological payload, e.g. the lipocalin mutein, and have scalable manufacturability while also maintaining desirable physical characteristics to facilitate delivery into the lungs of a patient.

Summary of the Invention

The present invention is based on pre-formulation studies, a phase I crossover clinical study in humans to assess the pharmacokinetics (PK effects) of an inhaled anti-IL-4 receptor alpha (IL-4Rα) human tear lipocalin, AZD1402, in healthy subjects and a two-part phase IIa study to assess efficacy and safety of AZD1402 administered to human patients with asthma as a dry

powder. The amino acid sequence of AZD1402 is shown in Table 70 as SEQ ID NO:1. AZD1402 antagonises the IL-4 receptor alpha (IL-4Rα) and is designed for inhalation.

Based on these studies, the present invention provides a method for treating asthma in a human subject, wherein the method comprises administering by inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, to said subject, wherein a nominal delivered dose of about 0.1mg to about 30 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

The invention also provides a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation by inhalation, wherein a nominal delivered dose of about 0.1mg to about 30 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In addition, the invention provides the use of a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of asthma in a human subject, wherein the treatment comprises administering the dry powder formulation by inhalation, wherein a nominal delivered dose of about 0.1mg to about 30 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In any of the above aspects of the invention, a nominal delivered dose of about 0.5 mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation.

In any of the above aspects of the invention, a nominal delivered dose of about 0.5 mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation.

In some embodiments, a nominal delivered dose of about 0.5mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 1mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 2mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 3mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 4mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 5mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 6mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 10mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation. In some embodiments, a nominal delivered dose of about 30mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject by oral inhalation.

In another aspect, the invention also provides a method for treating asthma in a human subject, wherein the method comprises administering by inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, to said subject, wherein a nominal metered dose of about 0.2mg to about 40 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

The invention also provides a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation by inhalation, wherein a nominal metered dose of about 0.2mg to about 40 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In addition, the invention provides the use of a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a

variant or fragment thereof, for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of asthma in a human subject, wherein the treatment comprises administering the dry powder formulation by inhalation, wherein a nominal metered dose of about 0.2mg to about 40 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In any of these aspects, a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject.

In any of these aspects, a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6 mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject.

In some embodiments, a nominal metered dose of about 0.55mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 0.6mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 1.1mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 2.2mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 3.3mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 4.4mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 5.5mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 6.6mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 11.1mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 12mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject. In some embodiments, a nominal metered dose of about 36mg of said lipocalin mutein, or variant or fragment thereof, may be administered to said subject.

In any of the aspects or embodiments of the invention described above, the lipocalin mutein, or variant or fragment thereof, may administered to said subject at least once per day. In some embodiments, the lipocalin mutein, or variant or fragment thereof, is administered to said subject once per day. In some embodiments, the lipocalin mutein, or variant or fragment thereof, is administered to said subject twice daily.

In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25 mg.

In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 2.5mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 5mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 10mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 15mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 20mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject once per day is about 25mg.

In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg.

In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg.
In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 1mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 2mg. In some

embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 3mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 4mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 5mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 6mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 10mg. In some embodiments, the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 30mg.

In some embodiments, a total nominal delivered dose of about 1 mg, about 2mg, about 6mg, about 20mg or about 60mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day, e.g. with one, two or more doses being administered to the subject per day, such that the total nominal delivered dose per day is about 1 mg, about 2mg, about 6mg, about 20mg or about 60mg.

In some embodiments, a total nominal delivered dose of about 1 mg, about 2mg, about 4mg, about 6mg, about 8mg, about 10mg, about 12mg, about 20mg or about 60mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day, e.g. with one, two or more doses being administered to the subject per day, such that the total nominal delivered dose per day is about 1 mg, about 2mg, about 4mg, about 6mg, about 8mg, about 10mg, about 12mg, about 20mg or about 60mg.

In some embodiments, a total nominal delivered dose of about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day, e.g. with one, two or more doses being administered to the subject per day, such that the total nominal delivered dose per day is about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg.

In some embodiments, a total nominal delivered dose of about 1 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 2 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 2.5 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of

about 4 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 5 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 6 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 8 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 10 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 12 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 15 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 20 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 25 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal delivered dose of about 60 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg.

In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg.

In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.6mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 1.1mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 2.2mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 3.3mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or

fragment thereof, administered to said subject twice per day is about 4.4mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 5.5mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 6.6mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 11.1mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 12mg. In some embodiments, the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 36mg.

In some embodiments, a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day, e.g. with one, two or more doses being administered to the subject per day, such that the total nominal metered dose per day is about 1.1mg, about 1.2mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg.

In some embodiments, a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 4.4mg, about 6.6mg, about 8.8mg, about 11mg, about 13.2mg, about 22.2mg, about 24mg or about 72mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day, e.g. with one, two or more doses being administered to the subject per day, such that the total nominal metered dose per day is about 1.1mg, about 1.2mg, about 2.2mg, about 4.4mg, about 6.6mg, about 8.8mg, about 11mg, about 13.2mg, about 22.2mg, about 24mg or about 72mg.

In some embodiments, a total nominal metered dose of about 1.1 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 1.2 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 2.2 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 4.4 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 6.6 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 8.8 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered

dose of about 11 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 13.2 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 22.2 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 24 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day. In some embodiments, a total nominal metered dose of about 72 mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

In any of the described aspects and embodiments of the invention, the formulation may include a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

In some embodiments of any of the described aspects of the invention, the dry powder formulation comprises trehalose, leucine and/or trileucine, either singly or in any combination thereof. For example, in some preferred embodiments, the dry powder formulation may comprise (i) trehalose, (ii) trehalose and leucine, (iii) trehalose, leucine and trileucine, or (iv) trehalose and trileucine.

In some embodiments of any of the described aspects of the invention, the dry powder formulation further comprises a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and a tonicity agent, such as sodium chloride.

In another aspect, the invention provides a dry powder formulation, wherein said formulation comprises an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

In some embodiments, the dry powder formulation comprises trehalose, leucine and/or trileucine, either singly or in any combination thereof. For example, in some preferred embodiments, the dry powder formulation may comprise (i) trehalose, (ii) trehalose and leucine, (iii) trehalose, leucine and trileucine, or (iv) trehalose and trileucine. In the most preferred embodiment, the dry powder formulation comprises trehalose, leucine and trileucine.

In some embodiments, the dry powder formulation further comprises a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and a tonicity agent, such as sodium chloride.

In some embodiments, the dry powder formulation further comprises a buffering agent, such as a histidine buffer, and a tonicity agent, such as sodium chloride.

In some embodiments, the dry powder formulation provides a nominal delivered dose of about 0.1mg to about 30 mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

In some embodiments, the dry powder formulation provides a nominal delivered dose about 0.5 mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

In some embodiments, the dry powder formulation provides a nominal delivered dose about 0.5 mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

In some embodiments, the dry powder formulation provides a nominal delivered dose about 2.5 mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

In some embodiments, the nominal delivered dose is about 0.5mg. In some embodiments, the nominal delivered dose is about 1mg. In some embodiments, the nominal delivered dose is about 2mg. In some embodiments, the nominal delivered dose is about 2.5mg. In some embodiments, the nominal delivered dose is about 3mg. In some embodiments, the nominal delivered dose is about 4mg. In some embodiments, the nominal delivered dose is about 5mg. In some embodiments, the nominal delivered dose is about 6mg. In some embodiments, the nominal delivered dose is about 10mg. In some embodiments, the nominal delivered dose is about 15mg. In some embodiments, the nominal delivered dose is about 20mg. In some embodiments, the nominal delivered dose is about 25mg. In some embodiments, the nominal delivered dose is about 30mg.

In some embodiments, the dry powder formulation comprises a nominal metered dose of about 0.2mg to about 40 mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

In some embodiments, the dry powder formulation provides a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

In some embodiments, the dry powder formulation provides a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

In some embodiments, the nominal metered dose is about 0.55mg. In some embodiments, the nominal metered dose is about 0.6mg. In some embodiments, the nominal metered dose is about 1.1mg. In some embodiments, the nominal metered dose is about 2.2mg. In some embodiments, the nominal metered dose is about 3.3mg. In some embodiments, the nominal metered dose is about 4.4mg. In some embodiments, the nominal metered dose is about 5.5mg. In some embodiments, the nominal metered dose is about 6.6mg. In some embodiments, the nominal metered dose is about 11.1mg. In some embodiments, the nominal metered dose is about 12mg. In some embodiments, the nominal metered dose is about 36mg.

In any of the aspects or embodiments of the invention, the dry powder formulation may include a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

The invention also provides a method for treating asthma in a human subject, wherein the method comprises administering by oral inhalation the dry powder formulation described in any of the embodiments above to said subject.

The invention also provides the dry powder formulation described in any of the embodiments above for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation to said subject by oral inhalation.

In addition, the invention provides the use of the dry powder formulation described in any of the embodiments above for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of asthma in a human subject, wherein the treatment comprises administering the dry powder formulation to said subject by oral inhalation.

Summary of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures in which:

Figure 1 shows the study flow chart for the phase I clinical trial described in Example 16. Test product A: 18 mg nominal delivered dose of AZD1402 nebulizer solution administered via the InnoSpire Go nebulizer. Test product B: 10 mg nominal delivered dose of AZD1402 inhalation powder administered via the Plastiape Monodose inhaler. Test product C: 30 mg nominal delivered dose of AZD1402 inhalation powder administered via the Plastiape Monodose inhaler.

Figure 2 shows the Geometric Mean (*/SD) Serum Concentration (ng/mL) of AZD1402 Versus Time Following Inhalation via InnoSpire Go Nebulizer (18 mg nominal delivered dose), Plastiape Monodose Inhaler (10 mg or 30 mg nominal delivered dose) (Pharmacokinetic Analysis Set-Linear Scale). A: Nebulizer solution administered via InnoSpire Go nebulizer (18 mg nominal delivered dose); B: Inhalation powder administered via Plastiape Monodose inhaler (10 mg nominal delivered dose); C: Inhalation powder administered via Plastiape Monodose inhaler (30 mg nominal delivered dose). SD: Standard Deviation. N: number of subjects. GEOMETRIC MEAN / SD: exp(mean[log{PK Conc}] – std[log{PK Conc}]). GEOMETRIC MEAN * SD: exp(mean[log{PK Conc}] + std[log{PK Conc)}]). Vertical lines represent the geometric mean */ SD.

Figure 3 shows the Geometric Mean (*/SD) Serum Concentration (ng/mL) of AZD1402 Versus Time Following Inhalation via InnoSpire Go Nebulizer (18 mg nominal delivered dose), Plastiape Monodose Inhaler (10 mg or 30 mg nominal delivered dose) (Pharmacokinetic Analysis Set - Semi-Logarithmic Scale). A: Nebulizer solution administered via InnoSpire Go nebulizer (18 mg nominal delivered dose); B: Inhalation powder administered via Plastiape Monodose inhaler (10 mg nominal delivered dose); C: Inhalation powder administered via Plastiape Monodose inhaler (30 mg nominal delivered dose). SD: Standard Deviation. N: number of subjects. GEOMETRIC MEAN / SD: exp(mean[log{PK Conc}] – std[log{PK Conc}]). GEOMETRIC MEAN * SD: exp(mean[log{PK Conc}] + std[log{PK Conc)}]). Vertical lines represent the geometric mean */ SD.

Figure 4 shows AZD1402 steady-state serum concentrations after 12 days of once-a-day and twice-a-day dosing with 5 and 1 mg doses, respectively.

Detailed Description of the Invention

Aspects and embodiments of the present invention will now be discussed with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

The present invention relates to a method of treating asthma in a human subject. The asthma may, for example, be moderate or severe asthma. In moderate asthma, the patient can talk in phrases, has an increased respiratory rate, the accessary muscles are not used, the pulse rate is 100-120 bpm, the oxygen saturation (on air) is 90-95% and the peak expiratory flow (PEF) is >50% predicted or best (see Pocket Guide for Asthma Management and Prevention, Global Initiative for Asthma, Updated 2020). In severe asthma, the patient talks in words, has a respiratory rate of >30/min, the accessory muscles are in use, the pulse rate is >120bpm, the oxygen saturation (on air) is <90%. In some embodiments, the asthma is allergic asthma.

The method of treating asthma comprises administering a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, comprising the amino acid sequence set forth in SEQ ID NO: 1.

By "therapeutically effective amount" it is meant a dose that produces the effects for which it is administered. A "therapeutically effective amount" of a lipocalin mutein as described herein may vary according to factors such as age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art. A therapeutically effective amount, when used in the present application, is also one in which any toxic or detrimental effects of the lipocalin mutein are outweighed by the therapeutically beneficial effects.

Interleukin-4 receptor alpha chain (IL-4Rα) is a type I transmembrane protein that can bind interleukin 4 and interleukin 13 to regulate IgE antibody production in B cells. Among T cells, the encoded protein also can bind interleukin 4 to promote differentiation of Th2 cells.

Lipocalin muteins

Lipocalin muteins that are specific for IL-4 receptor alpha (IL-4Rα), in particular human IL-4Rα are disclosed in International patent publications WO 2008/015239, WO 2011/154420, and WO 2013/087660. Human interleukin-4 receptor alpha chain may have the amino acid sequence of SWISS PROT Data Bank Accession No. P24394, which is shown as SEQ ID NO:4, or of fragments thereof. An illustrative example of a fragment of human interleukin-4 receptor alpha chain includes amino acids 26 to 232 of IL-4 receptor alpha.

The IL-4Rα specific lipocalin mutein having the amino acid sequence shown as SEQ ID NO:1 is a mutein of human tear lipocalin.

As used herein, a “mutein” refers to the exchange, deletion, or insertion of one or more nucleotides or amino acids, compared to the naturally occurring (wild-type) nucleic acid or protein

"reference" scaffold, which is preferably mature human tear lipocalin shown as SEQ ID NO: 3. Said "reference scaffold" also includes mutein, or fragment or variant thereof, as described herein.

The amino acid sequence of human tear lipocalin is provided by SWISS-PROT Data Bank Accession Number P31025, as shown in SEQ ID NO: 2. Mature human tear lipocalin does not include the N-terminal signal peptide that is included in the sequence of SWISS-PROT Accession Number P31025, i.e. it lacks the N-terminal signal peptide (amino acids 1-18) that is included in the sequence of SWISS-PROT Accession Number P31025. The amino acid sequence of mature human tear lipocalin is shown in SEQ ID NO:3.

The lipocalin mutein used in the present invention comprises SEQ ID NO:1 or is a variant or fragment thereof. The lipocalin mutein shown as SEQ ID NO:1 is a variant of mature human tear lipocalin shown as SEQ ID NO:3, which lacks the first four amino acids and includes *inter alia* the following amino acid substitutions at the positions corresponding to the sequence positions of the amino acid sequence of mature human tear lipocalin shown as SEQ ID NO: 3: Arg 26 → Ser, Glu 27 → Arg, Phe 28 → Cys, Glu 30 → Arg, Met 31 → Ala, Asn 32 → Val, Leu 33 → Tyr, Glu 34 → Asn, Met 55 → Ala, Leu 56 → Gln, Ile 57 → Arg, Ser 58 → Lys, Cys 61→ Trp, Glu 63 → Lys, Asp 80 → Ser, Lys 83 → Arg, Glu 104 → Leu, Leu 105 → Cys, His 106 → Pro and Lys 108 → Gln.

In some embodiments, the lipocalin mutein used in the present invention comprises SEQ ID NO:1 or is a variant or fragment thereof. The lipocalin mutein shown as SEQ ID NO:1 is a variant of mature human tear lipocalin shown as SEQ ID NO:3, which lacks the first four amino acids and includes *inter alia* the following amino acid substitutions at the positions corresponding to the sequence positions of the amino acid sequence of mature human tear lipocalin shown as SEQ ID NO: 3: Arg 26 → Ser, Glu 27 → Arg, Phe 28 → Cys, Glu 30 → Arg, Met 31 → Ala, Asn 32 → Val, Leu 33 → Tyr, Glu 34 → Asn, Val 53 → Phe, Met 55 → Ala, Leu 56 → Gln, Ile 57 → Arg, Ser 58 → Lys, Cys 61→ Trp, Glu 63 → Lys, Val 64 → Tyr, Ala 66 → Leu, Asp 80 → Ser, Lys 83 → Arg, Tyr 100 → His, Cys 101 → Ser, Glu 104 → Leu, Leu 105 → Cys, His 106 → Pro, Lys 108 → Gln, Arg 111 → Pro, Lys 114 → Trp and Cys 153 → Ser.

