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(54) Titre : PROCEDES D'AMPLIFICATION DES REPONSES IMMUNITAIRES
(54) Title: METHODS OF BOOSTING IMMUNE RESPONSES

(57) Abrégé/Abstract:
The present invention relates to immunogenic compositions, such as vaccines, comprising immunogenic polypeptides from Haemophilus influenzae and Moraxellacatarrhalis, for use in methodsof boosting an immune response and methods of treatment using same. More particularly, the invention relates to use of such immunogenic compositions in methods of treating or preventing exacerbation of chronic obstructive pulmonary disease.

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(54) Title: METHODS OF BOOSTING IMMUNE RESPONSES

(57) Abstract: The present invention relates to immunogenic compositions, such as vaccines, comprising immunogenic polypeptides from *Haemophilus influenzae* and *Moraxellacatarrhalis*, for use in methodsof boosting an immune response and methods of treatment using same. More particularly, the invention relates to use of such immunogenic compositions in methods of treating or preventing exacerbation of chronic obstructive pulmonary disease.

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METHODS OF BOOSTING IMMUNE RESPONSES

Field of the Invention

The present invention relates to immunogenic compositions, such as vaccines, comprising immunogenic polypeptides from *Haemophilus influenzae* and *Moraxella catarrhalis*, for use in a method of boosting an immune response and methods of treatment using same. More particularly, the invention relates to use of such immunogenic compositions in methods of treating or preventing exacerbation of chronic obstructive pulmonary disease.

Background of the Invention

Chronic Obstructive Pulmonary Disease (COPD), a common preventable disease, is characterised by persistent airflow limitation that is usually progressive. The airflow limitation is associated with an enhanced chronic inflammatory response in the airways and lungs to noxious particles of gases. The most important environmental risk factor for COPD is tobacco smoking, even though other factors, such as occupational exposure, may also contribute to the development of the disease [**1**]. It is a multi-component disease that manifests as an accelerated decline in lung function, with symptoms such as breathlessness on physical exertion, deteriorating health status and exacerbations.

The prevalence of COPD is increasing: worldwide, COPD (GOLD grade II and above) affects 10.1±4.8% of the population ≥40 years of age [**2**]. COPD is most prevalent in adults/elderly with a history of smoking [**3**]. It is the fourth leading cause of chronic morbidity and mortality in the United States and the first in terms of disease burden in China. Recent papers report that in 2015, COPD ranked third among the global age-standardised death rates for both sexes, with about 3·2 million patients dying of the disease [**4**].

Acute exacerbations and comorbidities contribute to the overall disease severity in individual COPD patients. An acute exacerbation of COPD (AECOPD) is an acute event characterised by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication [**1**]. AECOPD increases morbidity and mortality, leading to faster decline in lung function, poorer functional status [**5**].

The lungs are known to be colonised with different strains of bacteria [**6**, **7**]. In COPD patients, acquisition of new bacterial strains is believed to be an important cause of

AECOPD [**8**]. Although estimates vary widely, Non-Typeable *Haemophilus influenzae* (NTHi) appears to be the main bacterial pathogen associated with AECOPD (11-38%), followed by *Moraxella catarrhalis* (Mcat) (3-25%) and *Streptococcus pneumoniae* (4-9%) [[**7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18** and **18A**].

A wide range of pharmacologic (such as inhaled corticosteroids, bronchodilators, phosphodiesterase inhibitors, theophyllines, long-term antibiotics and mucolytics) and non-pharmacologic (such as lung volume reduction surgery, home oxygen, ventilatory support and pulmonary rehabilitation) interventions exist to manage or treat COPD, some with a positive impact on the AECOPD rate. However, these approaches may not be completely effective, even when targeted and used optimally. Therefore, there exists a need for further treatment regimens to manage or treat COPD, particularly AECOPD.

Summary of the Invention

The present Inventors have discovered improved regimens for boosting an immune response against Non-Typeable *Haemophilus influenzae* and *Moraxella catarrhalis*. Particularly the treatment regimens are vaccine regimens, for example, prime-boost regimens.

Thus, in a first aspect of the invention there is provided an immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof, for use in a method of boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* in a subject, the method comprising the step of administering the immunogenic composition to the subject in an amount sufficient to elicit an immune response, particularly in an amount sufficient to elicit a further or additional, immune response relative to the pre-existing immune response.

Particularly, the subject has a previous history of Chronic Obstructive Pulmonary Disease (COPD). Yet more particularly, the subject has a previous history of moderate and severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD). Thus, in certain embodiments the immunogenic composition is for use in a method of treating or preventing an acute exacerbation of chronic obstructive pulmonary disease (AECOPD), the

method comprising boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* by administering the immunogenic composition to the subject in an amount sufficient to elicit an immune response, particularly in an amount sufficient to elicit a further or additional, immune response relative to the pre-existing immune response. In an embodiment, the acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is associated with a bacterial infection. However, this is not intended to imply that a bacterial infection must have been identified by testing, for example, by bacterial culture.

Generally, the pre-existing immune response has been elicited by prior administration of at least two doses, for example a first dose and a second dose, of an immunogenic composition comprising PD, PE, PilA and UspA2 or fragments thereof. For example, the pre-existing immune response may result from primary immunisation of the subject with at least two doses of a vaccine comprising PD, PE, PilA, and UspA2, or fragments thereof.

In certain embodiments the immunogenic composition is administered six to 12 months after administration of the first of the at least two doses of vaccine. Subsequently, the immunogenic composition may be administered at regular intervals, for example, every six to 12 months. Thus, in one embodiment the immunogenic composition can be administered six to 12 months after administration of the first of the at least two doses of vaccine, and again six to 12 months later, for example on the anniversary of the first of the at least two doses of vaccine.

Particularly, the step of administering the immunogenic composition to the subject elicits an immune response against PD, PE, PilA and UspA2. More particularly, the immune response against PD, PE, PilA and UspA2 is sufficient to induce protective or therapeutic immunity against non-typeable *Haemophilus influenzae* or *Moraxella catarrhalis*. Yet more particularly, the immune response against PD, PE, PilA and UspA2 is sufficient to induce protective or therapeutic immunity against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis*. Still yet more particularly, the immune response against PD, PE, PilA and UspA2 is sufficient to reduce the frequency of AECOPD.

Particularly, the subject is a suitable mammal, preferably a human. The subject may be an adult human, for example, aged between 18 and 80 years of age, 18 and 70, 18 and

50, 18 and 40 or between 50 and 70 or between 40 and 80 years of age. In some embodiments, the immunogenic composition is for use in a subject having a smoking history, for example, a smoking history of at least ten pack years. For example, 1 pack-year is equal to smoking 20 cigarettes (1 pack) per day for 1 year, or 40 cigarettes per day for half a year, or 10 cigarettes per day for 2 years. The number of pack years is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked (since 1 pack is 20 cigarettes, this may also be calculated as follows: average number of cigarettes smoked per day multiplied by the number of years and divided by 20). In other embodiments, the immunogenic composition is for use in a subject having cystic fibrosis, for example, diagnosed by genetic test, blood test and/or sweat test.

Brief description of Figures:

Figure 1a. shows the immune response to PD and PilA following immunisation with two doses of an AS01E adjuvanted NTHi immunogenic composition comprising PD and PE-PilA fusion protein administered intramuscularly according to a 0, 2-month schedule in current and former smokers (50-70 years old) in a Phase 1 clinical trial (NTHi-003).

Figure 1b. shows a theoretical trend in vaccine efficacy based on interim analysis of clinical trial data. A theoretical trend for a lower vaccine efficacy 7 month post-vaccination compared to 4 months post-vaccination is shown.

Figure 2. shows the ABCD assessment tool.

Figure 3. exemplifies treatment regimens of the present invention. **Figure 3(a)** provides a three-dose regime with primary vaccination taking place at Day 1, Day 61 and Day 181. Annual booster doses may be provided following completion of the three-dose vaccination regime. **Figure 3(b)** provides a three-dose regime with primary vaccination taking place at Day 1, Day 61 with a booster at Day 361. Annual booster doses may be provided following completion of the three-dose vaccination regime.

Figure 4. Geometric mean concentrations of anti-PE (**Figure 4A**), anti-PilA (**Figure 4B**) and anti-PD (**Figure 4C**) in participants who received 2 doses of NTHi vaccine (AS01E adjuvanted immunogenic composition comprising PD and PE-PilA fusion protein) at either 10 µg or 30 µg antigen/dose adjuvanted with AS01E; PRE, pre-Dose 1; PI(D30), 30 days post-Dose 1; PI(D60),pre-Dose 2; PII(D90), 30 days post-Dose 2; PII(D180), pre-Dose 3;

PIII(D210), 30 days post-Dose 3; PIII(D420), 8 months post-Dose 3 in a Phase 1 clinical trial (NTHi-003) in current and former smokers (50-70 years old).

Figure 5. Number of PD (**Figure 5A**), PE (**Figure 5B**) and PilA (**Figure 5C**) specific CD4+ T-cells expressing the following markers: All (1st bar), CD40L (2nd bar), IL-2 (3rd bar), TNF-α (4th bar), IFN-γ (5th bar), IL-13 (6th bar) and IL-17 (7th bar) prior and after each vaccination with the 10-AS01E-adjuvanted formulations in a Phase 1 clinical trial (NTHi-003) in current and former smokers (50-70 years old).