Variants

As used herein, the term "variant" relates to derivatives of a protein or polypeptide that include mutations, for example by substitutions, deletions, insertions, and/or chemical modifications of an amino acid sequence or nucleotide sequence. In some embodiments, such mutations and/or chemical modifications do not reduce the functionality of the protein or peptide. Such substitutions may be conservative, i.e., an amino acid residue is replaced with a chemically similar amino acid residue. Examples of conservative substitutions are the replacements among the members of the following groups: 1) alanine, serine, and threonine; 2) aspartic acid and

glutamic acid; 3) asparagine and glutamine; 4) arginine and lysine; 5) isoleucine, leucine, methionine, and valine; and 6) phenylalanine, tyrosine, and tryptophan. Such variants include proteins or polypeptides, wherein one or more amino acids have been substituted by their respective D-stereoisomers or by amino acids other than the naturally occurring 20 amino acids, such as, for example, ornithine, hydroxyproline, citrulline, homoserine, hydroxylysine, norvaline. Such variants also include, for instance, proteins or polypeptides in which one or more amino acid residues are added or deleted at the N- and/or C-terminus such as a deletion of four amino acids from the N-terminus and/or two amino acids from the C-terminus. Generally, a variant has at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity with the native sequence protein or polypeptide. A variant preferably retains the biological activity, e.g. binding the same target, of the protein or polypeptide from which it is derived.

Thus, a variant of the lipocalin mutein comprising the amino acid set forth in SEQ ID NO:1 in accordance with the present invention has at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity with the amino acid sequence shown as SEQ ID NO:1 and retains the ability to bind to IL-4 receptor alpha, in particular human IL-4Rα, or a fragment thereof. Preferably, the variant of the lipocalin mutein is capable of inhibiting IL-4 from binding to IL-4Rα.

In some embodiments, a variant of the lipocalin mutein comprising the amino acid set forth in SEQ ID NO:1 in accordance with the present invention has at least about 50%, at least about 60%, at least about 65%, at least about 70%, at least about 72%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 79%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity with the amino acid sequence of mature human tear lipocalin, shown as SEQ ID NO:3 and retains the ability to bind to IL-4 receptor alpha, in particular human IL-4Rα, or a fragment thereof. Preferably, the variant of the lipocalin mutein is capable of inhibiting IL-4 from binding to IL-4Rα.

As used herein, the term “sequence identity” or “identity” denotes a property of sequences that measures their similarity or relationship. The term “sequence identity” or “identity” as used in the present disclosure means the percentage of pair-wise identical residues – following (homologous) alignment of a sequence of a protein or polypeptide of the disclosure with a

sequence in question – with respect to the number of residues in the longer of these two sequences. Sequence identity is measured by dividing the number of identical amino acid residues by the total number of residues and multiplying the product by 100.

A skilled artisan will recognize available computer programs, for example BLAST (Altschul et al., *Nucleic Acids Res*, 1997), BLAST2 (Altschul et al., *J Mol Biol*, 1990), FASTA (which uses the method of Pearson and Lipman (1988)), the TBLASTN program, of Altschul *et al.* (1990) supra, GAP (Wisconsin GCG package, Accelerys Inc, San Diego USA) and Smith-Waterman (Smith and Waterman, *J Mol Biol*, 1981), for determining sequence identity using standard parameters. The percentage of sequence identity can, for example, be determined herein using the program BLASTP, version 2.2.5, November 16, 2002 (Altschul et al., *Nucleic Acids Res*, 1997). In this embodiment, the percentage of homology is based on the alignment of the entire protein or polypeptide sequences (matrix: BLOSUM 62; gap costs: 11.1; cut off value set to 10^{-3}) including the polypeptide sequences, preferably using the wild-type protein scaffold as reference in a pairwise comparison. It is calculated as the percentage of numbers of "positives" (homologous amino acids) indicated as result in the BLASTP program output divided by the total number of amino acids selected by the program for the alignment. Sequence identity is commonly defined with reference to the algorithm GAP (Wisconsin GCG package, Accelerys Inc, San Diego USA). GAP uses the Needleman and Wunsch algorithm to align two complete sequences, maximising the number of matches and minimising the number of gaps, which are spaces in an alignment that are the result of additions or deletions of amino acids. Generally, default parameters are used, with a gap creation penalty equalling 12 and a gap extension penalty equalling 4.

Specifically, in order to determine whether an amino acid residue of the amino acid sequence of a lipocalin (mutein) is different from a lipocalin mutein having the amino acid sequence shown as SEQ ID NO:1, a skilled artisan can use means and methods well-known in the art, e.g., alignments, either manually or by using computer programs such as BLAST 2.0, which stands for Basic Local Alignment Search Tool, or ClustalW, or any other suitable program which is suitable to generate sequence alignments. Accordingly, SEQ ID NO:1 can serve as "reference sequence", while the amino acid sequence of a lipocalin different from the lipocalin mutein having the amino acid sequence shown as SEQ ID NO:1 described herein serves as "query sequence".

Fragments

The term "fragment" as used herein in connection with the lipocalin muteins of the disclosure relates to proteins or peptides derived from the lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO:1 that are N-terminally and/or C-terminally truncated, i.e.

lacking at least one of the N-terminal and/or C-terminal amino acids. Such a fragment may lack up to 1, up to 2, up to 3, up to 4, up to 5, up to 10, up to 15, up to 20, up to 25, or up to 30 (including all numbers in between) of the N-terminal and/or C-terminal amino acids. As an illustrative example, such a fragment may lack the one, two, three, or four N-terminal and/or one or two C-terminal amino acids. It is understood that the fragment is preferably a functional fragment of a full-length lipocalin (mutein), which means that it preferably comprises the binding pocket of the full length lipocalin (mutein) from which it is derived. As an illustrative example, such a functional fragment may comprise at least amino acids at positions 5-158, 1-156, 5-156, 5-153, 26-153, 5-150, 9-148, 12-140, 20-135, or 26-133 corresponding to the linear polypeptide sequence of mature human tear lipocalin. Such fragments may include at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 consecutive amino acids of the sequence shown as SEQ ID NO: 1 and are usually detectable in an immunoassay of the lipocalin mutein having the amino acid sequence SEQ ID NO:1. A fragment may have at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% amino acid sequence identity with the amino acid sequence shown as SEQ ID NO:1. Preferably the fragment retains the ability to bind to IL-4 receptor alpha, in particular human IL-4Rα, or to a fragment thereof. Preferably, the fragment of the lipocalin mutein is capable of inhibiting IL-4 from binding to IL-4Rα.

A “fragment” with respect to the corresponding target IL-4Rα of the disclosure (which is described in UniProt P24394 and shown as SEQ ID NO: 4, which does not include the 25-residue signal peptide) refers to N-terminally and/or C-terminally truncated IL-4Rα or protein domains of IL-4Rα. Fragments of IL-4Rα as described herein retain the capability of the full-length IL-4Rα to be recognized and/or bound by a lipocalin mutein of the disclosure. As an illustrative example, the fragment may be an extracellular domain of IL-4Rα, such as an extracellular domain comprising amino acid residues 26-232 of UniProt P24394, which is shown as SEQ ID NO:5.

Administered and delivered doses

In the method of treating asthma, a nominal delivered dose of about 0.1mg to about 30 mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject by oral inhalation of a dry powder formulation. In some embodiments, a nominal delivered dose of about 0.5 mg, about 1mg, about 3mg, about 10mg or about 30mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject by oral inhalation of a dry powder formulation. In some embodiments, a nominal delivered dose of about 0.5 mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of the

lipocalin mutein, or variant or fragment thereof, is administered to the subject by oral inhalation of a dry powder formulation. In some embodiments, the nominal delivered dose is about 0.5mg. In some embodiments, the nominal delivered dose is about 1mg. In some embodiments, the nominal delivered dose is about 2mg. In some embodiments, the nominal delivered dose is about 3mg. In some embodiments, the nominal delivered dose is about 4mg. In some embodiments, the nominal delivered dose is about 5mg. In some embodiments, the nominal delivered dose is about 6mg. In some embodiments, the nominal delivered dose is about 10mg. In some embodiments, the nominal delivered dose is about 30mg. The nominal delivered dose is the target amount of lipocalin mutein, or variant or fragment thereof, the subject receives when inhaling the dry powder formulation. For example, it may be the amount of lipocalin mutein, or variant or fragment thereof, delivered from the mouthpiece of a device used to administer the dry powder formulation. Calculation of the nominal delivered dose is based on dry powder inhalation device efficiencies.

The nominal metered dose is the target amount of lipocalin mutein, or variant or fragment thereof, present in the dry powder formulation for addition to an inhalation device. For example, the nominal metered dose may be the target amount of lipocalin mutein, or variant or fragment thereof, filled in a capsule as a dry powder formulation, wherein the target amount is the average fill weight x the average active pharmaceutical ingredient (API) content. In the method of treating asthma described herein, a nominal metered dose of about 0.2mg to about 40 mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject by oral inhalation of a dry powder formulation. In some embodiments, a nominal metered dose of about 0.65 mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject by oral inhalation of a dry powder formulation. In some embodiments, a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject by oral inhalation of a dry powder formulation.

Frequency of administration

The lipocalin mutein may be administered to the human subject at least once per day and preferably twice per day, i.e. twice daily. When a nominal delivered dose of about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject twice per day, i.e. twice daily, each dose of about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg is administered to the subject twice per day, i.e. twice daily. Thus, the total nominal delivered dose of the lipocalin mutein, or variant or fragment thereof, administered to the subject per day is about 1mg, about 2mg, about 6mg, about 20mg or about 60mg. When a nominal delivered dose of about 0.5mg, about 1mg, about

2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject twice per day, i.e. twice daily, each dose of about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg is administered to the subject twice per day, i.e. twice daily. Thus, the total nominal delivered dose of the lipocalin mutein, or variant or fragment thereof, administered to the subject per day is about 1mg, about 2mg, about 4mg, about 6mg, about 8mg, about 10mg, about 12mg, about 20mg or about 60mg. Similarly, when a nominal metered dose of about 0.65mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject twice per day, i.e. twice daily, each dose of about 0.65mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg is administered to the subject twice per day, i.e. twice daily. Thus, the total nominal metered dose of the lipocalin mutein, or variant or fragment thereof, administered to the subject per day is about 1.1mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg. When a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of the lipocalin mutein, or variant or fragment thereof, is administered to the subject twice per day, i.e. twice daily, each dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg is administered to the subject twice per day, i.e. twice daily. Thus, the total nominal metered dose of the lipocalin mutein, or variant or fragment thereof, administered to the subject per day is about 1.1mg, about 1.2mg, about 2.2mg, about 4.4mg, about 6.6mg, about 8.8mg, about 11mg, about 13.2mg, about 22.2mg, about 24mg or about 72mg.

In some embodiments, a total nominal delivered dose of the lipocalin mutein, or variant or fragment thereof, of about 1mg, about 2mg, about 6mg, about 20mg or about 60mg is administered to the subject per day. In some embodiments, a total nominal delivered dose of the lipocalin mutein, or variant or fragment thereof, of about 1mg, about 2mg, about 4mg, about 6mg, about 8mg, about 10mg, about 12mg, about 20mg or about 60mg is administered to the subject per day. In some embodiments, a total nominal metered dose of the lipocalin mutein, or variant or fragment thereof, of about 1.1mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg is administered to the subject per day. In some embodiments, a total nominal metered dose of the lipocalin mutein, or variant or fragment thereof, of about 1.1mg, about 1.2mg, about 2.2mg, about 4.4mg, about 6.6mg, about 8.8mg, about 11mg, about 13.2mg, about 22.2mg, about 24mg or about 72mg is administered to the subject per day. A total nominal delivered dose or a total nominal metered dose may be achieved by any number of doses per day, e.g. 1, 2, 3, 4, or 5 doses per day.

In some embodiments, a total nominal delivered dose of the lipocalin mutein, or variant or fragment thereof, of about 1mg, about 2mg, about 6mg, about 20mg or about 60mg may be achieved by administering a nominal delivered dose of the lipocalin mutein, or variant or fragment thereof of about 1mg, about 2mg, about 6mg, about 20mg or about 60mg to the subject once per day. Similarly, a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg of the lipocalin mutein, or variant or fragment thereof, may be achieved by administering a nominal metered dose of the lipocalin mutein, or variant or fragment thereof of about 1.1mg, about 1.2mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg to the subject once per day.

In some embodiments, a total nominal delivered dose of the lipocalin mutein, or variant or fragment thereof, of about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg may be achieved by administering a nominal delivered dose of the lipocalin mutein, or variant or fragment thereof of about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg to the subject once per day.

Dry powder inhalation

The lipocalin mutein according to the present invention is administered to the human subject by inhalation of a dry powder formulation. Suitably, the dry powder formulations are formulated for pulmonary delivery, including by inhalation via a dry powder inhaler (DPI). Inhalation of a dry powder formulation is usually by oral inhalation. The dry powder formulation may, for example, be present in a capsule.

Means and devices for inhaled administration of a dry powder formulation of the lipocalin mutein are known to the skilled person. Such means and devices include a single-dose dry powder inhaler, such as a capsule-based single-dose dry powder inhaler. Other means and devices suitable for directing inhaled administration of a dry powder formulation of a lipocalin mutein to the lung, such as a multi-dose dry powder inhaler, are also known in the art.

A preferred capsule-based dry powder inhaler is rotating capsule inhaler, such as a Plastiape Monodose inhaler, e.g. the RPC Plastiape S.p.A RS01 Monodose Model 7, which is a Class I CE-marked medical device according to the Medical Device Directive 93/42/EEC. This inhaler delivers drug formulations to the lung in dry powder form and is intended for self-administration by the patient at home, outside the home and in institutions, such as hospitals and clinics.

Dry powder formulation

Lipocalin muteins for use in the present invention will usually be administered in the form of a dry powder formulation, which may comprise at least one component in addition to the lipocalin

mutein. Thus, pharmaceutical compositions for use in accordance with the present invention may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient.

For example, in addition to the active agent, i.e. the lipocalin mutein, the dry powder formulation may comprise a stabiliser, an aerosol performance enhancer, a buffering agent and a tonicity agent, as described in more detail below.

As used herein, a "dry powder formulation" refers to a formulation that includes a plurality of solid microparticles in a powder composition that suitably contains less than about 20% moisture, more suitably less than 10% moisture, less than about 5-6% moisture, or less than about 3% moisture. As described herein, dry powder formulations can preferably be utilized for delivery via inhalation to a patient.

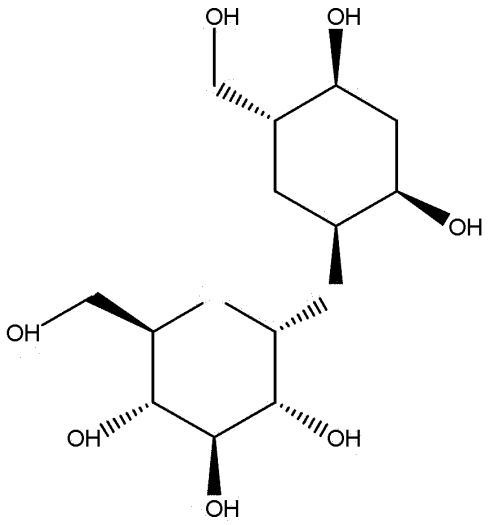
Microparticle

A "microparticle" as used herein refers to a solid particle having a size mass mean diameter (MMD) of less than 20 µm. Mass mean diameter is a measure of the mean particle size of the microparticles, measured using a suitable method, including for example centrifugal sedimentation, electron microscopy, light scattering, laser diffraction.

The dry powder formulations described herein suitably contain a plurality of microparticles. As used herein "plurality" refers to 2 or more of an item, and suitably refers to 5 or more, 10 or more, 50 or more, 100 or more, 500 or more, 1000 or more, etc.

Stabiliser

In suitable embodiments, the dry powder formulations described herein further comprise a stabiliser to aid in stabilizing the formulation, and in particular, in stabilizing the active agent. A stabiliser refers to an excipient that stabilizes an active agent (suitably a polypeptide) in a dry powder formulation, suitably by substituting for water at the active agent surface during drying, or otherwise impeding the degradation process, and forms an amorphous solid that includes the active agent. Examples of stabilisers include amorphous saccharides, polymeric sugars, buffers, salts, or synthetic polymers (e.g., poly-L-glycolic acid), as well as mixtures of such components. In suitable embodiments, the stabiliser is an amorphous saccharide. Most preferably, the stabilizer is the amorphous saccharide with high glass transition temperature, such as trehalose. The chemical structure of trehalose is provided below.

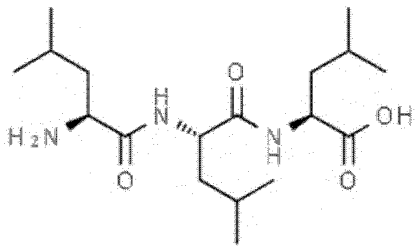


Suitably, trehalose is present at about 0.5% to about 99.5% (weight percentage, w/w %) of the dry powder formulation. In some embodiments, trehalose is present at about 5% to about 98% (w/w %) of the dry powder formulation. In preferred embodiments, trehalose is present at about 5% to about 95% (w/w %) of the dry powder formulation.

Aerosol performance enhancer

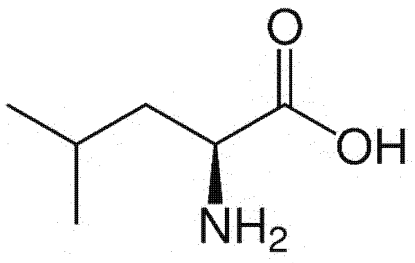
The dry powder formulations of the invention preferably include an aerosol performance enhancer, such as trileucine and/or leucine.

"Trileucine" as utilized herein refers to the chemical compound in which three leucine molecules are linked together in a peptide, as leucine-leucine-leucine (Leu-Leu-Leu), $C_{18}H_{35}N_3O_4$. The chemical structure of trileucine is provided below:



As used herein "leucine," whether present as a single amino acid or as an amino acid component of a peptide, refers to the amino acid leucine ($C_6H_{13}NO_2$), which may be a racemic

mixture or in either its D- or L-form, as well as modified forms of leucine (i.e., where one or more atoms of leucine have been substituted with another atom or functional group). The chemical structure of leucine is provided below:



The amounts of leucine and trileucine provided herein, unless otherwise stated, are provided as weight percentages (wt % or w/w %) of the formulations. As the dry powder formulations contain substantially little if any water, the weight components of the dry powder formulations are thus dry weight percentages of the final formulations.

In exemplary embodiments, the dry powder formulation comprises about 10% to about 45% leucine by weight (w/w %) or about 20% to about 45% leucine by weight (w/w %). In some embodiments, the dry powder formulation comprises about 40% leucine by weight (w/w %).

In exemplary embodiments, the dry powder formulation comprises about 2% to about 20% trileucine by weight (w/w %). In some embodiments, the dry powder formulation comprises about 20% trileucine by weight (w/w %).

In embodiments of the formulation comprising leucine, trileucine and an active agent, the leucine and trileucine are kept at a desired ratio range that provides the improved compressed bulk density characteristics described herein, as well as providing the desired microparticle characteristics that allow for improved storage and delivery. In some embodiments, the weight ratio of leucine and trileucine in the microparticles, i.e., leucine:trileucine, is about 0.01:1 to about 20:1. In preferred embodiments, the weight ratio of leucine:trileucine in the microparticles is less than about 4:1. Increasing the amount of trileucine present in the formulation still further is likely to have beneficial effects in regards to moisture robustness and maintaining particle morphology while scaling up.

Unless otherwise stated, the ratios described herein are expressed as ratios by weight% (*w/w* – also referred to as a "weight ratio" or w/w %), that is, weight of leucine:weight of trileucine in the formulations described herein. The ratios are achieved by providing a desired mg/mL concentration of leucine and trileucine in a feedstock, and then drying to remove the feedstock solvent resulting in an atomized microparticle where the starting concentration ratio (expressed in mg/mL), is maintained as a final ratio of leucine:trileucine by weight.

Exemplary weight percentages for leucine and trileucine that can be utilized in the dry powder formulations to achieve these ratios are described herein. Suitably, the dry powder formulations comprise about 5% to about 15% leucine by weight (w/w %) and about 1% to about 5% trileucine by weight (w/w %). In some embodiments, the dry powder formulations comprise about 8% to about 11% leucine by weight (w/w %) and about 2% to about 4% trileucine by weight (w/w %), and in some preferred embodiments, the dry powder formulations comprise about 10% leucine and about 2.6% trileucine by weight (w/w %).

The use of the combination of leucine and trileucine in a dry powder formulation allows for the reduction in the overall amount of leucine and/or trileucine required to prepare microparticles, as compared to dry powder formulations that contain only one of these components, while still providing the desired stability. This helps to overcome the solubility limitations of trileucine and leucine and provides higher throughput.

An exemplary process of preparing a dry powder formulation, in accordance with embodiments hereof, may take place as follows. A liquid feedstock containing the desired final components of the dry powder formulation are atomized using an atomizer, to a fine mist. The mist is then dried as described herein. The atomized droplets contain the dissolved components, initially as a liquid droplet. As the droplet dries, different components of the formulation begin to saturate and precipitate at varying rates. As described herein, a shell begins to form around an outer surface of the microparticles of the dry powder formulations. This shell suitably includes the leucine and trileucine components at an outer surface of the shell. It should be noted that leucine and trileucine become preferentially located at an outer surface of the microparticles, while smaller amounts of leucine and trileucine can also found throughout the microparticles. In embodiments, a higher concentration of leucine and trileucine are suitably found at or near the surface of the microparticles, rather than near the center of the microparticles. The presence of leucine and trileucine at the surface of the microparticles may be analysed by Time-of-Flight Secondary Ion Mass Spectometry (ToF-SIMS). In some embodiments, the center of the microparticles contain a substantial amount of the active agent, along with other excipient components as described herein, suitably in an amorphous form. As used herein, a “substantial amount” of the active agent means at least about 60% of the active agent (i.e., of the total active agent in the formulation) is located at or near the center of the microparticles, suitably at least about 70%, and more suitably at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, and in embodiments about 95%-100%, of the active agent is located at or near the center of the microparticles.

Buffering agent

Exemplary buffering agents that can be included in the dry powder formulations include various phosphate buffers, citrate buffers (such as sodium citrate), a histidine buffer, a glycine buffer, an

acetate buffer, and a tartrate buffer, as well as combinations of such buffers. Preferably, the buffering agent is a phosphate buffer. Most preferably, the dry powder formulation includes potassium dihydrogen phosphate and anhydrous disodium phosphate. In some embodiments, the dry powder formulation may comprise about 0.01% to 5% w/w buffer salt. In some embodiments, the dry powder formulation may comprise about 0.01% to 3% w/w buffer salt.

Buffers also provide control of the pH of the dry powder formulations, suitably maintaining a pH of between about pH 5 and about 8, for example, about pH 5 to about pH 6, about pH 5.5 to about pH 6.5, about pH 6 to about pH 7, about pH 6.5 to about pH 7.5, or about pH 7 to about pH 8.

In compositions and formulations that "consist essentially" of the recited ingredients, such compositions and formulations contain the recited components and those that do not materially affect the basic and novel characteristics of the claimed formulations. Components that do not materially affect the basic and novel characteristics of the claimed formulations are those that do not limit the ability of the leucine and trileucine to stabilize the dry powder formulations. Suitably, compositions and formulations that consist essentially of the recited ingredients specifically exclude other amino acids or tripeptide amino acids, but can include additional sugars, buffers, etc.

Tonicity agent

In some embodiments, the dry powder formulations described herein comprise a tonicity agent, such as sodium chloride. In some embodiments, the dry powder formulations comprise 0-15 w/w % sodium chloride. Suitably, the dry powder formulations comprise about 0.4-15 w/w % sodium chloride.

Microparticle size

The microparticles that make up the dry powder formulations described herein suitably have a specified mass median aerodynamic diameter (MMAD) when provided in aerosol form.

As used herein, "mass median aerodynamic diameter" or "MMAD" is a measure of the aerodynamic size of a dispersed microparticle. The aerodynamic diameter is used to describe an aerosolized powder in terms of its settling behaviour and is the diameter of a unit density sphere having the same settling velocity, in air, as the microparticle. The aerodynamic diameter encompasses particle shape, density and physical size of a microparticle. As used herein, MMAD refers to the midpoint or median of the aerodynamic particle size distribution of an aerosolized powder determined by cascade impaction, unless otherwise indicated. Suitably the microparticles of the dry powder formulations provided herein have a mass median aerodynamic diameter (MMAD) of at least 1 μm or greater, more suitably about 1 μm to about

10 μm, about 2 μm to about 8 μm, about 2 μm to about 7 μm, about 2 μm to about 6 μm, about 2 μm to about 5 μm, about 2 μm to about 4 μm, about 2 μm to about 3 μm, about 3 μm to about 4 μm, about 2 μm, or about 3 μm.

Suitably, the fine particle fraction (the fraction of particles emitted from an inhalation device having an aerodynamic particle diameter of less than 5 μm of the dry powder formulations) described herein is ≥ 50%, more suitably ≥ 60%. This fine particle fraction (FPF) may contribute to a low device retention of the dry powder formulations of less than 20%, suitably less than 15%, less than 10%, or less than 5%, remaining in a device following delivery to a patient.

Exemplary dry powder formulations

Some exemplary formulations are shown in the tables below:

Table 1 Drug product composition for AZD1402 inhalation powder, hard capsule 12 mg/MD, 10mg/DD

Components	Quantity (mg/g)	Quantity (mg/MD)[a]	Quantity (mg/DD)[b]	Function	Standard
AZD1402 (SEQ ID NO: 1)	600	12	10	Active	AstraZeneca
Potassium dihydrogen phosphate[c]	1.5	0.03	0.025	Buffering agent	European Pharmacopoeia (Ph Eur) or National Formulary (NF)
Disodium phosphate, anhydrous[c]	4.5	0.09	0.075	Buffering agent	Ph Eur or United States Pharmacopeia (USP)
Sodium chloride[c]	99	1.9	1.7	Tonicity agent	Ph Eur or USP
Sterile water for injection[c, d]	q.s	q.s	q.s	Processing agent[f]	Ph Eur or USP
Trehalose[e]	95	1.9	1.6	Stabilizer	Ph Eur or NF
Leucine	200	4	3.3	Aerosol performance enhancer	AstraZeneca, Ph Eur and USP
Nitrogen[d]	q.s	q.s	q.s	Processing agent[f]	Ph Eur or NF
Purified water[d]	q.s	q.s	q.s	Processing agent[f]	Ph Eur or USP
Size 3 HPMC capsule	NA	1psc	1psc	Pre-metering vessel	Ph Eur or USP

[a] MD = metered dose, extrapolated from product range data

[b] DD = delivered dose, measured value for AZD1402 and theoretical values for other components.

[c] From drug substance (AZD1402) solution. Component amount will vary due to variation in protein concentration between drug substance batches.

[d] Removed during processing.

[e] Trehalose is charged as trehalose dihydrate in the manufacturing process, the water is removed during processing. 95 mg of trehalose corresponds to 105 mg trehalose dihydrate. Amount of trehalose will vary due to variation in buffer salt amounts coming from drug substance solution.

[f] An agent used to prepare the composition which is absent from the final composition or is removed during processing of the composition.

q.s. *quantum satis*

NA Not applicable.

Table 2 Drug product composition for AZD1402 inhalation powder, hard capsule 1.1mg/MD, 1 mg/DD

Components	Quantity (mg/g)	Quantity (mg/MD)[a]	Quantity (mg/DD)[b]	Function	Standard
AZD1402 (SEQ ID NO: 1)	110	1.1	1.0	Active	AstraZeneca
Potassium dihydrogen phosphate[c]	0.29	0.0029	0.0026	Buffering agent	Ph Eur or NF
Disodium phosphate, anhydrous[c]	0.86	0.0086	0.0077	Buffering agent	Ph Eur or USP
Sodium chloride[c]	18	0.18	0.16	Tonicity agent	Ph Eur or USP
Water for injections/ Water for injection[c, d]	qs	qs	qs	Processing agent[f]	Ph Eur or USP
Trehalose[e]	745	7.45	6.70	Stabilizer	Ph Eur or NF
Leucine	100	1.0	0.90	Aerosol performance enhancer	AstraZeneca, Ph Eur and USP
Trileucine	26	0.26	0.23	Aerosol performance enhancer	AstraZeneca
Nitrogen[d]	qs	qs	qs	Processing agent[f]	Ph Eur or NF
Purified water[d]	qs	qs	qs	Processing agent[f]	Ph Eur or USP
Size 3 HPMC capsule	NA	1 unit	1 unit	Pre-metering vessel	Ph Eur or USP

[a] MD = metered dose, extrapolated from product range data

[b] DD = delivered dose, measured value for AZD1402 and theoretical values for other components.

[c] From drug substance (AZD1402) solution. Component amount will vary due to variation in protein concentration between drug substance batches.

[d] Removed during processing.

[e] Trehalose is charged as trehalose dihydrate in the manufacturing process, the water is removed during processing. 745 mg of trehalose corresponds to 822 mg trehalose dihydrate. Amount of trehalose will vary due to variation in buffer salt amounts coming from drug substance solution.

[f] An agent used to prepare the composition which is absent from the final composition or is removed during processing of the composition.

q.s. *quantum satis.*

NA Not applicable.

Table 3 Drug product composition for AZD1402 inhalation powder, hard capsule 3.3mg/MD, 3 mg/DD

Components	Quantity (mg/g)	Quantity (mg/MD)[a]	Quantity (mg/DD)[b]	Function	Standard
AZD1402 (SEQ ID NO: 1)	330	3.3	3	Active	AstraZeneca
Potassium dihydrogen phosphate[c]	0.88	0.0088	0.0079	Buffering agent	Ph Eur or NF
Disodium phosphate, anhydrous[c]	2.6	0.026	0.026	Buffering agent	Ph Eur or USP
Sodium chloride[c]	55	0.55	0.50	Tonicity agent	Ph Eur or USP
Water for injections/ Water for injection[c, d]	qs	qs	qs	Processing agent[f]	Ph Eur or USP
Trehalose[e]	486	4.86	4.37	Stabilizer	Ph Eur or NF
Leucine	100	0.10	0.90	Aerosol performance enhancer	AstraZeneca, Ph Eur and USP
Trileucine	26	0.26	0.23	Aerosol performance enhancer	AstraZeneca
Nitrogen[d]	qs	qs	qs	Processing agent[f]	Ph Eur or NF
Purified water[d]	qs	qs	qs	Processing agent[f]	Ph Eur or USP
Size 3 HPMC capsule	NA	1 unit	1 unit	Pre-metering vessel	Ph Eur or USP

[a] MD = metered dose, extrapolated from product range data

[b] DD = delivered dose, measured value for AZD1402 and theoretical values for other components.

[c] From drug substance (AZD1402) solution. Component amount will vary due to variation in protein concentration between drug substance batches.

[d] Removed during processing.

[e] Trehalose is charged as trehalose dihydrate in the manufacturing process, the water is removed during processing. 486 mg of trehalose corresponds to 536 mg trehalose dihydrate. Amount of trehalose will vary due to variation in buffer salt amounts coming from drug substance solution.

[f] An agent used to prepare the composition which is absent from the final composition or is removed during processing of the composition.

q.s. *quantum satis.*

NA Not applicable.

Table 4 Drug product composition for AZD1402 inhalation powder, hard capsule 11.1mg/MD, 10 mg/DD

Components	Quantity (mg/g)	Quantity (mg/MD)[a]	Quantity (mg/DD)[b]	Function	Standard
AZD1402 (SEQ ID NO: 1)	600	11.1	10	Active	AstraZeneca
Potassium dihydrogen phosphate[c]	1.6	0.030	0.026	Buffering agent	Ph Eur or NF
Disodium phosphate, anhydrous[c]	4.7	0.087	0.078	Buffering agent	Ph Eur or USP
Sodium chloride[c]	100	1.85	1.67	Tonicity agent	Ph Eur or USP
Water for injections/ Water for injection[c, d]	qs	qs	qs	Processing agent[f]	Ph Eur or USP
Trehalose[e]	168	2.94	2.79	Stabilizer	Ph Eur or NF
Leucine	100	1.85	1.67	Aerosol performance enhancer	AstraZeneca, Ph Eur and USP
Trileucine	26	0.48	0.43	Aerosol performance enhancer	AstraZeneca
Nitrogen[d]	qs	qs	qs	Processing agent[f]	Ph Eur or NF
Purified water[d]	qs	qs	qs	Processing agent[f]	Ph Eur or USP
Size 3 HPMC capsule	NA	1 unit	1 unit	Pre-metering vessel	Ph Eur or USP

[a] MD = metered dose, extrapolated from product range data

[b] DD = delivered dose, measured value for AZD1402 and theoretical values for other components.