Figure 6. The IgG responses induced against UspA2, PD, PE and PilA in mice by tetravalent NTHi-Mcat (PD-PEPilA-UspA2) vaccine are shown in **Figure 6A** (UspA2), **Figure 6B** (PD, protein D), **Figure 6C** (PE, protein E), **Figure 6D** (PilA) and **Figure 6E** (PE, PilA and PD) respectively. No major impact of the addition of UspA2 on PD and PEPilA immunogenicity in AS01E was observed.

Figure 7. Bactericidal responses induced by UspA2. The anti-*Moraxella catarrhalis* bactericidal assay was performed against strains expressing a homologous (25238) or a heterologous (F10) UspA2. UspA2 induced high bactericidal titres against both strains.

Figure 8. The IgG responses induced against UspA2, PD, PE and PilA in mice by NTHi-Mcat vaccine (PD-PEPilA-UspA2) are shown in **Figure 8A** (PD, protein D), **Figure 8B** (PE, protein E), **Figure 8C** (PilA) and **Figure 8D** (UspA2) respectively in the Phase 1 study in healthy adults aged 18-40 years and in current and former smokers aged 50-70 years (NTHI MCAT-001).

Description of the Invention

COPD is characterised by progressive worsening of airflow limitation and a decline in pulmonary function and is complicated by acute exacerbations (AECOPD) which are transient and apparently stochastic periods of increased COPD symptoms requiring additional medical treatment and often hospitalisation. An “acute exacerbation" has its normal meaning in the art, referring to an abrupt or sudden worsening of a patient's COPD symptoms beyond their usual normal day-to-day variations and state and requiring urgent care. Acute exacerbations may be triggered by a variety of stimuli including exposure to pathogens, such as bacteria and viruses, inhaled irritants such as smoke from cigarettes,

allergens, or pollutants. COPD patients with a documented history of one or more acute exacerbations have an increased risk of subsequent exacerbations, particularly bacterial exacerbation. The term "bacterial exacerbation" refers to an acute exacerbation associated with a positive bacterial pathogen on routine culture (for example, *Haemophilus influenza* and/or *Moraxella catarrhalis*) or a total aerobic CFU (colony forming units) count greater than or equal to 10^7 cells. Without wishing to be bound by theory, the treatment regimens reduce the risk of such bacterial exacerbations happening by inducing an immune response in a subject characterised by an increase in the level of antibodies that prevent or reduce the risk of infection by and/or colonisation of the subject, particularly the subject's airways, with *Haemophilus influenza* and *Moraxella catarrhalis*. In so doing, the present invention reduces the frequency, duration or severity of an acute exacerbation of COPD and/or reduces the frequency, duration or severity of one or more symptoms of an acute exacerbation of COPD. A reduction in frequency, duration or severity of acute exacerbation or one or more symptoms of acute exacerbation may be measured by clinical observation by an ordinarily skilled doctor or clinician. A reduction in frequency, duration or severity is determined relative to the frequency, duration or severity of an acute exacerbation or symptom in the same subject not treated according to the methods of the present invention. Suitable clinical observations by an ordinarily skilled clinician may include objective measures of lung function, as well as the frequency with which medical intervention is required. Subjective self-evaluation by the subject may also be used as a measure, for example, using an FDA-recognized subject reported outcome tool or the Exacerbations from Pulmonary Disease Tool (EXACT-PRO).

Following immunisation schedules using two-doses of an investigational NTHi-Mcat vaccine, the inventors have observed that the antibody response in subjects peaks one month post administration of the 2nd dose (Figure 1a). Following this peak, a decline in the level of vaccine specific antibodies is observed 4 to 5 months after administration of the 2nd dose at which point the level of circulating antibodies stabilises during the next months (Figure 1a; Example 4 Table 9). A theoretical trend for a lower vaccine efficacy 7 month post-vaccination compared to 4 months post-vaccination was predicted (Figure 1b). Whilst persistence of the response is observed up to 1 year after administration of the 2nd dose, the inventors have developed improved treatment regimens comprising a third, or a booster dose of vaccine to improve the immune response providing increased levels of antibody compared to a two-dose immunisation schedule.

Thus, the present invention relates to immunogenic compositions for use in methods of boosting pre-existing immune responses against non-typeable *Haemophilus influenzae* and/or *Moraxella catarrhalis*. As a result, the treatment regimens reduce or inhibit acute exacerbation of chronic obstructive pulmonary disease (AECOPD) in a subject. Particularly suitable immunogenic compositions are described in the following pages and generally will comprise (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof. Particularly the treatment regimens improve the humoral immune response and more particularly, increase or "boost" the level of anti-PD, anti-PE, anti-PilA and anti-UspA2 antibodies.

In a first embodiment of the present invention there is provided an immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof, for use in a method of boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* in a subject, the method comprising the step of administering the immunogenic composition to the subject in an amount sufficient to elicit an immune response. More particularly, there is provided an immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof, for use in a method of treating or preventing an acute exacerbation of chronic obstructive pulmonary disease (AECOPD) in a subject, the method comprising boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* by administering the immunogenic composition to the subject in an amount sufficient to elicit an immune response. More particularly to increase the pre-existing immune response.

The term "pre-existing immunity" refers to a subject that has previously been exposed to a particular antigen or antigens and thus has a detectable serum antibody titer against the antigen(s) of interest. In contrast, the term "naive" refers to a subject that has not been previously exposed to a particular antigen or antigens and does not have a

detectable serum antibody titer against the antigen(s) of interest. The presence of pre-existing immunity may be verified, if necessary, by conventional methods known in the art. For example, a subject with pre-existing immunity, in other words a “seropositive subject”, can be identified by the presence of antibodies or other immune markers in serum, which indicate prior exposure to a particular antigen. In relation to the present invention, the pre-existing immunity results from prior vaccination or “priming” of the subject against *Haemophilus influenzae* and *Moraxella catarrhalis* by sequential administration of at least two doses, a first dose and a second dose, of an immunogenic composition comprising PD, PE, PilA and UspA2, or fragments thereof. Priming typically involves administration of the first dose of an immunogenic composition comprising PD, PE, PilA and UspA2, or fragments thereof at a first time point, followed by administration of the second dose of an immunogenic composition comprising PD, PE, PilA and UspA2, or fragments thereof at a second time point. The first and second time points will generally be separated by at least two weeks, and typically by approximately 8 weeks (two months or 60 days). If the first time point may is referred to as ‘Day 1’, a second time point 60 days later will be referred to as ‘Day 61’.

Following such priming, a third dose of an immunogenic composition is administered to improve, stimulate or expand the pre-existing immune response, for example, to stimulate an immune response that results in an increase in the levels of anti-PD, anti-PE, anti-PilA and anti-UspA2 antibodies, by way of non-limiting example, IgA, IgG or IgE. When the third dose is administered around a year after the first dose, it may generally be referred to as a booster dose. In some embodiments, the term “boost” or "boosting" is meant to include situations in which the concentration of vaccine specific antibodies such as IgG, IgG1, and IgG3 are significantly increased in treatment groups compared with corresponding placebo treatment groups. Such immune boosting may be effective in preventing or treating an acute exacerbation of chronic obstructive pulmonary disease (AECOPD) in a subject. Particularly, the third or booster dose is administered in an amount sufficient to elicit a further or additional immune response relative to, or when compared with, the pre-existing immune response.

The third dose may be administered to the patient at least six months after administration of the first dose of a vaccine, for example on or about Day 181. The third dose, in certain embodiments referred to as a booster dose, may be administered at least six, at least seven, at least eight, at least nine, at least ten, at least eleven or at least twelve

months after the first dose. For example, the third dose may be administered in the range of from between six to seven months, six to eight months, six to nine months, six to 10 months, six to 11 months or six to 12 months after the first dose. For example, on or about, day 181, day 211, day 241, day 271, day 301, day 331, day 361 or day 391. Particularly, in the range of from between five months to twelve and a half (12.5) months, five and a half (5.5) to twelve and a half (12.5) months, six to twelve months, from seven to twelve months, from eight to twelve months, from nine to twelve months, from ten to twelve months or from eleven to twelve months. For example, the third dose may be administered in the range of seven to twelve and a half (12.5) months, eight to twelve and a half (12.5) months, nine to twelve and a half (12.5) months, ten to twelve and a half (12.5) months or eleven to twelve and a half (12.5) months after the first dose. Particularly in the range of from between day 166 to day 391, day 181 to day 361, day 241 to day 361, day 271 to day 361, day 301 to day 361 or day 331 to day 391, particularly day 331 to day 365.

In one embodiment the immunogenic composition (e.g. third dose) is administered six to 13 months (e.g. administered between six and 12 months) after administration of the first of the at least two doses of vaccine. For example, the immunogenic composition (e.g. third dose) may be administered six months after administration of the first of the at least two doses of vaccine. For example, the immunogenic composition (e.g. third dose) may be administered 12 months after administration of the first of the at least two doses of vaccine.