[c] From drug substance solution. Component amount will vary due to variation in protein concentration between drug substance batches.

[d] Removed during processing.

[e] Trehalose is charged as trehalose dihydrate in the manufacturing process, the water is removed during processing. 168 mg of trehalose corresponds to 185 mg trehalose dihydrate. Amount of trehalose will vary due to variation in buffer salt amounts coming from drug substance solution.

qs quantum satis.

NA Not applicable.

Thus, in some preferred embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose, leucine and trileucine. In some embodiments, the dry powder formulation further comprises a buffering agent, such as a

phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose, leucine and trileucine, wherein said formulation provides a nominal delivered dose of about 0.5 mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose, leucine and trileucine, wherein said formulation provides a nominal delivered dose of about 0.5 mg, about 1mg, about 2 mg, about 3mg, about 4mg, about 5mg, about 6 mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose, leucine and trileucine, wherein said formulation provides a nominal delivered dose of about 2.5 mg, about 5mg, about 10 mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

In other preferred embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and leucine. In some embodiments, the dry powder formulation further comprises a buffering agent, such as a phosphate buffer, e.g.

potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and leucine, wherein said formulation provides a nominal delivered dose of about 0.5 mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and leucine, wherein said formulation provides a nominal delivered dose of about 0.5 mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and leucine, wherein said formulation provides a nominal delivered dose of about 2.5 mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

In other preferred embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and trileucine. In some embodiments, the dry powder formulation further comprises a buffering agent, such as a phosphate buffer, e.g.

potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and trileucine, wherein said formulation provides a nominal delivered dose of about 0.5 mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Thus, in some embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and trileucine, wherein said formulation provides a nominal delivered dose of about 2.5 mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation, wherein the formulation may further comprise a buffering agent, such as a phosphate buffer, e.g. potassium dihydrogen phosphate and/or anhydrous disodium phosphate as a buffering agent, and/or a tonicity agent, such as sodium chloride.

Each of the individual components may be present in the amounts and/or ratios described herein.

In some embodiments, the dry powder formulation of the invention may be as shown in one of Tables 5-16 below, where AZD1402 is an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a fragment or variant thereof.

Table 5 Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	60
PBS pH=7.4	10.7
Leucine	20
Trehalose	9.3

Table 6 Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	11
PBS pH=7.4	1.9
Leucine	10
Trileucine	2.6
Trehalose	74.5

Table 7 Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	33
PBS pH=7.4	5.8
Leucine	10
Trileucine	2.6
Trehalose	48.6

Table 8 Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	60
PBS pH=7.4	11.1
Leucine	10
Trileucine	2.6
Trehalose	16.3

Table 9: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	60.00
PBS pH=7.4 Components of PBS - NaCl Na_2HPO_4 KH_2PO_4	10.43 9.82 0.46 0.16
Leucine	20.00
Trehalose	9.13

Table 10: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	11.00
PBS pH=7.4 Components of PBS - NaCl Na_2HPO_4 KH_2PO_4	1.91 1.85 0.08 0.03
Leucine	10.00
Trileucine	2.60
Trehalose	74.49

Table 11: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	33.00
PBS pH=7.4 Components of PBS - NaCl	5.74 5.04

Na_2HPO_4	0.25
KH_2PO_4	0.09
Leucine	10.00
Trileucine	2.60
Trehalose	48.66

Table 12: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	60.00
PBS pH=7.4	10.43
Components of PBS -	
NaCl	9.82
Na_2HPO_4	0.46
KH_2PO_4	0.16
Leucine	10.00
Trileucine	2.60
Trehalose	16.97

Table 13: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	10.0
Buffer	0.11
Tonicity agent	1.8
Leucine	40.0
Trehalose	48.1

Table 14: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402	10.0
Buffer	0.11
Tonicity agent	1.8
Trileucine	20.0
Trehalose	68.1

Table 15: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	50
Histidine pH=5.5	2.95
NaCl	8.63
Leucine	10
Trileucine	2.6
Trehalose	25.82

Table 16: Composition of an exemplary formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	10
Histidine pH=5.5	0.59
NaCl	1.73
Leucine	10
Trileucine	2.6
Trehalose	75.08

Thus, in some preferred embodiments, the dry powder formulation of the invention comprises: an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose, leucine and trileucine. In some embodiments, the dry powder formulation further comprises a buffering agent, such as a phosphate buffer.

In other preferred embodiments, the dry powder formulation comprises an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, trehalose and leucine. In some embodiments, the dry powder formulation further comprises a buffering agent, such as a phosphate buffer.

The dry powder formulation comprising the lipocalin mutein may be administered alone or in combination with other treatments, either simultaneously or sequentially. For example, the lipocalin mutein may be administered in combination with corticosteroids, such as inhaled corticosteroids (ICS), long-acting beta 2 agonists (LABA) and/or long-acting muscarinic antagonists (LAMA). In some embodiments, the lipocalin mutein may be administered in combination with low or medium dose ICS, inhaled LABA and/or inhaled LAMA. Low medium and high daily doses of ICS are described in the Pocket Guide for Asthma Management and Prevention, Global Initiative for Asthma, Updated 2020.

Systemic exposure

As used herein "systemic exposure" means that a substantive portion of the inhaled lipocalin mutein enters the circulatory system and, optionally, that the entire body may be affected by the lipocalin mutein. Systemic exposure may mean that the amount of the lipocalin mutein that enters the circulatory system is quantifiable. Systemic exposure may equate to the concentration of lipocalin mutein that enters the bloodstream that is quantifiable. This exposure can be represented by the blood (serum, plasma or whole blood) concentration of the lipocalin mutein which can be measured over time and recorded by a range of parameters including the area under the curve (AUC). Systemic exposure to lipocalin mutein can also impact biomarkers, the levels of which can correlate directly to concentration of lipocalin mutein and therefore to systemic exposure. The term "quantifiable" or "detectable," when used in connection with systemic exposure, refers to the exposure represented by the blood (serum, plasma or whole blood) concentration of the lipocalin mutein or by the levels of biomarkers measurable by one or more analytical methods known in art. Such analytical methods include, but are not limited to, ELISA, competitive ELISA, fluorescence titration, calorimetric methods, mass spectrometry (MS), and chromatography methods, such as high-performance liquid chromatography (HPLC). It is also understood measurements performed using such analytical

methods are associated with detection limits, such as instrument detection limit, method detection limits, and limit of quantification.

Pharmacokinetic properties

The results presented herein demonstrate that administration of a nominal delivered dose to the lungs of 10mg of the lipocalin mutein by inhalation of a dry powder formulation results in a comparable systemic exposure of the lipocalin mutein as administration of a nominal delivered dose of 18mg of the lipocalin mutein by inhalation of a nebulised formulation. Systemic exposure in this context was measured by C_{max}, AUC and AUC_{last}, as defined in Table 69. Therefore, administration of the lipocalin mutein by inhalation of a dry powder formulation advantageously achieves the same level of systemic exposure as almost twice the dose administered by inhalation of a nebulised formulation. Similarly, systemic exposure to the lipocalin mutein, as measured by C_{max}, AUC and $AUC_{last,}$ was approximately 73-78% lower following administration of an nominal delivered dose of 18mg of the lipocalin mutein by inhalation of a nebulised formulation or administration of an nominal delivered dose to the lungs of 10mg of the lipocalin mutein by inhalation of a dry powder formulation than following administration of an nominal delivered dose to the lungs of 30mg of the lipocalin mutein by inhalation of a dry powder formulation.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise” and “include”, and variations such as “comprises”, “comprising”, and “including” will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a lipocalin mutein" includes one or more lipocalin muteins.

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range. The term, however, also includes the concrete number, e.g., “about 20” includes 20.

The term ‘at least about’ as used herein includes the concrete number e.g. ‘at least about 6’ includes 6.

The features disclosed in the foregoing description, or in the following claims, or in the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for obtaining the disclosed results, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention.

For the avoidance of any doubt, any theoretical explanations provided herein are provided for the purposes of improving the understanding of a reader. The inventors do not wish to be bound by any of these theoretical explanations.

Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Examples

Table 17. Nominal Metered and Delivered doses

Example	Formulation	Drug load (w/w %)	Fill weight (mg)	Nominal metered dose (mg)	Nominal delivered dose (mg)
1	T	85	20	17	
2	T	2	20	0.4	
3	T	60	20	12	
4	TL	2	10	0.4	
5	TL	60	20	12	10
6	TL	60	40	12	
7	TLT	2	10	0.2	0.15
8	TLT	60	40	24	22
9	TLT	11	10	1.1	1
10	TLT	33	10	3.3	3
11	TLT	60	18.5	11.1	10
12	TT (3 w/w %Trileu)	5	10	0.5	
13	TT (5 w/w % Trileu)	5	10	0.5	

16	TLTH	10	10	1.0	0.9
17	TLTH	50	18.5	9.3	8.4

API: active pharmaceutical ingredient; T: trehalose; TL: trehalose and leucine; TLT: trehalose, leucine and trileucine; TT: trehalose and trileucine

EXAMPLE 1 – AEROSOL PERFORMANCE CHARACTERISTICS OF 85 W/W% AZD1402 AND BUFFER SALTS FORMULATION

The following example evaluates the aerosol performance of a formulation comprising AZD1402 and buffer salts in a dry powder inhaler device. The aerosol performance outputs listed in Table 19 were tested on a formulation with a ratio of AZD1402: PBS of not less than (NLT) 5.2:1. In all of Examples 1-15 all product performance characterization was completed using a Monodose RS01 device, with size 3 capsules. Next Generation Impactor (NGI) analysis was performed at an air flow rate of 60 L/min.

Table 18: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	85
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	14.1
Trehalose	0.9

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/ml. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by diluting AZD1402 solution with water. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate, 1.85 ml/min; atomizer air, 2150 liters/hr; and drying gas flow, 23 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 19.

Table 19: Particle Parameters Analyzed

Analysis	Instrument	Result 85 w/w% drug load
Residual Moisture Content	Oven KF	3.3%

Primary Particle Size Distribution	Mastersizer 3000	d.50: 2.02µm
Glass Transition Temperature (Tg)	DSC	67°C

Cascade impaction testing was performed as per United States Pharmacopeia Chapter 601 (USP <601>) to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 20 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

In all of Examples 1-15 an enzyme linked immunosorbent assay (ELISA) is used to demonstrate the binding activity of AZD1402 to its specified target IL-4 receptor. The method is an antibody capture, antigen excess assay and relies on the fact that AZD1402 can be immobilised on to a 96 well plate coated with an antigen, recombinant human sIL-4 alpha receptor. The immobilised AZD1402 is detected by adding a primary anti-AZD1402 antibody and subsequently a secondary anti-antibody labelled with a HRP enzyme is added. The HRP enzyme catalyses the reaction of a substrate to produce a coloured product. The amount of coloured product is directly proportional to the amount of secondary antibody present, which is in turn directly proportional to the amount of AZD1402 captured by the antigen. Standards of various known concentrations of AZD1402 are incubated with excess antigen and a calibration curve is constructed. The resulting log concentration versus absorbance response is plotted with a 4parameter logistic (4PL) curve fitting.

The results including 12 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 20.

Table 20: Characterization results

Analysis	Initial Result	Stability 12 months 25°C/60% RH	Stability 12 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	3.3 µm	3.5 µm	3.5 µm
FPD (fine particle dose)	9.9 mg	9.6 mg	9.7 mg
% retention in capsule and device	8.8%	8.7%	8.0%

% FPF (fine particle fraction) < 5µm % of DD	73.1%	70.3%	70.9%
Delivered Dose (mg)	Not analysed	Not analysed	Not analysed
Bioactivity (ELISA) (% of ref[a])	Not analysed	96%	90%

[a] Bioactivity is measured against a reference sample.

EXAMPLE 2 – AEROSOL PERFORMANCE CHARACTERISTICS OF 2 W/W% AZD1402 IN A TREHALOSE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 22 were tested on a formulation with a drug load of 2 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 21: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	2
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	0.4
Trehalose	97.6

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 40-50 mg/mL by first dissolving the trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr; and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 22.

Table 22: Particle Parameters Analyzed

Analysis	Instrument	Result 2 w/w % drug load
Residual Moisture Content	Oven KF	1.7%
Primary Particle Size Distribution	Mastersizer 3000	d.50: 1.69 µm
Glass Transition Temperature (Tg)	DSC	93°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 20 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The results of the aerosol analysis are summarized in Table 23. This formulation was not followed on stability due to initial results indicating inadequate properties.

Table 23: Characterization results

Analysis	Initial Result	Stability 25°C/60% RH	Stability 40°C/75% RH
MMAD (median mass aerodynamic diameter)	3.6 µm	Not analysed	Not analysed
FPD (fine particle dose)	174 µg	Not analysed	Not analysed
% retention in capsule and device	29.6%	Not analysed	Not analysed
% FPF (fine particle fraction) < 5um	70.3%	Not analysed	Not analysed

Delivered Dose (mg)	Not analysed	Not analysed	Not analysed
Bioactivity (ELISA) (% of ref[a])	87%	Not analysed	Not analysed

[a] Bioactivity is measured against a reference sample.

EXAMPLE 3 – AEROSOL PERFORMANCE CHARACTERISTICS OF 60 W/W% AZD1402 IN A TREHALOSE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 25 were tested on a formulation with a drug load of 60 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 24: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	60
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	10.6
Trehalose	29.4

AZD1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 40-50 mg/mL by first dissolving the trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr, and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 25.

Table 25: Particle Parameters Analyzed

Analysis	Instrument	Result
Residual Moisture Content	Oven KF	2.4%
Primary Particle Size Distribution	Laser diffraction	d.50: 2.03 µm
Glass Transition Temperature (Tg)	DSC	84°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 20 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 26.

Table 26: Characterization results

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	3.2 µm	3.2 µm	3.1 µm
FPD (fine particle dose)	7.1 mg	7.6 mg	7.4 mg
% retention in capsule and device % of TD	8.4%	8.4%	8.3%
% FPF (fine particle fraction) < 5um % of DD	73.8%	75.0%	75.5%
Delivered Dose (mg)	Not analysed	Not analysed	Not analysed
Bioactivity (ELISA) (% of ref[a])	80%	87%	89%

[a] Bioactivity is measured against a reference sample.

EXAMPLE 4 – AEROSOL PERFORMANCE CHARACTERISTICS OF 2 W/W% AZD1402 IN TREHALOSE-LEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose and leucine in a dry powder inhaler device. The aerosol performance outputs listed in Table 28 were tested on a formulation with a drug load of 2 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 27: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	2
PBS pH=7.4 (4mM Na/K phosphate, 155mM	0.4

NaCl, pH 7.4)	
Leucine	20
Trehalose	77.6

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr, and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 28.

Table 28: Particle Parameters Analyzed

Analysis	Instrument	Result 2 w/w% drug load
Residual Moisture Content	Oven KF	0.9%
Primary Particle Size Distribution	Malvern 3000	d.50: 1.63µm
Glass Transition Temperature (Tg)	DSC	95.5°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one

size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 29.

Table 29: Results of Aerosol Characterization

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	2.9 µm	3.0 µm	3.1 µm
FPD (fine particle dose)	113 µg	108 µg	98 µg
% retention in capsule and device (% of TD)	12.6%	10.7%	23.2%
% FPF (fine particle fraction) < 5um (% of DD)	83.9%	73.7%	61.9%
Delivered Dose (mg)	145 µg	144 µg	127 µg
Bioactivity (ELISA) (% of ref[a])	98%	93%	103%

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 5 – AEROSOL PERFORMANCE CHARACTERISTICS OF 60 W/W% AZD1402 IN LEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 31 were tested on a formulation with a drug load of 60 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 30: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	60
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	10.7
Leucine	20
Trehalose	9.3

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 40-50 mg/mL by first dissolving the trileucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate 6.0 ml/min; atomizer air 6.5 kg/h; and drying gas flow 40 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation. This formulation had adequate chemical and physical stability, including bioactivity, and aerosol performance throughout the storage period at a time when sourcing of trileucine was an issue.

Data from physical powder characteristics attributes are listed in Table 31.

Table 31: Particle Parameters Analyzed

Analysis	Instrument	Result 60 w/w % drug load
Residual Moisture Content	Oven KF	2.0%
Primary Particle Size Distribution	Malvern	d.50: 2.29 µm
Glass Transition Temperature (Tg)	DSC	Not analyzed

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device.

The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 20 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 20 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 12 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 32.