The acute exacerbation of chronic obstructive pulmonary disease (AECOPD) associated with a bacterial infection may be defined by: (a) a positive bacterial pathogen on culture of an induced or spontaneous sputum sample obtained from a subject; and/or (b) a total aerobic CFU count greater than or equal to 10^7 bacterial cells; and/or (c) the presence of increased sputum purulence. Bacterial infection may also be determined by molecular detection, for example using polymerase chain reaction (PCR), sequencing of selected genes, particularly genes that are heterogeneous between strains of a species, such as the P2 gene of NTHi. Particularly, the bacterial exacerbation may be associated with a bacterial infection with: (a) *Haemophilus influenzae*, particularly non-typeable *Haemophilus influenzae* (NTHi), (b) *Moraxella catarrhalis* or (c) *Haemophilus influenzae*, particularly non-typeable *Haemophilus influenzae* (NTHi), and *Moraxella catarrhalis*, for example, as determined by positive bacterial culture.

The immunogenic compositions administered as the first, second and booster dose may be the same (homologous) or different (heterologous) but preferably they will comprise an immunologically effective amount of: (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof. By way of non-limiting example, heterologous immunogenic compositions may differ in terms of amount of antigen, formulation, adjuvant, vector, etc. Generally, the immunogenic compositions will be the same, i.e., in terms of formulation, antigen content, excipients, etc.

In certain embodiments, the immunogenic composition is for use in a method of protecting a subject against an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi. In certain embodiments, the immunogenic composition is for use in a method of reducing the risk of an exacerbation of chronic obstructive pulmonary disease (COPD) in a subject, particularly an exacerbation associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi. In certain embodiments, the immunogenic composition is for use in a method of protecting a subject against an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Moraxella catarrhalis*. In certain embodiments, the immunogenic composition is for use in a method of protecting a subject against an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi and *Moraxella catarrhalis*.

In certain embodiments, the immunogenic composition is for use in a method of reducing the severity of or delaying the onset of at least one symptom associated with an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi. In certain embodiments, the immunogenic composition is for use in a method of reducing the severity of or delaying the onset of at least one symptom associated with an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Moraxella catarrhalis*. In certain embodiments, the immunogenic composition is for use in a method of reducing the severity of or delaying the onset of at least one symptom associated with an exacerbation of chronic obstructive pulmonary disease (COPD)

associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi and *Moraxella catarrhalis*.

The invention further provides an immunogenic composition for use in a prime-boost immunisation method to treat or prevent an exacerbation of chronic obstructive pulmonary disease (COPD) associated with a bacterial infection by *Haemophilus influenzae* and *Moraxella catarrhalis* in a subject, the method comprising the steps of:
(a) administering to a subject a first dose of the immunogenic composition; and
(b) administering to the subject a second dose of the immunogenic composition; and
(c) administering to the subject a third dose of the immunogenic composition; wherein the immunogenic composition comprises: (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

The subject may be any suitable mammal but preferably is a human. The subject may be an adult human, for example, aged between 18 and 40 or between 50 and 70 or between 40 and 85 years of age. The subject has a previous history of Chronic Obstructive Pulmonary Disease (COPD), particularly, a previous history of moderate and severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD). For example, a confirmed diagnosis of COPD, categorised as moderate, severe, or very severe according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification. The Global Strategy for the Diagnosis, Management and Prevention of COPD prepared by GOLD state that COPD should be considered in any patient with dyspnea, chronic cough or sputum production, and/or a history of exposure to risk factors for the disease, such as tobacco smoking, occupation, or pollutants. A spirometry assessment, measuring airflow limitation, is required to establish diagnosis. The classification of airflow limitation severity in COPD outlined in the GOLD strategy is shown in Table 1.

Table 1. Classification of airflow limitation severity in COPD (Based on post-bronchodilator FEV_1) In patients with $FEV_1/FVC < 0.70$

GOLD 1	Mild	$FEV_1 \geq 80\%$ predicted
GOLD 2	Moderate	$50\% \leq FEV_1 < 80\%$ predicted
GOLD 3	Severe	$30\% \leq FEV_1 < 50\%$ predicted

GOLD 4	Very Severe	FEV_1 < 30% predicted

COPD assessment also includes analysis of patient symptoms, and this can be performed using comprehensive disease-specific health status questionnaires such as the Chronic Respiratory Questionnaire (CRQ) and St. George's Respiratory Questionnaire (SGRQ). For routine practice the COPD Assessment Test (CAT™) and The COPD Control Questionnaire (The CCQ©) have been developed. The CAT™ and CCQ© tests do not categorise patients for the purpose of treatment, however for the SRGQ assessment a symptom score ≥ 25 may be used as the threshold for considered regular treatment for breathlessness. The equivalent threshold for the CAT™ is 10. A simple assessment of breathlessness is the Modified British Medical Research Council (mMRC) Questionnaire. According to the GOLD strategy, of the patients classified at the GOLD 2 (moderate) stage, approximately 20% may experience frequent exacerbations requiring antibiotic and/or systemic corticosteroid therapy in addition to regular maintenance therapy. The risk of exacerbations is significantly higher for patients classified as GOLD 3 (severe) and GOLD 4 (very severe). The "ABCD" assessment tool is further used to understand a COPD patient's severity of disease. This assessment combines the patient's spirometry analysis with their exacerbation history and symptom assessment to give a spirometric grade combined with an "ABCD" group. The ABCD assessment tool is shown in Figure 2. In some embodiments, the subject has GOLD 2 (moderate), GOLD 3 (severe) or GOLD 4 (very severe) COPD status. The subject may be one that has experienced at least one (e.g. 2 or more, 3 or more) episodes of acute exacerbation in chronic obstructive pulmonary disease (AECOPD), particularly at least one (e.g. 2 or more, 3 or more) episodes of acute exacerbation in chronic obstructive pulmonary disease (AECOPD) within a period of 12 months. Yet more particularly the subject has experienced at least one (e.g. 2 or more, 3 or more) episode of acute exacerbation in chronic obstructive pulmonary disease (AECOPD) in the preceding 12 months. The subject may be a subject having bronchiectasis. In certain embodiments the subject has experienced an acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and failed to achieve resolution of symptoms after antibiotic therapy.

The skilled person will also understand that the invention is also applicable to methods of treatment. The invention therefore also provides: a method for the treatment or prevention of an acute exacerbation of chronic obstructive pulmonary disease (AECOPD) in a subject comprising administering to said subject an immunogenic composition

comprising an immunologically effective amount of (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

The present invention also provides a method of immunising a subject against *Haemophilus influenza* and *Moraxella catarrhalis* infection to treat or prevent an exacerbation of chronic obstructive pulmonary disease (COPD) comprising, administering to the subject an immunogenic composition comprising an immunologically effective amount of (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

The present invention also provides a method of inducing an immune response to *Haemophilus influenza* and *Moraxella catarrhalis* in a subject to treat or prevent an exacerbation of chronic obstructive pulmonary disease (COPD), the method comprising administering to the subject an immunogenic composition comprising an immunologically effective amount of (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

The invention further provides a prime-boost immunization method for inducing an immune response to *Haemophilus influenzae* and *Moraxella catarrhalis* to treat or prevent an exacerbation of chronic obstructive pulmonary disease (COPD), the method comprising the steps of:

(a) administering to a subject a first immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof; and

(b) administering to the subject a second immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a

fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof; and

(c) administering to the subject a third immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof; wherein at least one of the first, second or third immunogenic compositions. In some embodiments, the first, second and third immunogenic compositions are heterologous compositions. In other embodiments, the first, second and third immunogenic compositions are homologous compositions.

In certain embodiments there is provided a vaccination protocol comprising administering a first, a second and a third immunologically effective dose of an immunogenic composition to a subject, wherein the third dose of the immunogenic composition is administered at least 6 months after administration of the first dose of the immunogenic composition, wherein the immunogenic composition comprises (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

In certain embodiments, the method is a method of protecting a subject against an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi. In certain embodiments, the method is a method of protecting a subject against an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Moraxella catarrhalis*. In certain embodiments, the method is a method of protecting a subject against an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi and *Moraxella catarrhalis*.

In certain embodiments, the method is a method of reducing the severity of or delaying the onset of at least one symptom associated with an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi. In certain embodiments, the method is a

method of reducing the severity of or delaying the onset of at least one symptom associated with an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Moraxella catarrhalis*. In certain embodiments, the method is a method of reducing the severity of or delaying the onset of at least one symptom associated with an exacerbation of chronic obstructive pulmonary disease (COPD) associated with or caused by a bacterial infection with *Haemophilus influenza,* particularly NTHi and *Moraxella catarrhalis*.

In other embodiments, the present invention provides immunogenic compositions and vaccines for use in the manufacture of a medicament for treating or preventing an exacerbation of chronic obstructive pulmonary disease (COPD) associated with a bacterial infection in a subject caused by *Haemophilus influenza,* particularly NTHi and *Moraxella catarrhalis*.

By way of non-limiting example, Figures 3(a) and 3(b) provide a schematic of generalised regimens of the invention.

Immunogenic compositions

The term “immunogenic composition” broadly refers to any composition that may be administered to elicit an immune response, such as an antibody or cellular immune response, against an antigen present in the composition. Thus, compositions of the invention are immunogenic. When the immunogenic compositions prevent, ameliorate, palliate or eliminate disease from the subject, then such compositions may be referred to as a vaccine. Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat infection), but will typically be prophylactic. In certain embodiments, the immunogenic composition is a vaccine. The term “antigen” refers to a substance that, when administered to a subject, elicits an immune response directed against the substance. In the context of the present invention, PD, PE, PilA, UspA2 (including fragments thereof) are antigens. Preferably the PD, PE, Pila and UspA2 antigens are recombinant antigens prepared or manufactured using recombinant DNA technology. Particularly, when administered to a subject the immunogenic composition elicits an immune response directed against PD, PE, PilA, UspA2. Particularly the immune response directed against PD, PE, PilA, UspA2 is protective, that is, it can prevent or reduce infection or colonisation caused by *Haemophilus influenzae* and/or *Moraxella catarrhalis*.

Protein D (PD)

The immunogenic composition for use in the invention comprises protein D or an immunogenic fragment thereof from *Haemophilus influenza*. Protein D (PD) is a highly conserved 42 kDa surface lipoprotein found in all *Haemophilus influenzae*, including nontypeable *Haemophilus influenzae*. Inclusion of this protein in the immunogenic composition may provide a level of protection against *Haemophilus influenzae* related otitis media [**19**]. Suitable amino acid sequences for PD include, for example, the protein D sequence from Figure 9 of EP 0594610 (Figure 9a and 9b together, 364 amino acids) and as described in WO91/18926 or WO00/56360 (disclosed herein as SEQ ID NOs: 1 and 2. Other suitable proteins may be encoded by, for example, Genbank accession numbers: X90493 (SEQ ID NO:3), X90489 (SEQ ID NO:4), X90491 (SEQ ID NO:5), Z35656 (SEQ ID NO:6), Z35657 (SEQ ID NO:7), Z35658 (SEQ ID NO:8), M37487 (SEQ ID NO:9).

One skilled in the art will further recognise that immunogenic compositions may comprise polypeptides having sequence identity to Protein D provided that such polypeptides are capable of generating an immune response to Protein D, for example, they comprise one or more epitopes of protein D. Thus, immunogenic compositions may comprise an isolated immunogenic polypeptide having sequence identity of at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to SEQ ID NO:1 wherein the isolated immunogenic polypeptide is capable of eliciting an immune response against SEQ ID NO:1, particularly an immune response that results in the formation of antibodies that bind to SEQ ID NO:1.

Protein E (PE)

Protein E is an outer membrane lipoprotein with adhesive properties. It plays a role in the adhesion/invasion of non-typeable *Haemophilus influenzae* (NTHi) to epithelial cells [**20**, **21**, **22**]. It is highly conserved in both encapsulated *Haemophilus influenzae* and non-typeable *Haemophilus influenzae* and has a conserved epithelial binding domain [**23**]. Thirteen different point mutations have been described in different *Haemophilus* species when compared with *Haemophilus influenzae* Rd as a reference strain. Its expression is observed on both logarithmic growing and stationary phase bacteria (WO2007/084053). Protein E is also involved in human complement resistance through binding vitronectin. [**24**]. PE, by the binding domain PKRYARSVRQ YKILNCANYH LTQVR (SEQ ID NO:10, corresponding to amino acids 84-108 of SEQ ID NO:11), binds vitronectin which is an important inhibitor of the terminal complement pathway [**24**].

Protein E from *H. influenza* (also referred to as: "protein E", "Prot E" and "PE") may consist of or comprise the amino acid sequence of SEQ ID NO:11 (corresponding to SEQ ID NO:4 of WO2012/139225A1). One skilled in the art will further recognise that immunogenic compositions may comprise polypeptides having sequence identity to Protein E provided that such polypeptides are capable of generating an immune response to Protein E, for example, they comprise one or more epitopes of Protein E. Thus, immunogenic compositions may comprise an isolated immunogenic polypeptide having sequence identity of at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to SEQ ID NO:11 wherein the isolated immunogenic polypeptide is capable of eliciting an immune response against SEQ ID NO:11, particularly an immune response that results in the formation of antibodies that bind to SEQ ID NO:11. The immunogenicity of PE polypeptides may be measured as described in WO2012/139225A1 herein incorporated by reference.

Pilin A (PilA)

Pilin A (PilA) is likely the major pilin subunit of *H. influenzae* Type IV Pilus (Tfp) involved in twitching motility [**25**]. NTHi PilA is a conserved adhesin expressed in vivo. It has been shown to be involved in NTHi adherence, colonization and biofilm formation [**26**]. PilA may consist of or comprise the protein sequence of SEQ ID NO:12 (corresponding to SEQ ID NO. 58 of WO2012/139225A1). One skilled in the art will further recognise that immunogenic compositions may comprise polypeptides having sequence identity to Pilin A provided that such polypeptides are capable of generating an immune response to PilA, for example, they comprise one or more epitopes of PilA. Thus, immunogenic compositions may comprise an isolated immunogenic polypeptide having sequence identity of at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to SEQ ID NO:12 wherein the isolated immunogenic polypeptide is capable of eliciting an immune response against SEQ ID NO:12, particularly an immune response that results in the formation of antibodies that bind to SEQ ID NO:12. The immunogenicity of PilA polypeptides may be measured as described in WO2012/139225A1 herein incorporated by reference.

Ubiquitous surface protein A2 (UspA2)

As used herein "UspA2" means Ubiquitous surface protein A2 from *Moraxella catarrhalis* (*M. catarrhalis*; Mcat). Ubiquitous surface protein A2 is a trimeric autotransporter

identified in *Moraxella catarrhalis* that appears as a lollipop-shared structure in electron micrographs [**27**]. It is composed of an N-terminal head, followed by a stalk which ends in an amphipathic helix and a C-terminal membrane domain [**27**]. UspA2 contains a very well conserved domain [**28**], which is recognized by a monoclonal antibody that was shown protective upon passive transfer in a mouse *Moraxella catarrhalis* challenge model [**29**]. UspA2 has been shown to interact with host structures and extracellular matrix proteins like fibronectin [**30**] and laminin [**31**] suggesting it can play a role at an early stage of *Moraxella catarrhalis* infection. UspA2 also seems to be involved in the ability of *Moraxella catarrhalis* to resist the bactericidal activity of normal human serum [**32**]. It (i) binds the complement inhibitor C4bp, enabling *Moraxella catarrhalis* to inhibit the classical complement system, (ii) prevents activation of the alternative complement pathway by absorbing C3 from serum and (iii) interferes with the terminal stages of the complement system, the Membrane Attack Complex (MAC), by binding the complement regulator protein vitronectin [**33**]. UspA2 may consist of or comprise the amino acid sequence of SEQ ID NO:13 from ATCC 25238 as well as sequences with at least or exactly 63%, 66%, 70%, 72%, 74%, 75%, 77%, 80%, 84%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity, over the entire length, to SEQ ID NO: 13.

UspA2 as described in SEQ ID NO:13 contains a signal peptide at amino acids 1 to 29 (SEQ ID NO:14), a laminin binding domain at amino acids 30 to 177 (SEQ ID NO: 15), a fibronectin binding domain at amino acids 165 to 318 (SEQ ID NO:16) (Tan et al. JID 192: 1029-38 (2005)), a C3 binding domain at amino acids 30 to 539 (SEQ ID NO:17) (WO2007/018463) or a fragment of amino acids 30 to 539 of SEQ ID NO: 10, for example, amino acids 165 to 318 of SEQ ID NO: 1 (Hallström T et al. J. Immunol. 186: 3120-3129 (2011)), an amphipathic helix at amino acids 519 to 564 (SEQ ID NO:18) or amino acids 520-559 (SEQ ID NO:19), (identified using different prediction methods) and a C terminal anchor domain at amino acids 576 to 630 (SEQ ID NO:20) (Brooks et al., Infection & Immunity, 76(11), 5330-5340 (2008)). UspA2 amino acid differences have been described for various *Moraxella catarrhalis* species. See for example, J Bacteriology 181(13):4026-34 (1999), Infection and Immunity 76(11):5330-40 (2008) and PLoS One 7(9):e45452 (2012).

UspA2 may consist of or comprise an amino acid sequence that differs from SEQ ID NO:13 at any one or more amino acid selected from the group consisting of: AA (amino acid) 30 to 298, AA 299 to 302, AA 303 to 333, AA 334 to 339, AA 349, AA 352 to 354, AA

368 to 403, AA 441, AA 451 to 471, AA 472, AA474 to 483, AA 487, AA 490, AA 493, AA 529, AA 532 or AA 543. UspA2 may consist of or comprise an amino acid sequence that differs from SEQ ID NO:13 in that it contains an amino acid insertion in comparison to SEQ ID NO:13. UspA2 may consists of or comprise an amino acid sequence that differs from SEQ ID NO:13 at any one of the amino acid differences in SEQ ID NO: 21 through SEQ ID NO: 57. For example, SEQ ID NO:13 may contain K instead of Q at amino acid 70, Q instead of G at amino acid 135 and/or D instead of N at amino acid 216. Further amino acid sequences of UspA2 from 38 strains of *Moraxalla catarrhalis* are provided as SEQ ID NOs: 21 to 57. WO2015/125118A1 describes compositions comprising *Moraxella catarrhalis* (*M.catarrhalis*, Mcat) Ubiquitous surface protein A2 (UspA2).