Table 32: Results of Aerosol Characterization

Analysis	Initial Result	Stability 12 months 25°C/60% RH	Stability 12 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	3.2 µm	2.8 µm	Not analysed
FPD (fine particle dose)	6.75 mg	7.34 mg	Not analysed
% retention in capsule and device	9.1%	7.2%	Not analysed
% FPF (fine particle fraction) < 5um (% of DD)	69.3%	73.0%	Not analysed
Delivered Dose (mg)	10.4 mg	10.9 mg	Not analysed
Bioactivity (ELISA) (% of ref[a])	94%	108%	Not analysed

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 6 – AEROSOL PERFORMANCE CHARACTERISTICS OF 60 W/W% AZD1402 IN TREHALOSE-LEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 34 were tested on a formulation with a drug load of 60 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 33: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	60
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	10.6
Leucine	20
Trehalose	9.4

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 40 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr, and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 34.

Table 34: Particle Parameters Analyzed

Analysis	Instrument	Result 60 w/w% drug load
Residual Moisture Content	Oven KF	1.1/1.4%

Primary Particle Size Distribution	Malvern 3000	d.50: 2.09 µm
Glass Transition Temperature (Tg)	DSC	92.5 °C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 40 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 40 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 35.

Table 35: Characterization results

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	3.4 µm	3.5 µm	3.6 µm
FPD (fine particle dose)	14.2 mg	13.4 mg	13.9 mg
% retention in	7.5%	8.9%	8.4%

capsule and device (% of TD)			
% FPF (fine particle fraction) < 5um (% of DD)	70.9%	67.9%	68.0%
Delivered Dose (mg)	22.3 mg	22.8 mg	22.8 mg
Bioactivity (ELISA) (% of ref[a])	91%	104%	110%

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 7 – AEROSOL PERFORMANCE CHARACTERISTICS OF 2 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 37 were tested on a formulation with a drug load of 2 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 36: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	2
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	0.4
Leucine	10
Trileucine	2.6
Trehalose	85

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr, and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content, particle properties and improved physical robustness, such as

moisture resistance, as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 37.

Table 37: Particle Parameters Analyzed

Analysis	Instrument	Result 2 w/w% drug load
Residual Moisture Content	Oven KF	1.1%
Primary Particle Size Distribution	Malvern 3000	d.50: 1.95µm
Glass Transition Temperature (Tg)	DSC	96.0°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 38.

Table 38: Characterization results

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	2.7 µm	2.8 µm	2.7 µm
FPD (fine particle dose)	112 µg	108 µg	114 µg
% retention in capsule and device % of TD	9.7 %	8.9 %	8.5 %
% FPF (fine particle fraction) < 5um % of DD	71.8 %	81.3 %	80.2 %
Delivered Dose (mg)	0.15 mg	0.16 mg	0.15 mg
Bioactivity (ELISA) (% of ref)	78 %	Not analysed	90 %

EXAMPLE 8 – AEROSOL PERFORMANCE CHARACTERISTICS OF 60 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 40 were tested on a formulation with a drug load of 60 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 39: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	60
PBS pH=7.4	10.6

(4mM Na/K phosphate, 155mM NaCl, pH 7.4)	
Leucine	10
Trileucine	2.6
Trehalose	16.8

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 40 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr, and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 40.

Table 40: Particle Parameters Analyzed

Analysis	Instrument	Result 60 w/w% drug load
Residual Moisture Content	Oven KF	1.4%
Primary Particle Size Distribution	Malvern 3000	d.50: 1.94
Glass Transition Temperature (Tg)	DSC	88°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 40 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 40 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 41.

Table 41: Results of Aerosol Characterization

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	3.2 µm	3.3 µm	3.5 µm
FPD (fine particle dose)	13.9 mg	13.6 mg	12.6 mg
% retention in capsule and device % of TD	6.8 %	8.2 %	8.0 %
% FPF (fine particle fraction) < 5um % of DD	67.9 %	67.6 %	62.0 %
Delivered Dose (mg)	22.0 mg	22.6 mg	22.7 mg
Bioactivity (ELISA) (% of ref[a])	90 %	Not analysed	109 %

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 9 – AEROSOL PERFORMANCE CHARACTERISTICS OF 11 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 43 were tested on a formulation with a drug load of 11 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 42: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	11
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	2.0
Leucine	10
Trileucine	2.6
Trehalose	74.4

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate, 6.0 ml/min; atomizer gas 9,5 kg/h; and drying gas flow 40 kg/hr. The parameters were selected to achieve an acceptable, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity for a dry powder formulation intended for inhalation. This formulation displayed good aerosol performance, improved physical robustness, such as moisture resistance, bioactivity and was physically and chemically stable throughout the storage period.

Data from physical powder characteristics attributes are listed in Table 43.

Table 43: Particle Parameters Analyzed

Analysis	Instrument	Result 11 w/w % drug load
Residual Moisture Content	Oven KF	1.1%
Primary Particle Size Distribution	Malvern 3000n	d.50: 2.00 µm

Glass Transition Temperature (Tg)	DSC	91.3°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 44.

Table 44: Results of Aerosol Characterization

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	2.5 µm	2.7 µm	2.8 µm
FPD (fine particle dose)	704 µg	668 µg	646 µg
% retention in capsule and device % of TD	10.2%	10.2 %	11.0 %
% FPF (fine particle fraction) < 5um % of	75.7 %	71.7 %	70.9 %

DD			
Delivered Dose (mg)	1.04 mg	1.02 mg	1.00 mg
Bioactivity (ELISA) (% of ref[a])	105 %	82 %	86 %

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 10 – AEROSOL PERFORMANCE CHARACTERISTICS OF 33 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 46 were tested on a formulation with a drug load of 33 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 45: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	33
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	6.1
Leucine	10
Trileucine	2.6
Trehalose	48.3

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate, 6.0 ml/min; atomizer gas 9.5 kg/h; and drying gas flow 40 kg/hr. The parameters were selected to achieve an acceptable water content and particle properties as well to maintain bioactivity for a dry powder formulation intended for inhalation. This formulation displayed good aerosol performance, improved physical robustness, such as moisture resistance, bioactivity and was physically and chemically stable throughout the storage period.

Data from physical powder characteristics attributes are listed in Table 46.

Table 46: Particle Parameters Analyzed

Analysis	Instrument	Result 33 w/w % drug load
Residual Moisture Content	Oven KF	1.5%
Primary Particle Size Distribution	Malvern 3000	d.50: 2.16µm
Glass Transition Temperature (Tg)	DSC	89.0°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 47.

Table 47: Results of Aerosol Characterization

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH

MMAD (median mass aerodynamic diameter)	2.6 µm	2.8 µm	2.8 µm
FPD (fine particle dose)	2230 µg	2002 µg	2065 µg
% retention in capsule and device	9.5%	12.1%	10.8%
FPF (fine particle fraction) < 5um (% of DD)	77.0%	72.4%	72.4%
Delivered Dose (mg)	2.95 mg	3.01 mg	3.04 mg
Bioactivity (ELISA) (% of ref[a])	101 %	74 %	83 %

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 11 – AEROSOL PERFORMANCE CHARACTERISTICS OF 60 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 49 were tested on a formulation with a drug load of 60 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 48: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	60
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	11.1
Leucine	10
Trileucine	2.6
Trehalose	16.3

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine, trileucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate, 6.0 ml/min; atomizer gas 9.5 kg/h; and drying gas flow 40 kg/hr. The parameters were selected to achieve an acceptable water, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity for a dry powder formulation intended for inhalation. This formulation displayed good aerosol performance, improved physical robustness, such as moisture resistance, bioactivity and was physically and chemically stable throughout the storage period.

Data from physical powder characteristics attributes are listed in Table 49.

Table 49: Particle Parameters Analyzed

Analysis	Instrument	Result 60w/w% drug load
Residual Moisture Content	Oven KF	2.0%
Primary Particle Size Distribution	Malvern 3000	2.13µm
Glass Transition Temperature (Tg)	DSC	76.8°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 18.5 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 18.5 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min

(corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 6 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 50.

Table 50: Results of Aerosol Characterization

Analysis	Initial Result	Stability 6 months 25°C/60% RH	Stability 6 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	2.7 µm	2.9 µm	3.0 µm
FPD (fine particle dose)	6.98 mg	6.71 mg	7.06 mg
% retention in capsule and device	10.2%	9.5%	9.0%
FPF (fine particle fraction) < 5um (% of DD)	76.3%	73.0%	73.1%
Delivered Dose (mg)	10.11 mg	9.91 mg	9.85 mg
Bioactivity (ELISA) (% of ref[a])	111%	117%	119%

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 12 – AEROSOL PERFORMANCE CHARACTERISTICS OF 5 W/W% AZD1402 IN 3W/W% TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 52 were tested on a formulation with a drug load of 5 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 51: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	5
PBS pH=7.4	0.9

(4mM Na/K phosphate, 155mM NaCl, pH 7.4)	
Trileucine	3.0
Trehalose	91.1

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the trileucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr; and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity, for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 52.

Table 52: Particle Parameters Analyzed

Analysis	Instrument	Result 5 w/w% drug load
Residual Moisture Content	Oven KF	1.1%
Primary Particle Size Distribution	Laser diffraction	d.50: 1.87 µm
Glass Transition Temperature (Tg)	DSC	97.4°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose

collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 2 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 53.

Table 53: Results of Aerosol Characterization

Analysis	Initial Result	Stability 2 months 25°C/60% RH	Stability 2 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	2.6 µm	Not analysed	2.6 µm
FPD (fine particle dose)	310 µg	Not analysed	316 µg
% retention in capsule and device	9.7%	Not analysed	8.2%
% FPF (fine particle fraction) < 5um (% of DD)	77.3%	Not analysed	77.1%
Delivered Dose (mg)	455 µg	Not analysed	435 µg
Bioactivity (ELISA) (% of ref)	Not analysed	Not analysed	Not analysed

EXAMPLE 13 – AEROSOL PERFORMANCE CHARACTERISTICS OF 5 W/W% AZD1402 IN 5 W/W% TRILEUCINE FORMULATION

The following example evaluates the aerosol performance of a formulation comprising trehalose in a dry powder inhaler device. The aerosol performance outputs listed in Table 55 were tested on a formulation with a drug load of 5 w/w% and a ratio of AZD1402: PBS of NLT 5.2:1.

Table 54: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	5
PBS pH=7.4 (4mM Na/K phosphate, 155mM NaCl, pH 7.4)	0.9
Trileucine	5
Trehalose	89.1

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the trileucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried, using an outlet temperature of 65°C, feedstock feed rate 2.0 ml/min; atomizer air 2150 liters/hr; and drying gas flow 23 kg/hr. The parameters were selected to achieve an acceptable water content, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity, for a dry powder formulation intended for inhalation.

Data from physical powder characteristics attributes are listed in Table 55.

Table 55: Particle Parameters Analyzed

Analysis	Instrument	Result 5w/w% drug load
Residual Moisture Content	Oven KF	1.1%
Primary Particle Size Distribution	Malvern 3000	d.50: 1.99 µm
Glass Transition Temperature (Tg)	DSC	94.8°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry

powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis including 2 months stability for capsules stored individually in a sealed aluminum pouch are summarized in Table 56.

Table 56: Results of Aerosol Characterization

Analysis	Initial Result	Stability 2 months 25°C/60% RH	Stability 2 months 40°C/75% RH
MMAD (median mass aerodynamic diameter)	2.6 µm	Not analysed	2.6 µm
FPD (fine particle dose)	323µg	Not analysed	314 µg
% retention in capsule and device	7.8%	Not analysed	7.5%
% FPF (fine particle fraction) < 5um (% of DD)	85.7%	Not analysed	79.8%
Delivered Dose (mg)	433 µg	Not analysed	435 µg
Bioactivity (ELISA) (% of ref[s])	Not analysed	Not analysed	Not analysed

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 14 – AEROSOL PERFORMANCE CHARACTERISTICS OF 10 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION AND HISTIDINE BUFFER

The following example evaluates the aerosol performance of a formulation comprising trehalose, leucine and trileucine in a dry powder inhaler device. The aerosol performance outputs listed in Table 58 were tested on a formulation with a drug load of 10 w/w% and a ratio of AZD1402: Histidine of NLT 4.1:1.

Table 57: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	10
Histidine pH=5.5	0.59
NaCl	1.73
Leucine	10
Trileucine	2.6
Trehalose	75.08

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate, 6.0 ml/min; atomizer gas 9,5 kg/h; and drying gas flow 40 kg/hr. The parameters were selected to achieve an acceptable, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity for a dry powder formulation intended for inhalation. This formulation displayed good aerosol performance, improved physical robustness, such as moisture resistance, bioactivity and was physically and chemically stable throughout the storage period.

Data from physical powder characteristics attributes are listed in Table 58.

Table 58: Particle Parameters Analyzed

Analysis	Instrument	Result 10w/w% drug load
Residual Moisture Content	Oven KF	0.6%
Primary Particle	Malvern 3000n	d.50: 1.93 µm

Size Distribution		
Glass Transition Temperature (Tg)	DSC	84.2°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 10 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis for capsules stored individually in a sealed aluminum pouch are summarized in Table 59.

Table 59: Results of Aerosol Characterization

Analysis	Initial Result
MMAD (median mass aerodynamic diameter)	2.64 µm
FPD (fine particle dose)	730 µg
% retention in capsule and device % of TD	8 %
% FPF (fine particle	77 %

fraction) < 5um % of DD	
Delivered Dose (mg)	0.993 mg
Bioactivity (ELISA) (% of ref)[a]	112 %

[a] Bioactivity is measured against a reference sample and there is variability in the method.

EXAMPLE 15 – AEROSOL PERFORMANCE CHARACTERISTICS OF 50 W/W% AZD1402 IN TREHALOSE-LEUCINE-TRILEUCINE FORMULATION AND HISTIDINE BUFFER

The following example evaluates the aerosol performance of a formulation comprising trehalose, leucine and trileucine in a dry powder inhaler device. The aerosol performance outputs listed in Table 61 were tested on a formulation with a drug load of 50 w/w% and a ratio of AZD1402: Histidine of NLT 4.1:1.

Table 60: Composition of formulation

Component	Amount (w/w%)
AZD1402 (SEQ ID NO: 1)	50
Histidine pH=5.5	2.95
NaCl	8.63
Leucine	10
Trileucine	2.6
Trehalose	25.82

AZD 1402 was initially in a liquid formulation at a concentration of NLT 50 mg/mL. A feed stock solution was prepared at a total concentration of solids at 50 mg/mL by first dissolving the leucine and trehalose in water and then adding into the AZD1402 solution. The feed solution was spray dried using an outlet temperature of 65°C, feedstock feed rate, 6.0 ml/min; atomizer gas 9,5 kg/h; and drying gas flow 40 kg/hr. The parameters were selected to achieve an acceptable, particle properties and improved physical robustness, such as moisture resistance, as well to maintain bioactivity for a dry powder formulation intended for inhalation. This formulation displayed good aerosol performance, improved physical robustness, such as moisture resistance, bioactivity and was physically and chemically stable throughout the storage period.

Data from physical powder characteristics attributes are listed in Table 61.

Table 61: Particle Parameters Analyzed

Analysis	Instrument	Result 50 w/w % drug load
Residual Moisture Content	Oven KF	1.1%
Primary Particle Size Distribution	Malvern 3000n	d.50: 2.16 µm
Glass Transition Temperature (Tg)	DSC	76.8°C

Cascade impaction testing was performed as per USP <601> to measure the aerosol performance of the spray dried formulations when delivered from a dry powder inhaler device. The cascade impactor apparatus used was the Next Generation Impactor (NGI; USP41, Chapter <601>). For the aerosol measurements made in this example, one size 3 HPMC capsule containing 18.5 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the NGI at an airflow rate of 60 L/min as per USP methodology. Samples from each stage of the NGI were recovered and assayed for AZD1402 content.

The delivered dose testing of the spray dried formulations, when delivered from the Monodose dry powder inhaler device, was performed as per USP <601> while using a different dose collection unit. The powder aerosol is collected in a glass apparatus, an impinger, equipped with an inflatable inlet for tightness and a sintered glass filter (pore size 40 to 100 µm), (also described in Hugosson S, et al, Pharm Forum 1993;19(No 3):5458-5466.). In this example, one size 3 HPMC capsule containing 18.5 mg of the spray dried powder formulation was dispersed from the dry powder inhaler device and delivered into the impinger at an airflow rate of 60 L/min (corresponding to a pressure drop of 4 kPa over the inhaler) as per USP methodology. A sample from the impinger is recovered and assayed for AZD1402 content.