Immunogenic fragments

In certain embodiments, immunogenic fragments of the above proteins may also be used. As used herein the term "fragment" refers to a sequence that is a subset of another sequence. The term is used to refer to a part or portion of an intact or complete wild-type polypeptide but which comprise fewer amino acid residues than the intact or complete wild-type polypeptide. Thus, the term refers to truncated or shorter amino acid sequences corresponding to one or more regions of a wild-type or reference polypeptide and it is to be understood that as used herein, the term fragment excludes reference to the full-length or wild-type polypeptide sequence. One example of a fragment is an epitope sequence. A fragment or subsequence of an amino acid sequence can be any number of residues less than that found in the naturally occurring, or reference, polypeptide. However, it will be clear to one skilled in the art that, in the context of the present invention, any such immunogenic fragments must be capable of eliciting an immune response against the full length polypeptide, particularly an immune response that results in the formation of antibodies capable of binding to the full length polypeptide. Fragments of a protein can be produced using techniques known in the art, e.g. recombinantly, by proteolytic digestion, or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide.

The fragments should comprise at least n consecutive amino acids from the sequences and, depending on the particular sequence, n is 7 or more (e.g. 7, 10, 15, 20, 25, 30 or 50 or more). The fragments may comprise an amino acid sequence of from 7 amino acid residues up to 10, up to 15, up to 20, up to 30 or up to 50 consecutive amino

acid residues. The fragments may comprise an amino acid sequence of more than 7 amino acid residues but less than 50, less than 40, less than 30, less than 25, less than 20, less than 15 or less than 10 consecutive amino acid residues. Preferred fragments may comprise one or more epitopes from the sequence. To the extent that such fragments are used, they will share the immunogenic properties of the naturally occurring, or reference, polypeptide, more particularly the 'immunogenic property' (or properties) in the context of the present invention, is the ability to elicit a therapeutic immune response against nontypeable *Haemophilus influenzae* or *Moraxella catarrhalis* (e.g. provide or induce a protective effect which is at least 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of that shown by the relevant or corresponding nontypeable *Haemophilus influenzae* or *Moraxella catarrhalis* sequence referred to in the sequence listing).

It will be clear to those skilled in the art that, whilst such fragments are truncated or shorter fragments of a reference sequence, such fragments may be modified to comprise additional sequences not found in the reference polypeptide, for example, to form fusion polypeptides, include 'tag' sequences such as His tags or Glutathione S- transferase (GST) tags, linker sequences and the like. Thus, in such modified fragments the amino group of the N terminal amino acid of the fragment is not linked by a peptide bond to the carboxyl group of an amino acid to which it would be linked in the reference polypeptide from which it is derived and/or the carboxyl group of the C terminal amino acid of the fragment is not linked by a peptide bond to the amino group of an amino acid to which it would be linked in the reference polypeptide from which it is derived.

Particular immunogenic fragments of Protein D comprise or consist of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of, for example, SEQ ID NO: 1 or 2. Preferably, the immunogenic fragments elicit antibodies that can bind to SEQ ID NO:1 or 2. In particular embodiments, a protein D immunogenic fragment sequence may comprise (or consist) of the protein D fragment described in EP0594610 which begins at the sequence SSHSSNMANT (SEQ ID NO:58), and lacks the 19 N-terminal amino acids from Fig 9 of EP0594610, optionally with the tripeptide MDP from NS1 fused to the N-terminal of said protein D fragment (348 amino acids) (SEQ ID NO:2). The protein D or fragment of protein D may be lipidated or un-lipidated. Particularly, the Protein D or fragment of Protein D is un-lipidated. The immunogenic composition may comprise or consist of an immunogenic fragment of Protein D, suitably an isolated immunogenic polypeptide having at least 70%,

80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:1

Particular immunogenic fragments of Protein E comprise or consist of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of SEQ ID NO:11. Preferably, the immunogenic fragments elicit antibodies that can bind to SEQ ID NO: 11. The immunogenic composition may comprise an immunogenic fragment of Protein E, suitably an isolated immunogenic polypeptide having at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:59 (corresponding to SEQ ID NO: 125 of WO2012/139225A1): SEQ ID NO:59: Amino acids 20-160 of Protein E.

Particular immunogenic fragments of PilA comprise or consist of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of SEQ ID NO:12. Preferably, the immunogenic fragments elicit antibodies that can bind to SEQ ID NO:12. The immunogenic composition may comprise an immunogenic fragment of Pilin A, suitably an isolated immunogenic polypeptide having at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO:60 (corresponding to Seq ID No. 127 of WO2012/139225A1): SEQ ID NO:60 Amino acids 40-149 of PilA from H. influenzae strain 86-028NP.

Immunogenic fragments of UspA2 comprise immunogenic fragments of at least 450 contiguous amino acids of SEQ ID NO: 1, 490 contiguous amino acids of SEQ ID NO: 13 (for example, the UspA2 fragment of MC-004 or MC-005), 511 contiguous amino acids of SEQ ID NO: 13 (for example, the UspA2 fragment of construct MC-001, MC-002, MC-003 or MC-004), 534 contiguous amino acids of SEQ ID NO: 13 (for example, the UspA2 fragment of MC-009 or MC-011) or 535 contiguous amino acids of SEQ ID NO: 13 (for example, the UspA2 fragment of MC-007, MC-008 or MC-010). The immunogenic fragments may elicit antibodies which can bind SEQ ID NO: 13.

Immunogenic fragments of UspA2 may comprise immunogenic fragments of at least 450, 490, 511, 534 or 535 contiguous amino acids of SEQ ID NO: 13. Immunogenic fragments of UspA2 may comprise immunogenic fragments of UspA2, for example any of the UspA2 constructs MC-001 (SEQ ID NO:61), MC-002 (SEQ ID NO:62), MC-003 (SEQ ID NO:63), MC-004 (SEQ ID NO:64), MC-005 (SEQ ID NO:65), MC-006 (SEQ ID NO:66), MC-007 (SEQ ID NO:67), MC-008 (SEQ ID NO:68), MC-009 (SEQ ID NO:69), MC-010

(SEQ ID NO:70) or MC-011 (SEQ ID NO:71). UspA2 constructs MC-001 to MC-011 are further described in WO2015/125118. The immunogenic fragments may elicit antibodies which can bind the full length sequence from which the fragment is derived.

In another aspect of the invention, the immunogenic composition comprises an immunogenic fragment of UspA2, suitably an isolated immunogenic polypeptide with at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to a polypeptide selected from the group consisting of MC-001 (SEQ ID NO:61), MC-002 (SEQ ID NO:62), MC-003 (SEQ ID NO:63), MC-004 (SEQ ID NO:64), MC-005 (SEQ ID NO:65), MC-006 (SEQ ID NO:66), MC-007 (SEQ ID NO:67), MC-008 (SEQ ID NO:68), MC-009 (SEQ ID NO:69), MC-010 (SEQ ID NO:70) or MC-011 (SEQ ID NO:71) for example MC009 SEQ ID NO:69 (corresponding to Seq ID NO: 69 of WO2015/125118A1). In an embodiment, an immunogenic fragment of UspA2 contains a laminin binding domain and a fibronectin binding domain. In an additional embodiment, an immunogenic fragment of UspA2 contains a laminin binding domain, a fibronectin binding domain and a C3 binding domain. In a further embodiment, an immunogenic fragment of UspA2 contains a laminin binding domain, a fibronectin binding domain, a C3 binding domain and an amphipathic helix. Immunogenicity of UspA2 polypeptides may be measured as described in WO2015/125118A1; the contents of which are incorporated herein by reference.

Fusions

The polypeptides described herein can also be provided in other forms, such as in the form of a fusion protein. Particularly, Protein E and Pilin A may be provided in the form of a fusion protein (PE-PilA). Suitable fusions are disclosed in WO2012/139225 and a preferred fusion is SEQ ID NO:72 (corresponding to sequence number 194 of WO2012/139225). Thus, the immunogenic composition may comprise a polypeptide having at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 72 and/or 73.

Thus, in particular embodiments of the invention, the immunogenic composition comprises both Protein E and PilA in the form of a fusion protein, suitably an isolated immunogenic polypeptide with at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% to LVL-735, wherein the signal peptide has been removed, SEQ ID NO. 73 (Corresponding to Seq ID No. 219 of WO2012/139225A1). As used herein “signal peptide” refers to a short (less than 60 amino acids, for example, 3 to 60 amino

acids) polypeptide present on precursor proteins (typically at the N terminus), and which is typically absent from the mature protein. The signal peptide (sp) is typically rich in hydrophobic amino acids. The signal peptide directs the transport and/or secretion of the translated protein through the membrane. Signal peptides may also be called targeting signals, transit peptides, localization signals, or signal sequences. For example, the signal sequence may be a co-translational or post-translational signal peptide. The immunogenicity of Protein E (PE) and Pilin A (PilA) polypeptides may be measured as described in WO2012/139225A1; the contents of which are incorporated herein by reference.

Particular immunogenic compositions for use in the present invention will comprise (1) protein D, (2) a PE-PilA fusion protein and (3) UspA2. In certain embodiments, the immunogenic composition for use in the present invention comprise a recombinant UspA2 protein having at least 95% sequence identity to SEQ ID NO: 69, a recombinant Protein D protein having at least 95% sequence identity to SEQ ID NO:1 and a recombinant PE-PilA fusion protein having at least 95% sequence identity to SEQ ID NO: 72. Immunogenic compositions for use in the present invention may comprise (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 10 µg of UspA2 and an (4) adjuvant, particularly AS01E. Immunogenic compositions for use in the present invention may comprise (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 3.3 µg of UspA2 and an (4) adjuvant, particularly AS01E. Particularly, the PE-PilA fusion protein is the LVL735 construct (SEQ ID NO:72), as described in WO2012/139225. Particularly the UspA2 protein is the MC009 construct (SEQ ID NO:69), as described in WO2015125118. In certain embodiments, the immunogenic composition for use in the present invention comprise (1) 10 µg of a recombinant UspA2 protein of SEQ ID NO: 69, (2) 10 µg of a recombinant Protein D protein of SEQ ID NO:1 and (3) 10 µg of a recombinant PE-PilA fusion protein of SEQ ID NO: 72. In certain embodiments, the immunogenic composition for use in the present invention comprise (1) 3.3 µg of a recombinant UspA2 protein of SEQ ID NO: 69, (2) 10 µg of a recombinant Protein D protein of SEQ ID NO:1 and (3) 10 µg of a recombinant PE-PilA fusion protein of SEQ ID NO: 72. In other embodiments, the immunogenic composition for use in the present invention consists essentially of (1) 10 µg of a recombinant UspA2 protein of SEQ ID NO: 69, (2) 10 µg of a recombinant Protein D protein of SEQ ID NO:1, (3) 10 µg of a recombinant PE-PilA fusion protein of SEQ ID NO: 72 and (4) adjuvant AS01E. In other embodiments, the immunogenic composition for use in the present invention consists essentially of (1) 3.3 µg of a recombinant UspA2 protein of SEQ ID NO: 69, (2) 10 µg of a

recombinant Protein D protein of SEQ ID NO:1, (3) 10 µg of a recombinant PE-PilA fusion protein of SEQ ID NO: 72 and (4) adjuvant AS01E.

Formulations

Immunogenic compositions of the invention will generally comprise a pharmaceutically acceptable carrier. A 'pharmaceutically acceptable carrier' is a carrier that does not itself induce the production of antibodies. Such carriers are well known to those of ordinary skill in the art and include, by way of non-limiting example, polysaccharides, polylactic acids, polyglycolic acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Immunogenic compositions may also contain diluents, such as water, saline, glycerol, and the like. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical diluent. Such compositions may also include, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, etc. The pH of the composition may be between pH 6 and pH 8, particularly about pH 7. Stable pH may be maintained by the use of a buffer. Compositions may include an antimicrobial and/or a detergent such as Tween (polysorbate).

Suitable immunogenic compositions may be in aqueous form, for example, as a solution or suspension or in a dried form, for example, lyophilised. Dried or lyophilised compositions are generally reconstituted with a liquid medium prior to injection. For lyophilisation, a stabiliser such as a sugar alcohol (e.g. mannitol) and/or a disaccharide (e.g. sucrose or trehalose) may be included. Immunogenic compositions are preferably sterile and may also be pyrogen-free. Compositions may be isotonic with respect to the subject's body.

Immunogenic compositions may be prepared in various forms, in vials or as injectables in ready filled syringes, either with or without needles. Syringes generally contain a single dose of the composition, whilst a vial may contain a single dose or multiple doses. Compositions may be prepared for pulmonary administration, for example, as a fine powder or a spray for administration using an inhaler. Other forms for administration are known to the skilled person including, by way of non-limiting example, solid dosage forms, suppositories and pessaries, compositions for nasal, aural or ocular administration such as sprays, drops, gels or powders.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of antigens. The term “immunologically effective amount” refers to the amount of an antigen or antigens needed to stimulate or achieve the desired immunologic effect, particularly a cellular (T cell) response, a humoral (B cell or antibody) response, or both, as measured by standard assays known to one skilled in the art. This amount may vary depending upon the health and physical condition of the subject to be treated, age, capacity of the individual's immune system to synthesise antibodies, degree of protection desired, formulation and the like. One skilled in the art understands that the immunologically effective amount is the amount of antigen administered to a subject in a single dose and that the amount can be determined through routine trials, such as clinical or dose-ranging trials, and may fall within a range.

The amount of each individual protein antigen in a single dose of immunogenic composition will generally be from 1 µg (0.001 mg) to 120 µg (0.120 mg). The typical amount of immunogenic polypeptide or immunogenic fragment thereof from *Moraxella catarrhalis* may be expected to lie in the range of from about 1 µg (0.001 mg) to 120 µg (0.120 mg). More particularly in the range of from about 2.5 µg (0.0025 mg) to about 30 µg (0.03 mg), yet more particularly, in the range of from about 2.5 µg (0.0025 mg) to about 3.5 µg (0.0035 mg) of protein, for example about 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4 or 3.5 µg of protein. In general, the typical amount of the immunogenic polypeptide or immunogenic fragment thereof from *H. influenzae* may be expected to lie in the range of from about 5 µg (0.005 mg) to about 50 µg (0.05 mg) of protein, for example about 9.5, 9.6, 9.7, 9.8, 9.9, 10,10.1, 10.2, 10.3, 10.4, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 20, 25, 30, 35, 40, 45 or 50 µg of protein. The skilled person understands that in a multi-component immunogenic composition, i.e. one containing at least two different antigens, the immunologically effective amount of each antigen is likely to be different and therefore represents a proportion of the total amount of protein antigen per dose. By way of non-limiting example, an immunogenic composition that comprises an immunologically effective amount, X µg, of a first antigen and an immunologically effective amount, Y µg, of a second antigen will comprise X+Y µg of total protein antigen per dose.

Immunogenic compositions will generally comprise one or more adjuvants. As used herein, “adjuvant” means a compound or substance (or combination of compounds or substances) that, when administered to a subject in conjunction with an antigen or antigens, for example as part of an immunogenic composition or vaccine, increases or enhances the

subject's immune response to the administered antigen or antigens (compared to the immune response obtained in the absence of adjuvant).

Suitable adjuvants include an aluminum salt such as aluminum hydroxide gel or aluminum phosphate or alum, but may also be a salt of calcium, magnesium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatized saccharides, or polyphosphazenes. In one embodiment, the protein may be adsorbed onto aluminium phosphate. In another embodiment, the protein may be adsorbed onto aluminium hydroxide. In a third embodiment, alum may be used as an adjuvant.

Suitable adjuvant systems which promote a predominantly Th1 response include: non-toxic derivatives of lipid A, Monophosphoryl lipid A (MPL) or a derivative thereof, particularly 3-de-O-acylated monophosphoryl lipid A (3D-MPL) (for its preparation see GB 2220211 A); and a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with either an aluminum salt (for instance aluminum phosphate or aluminum hydroxide) or an oil-in-water emulsion. In such combinations, antigen and 3D-MPL are contained in the same particulate structures, allowing for more efficient delivery of antigenic and immunostimulatory signals. Studies have shown that 3D-MPL is able to further enhance the immunogenicity of an alum-adsorbed antigen (Thoelen et al. Vaccine (1998) 16:708-14; EP 689454-B1).

AS01 is an Adjuvant System containing MPL (3-O-desacyl-4'- monophosphoryl lipid A), QS21 ((Quillaja saponaria Molina, fraction 21) Antigenics, New York, NY, USA) and liposomes. AS01B is an Adjuvant System containing MPL, QS21 and liposomes (50 µg MPL and 50 µg QS21). AS01E is an Adjuvant System containing MPL, QS21 and liposomes (25 µg MPL and 25 µg QS21). In one embodiment, the immunogenic composition or vaccine comprises AS01. In another embodiment, the immunogenic composition or vaccine comprises AS01B or AS01E. In a particular embodiment, the immunogenic composition or vaccine comprises AS01E.

AS02 is an Adjuvant System containing MPL and QS21 in an oil/water emulsion. AS02V is an Adjuvant System containing MPL and QS21 in an oil/water emulsion (50 µg MPL and 50 µg QS21).

AS03 is an Adjuvant System containing α-Tocopherol and squalene in an oil/water (o/w) emulsion. AS03A is an Adjuvant System containing α-Tocopherol and squalene in an o/w emulsion (11.86 mg tocopherol). AS03B is an Adjuvant System containing α-Tocopherol and squalene in an o/w emulsion (5.93 mg tocopherol). AS03C is an Adjuvant System containing α-Tocopherol and squalene in an o/w emulsion (2.97 mg tocopherol). In one embodiment, the immunogenic composition or vaccine comprises AS03.