The results of the aerosol analysis for capsules stored individually in a sealed aluminum pouch are summarized in Table 62.

Table 62: Results of Aerosol Characterization

Analysis	Initial Result
MMAD (median mass aerodynamic	2.86 µm

diameter)	
FPD (fine particle dose)	6.7 µg
% retention in capsule and device % of TD	7 %
% FPF (fine particle fraction) < 5um % of DD	75 %
Delivered Dose (mg)	10.12 mg
Bioactivity (ELISA) (% of ref)[a]	111 %

[a] Bioactivity is measured against a reference sample and there is variability in the method.

Example 16. A Randomized Open-label, 3-period, 3-treatment, Crossover Study to Assess the Effect of Inhalation Device and Formulation on Pharmacokinetics Following a Single Inhaled Dose of AZD1402 in Healthy Subjects

2.1 Study and Dose Rationale

This study was intended to assess the effect of an inhalation powder (delivered via the Plastiape Monodose inhaler) on the PK characteristics of AZD1402 and to compare it to the nebulizer solution (delivered via the InnoSpire Go) that has been administered in studies conducted to date. The study results provide information on the PK profile of the study material to be used in further clinical development. Since PK assessments were the primary objective, an open-label design was selected. The study was conducted in healthy subjects to minimise the effects of concomitant disease states or medications on study measurements. Subjects received all 3 test products in a randomized order.

The InnoSpire Go nebulizer nominal delivered dose of 18 mg (test product A) was selected since it was safe and well-tolerated in the studies conducted to date, clinically relevant for the future development program and provided measurable serum concentrations up to 18 hours.

The Plastiape Monodose dry powder inhaler (DPI) nominal delivered dose of 10 mg (Treatment B) was selected to deliver an equivalent lung dose to Treatment A of 9 mg (based on calculated nebulizer and DPI inhalation device efficiencies). The Plastiape Monodose inhaler nominal

delivered dose of 30 mg (test product C) was selected to evaluate the dose proportionality of the inhalation formulation and Plastiape Monodose inhaler device.

The selected doses were well below the InnoSpire Go nebulizer nominal delivered doses of up to 160 mg that have been administered to healthy subjects in a single ascending dose study and which were safe and well tolerated (NCT03384290).

2.2 Overall Study Design and Plan: Description

This study was a randomized, open-label, 3-period, 3-treatment, single-dose, single-centre, crossover study. Eighteen healthy male and female subjects were to be randomized in this study to ensure that at least 12 subjects were evaluable. A subject was considered to be evaluable if the subject completed all 3 Treatment Periods with no important protocol deviations. Each subject received all 3 doses of the IMP; no placebo was used in this study.

Subjects were randomized to 1 of 6 treatment sequences and received the 3 single dose test products of AZD1402 listed below.

- Test product A: 18 mg nominal delivered dose of AZD1402 nebulizer solution administered via the InnoSpire Go nebulizer.
- Test product B: 10 mg nominal delivered dose of AZD1402 inhalation powder administered via a Plastiape Monodose inhaler.
- Test product C: 30 mg nominal delivered dose of AZD1402 inhalation powder administered via a Plastiape Monodose inhaler.

The study comprised:

- A Screening Period of up to 28 days before the first administration of AZD1402.
- Three Treatment Periods during which subjects were resident at the Clinical Unit from the day before dosing with AZD1402 (Day -1) until at least 48 hours after dosing and discharged on Day 3.
- A Follow-up Visit at 10 to 12 days after the last administration of AZD1402 in Treatment Period 3.

Each Treatment Period was separated by a minimum washout period of 5 days between doses. All subjects signed an ICF before they participated in any specific study related procedures. Subjects attended a Screening Visit within 28 days before receiving their first dose of AZD1402. If they were eligible, they returned for Treatment Period 1 when they had baseline assessments and received 1 of 3 test products in a randomized order. For each Treatment Period, the

subjects received a single dose of IMP in the morning of Day 1 and had further assessments for 48 hours after dosing.

The study flow chart is shown in Figure 1.

Table 63 Identity of the Investigational Medicinal Products

	Test Product A	Test Product B	Test Product C
Supplier:	AstraZeneca	AstraZeneca	AstraZeneca
Formulation:	AZD1402 nebulizer solution	AZD1402 inhalation powder	AZD1402 inhalation powder
Strength/concentration:	10 mg/mL, 5 mL	12 mg (1 × 12 mg capsule)	36 mg (3 × 12 mg capsules)
Estimated Delivered Dose:	18 mg	10 mg	30 mg
Route of administration:	Oral inhalation	Oral inhalation	Oral inhalation
Specific device for drug administration:	InnoSpire Go nebulizer	Plastiape Monodose inhaler	Plastiape Monodose inhaler
Regimen:	Single Dose	Single dose	Single dose

An example of the components used to produce the 12 mg capsules for this study (12mg/MD) is listed in Table 1 above.

2.3 Selection and Timing of Dose for Each Subject

In each Treatment Period, the subject received a single inhaled dose of AZD1402. AZD1402 was administered as a nebulizer solution via the InnoSpire Go nebulizer for test product A (Treatment A) and as an inhalation powder via a Plastiape Monodose inhaler for test product B (Treatment B) and test product C (Treatment C).

The dose was administered after an overnight fast of at least 10 hours. Subjects were allowed to drink water to prevent dehydration until 1 hour before IMP administration. Water was allowed *ad libitum* from 1 hour after IMP administration and a light breakfast was provided 2 hours after IMP administration. All subjects received the test products in a randomized sequence.

2.4 Disposition of Subjects

A total of 18 subjects were randomly assigned to 1 of the 6 treatment groups, as planned. All randomized subjects received single doses of all 3 Investigational Medicinal Products (IMPs) (i.e., Treatments A, B and C of AZD1402) during 3 Treatment Periods. Each Treatment Period was separated by a minimum washout period of 5 days between doses, as planned.

Each of the 18 randomized subjects completed all 3 Treatment Periods. No subject was withdrawn from the study, or discontinued treatment.

2.5 Demographics

All subjects included in the study were males. A majority of the subjects were White (77.8%).

No relevant differences were observed in the subject demographic characteristics across the

6 treatment sequences at baseline. The differences in mean age across the treatment sequences were not considered to be meaningful due to the small number of subjects in the study, and in turn, in each treatment sequence.

2.6 Analysis of Pharmacokinetic Data

2.6.1 Serum Concentrations

Summary statistics of the serum concentrations of AZD1402, following a single inhaled dose via InnoSpire Go nebulizer (18 mg delivered dose) or Plastiape Monodose inhaler (10 mg or 30 mg delivered dose) for each subject and time point are presented in Figures 2 and 3.

The geometric mean serum AZD1402 concentration *versus* time profiles are presented in Figure 2 (linear scale) and Figure 3 (semi-logarithmic scale). Where actual collection time of a sample deviated >10% from nominal time the result was excluded from summary statistics and geometric mean plots.

2.6.2 Serum Pharmacokinetic Parameters of AZD1402

Summary statistics of the serum AZD1402 PK parameters are presented in Table 64. The statistical analyses of key PK parameters are presented in Tables 65 and 66.

One profile from Period 1, E0001133, Treatment C had a positive pre-dose concentration of 1.54 ng/mL. This concentration was <5% Cmax and was excluded from PK analysis.

For two profiles no or limited PK parameters were calculable: E0001133, Treatment A was fully below limit of quantification (BLQ) over the entire sampling interval and thus no PK parameters were calculable and E0001143, Treatment A had only one quantifiable concentration and therefore only Cmax and tmax were calculable.

For 6 profiles AUC, AUC/D, CL/F, Vz/F and MRT values were excluded from summary statistics due to >20% AUC being extrapolated: E0001110, Treatment B; E0001114, Treatments A and B; E0001116, Treatments B and C and E0001118, Treatment B. One profile (E0001135, Treatment C) had adjusted R-squared<0.8 t½λz and t½λz dependent parameters were excluded from summary statistics.

Following inhalation of AZD1402 either via nebulizer (Treatment A, 18 mg delivered dose) or Plastiape Monodose Inhaler (Treatment B, 10 mg delivered dose or Treatment C, 30 mg delivered dose) a sustained and relatively prolonged absorption was observed with a similar

median tmax of 3.00 to 3.50 h. Individual tmax ranged from 1.98 to 8.27 h and the ranges were similar across all three treatments.

After reaching Cmax, serum concentrations of AZD1402 declined in an essentially monophasic manner with similar mean t½λz values across all three groups: 6.919 h, 7.457 h and 6.912 h for Treatments A, B and C respectively. The regression analyses applied to the data were generally a good fit (adjusted R-squared>0.8) and were considered to adequately reflect the decline in concentrations.

The t½λz for the majority of profiles following Treatments A and B and for 3 profiles following Treatment C were calculated over a period of less than 3 times the resultant half-life. Since the profiles were generally monophasic and the t½λz across all 3 groups were consistent, no data were excluded due to half-life span.

The mean CL/F and Vz/F values were similar for both Treatment B and C with the Plastiape Monodose inhaler but were higher following administration via InnoSpire Go nebulizer (Treatment A). These data, combined with higher dose normalised exposures following Treatments B and C, are consistent with lower bioavailability of AZD1402 when dosed as the Innospire Go formulation.

Inter-subject variability in AZD1402 was moderate to high for AUC (48.79%, 35.84% and 62.74% for Treatments A, B and C respectively), high for AUClast (51.86%, 57.71% and 68.69% for Treatments A, B and C respectively) and high for Cmax (101.1%, 47.76% and 67.82% for Treatments A, B and C respectively).

Exposure to AZD1402, as judged by Cmax and AUC, was similar between Treatments A and B, and mean AUClast for Treatment A was slightly higher than for Treatment B. Inferential statistical analysis showed that in comparison between Treatments A and B the geometric LSmean ratios for Cmax and AUC were 105.45%, 102.39% and the geometric LS mean ratio for AUClast was 122.21% respectively. For all 3 parameters the 90% CIs of the geometric LS means included 100%.

Exposure (Cmax, AUC, and AUClast) following Treatment A was approximately 27% of that after Treatment C: geometric LS mean ratio (90% CI) was 27.47% (18.90, 39.94), 27.09% (20.25, 36.24), and 26.50% (19.40, 36.20) for Cmax, AUC, and AUClast, respectively.

Exposure (Cmax, AUC, and AUClast) following Treatment B was approximately 22-26% of that after Treatment C: geometric LS mean ratio (90%CI) was 26.05% (18.03, 37.64), 26.46% (19.72, 35.50), and 21.68% (16.04, 29.32) for Cmax, AUC, and AUClast, respectively.

An assessment of dose proportionality for the two dry powder Treatments B and C gave slope estimates for Cmax and AUC of 1.196 and 1.259, respectively, and the corresponding 90% CIs

contained the unit of 1, suggesting an approximately dose-proportional increases. However, the slope estimate for AUClast was slightly above 1, with value of 1.368 (90%CI: 1.222, 1.614).

Table 64 Summary Pharmacokinetic Parameters of AZD1402 for each Treatment (Pharmacokinetic Analysis Set)

Parameter (unit)	Summary Statistic	A Innospire Go Nebulizer 18 mg	B Plastiape Monodose Inhaler 10 mg	C Plastiape Monodose Inhaler 30 mg
AUC (ng.h/mL)	n	15	14	16
	Geometric Mean	186.5	186.4	715.3
	(CV%)	(48.79)	(35.84)	(62.74)
AUC/D	n	15	14	16
(ng.h/mL/mg)	Geometric Mean	10.36	18.64	23.84
	(CV%)	(48.79)	(35.84)	(62.74)
AUClast	n	16	18	18
(ng.h/mL)	Geometric Mean	172.0	142.0	654.6
	(CV%)	(51.86)	(57.71)	(68.69)
AUClast /D	n	16	18	18
(ng.h/mL/mg)	Geometric Mean	9.558	14.20	21.82
	(CV%)	(51.86)	(57.71)	(68.69)
Cmax	n	17	18	18
(ng/mL)	Geometric Mean	14.50	14.02	53.81
	(CV%)	(101.1)	(47.76)	(67.82)
Cmax /D	n	17	18	18
(ng/mL/mg)	Geometric Mean	0.8054	1.402	1.794
	(CV%)	(101.1)	(47.76)	(67.82)
tmax	n	17	18	18
(h)	Median	3.03	3.50	3.00
	(Min, Max)	(1.98, 6.05)	(1.98, 7.98)	(1.98, 8.27)
t½λz	n	16	18	17
(h)	Arithmetic Mean	6.919	7.457	6.912
	(SD)	(4.169)	(5.016)	(1.964)
λz	n	16	18	17
(1/h)	Arithmetic Mean	0.12086	0.11748	0.10940
	(SD)	(0.045113)	(0.047808)	(0.036362)
MRT	n	15	14	16
(h)	Arithmetic Mean	10.21	10.50	11.65
	(SD)	(2.730)	(2.818)	(3.584)
CL/F	n	15	14	16
(L/h)	Arithmetic Mean	106.0	56.78	50.36
	(SD)	(48.15)	(20.95)	(39.95)
Vz/F)	n	15	14	16
(L)	Arithmetic Mean	888.8	477.6	461.1
	(SD)	(392.5)	(145.3)	(236.2)

AUC = area under the serum concentration-time curve from time zero extrapolated to infinity; AUC/D = area under the serum concentration-time curve from time zero extrapolated to infinity divided by dose; AUClast = area under the serum concentration-curve from time zero to time of last quantifiable concentration; AUClast/D = area under the serum concentration-curve from time zero to time of last quantifiable concentration divided by dose; Cmax = maximum observed serum concentration; Cmax/D = maximum observed serum concentration divided by dose; tmax = time to reach maximum observed concentration; t½λz = half-life terminal associated with the terminal slope (λz) of a semi-logarithmic concentration time curve; λz= terminal elimination rate constant; MRT = mean residence time of the unchanged drug in the systemic circulation from zero to infinity; CL/F = apparent total body clearance of the drug from serum after extravascular administration; Vz/F = apparent volume of distribution during the terminal phase after extravascular administration.

Table 65 Statistical Comparison of Key Pharmacokinetic Parameters of AZD1402 (Pharmacokinetic Analysis Set)

Parameter (Unit)	Treatment	N	n	Geometric LS mean	90%CI	Pair	Ratio (%)	Pairwise Comparisons 90%CI
Cmax (ng/mL)	A	18		14.78	[11.30, 19.35]			
	B	18	17	14.02	[10.81, 18.19]	A/B	105.45	[72.53, 153.32]
AUC (ng.h/mL)	A	18		188.77	[153.32, 232.42]			
	B	18	12	184.37	[148.63, 228.69]	A/B	102.39	[75.89, 138.15]
AUClast (ng.h/mL)	A	18		173.48	[138.15, 217.83]			
	B	18	16	141.95	[114.69, 175.70]	A/B	122.21	[89.45, 166.95]
Cmax (ng/mL)	A	18		14.78	[11.30, 19.35]			
	C	18	17	53.81	[41.48, 69.81]	A/C	27.47	[18.90, 39.94]
AUC (ng.h/mL)	A	18		188.77	[153.32, 232.42]			
	C	18	13	696.83	[569.68, 852.37]	A/C	27.09	[20.25, 36.24]
AUClast (ng.h/mL)	A	18		173.48	[138.15, 217.83]			
	C	18	16	654.64	[528.89, 810.28]	A/C	26.50	[19.40, 36.20]
Cmax (ng/mL)	B	18		14.02	[10.81, 18.19]			
	C	18	18	53.81	[41.48, 69.81]	B/C	26.05	[18.03, 37.64]
AUC (ng.h/mL)	B	18		184.37	[148.63, 228.69]			
	C	18	13	696.83	[569.68, 852.37]	B/C	26.46	[19.72, 35.50]
AUClast (ng.h/mL)	B	18		141.95	[114.69, 175.70]			
	C	18	18	654.64	[528.89, 810.28]	B/C	21.68	[16.04, 29.32]

A: Nebulizer solution administered via InnoSpire Go nebulizer (18 mg estimated delivered dose)

B: Inhalation powder administered via Plastiape Monodose inhaler (10 mg estimated delivered dose

C: Inhalation powder administered via Plastiape Monodose inhaler (30 mg estimated delivered dose

CI: confidence interval; LS: least-squares. n: all subjects included in the statistical comparison analysis; N: all subjects in the Pharmacokinetic analysis set. Only the data for the comparison under investigation are included in the statistical analysis. Result based on analysis of variance (ANOVA) of log transformed PK parameter with treatment, sequence, period and subject within sequence as fixed effects. Geometric mean ratio and corresponding CI are back-transformed and presented as percentages. Geometric LS mean and corresponding 90% CI are also back transformed.

Table 66 Statistical Analysis of Dose Proportionality for Dry Powder Plastiape Monodose Inhaler administration of AZD1402 Treatments B and C (Pharmacokinetic Analysis Set)

		Slope			Intercept			Coefficient of Determination
Parameter (Unit)	n	Estimate	SE	90%CI	Estimate	SE	90%CI	
Cmax (ng/mL)	18	1.196	0.202	(0.855, 1.538)	-0.319	0.590	(-1.314, 0.677)	74.8546
AUC (ng.h/mL)	18	1.259	0.172	(0.966, 1.552)	2.060	0.510	(1.194, 2.926)	53.9994
AUClast (ng.h/mL)	18	1.368	0.145	(1.122, 1.614)	1.635	0.432	(0.907, 2.363)	50.8133

CI: confidence interval. SE: standard error. n: number of data points used in regression

Model: The linear model used was log (Y) = intercept + slope * log (dose), where Y is the parameter estimate; the natural logarithm has been used. Exponentiation of both sides of this equation produces the usual form of the power model: Y = exp(intercept) * (dose) ^ slope. The slope parameter is obtained via linear least squares.

2.6.3 Pharmacokinetic Conclusions

- Following single doses of AZD1402 nebulizer solution via InnoSpire Go nebulizer or inhalation powder via Plastiape Monodose inhaler a steady absorption was observed; median tmax ranged from 3.00 to 3.50 hours and mean t1/2λz was approximately 7 hours, similar across treatments.
- Systemic exposure to AZD1402 as judged by Cmax, AUC and AUClast, was comparable between InnoSpire Go nebulizer with 18 mg nominal delivered dose and Plastiape Monodose inhaler with 10 mg nominal delivered dose.
- Systemic exposure to AZD1402, as judged by Cmax, AUC and AUClast, following administration via InnoSpire Go nebulizer with 18 mg nominal delivered dose or Plastiape Monodose inhaler with a 10 mg nominal delivered dose was approximately 73% - 78% lower than that following administration via the Plastiape Monodose inhaler with 30 mg nominal delivered dose.
- Cmax and AUC of AZD1402 following single inhalation of dry powder via Plastiape Monodose inhaler increased in a broadly dose-proportional manner over the 10 to 30 mg nominal delivered dose.

2.7 Safety Conclusions

Table 67 summarises AEs reported for the 3 single dose treatments: A (18 mg once daily), B (10 mg once daily) and C (30 mg once daily), and overall.

A total of 13 subjects (72.2% of 18 treated subjects) experienced at least 1 TEAE during the study. The most frequently reported TEAEs (>2 subjects) by preferred term were cough (2 subjects with episodes reported while on Treatment A, 1 while on Treatment B and 4 while on Treatment C) and headache (2 subjects each with episodes reported while on Treatment A and Treatment B, and 1 while on Treatment C).

The majority of subjects (72.2%) experienced TEAEs that were mild. The remaining subjects experienced TEAEs that were moderate: 2 (11.1%) subjects while on Treatment A, 3 (16.7%) subjects while on Treatment B and 4 (22.2%) subjects while on Treatment C.

One subject, a healthy volunteer who had a past history of childhood asthma and ongoing allergies to dust mites, cats and tree bark, had a suspected unexpected serious adverse reaction (SUSAR) of respiratory tract inflammation during Treatment Period 3 after inhalation of Treatment C (AZD1402 inhalation powder with a nominal dose delivery of 30 mg). The subject's FEV1 predicted decreased and C-reactive protein levels were elevated outside the normal range, both of which recovered to pre-dose levels when the subject recovered in 21 days of SUSAR onset. This SUSAR was of moderate intensity and considered to be related to the IMP by the Investigator.

Overall, the results of this study show that AZD1402 administered by inhalation in a DPI form is safe in healthy volunteers and merits further testing in patients having lung disease, e.g. asthma.

Table 67 Summary of AEs reported for the 3 single dose treatments

System Organ Class Preferred Term	A Innospire Go Nebuliser 18 mg (N=18) n (%)	B Plastiape Monodose Inhaler 10 mg (N=18) n (%)	C Plastiape Monodose Inhaler 30 mg (N=18) n (%)	Total (N=18) n (%)
Overall	**8 (44.4)**	**7 (38.9)**	**7 (38.9)**	**13 (72.2)**
Respiratory, thoracic and mediastinal disorders	**4 (22.2)**	**2 (11.1)**	**5 (27.8)**	**9 (50.0)**
Cough	2 (11.1)	1 (5.6)	4 (22.2)	6 (33.3)
Oropharyngeal pain	1 (5.6)	0 (0.0)	1 (5.6)	2 (11.1)
Throat irritation	1 (5.6)	1 (5.6)	0 (0.0)	2 (11.1)
Dyspnoea	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)
Respiratory tract inflammation	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)
General disorders and administration site conditions	**3 (16.7)**	**1 (5.6)**	**2 (11.1)**	**5 (27.8)**
Pyrexia	1 (5.6)	1 (5.6)	0 (0.0)	2 (11.1)
Asthenia	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)
Chest discomfort	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)
Chills	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Fatigue	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Feeling cold	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Vessel puncture site pain	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Nervous system disorders	**3 (16.7)**	**2 (11.1)**	**1 (5.6)**	**5 (27.8)**

System Organ Class Preferred Term	A Innospire Go Nebuliser 18 mg (N=18) n (%)	B Plastiape Monodose Inhaler 10 mg (N=18) n (%)	C Plastiape Monodose Inhaler 30 mg (N=18) n (%)	Total (N=18) n (%)
Headache	2 (11.1)	2 (11.1)	1 (5.6)	5 (27.8)
Dizziness	2 (11.1)	0 (0.0)	0 (0.0)	2 (11.1)
Dizziness postural	1 (5.6)	1 (5.6)	0 (0.0)	1 (5.6)
Gastrointestinal disorders	**3 (16.7)**	**0 (0.0)**	**1 (5.6)**	**4 (22.2)**
Diarrhoea	1 (5.6)	0 (0.0)	1 (5.6)	2 (11.1)
Mouth ulceration	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Nausea	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Vomiting	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)
Immune system disorders	**0 (0.0)**	**1 (5.6)**	**1 (5.6)**	**2 (11.1)**
Seasonal allergy	0 (0.0)	1 (5.6)	1 (5.6)	2 (11.1)
Injury, poisoning and procedural complications	**0 (0.0)**	**1 (5.6)**	**1 (5.6)**	**2 (11.1)**
Contusion	0 (0.0)	1 (5.6)	1 (5.6)	2 (11.1)
Musculoskeletal and connective tissue disorders	**1 (5.6)**	**1 (5.6)**	**0 (0.0)**	**2 (11.1)**
Arthralgia	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)
Back pain	1 (5.6)	0 (0.0)	0 (0.0)	1 (5.6)
Skin and subcutaneous tissue disorders	**0 (0.0)**	**1 (5.6)**	**1 (5.6)**	**2 (11.1)**
Dry skin	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)
Night sweats	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)
Investigations	**0 (0.0)**	**1 (5.6)**	**0 (0.0)**	**1 (5.6)**
Transaminases increased	0 (0.0)	1 (5.6)	0 (0.0)	1 (5.6)
Renal and urinary disorders	**0 (0.0)**	**0 (0.0)**	**1 (5.6)**	**1 (5.6)**
Micturition urgency	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)

2.8 Discussions

AZD1402 is a recombinant, monospecific, Anticalin® (modified lipocalin) protein targeting the interleukin 4 receptor alpha (IL-4Rα) and is being developed as inhaled therapy for the treatment of moderate to severe persistent asthma for patients that are not controlled on standard of care therapies. This was a randomized open-label, 3-period, 3-treatment, crossover study to assess the effect of inhalation device and formulation on PK following a single inhaled dose of AZD1402 in healthy subjects. This study was intended to assess the effect of an inhalation powder in 2 different doses (delivered via the Plastiape Monodose inhaler) on the PK

characteristics of AZD1402 and to compare it to the nebulizer solution (delivered via the InnoSpire Go) that has been administered in the clinical studies conducted to date.

Exposure to AZD1402, as judged by Cmax and AUC, was similar between Treatments A and B and the mean AUClast for Treatment A was slightly higher than for Treatment B. Inferential statistical analysis showed that in comparison between Treatments A and B the geometric LS mean ratios for Cmax and AUC were 105.45%, 102.39% and the geometric LS mean ratio for AUClast was 122.21% respectively. For all 3 parameters the 90% CIs of the geometric LS means included 100%.

Drug exposure (Cmax, AUC, and AUClast) following Treatment A was approximately 27% of that after Treatment C: geometric LS mean ratio (90% CI) was 27.47% (18.90, 39.94), 27.09% (20.25, 36.24), and 26.50% (19.40, 36.20) for Cmax, AUC, and AUClast, respectively.

Drug exposure (Cmax, AUC, and AUClast) following Treatment B was approximately 22-26% of that after Treatment C: geometric LS mean ratio (90%CI) was 26.05% (18.03, 37.64), 26.46% (19.72, 35.50), and 21.68% (16.04, 29.32) for Cmax, AUC, and AUClast, respectively.

Assessment of dose proportionality for the two dry powder Treatments B and C gave slope estimates for Cmax and AUC of 1.196 and 1.259, respectively, and the corresponding 90% Cis contained the unit of 1, suggesting approximately dose-proportional increases. However, the slope estimate for AUClast was slightly above 1, with value of 1.368 (90%CI: 1.222, 1.614).

The selected doses (InnoSpire Go nebulizer nominal delivered dose of 18 mg [Treatment A], Plastiape Monodose inhaler nominal delivered dose of 10 mg [Treatment B), and Plastiape Monodose inhaler nominal delivered dose of 30 mg [Treatment C]) were well below the InnoSpire Go nebulizer nominal delivered doses of up to 160 mg that have been administered to healthy subjects in a SAD study (PRS-060-PCS_06_17) and that were safe and well tolerated.

2.10 Conclusions

- AZD1402 was steadily absorbed following single dose of nebulizer solution via InnoSpire Go nebulizer or inhalation powder via Plastiape Monodose inhaler; median Tmax ranged from 3.00 to 3.50 hours and mean t1/2λz was approximately 7 hours, similar across treatments.
- Systemic exposure to AZD1402 was comparable between InnoSpire Go nebulizer with 18 mg nominal delivered dose and Plastiape Monodose inhaler with 10 mg nominal delivered dose.
- Systemic exposure to AZD1402 using InnoSpire Go nebulizer with 18 mg nominal delivered dose or Plastiape Monodose inhaler with 10 mg nominal delivered dose was lower than that using Plastiape Monodose inhaler with 30 mg nominal delivered dose.

Cmax and AUC of AZD1402 following single inhalation of dry powder via Plastiape Monodose inhaler increased in a broadly dose-proportional manner over the 10 to 30 mg nominal delivered dose.

- Overall, the safety of AZD1402 administered by inhalation in a DPI form merits further testing in patients having lung disease, e.g. asthma.

Example 17 A Two-part Phase IIa Randomised, Double-blind, Placebo-controlled, Dose-ranging, Multicentre Study to Assess Efficacy and Safety of Three Inhaled Dose Levels of AZD1402 Administered as a Dry Powder Twice Daily for Four Weeks in Adults with Asthma on Medium Dose Inhaled Corticosteroids

This is a randomised, placebo-controlled, double-blinded, multi-centre, 2-part study to assess the efficacy and safety of inhaled AZD1402. Part 1 will be performed in a lead-in cohort for each dose level to evaluate the safety and pharmacokinetic (PK) in a population with asthma controlled on medium dose inhaled corticosteroids (ICS)-long acting beta agonists (LABA) before progressing to dosing in adults with asthma who are uncontrolled on medium dose ICS-LABA in Part 2. Part 2 will be initiated for each dose level following evaluation of safety and PK at the relevant dose level in Part 1. The entire study period for each participant in both Parts 1 and 2, is approximately 3.5 months; a 2-week Screening Period, a 4 week Run-in Period, 4 weeks of Treatment Period, and 4 weeks of Follow-Up Period.

Part 1 of the study will be randomised, double blind, placebo-controlled, and conducted in parallel for the 2 lower dose levels (Part 1a) followed by an unblinded safety review and escalation to the highest dose (Part 1b) dependent on the outcome of the safety review.

Part 1a will consist of 30 participants who will be randomised 1:1:1 to receive 1 of the 2 lower AZD1402 dry power inhaler (DPI) doses (1 or 3 mg nominal delivered doses) or placebo in parallel. Part 1b will consist of 15 participants who will be randomised 2:1 to receive the highest AZD1402 DPI dose (10 mg nominal delivered dose) or placebo.

Examples of the components which could be used to produce the 1 mg, 3mg and 10mg capsules for this study are listed in Tables 2, 3 and 4 above.

Part 1a lead-in cohort

• AZD1402 oral inhalation 1 mg nominal delivered dose twice daily (BID) via DPI

• AZD1402 oral inhalation 3 mg nominal delivered dose BID via DPI

• Placebo oral inhalation BID via DPI

Part 1b lead-in cohort

• AZD1402 oral inhalation 10 mg nominal delivered dose BID via DPI

• Placebo oral inhalation BID via DPI

Part 2 will be randomised, double blind, placebo controlled and will include 360 participants to evaluate 3 inhaled dose levels of AZD1402 versus placebo.

Apart from the scheduled clinic visits, the 4 weeks of dosing in Part 2 of the study will be at home. Part 2a, which includes the 2 lower dose levels (1 and 3 mg nominal delivered doses) will be started in parallel after the unblinded safety review for Part 1a. The higher dose of 10 mg nominal delivered dose (Part 2b) will be included in Part 2 following the unblinded review of Part 1b, depending on the outcome of the safety review. Once Part 2b starts, all 3 dose levels (and placebo) will run in parallel.

Part 2 will include:

• AZD1402 oral inhalation 1 mg nominal delivered dose BID via DPI

• AZD1402 oral inhalation 3 mg nominal delivered dose BID via DPI

• AZD1402 oral inhalation 10 mg nominal delivered dose BID via DPI

• Placebo oral inhalation BID via DPI

EXAMPLE 18 – Exposure profile after once-a-day dosing of AZD1402

In order to predict the exposure profile after once-a-day dosing of AZD1402, the intravenous (i.v.) exposure data from the single dose study NCT03384290 (described in Example 2 of WO 2020/200960) was fitted to a 2-compartment pharmacokinetic model using Nonmem (version 7.3.0). The absorption rate of inhaled AZD1402 from the lung was subsequently modelled based on inhaled exposure data from studies NCT03384290 (single ascending dose (SAD) study described in Example 2 of WO 2020/200960), NCT03574805 (multiple ascending dose (MAD) study described in Examples 3 and 4 of WO 2020/200960) and NCT03921268, which is described in Example 16 above. The final model was used to simulate PK serum exposures after once and twice-a-day dosing for a range of doses. Figure 4 shows that a 5-fold higher once-a-day nominal delivered dose (of 5 mg) is needed to achieve the same daily minimum serum concentration as a twice-a-day nominal delivered dose of 1 mg. A 5-fold higher once-a-day dose (or 2.5-fold the total daily dose) than the twice-a-day is predicted to be needed also for other doses as summarised in the table below.