AS04 is an Adjuvant System containing MPL (50 µg MPL) adsorbed on an aluminum salt (500 µg Al^{3+}). In one embodiment, the immunogenic composition or vaccine comprises AS04.

A system involving the use of QS21 and 3D-MPL is disclosed in WO 94/00153. A composition wherein the QS21 is quenched with cholesterol is disclosed in WO 96/33739. An additional adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210. In one embodiment the immunogenic composition additionally comprises a saponin, which may be QS21. The formulation may also comprise an oil in water emulsion and tocopherol (WO 95/17210). Unmethylated CpG containing oligonucleotides (WO 96/02555) and other immunomodulatory oligonucleotides (WO 0226757 and WO 03507822) are also preferential inducers of a TH1 response and are suitable for use in the present invention.

Additional adjuvants are those selected from the group of metal salts, oil in water emulsions, Toll like receptor agonists, (in particular Toll like receptor 2 agonist, Toll like receptor 3 agonist, Toll like receptor 4 agonist, Toll like receptor 7 agonist, Toll like receptor 8 agonist and Toll like receptor 9 agonist), saponins or combinations thereof.

Possible excipients include arginine, pluronic acid and/or polysorbate. In a preferred embodiment, polysorbate 80 (for example, TWEEN (a US registered trademark) 80) is used. In a further embodiment, a final concentration of about 0.03% to about 0.06% is used. Specifically, a final concentration of about 0.03%, 0.04%, 0.05% or 0.06% polysorbate 80 (w/v) may be used.

Formulations comprising the immunogenic compositions of the invention may be adapted for administration by an appropriate route, for example, by the intramuscular,

sublingual, transcutaneous, intradermal or intranasal route. Such formulations may be prepared by any method known in the art.

Kits

The invention further provides kits for use in the methods of the invention comprising a first container comprising a lyophilised immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof and a second container comprising a liquid comprising AS01E. In certain particular embodiments, the kit further comprises a buffer. In certain other embodiments, the kit further comprises instructions for use.

General

The term "comprising" encompasses "including" e.g. a composition "comprising" X may include something additional e.g. X + Y. The word "substantially" does not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. In some implementations, the term "comprising" refers to the inclusion of the indicated active agent, such as recited polypeptides, as well as inclusion of other active agents, and pharmaceutically acceptable carriers, excipients, emollients, stabilizers, etc., as are known in the pharmaceutical industry. In some implementations, the term "consisting essentially of" refers to a composition, whose only active ingredient is the indicated active ingredient(s), for example antigens, however, other compounds may be included which are for stabilizing, preserving, etc. the formulation, but are not involved directly in the therapeutic effect of the indicated active ingredient. Use of the transitional phrase "consisting essentially" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim, and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. See, In re Herz, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976) (emphasis in the original); see also MPEP § 2111.03. Thus, the term "consisting essentially of" when used in a claim of this invention is not intended to be interpreted to be equivalent to "comprising". The term "consisting of" and variations thereof means limited to" unless expressly specified otherwise. In certain territories, the term "comprising an active ingredient consisting of" may be used in place of "consisting essentially". The term "about" in relation to a numerical value x means, for example, x ± 10%, x ± 5%, x ± 4%, x ± 3%, x ± 2%, x ± 1%. The word "substantially" does not exclude

"completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention. Where methods refer to steps of administration, for example as (a), (b), (c), etc., these are intended to be sequential, i.e., step (c) follows step (b) which is preceded by step (a). Antibodies will generally be specific for their target, i.e., they will have a higher affinity for the target than for an irrelevant control protein, such as bovine serum albumin.

Identity between polypeptides may be calculated by various algorithms. For example, the Needle program, from the EMBOSS package (Free software; EMBOSS: The European Molecular Biology Open Software Suite (2000). Trends in Genetics 16(6): 276—277) and the Gap program from the GCG® package (Accelrys Inc.) may be used. This Gap program is an implementation of the Needleman-Wunsch algorithm described in: Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453. The BLOSUM62 scoring matrix has been used, and the gap open and extension penalties were respectively 8 and 2.

Looking at the computed alignment, identical residues between two compared sequences can be observed. A percentage of identity can be computed by (1) calculating the number of identities divided by the length of the alignment, multiplied by 100 (for example, for the Needle program analysis), (2) calculating the number of identities divided by the length of the longest sequence, multiplied by 100, (3) calculating the number of identities divided by the length of the shortest sequence, multiplied by 100, or (4) calculating the number of identities divided by the number of aligned residues, multiplied by 100 (a residue is aligned if it is in front of another) (for example, for the Gap program analysis).

Generally, sequence identity is calculated over the entire length of the reference sequence, for example the full-length or wild-type sequence. Amino acid substitution may be conservative or non-conservative. In some embodiments, amino acid substitution is conservative. Substitutions, deletions, additions or any combination thereof may be combined in a single variant so long as the variant is an immunogenic polypeptide. Embodiments herein relating to "vaccine compositions" of the invention are also applicable to embodiments relating to "immunogenic compositions" of the invention, and vice versa.

All references or patent applications cited within this patent specification are incorporated by reference herein.

Aspects of the Invention

The following clauses describe additional embodiments of the invention:

Embodiment 1. An immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof, for use in a method of boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat) in a subject, wherein the pre-existing immune response has been elicited by previous administration of at least two doses of a vaccine comprising PD, PE, PilA and UspA2, the method comprising the step of administering the immunogenic composition to the subject in an amount sufficient to elicit an immune response in the subject against PD, PE, PilA and UspA2, particularly in an amount sufficient to elicit a further or additional, immune response against PD, PE, PilA and UspA2 relative to the pre-existing immune response.

Embodiment 2. The immunogenic composition for use of Embodiment 1, wherein the subject has a previous history of Chronic Obstructive Pulmonary Disease (COPD).

Embodiment 3. The immunogenic composition for use of Embodiment 2, wherein the subject has a previous history of moderate and severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD).

Embodiment 4. The immunogenic composition for use of Embodiment 2 or 3 wherein the immunogenic composition is administered six to 12 months after administration of the first of the at least two doses of vaccine.

Embodiment 5. The immunogenic composition for use of Embodiment 4 wherein the immunogenic composition is subsequently administered every 12 months on the anniversary of administration of the first of the at least two doses of vaccine.

Embodiment 6. The immunogenic composition for use of any preceding Embodiment, wherein the immune response against PD, PE, PilA and UspA2 is sufficient to induce

protective or therapeutic immunity against non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat).

Embodiment 7. The immunogenic composition for use of any preceding Embodiment, wherein the immune response against PD, PE, PilA and UspA2 is sufficient to reduce the frequency of AECOPD.

Embodiment 8. The immunogenic composition for use of Embodiment 6 or 7, wherein the subject is a human.

Embodiment 9. The immunogenic composition for use of Embodiment 8, wherein the subject is an adult human aged between 18 and 40 or between 50 and 70 or between 40 and 80 years of age.

Embodiment 10. The immunogenic composition for use of Embodiment 9, wherein the subject has a smoking history of at least ten pack years.

Embodiment 11. The immunogenic composition for use of any preceding Embodiment wherein the UspA2 is at least 63%, 66%, 70%, 72%, 74%, 75%, 77%, 80%, 84%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical, over the entire length, to SEQ ID NO: 13.

Embodiment 12. The immunogenic composition for use of any preceding Embodiment wherein the UspA2 consists essentially of an immunogenic fragment of UspA2 selected from the group consisting of amino acids 30-540 of SEQ ID NO. 13 (SEQ ID NO: 61, 62, 63 or 64), amino acids 31-540 of SEQ ID NO: 13 (SEQ ID NO: 71), amino acids 30-519 of SEQ ID NO: 13 (SEQ ID NO: 65 or 66), amino acids 30-564 of SEQ ID NO: 13 (SEQ ID NO: 67 or 68) and amino acids 31-564 of SEQ ID NO: 13 (SEQ ID NO: 69 or 70).

Embodiment 13. The immunogenic composition for use of any preceding Embodiment wherein PE and PilA are present as a fusion protein, particularly SEQ ID NO:72 or SEQ ID NO:73.

Embodiment 14. The immunogenic composition for use of any preceding Embodiment wherein the immunogenic composition comprises UspA2 (SEQ ID NO: 69), Protein D (SEQ ID NO:1) and a PE-PilA fusion protein (SEQ ID NO: 72).

Embodiment 15. The immunogenic composition for use of any preceding Embodiment further comprising an adjuvant, particularly the adjuvant AS01E.

Embodiment 16. The immunogenic composition for use of Embodiment 14, comprising (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 10 µg of UspA2 and (4) adjuvant AS01E.

Embodiment 17. The immunogenic composition for use of Embodiment 14 comprising, (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 3.3 µg of UspA2 and (4) adjuvant AS01E.

Embodiment 18. A vaccination protocol comprising administering a first, a second and a third immunologically effective dose of an immunogenic composition to a subject, wherein the third dose of the immunogenic composition is administered at least 6 months after administration of the first dose of the immunogenic composition, wherein the immunogenic composition comprises (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

The following clauses also describe additional embodiments of the invention:

Embodiment 1a. A method of boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* in a subject, the method comprising the step of administering an immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof to the subject in an amount sufficient to elicit a further or additional, immune response relative to the pre-existing immune response.