Table 68

Twice a day delivered DPI dose (total daily dose, mg)	Once a day delivered DPI dose (mg)
0.5 (1)	2.5
1 (2)	5
2 (4)	10
3 (6)	15
4 (8)	20
5 (10)	25

Table 69 List of Abbreviations and Definitions of Terms

Abbreviation or special term	Explanation
AE	Adverse event
ATS	American Thoracic Society
AUC	Area under serum concentration time curve from time zero extrapolated to infinity
AUC/D	Area under the serum concentration-time curve from time zero extrapolated to infinity divided by dose
AUClast	Area under the serum concentration-curve from time zero to time of last quantifiable concentration
AUClast/D	Area under the serum concentration-time curve from time zero to time of last quantifiable concentration divided by dose
BLQ	Below limit of quantification
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
CL/F	Apparent total body clearance of drug from serum after extravascular administration
Cmax	Maximum observed serum concentration
Cmax/D	Maximum observed serum concentration divided by dose
CRF	Case report form
CSA	Clinical study agreement
CSP	Clinical study protocol
CSR	Clinical study report
DAE	Adverse events leading to the discontinuation of investigational product
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
ECG	Electrocardiogram
EDV	Early Discontinuation Visit
ERS	European Respiratory Society

Abbreviation or special term	Explanation
FeNO	Exhaled nitric oxide
FEV1	Forced expiratory volume in the first second
FIH	First-in-human
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HIV	Human immunodeficiency virus
HPC	High positive control
λz	Terminal rate constant
IB	Investigator's Brochure
ICD	Informed consent document
ICH	International Council for Harmonisation
ICS	Inhaled corticosteroid
IgE	Immunoglobulin E
IGRA	Interferon gamma release assay
IL-4Rα	Interleukin 4 receptor alpha subunit
IL	Interleukin
IMP	Investigational medicinal product (active treatments and placebo)
INF	Interferon
ISRB	Innovative Medicines Safety Review Board
LABA	Long-acting beta 2 agonist
LLOQ	Lower limit of quantification
LPC	Low positive control
MedDRA	Medical Dictionary for Regulatory Activities
MRD	Minimal required dilution
MRT	Mean residence time of the unchanged drug in the systemic circulation from zero

Abbreviation or special term	Explanation
ms	Milliseconds
MSD	Meso Scale Discovery
NIMP	Non-investigational medical products
PD	Pharmacodynamics
pECG	Paper print-out ECG
PI	Principal Investigator
PK	Pharmacokinetics
PR (PQ)	ECG interval measured from the onset of the P wave to the onset of the QRS
	complex
QRS	ECG interval measured from the onset of the QRS complex to the J point.
QT	ECG interval measured from the onset of the QRS complex to the end of the T wave
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
RR	The time between corresponding points on 2 consecutive R waves on ECG
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
SRC	Safety Review Committee
SS	Study synopsis document
SUSAR	Suspected unexpected serious adverse reaction
TA	Therapeutic area
TB	Tuberculosis
t½λz	t½λz Half-life associated with the terminal slope (λz) of a semi-logarithmic concentration-time curve
TEAE	TEAE Treatment emergent adverse event
Tlc	Tlc Tear lipocalin
t_{max}	tmax Time to reach maximum observed serum concentration

Abbreviation or special term	Explanation
ULN	ULN Upper limit of normal
Vz/F	Vz/F Apparent volume of distribution during the terminal phase (extravascular administration)
WAD	Window Allowance Document

References

A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. The entirety of each of these references is incorporated herein.

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Sequences

Table 70

SEQ ID NO:	Description	Protein/DNA	Sequence
1	Amino acid sequence of the human tear lipocalin mutein PRS-060/AZD1402	Protein	ASDEEIQDVSGTWYLKAMTVDSRCPRAVY NSVTPMTLTTLEGGNLEAKFTAQRKGRWQ KYKLVLEKTDEPGKYTASGGRHVAYIIRSH VKDHYIFHSEGLCPGQPVPGVWLVGRDPK NNLEALEDFEKAAGARGLSTESILIPRQSET SSPGSD
2	Human tear lipocalin	Protein	MKPLLLAVSLGLIAALQAHHLLASDEEIQDV SGTWYLKAMTVDREFPEMNLESVTPMTLT TLEGGNLEAKVTMLISGRCQEVKAVLEKTD EPGKYTADGGKHVAYIIRSHVKDHYIFYCE GELHGKPVRGVKLVGRDPKNNLEALEDFE KAAGARGLSTESILIPRQSETCSPGSD
3	Mature form of human tear lipocalin	Protein	HHLLASDEEIQDVSGTWYLKAMTVDREFP EMNLESVTPMTLTTLEGGNLEAKVTMLISG RCQEVKAVLEKTDEPGKYTADGGKHVAYII RSHVKDHYIFYCEGELHGKPVRGVKLVGR DPKNNLEALEDFEKAAGARGLSTESILIPRQ SETCSPGSD
4	Amino acid sequence of human interleukin-4 receptor alpha chain	Protein	MKVLQEPTCVSDYMSISTCEWKMNGPTNC STELRLLYQLVFLLSEAHTCIPENNGGAGC VCHLLMDDVVSADNYTLDLWAGQQLLWK GSFKPSEHVKPRAPGNLTVHTNVSDTLL LTWSNPYPPDNYLYNHLTYAVNIWSENDP ADFRIYNVTYLEPSLRIAASTLKSGISYRAR VRAWAQCYNTTWSEWSPSTKWHNSYREP FEQHLLLGVSVSCIVILAVCLLCYVSITKIKK EWWDQIPNPARSRLVAIIIQDAQGSQWEKR SRGQEPAKCPHWKNCLTKLLPCFLEHNMK RDEDPHKAAKEMPFQGSGKSAWCPVEISK TVLWPESISVVRCVELFEAPVECEEEEEVE EEKGSFCASPESSRDDFQEGREGIVARLT ESLFLDLLGEENGGFCQQDMGESCLLPPS GSTSAHMPWDEFPSAGPKEAPPWGKEQP LHLEPSPPASPTQSPDNLTCTETPLVIAGN PAYRSFSNSLSQSPCPRELGPDPLLARHLE EVEPEMPCVPQLSEPTTVPQPEPETWEQIL RRNVLQHGAAAAPVSAPTSGYQEFVH AVEQGGTQASAVVGLGPPGEAGYKAFSSL LASSAVSPEKCGFGASSGEEGYKPFQDLIP GCPGDPAPVPVPLFTFGLDREPPRSPQSS HLPSSSPEHLGLEPGEKVEDMPKPPLPQE QATDPLVDSLGSGIVYSALTCHLCGHLKQC HGQEDGGQTPVMASPCCGCCCGDRSSPP TTPLRAPDPSPGGVPLEASLCPASLAPSGI SEKSKSSSSFHPAPGNAQSSSQTPKIVNFV

			SVGPTYMRVS
5	Amino acid sequence of extracellular domain of human interleukin-4 receptor alpha chain	Protein	MKVLQEPTCVSDYMSISTCEWKMNGPTNC STELRLLYQLVFLLSEAHTCIPENNGGAGC VCHLLMDDVVSADNYTLDLWAGQQLLWK GSFKPSEHVKPRAPGNLTVHTNVSDTLL LTWSNPYPPDNYLYNHLTYAVNIWSENDP ADFRIYNVTYLEPSLRIAASTLKSGISYRAR VRAWAQCYNTTWSEWSPSTKWHNSYREP FEQH

The following numbered clauses, describing aspects of the invention, are part of the description.

1. A method for treating asthma in a human subject, wherein the method comprises administering by inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, to said subject, wherein a nominal delivered dose of about 0.1mg to about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

2. The method of clause 1, wherein a nominal delivered dose of about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

3. A method for treating asthma in a human subject, wherein the method comprises administering by inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, to said subject, wherein a nominal metered dose of about 0.2mg to about 40mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

4. The method of clause 3, wherein a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject.

5. The method of any one of the preceding clauses, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject at least once per day.

6. The method of clause 5, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject twice daily.

7. The method of clause 6, wherein the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg.

8. The method of clause 5 or 6, wherein a total nominal delivered dose of about 1mg, about 2mg, about 6mg, about 20mg or about 60mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

9. The method of clause 6, wherein the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg.

10. The method of clause 5 or 6, wherein a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

11. The method of any one of the preceding clauses, wherein said formulation includes a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

12. The method of any one of the preceding clauses, wherein the dry powder formulation comprises trehalose.

13. The method of any one of the preceding clauses, wherein the dry powder formulation comprises leucine.

14. The method of clause 12 or 13, wherein the dry powder formulation comprises trileucine.

15. The method of clause 14, wherein the dry powder formulation comprises trehalose, leucine and trileucine.

16. A dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation by inhalation, wherein a nominal delivered dose of about 0.1mg to about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

17. The dry powder formulation for use according to clause 16, wherein a nominal delivered dose of about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

18. A dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation by inhalation, wherein a nominal metered dose of about 0.2mg to about 40mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

19. The dry powder formulation for use according to clause 18, wherein a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject.

20. The dry powder formulation for use according to any one of clauses 16 to 19, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject at least once per day.

21. The dry powder formulation for use according to clause 20, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject twice daily.

22. The dry powder formulation for use according to clause 21, wherein the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg.

23. The dry powder formulation for use according to clause 20 or 21, wherein a total nominal delivered dose of about 1mg, about 2mg, about 6mg, about 20mg or about 60mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

24. The dry powder formulation for use according to clause 21, wherein the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about

36mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject twice per day.

25. The dry powder formulation for use according to clause 20 or 21, wherein a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 6.6mg, about 22.2mg, about 24mg or about 72mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

26. The dry powder formulation for use according to any one of clauses 16 to 25, wherein said formulation includes a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

27. The dry powder formulation for use according to any one of clauses 16 to 26, wherein the dry powder formulation comprises trehalose.

28. The dry powder formulation for use according to any one of clauses 16 to 27, wherein the dry powder formulation comprises leucine.

29. The dry powder formulation for use according to clause 27 or 28, wherein the dry powder formulation comprises trileucine.

30. The dry powder formulation for use according to clause 29, wherein the dry powder formulation comprises trehalose, leucine and trileucine.

31. A dry powder formulation, wherein said formulation comprises an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO:1, or a variant or fragment thereof.

32. The dry powder formulation of clause 31, wherein said formulation comprises trehalose.

33. The dry powder formulation of clause 31 or 32, wherein the dry powder formulation comprises leucine.

34. The dry powder formulation of clause 32 or 33, wherein the dry powder formulation comprises trileucine.

35. The dry powder formulation of clause 34, wherein the dry powder formulation comprises trehalose, leucine and trileucine.

36. The dry powder formulation of any one of clauses 31-35, wherein said formulation provides a nominal delivered dose of about 0.1mg to about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

37. The dry powder formulation of clause 36, wherein said formulation provides a nominal delivered dose about 0.5mg, about 1mg, about 3mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

38. The dry powder formulation of any one of clauses 31-37, wherein said formulation comprises a nominal metered dose of about 0.2mg to about 40mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

39. The dry powder formulation of clause 38, wherein said formulation comprises a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 3.3mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

40. The dry powder formulation of any one of clauses 31-39, wherein said formulation includes a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

41. A method for treating asthma in a human subject, wherein the method comprises administering by oral inhalation the dry powder formulation according to any one of clauses 31-40.

42. The dry powder formulation of any one of clauses 31-40 for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation to said subject by oral inhalation.

43. The use of the dry powder formulation of any one of clauses 31-40 for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of asthma in a human subject, wherein the treatment comprises administering the dry powder formulation by oral inhalation.

Claims

1. A method for treating asthma in a human subject, wherein the method comprises administering by inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, to said subject, wherein a nominal delivered dose of about 0.1mg to about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

2. The method of claim 1, wherein a nominal delivered dose of about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

3. A method for treating asthma in a human subject, wherein the method comprises administering by inhalation a dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof, to said subject, wherein a nominal metered dose of about 0.2mg to about 40mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

4. The method of claim 3, wherein a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject.

5. The method of any one of the preceding claims, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject at least once per day.

6. The method of claim 5, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject twice daily.

7. The method of claim 6, wherein the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg.

8. The method of claim 5 or 6, wherein a total nominal delivered dose of about 1mg, about 2mg, about 4mg, about 6mg, about 8mg, about 10mg, about 12mg, about 20mg or about 60mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

9. The method of claim 6, wherein the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg.

10. The method of claim 5 or 6, wherein a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 4.4mg, about 6.6mg, about 8.8mg, about 11mg, about 13.2mg, about 22.2mg, about 24mg or about 72mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

11. The method of claim 1, wherein a nominal delivered dose of about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject once per day.

12. The method of any one of the preceding claims, wherein said formulation includes a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

13. The method of any one of the preceding claims, wherein the dry powder formulation comprises trehalose.

14. The method of any one of the preceding claims, wherein the dry powder formulation comprises leucine.

15. The method of claim 13 or 14, wherein the dry powder formulation comprises trileucine.

16. The method of claim 15, wherein the dry powder formulation comprises trehalose, leucine and trileucine.

17. A dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation by inhalation, wherein a nominal delivered dose of about 0.1mg to about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

18. The dry powder formulation for use according to claim 17, wherein a nominal delivered dose of about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation.

19. A dry powder formulation comprising a therapeutically effective amount of an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein, or a variant or fragment thereof, for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation by inhalation, wherein a nominal metered dose of about 0.2mg to about 40mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject by oral inhalation, and wherein the anti-IL-4 receptor alpha (IL-

4Rα) lipocalin mutein comprises the amino acid sequence set forth in SEQ ID NO: 1, or a variant or fragment thereof.

20. The dry powder formulation for use according to claim 19, wherein a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject.

21. The dry powder formulation for use according to any one of claims 17 to 20, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject at least once per day.

22. The dry powder formulation for use according to claim 21, wherein said lipocalin mutein, or variant or fragment thereof, is administered to said subject twice daily.

23. The dry powder formulation for use according to claim 22, wherein the nominal delivered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg.

24. The dry powder formulation for use according to claim 21 or 22, wherein a total nominal delivered dose of about 1mg, about 2mg, about 4mg, about 6mg, about 10mg, about 12mg, about 20mg or about 60mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

25. The dry powder formulation for use according to claim 22, wherein the nominal metered dose for each dose of said lipocalin mutein, or variant or fragment thereof, administered to said subject twice per day is about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, about 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject twice per day.

26. The dry powder formulation for use according to claim 21 or 22, wherein a total nominal metered dose of about 1.1mg, about 1.2mg, about 2.2mg, about 4.4mg, about 6.6mg, about 8.8mg, about 11mg, about 13.2mg, about 22.2mg, about 24mg or about 72mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject per day.

27. The dry powder formulation for use according to claim 17, wherein a nominal delivered dose of about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, is administered to said subject once per day.

28. The dry powder formulation for use according to any one of claims 17 to 27, wherein said formulation includes a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

29. The dry powder formulation for use according to any one of claims 17 to 28, wherein the dry powder formulation comprises trehalose.

30. The dry powder formulation for use according to any one of claims 17 to 29, wherein the dry powder formulation comprises leucine.

31. The dry powder formulation for use according to claim 29 or 30, wherein the dry powder formulation comprises trileucine.

32. The dry powder formulation for use according to claim 31, wherein the dry powder formulation comprises trehalose, leucine and trileucine.

33. A dry powder formulation, wherein said formulation comprises an anti-IL-4 receptor alpha (IL-4Rα) lipocalin mutein comprising the amino acid sequence set forth in SEQ ID NO:1, or a variant or fragment thereof.

34. The dry powder formulation of claim 33, wherein said formulation comprises trehalose.

35. The dry powder formulation of claim 33 or 34, wherein the dry powder formulation comprises leucine.

36. The dry powder formulation of claim 34 or 35, wherein the dry powder formulation comprises trileucine.

37. The dry powder formulation of claim 36, wherein the dry powder formulation comprises trehalose, leucine and trileucine.

38. The dry powder formulation of any one of claims 3337, wherein said formulation provides a nominal delivered dose of about 0.1mg to about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

39. The dry powder formulation of claim 38, wherein said formulation provides a nominal delivered dose about 0.5mg, about 1mg, about 2mg, about 3mg, about 4mg, about 5mg, about 6mg, about 10mg or about 30mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

40. The dry powder formulation of claim 38, wherein said formulation provides a nominal delivered dose about 2.5mg, about 5mg, about 10mg, about 15mg, about 20mg or about 25mg of said lipocalin mutein, or variant or fragment thereof, when said formulation is administered to said subject by oral inhalation.

41. The dry powder formulation of any one of claims 33-40, wherein said formulation comprises a nominal metered dose of about 0.2mg to about 40mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

42. The dry powder formulation of claim 41, wherein said formulation comprises a nominal metered dose of about 0.55mg, about 0.6mg, about 1.1mg, about 2.2mg, about 3.3mg, 4.4mg, about 5.5mg, about 6.6mg, about 11.1mg, about 12mg or about 36mg of said lipocalin mutein, or variant or fragment thereof, for administration to said subject by oral inhalation.

43. The dry powder formulation of any one of claims 33-42, wherein said formulation includes a plurality of microparticles, the microparticles comprising said lipocalin mutein, or variant or fragment thereof.

44. A method for treating asthma in a human subject, wherein the method comprises administering by oral inhalation the dry powder formulation according to any one of claims 33-43.

45. The dry powder formulation of any one of claims 33-43 for use in a method for treatment of asthma in a human subject, wherein the method comprises administering the dry powder formulation to said subject by oral inhalation.

46. The use of the dry powder formulation of any one of claims 33-43 for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of asthma in a human subject, wherein the treatment comprises administering the dry powder formulation by oral inhalation.