Embodiment 2a. The method according to Embodiment 1a wherein the pre-existing immune response has been elicited by prior administration of at least two doses of the immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

Embodiment 3a. The method according to Embodiment 1a or 2a, wherein the subject has a previous history of Chronic Obstructive Pulmonary Disease (COPD).

Embodiment 4a. The method according to Embodiment 3a, wherein the subject has a previous history of moderate and severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD).

Embodiment 5a. The method according to Embodiment 2a, 3a or 4a wherein the immunogenic composition is administered six to 13 months (e.g. administered between six and 12 months; administered at six months; or administered at 12 months) after administration of the first of the at least two doses of vaccine.

Embodiment 6a. The method according to Embodiment 5a wherein the immunogenic composition is subsequently administered every 12 months on the anniversary of administration of the first of the at least two doses of vaccine.

Embodiment 7a. The method according to any preceding Embodiment 1a to 6a, wherein the further or additional immune response is sufficient to induce protective or therapeutic immunity against non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat).

Embodiment 8a. The method according to any preceding Embodiment 1a to 7a, wherein the immune response is against PD, PE, PilA and UspA2 and is sufficient to reduce the frequency of AECOPD.

Embodiment 9a. The method according to Embodiment 7a or 8a, wherein the subject is a human.

Embodiment 10a. The method according to Embodiment 9a, wherein the subject is an adult human aged between 18 and 40 or between 50 and 70 or between 40 and 80 years of age.

Embodiment 11a. The method according to Embodiment 9a, wherein the subject has a smoking history of at least ten pack years.

Embodiment 12a. The method according to any preceding Embodiment 1a to 11a wherein the UspA2 is at least 63%, 66%, 70%, 72%, 74%, 75%, 77%, 80%, 84%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical, over the entire length, to SEQ ID NO: 13.

Embodiment 13a. The method according to any preceding Embodiment 1a to 12a wherein the UspA2 consists essentially of an immunogenic fragment of UspA2 selected from the group consisting of amino acids 30-540 of SEQ ID NO. 13 (SEQ ID NO: 61, 62, 63 or 64), amino acids 31-540 of SEQ ID NO: 13 (SEQ ID NO: 71), amino acids 30-519 of SEQ ID NO: 13 (SEQ ID NO: 65 or 66), amino acids 30-564 of SEQ ID NO: 13 (SEQ ID NO: 67 or 68) and amino acids 31-564 of SEQ ID NO: 13 (SEQ ID NO: 69 or 70).

Embodiment 14a. The method according to any preceding Embodiment 1a to 13a wherein PE and PilA are present as a fusion protein, particularly SEQ ID NO:72 or SEQ ID NO:73.

Embodiment 15a. The method according to any preceding Embodiment 1a to 14a wherein the immunogenic composition comprises UspA2 (SEQ ID NO: 69), Protein D (SEQ ID NO:1) and a PE-PilA fusion protein (SEQ ID NO: 72).

Embodiment 16a. The method according to any preceding Embodiment 1a to 15a further comprising an adjuvant, particularly the adjuvant AS01E.

Embodiment 17a. The method according to Embodiment 15a wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 10 µg of UspA2 and (4) adjuvant AS01E.

Embodiment 18a. The method according to Embodiment 15a wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 3.3 µg of UspA2 and (4) adjuvant AS01E.

Embodiment 19a. A vaccination protocol comprising administering a first, a second and a third immunologically effective dose of an immunogenic composition to a subject, wherein the third dose of the immunogenic composition is administered at least 6 months after administration of the first dose of the immunogenic composition, wherein the immunogenic

composition comprises (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

Embodiment 20a. The vaccination protocol according to Embodiment 19a wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 10 µg of UspA2 and (4) adjuvant AS01E.

Embodiment 21a. The vaccination protocol according to Embodiment 19a wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 3.3 µg of UspA2 and (4) adjuvant AS01E.

Embodiment 22a. The vaccination protocol according to Embodiment 20a or 21a wherein the immune response against PD, PE, PilA and UspA2 is sufficient to induce protective or therapeutic immunity against non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat).

Embodiment 23a. The vaccination protocol according to Embodiment 20a, 21a or 22a wherein the immune response against PD, PE, PilA and UspA2 is sufficient to reduce the frequency of AECOPD.

Embodiment 24a. The vaccination protocol according to Embodiment 23a wherein the subject is a human.

Embodiment 25a. The vaccination protocol according to Embodiment 24a wherein the subject is an adult human aged between 18 and 40 or between 50 and 70 or between 40 and 80 years of age.

Embodiment 26a. The vaccination protocol according to Embodiment 25a wherein the subject has a smoking history of at least ten pack years.

In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only, and are not to be construed as limiting the scope of the invention in any manner.

Examples

Example 1: Immunogenicity of PD, PE and PilA in human adults

An NTHi multi-component investigational vaccine was prepared based on a combination of three selected conserved surface proteins from NTHi presented as two vaccine antigens: (1) a free recombinant protein D (PD) and (2) a recombinant fusion of protein E and Pilin A (PE-PilA). The vaccine was presented as a lyophilized cake to be reconstituted with AS01E. After preparation, the appropriate injection volume (0.5 mL) of each vaccine dose was administered intramuscularly in the deltoid muscle of the non-dominant arm. An isotonic saline solution (0.9% NaCl) was used as placebo. The study vaccine was adjuvanted with AS01E. AS01E is an Adjuvant System containing 25 µg each of 3-O-desacyl-4′-monophosphoryl lipid A (MPL; GSK Vaccines, Rixensart, Belgium), *Quillaja saponaria* Molina fraction 21 (QS-21; Licensed by GSK from Antigenics Inc, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation) and liposome.

Current and former smokers aged from 50–70 years old were enrolled in a Phase I study (NTHi-003) and received two doses of the NTHi vaccine (10 µg or 30 µg of each antigen) at Day 1 and Day 61 (according to a 0, 2-month schedule). The antibody and cell mediated immune responses (CMI) to PD, PE and PilA induced by the vaccine formulation was evaluated prior to vaccination and at 30 days after each vaccination. Blood samples were taken for immunogenicity testing at 30 days after each vaccination (i.e. Days 0, 30, 60, 90, 180, 210 and 420). The anti-PD, anti-PE and anti-PilA antibody concentrations were measured by ELISA, using standardized procedures. The cut-off of the assays was 100 ELISA units (EU)/mL, 8 EU/mL and 7 EU/mL for anti-PD, anti-PE and anti-PilA, respectively (Figures 4A, 4B and 4C). Note: The anti-PD ELISA used in this study had been validated in 2001 (cut-off =100 EU/mL) and did not meet the latest validation standards. Therefore, sera samples were retested with the validated 2014 anti-PD ELISA (cut-off =153 EU/mL). CMI responses (antigen-specific CD4+and CD8+T-cells) were measured by flow cytometry using intracellular cytokine staining (ICS) on frozen peripheral blood mononuclear cells (PBMCs), following an adaptation of previously described methods [Moris P, van der Most R, Leroux-Roels I, Clement F, Drame M, Hanon E, et al.H5N1 influenza vaccine formulated with AS03 A induces strong cross-reactive and polyfunctional CD4 T-cell responses. J Clin Immunol 2011;31:443–54.]. After PBMC stimulation with the relevant antigens, the frequency of CD4+ and/or CD8+ T-cells expressing a selected set of cytokines (IL-2, IL-13, IL-17, IFN-γ, TNF-α and CD40L) or a selected combination of cytokines was evaluated (Figures 5A, 5B and 5C).

Example 2: Immunogenicity of UspA2 in combination with PD and PE-PilA NTHi antigens in Balb/c mice.

Immunization Protocol

Groups of 25 female Balb/c mice were immunized by the intramuscular (IM) route at days 0, 14 and 28 with 50 µl of the following formulations:
1 µg of UspA2 construct MC-009, 1 µg of PD, 1 µg PEPilA construct LVL-735 adjuvanted with AS01E (50/50 per ml QS21/MPL).

ELISA to measure anti-UspA2 antibodies

Anti-UspA2, anti-PE, anti-PilA and anti-PD IgG levels were determined in individual sera collected at days 28 and 42 as follows: Plates were coated overnight at 4°C with 100 µl per well of either (1) UspA2 construct MC-009 at 4 µg/ml in carbonate buffer pH 9.6, (2) 2 µg/ml of PE in carbonate buffer pH 9.6, (3) 4 µg/ml of PilA in carbonate buffer pH 9.6 or (4) 8 µg/ml of PD in carbonate buffer pH 9.6.

The plates were washed three times with 0.09% NaCl, 0.05% polysorbate 20 (TWEEN-20; TWEEN is a trademark of Croda International PLC). After washing, serial two fold dilutions of sera were added to micro-wells in PBS TWEEN-20 0.05%. The plates were placed at room temperature for 30 minutes with shaking. After washing, anti-mouse IgG antibodies (Jackson 115-035-003) conjugated to peroxidase (100 µl per well) were added, and the plates were placed at room temperature for 30 minutes with shaking. Plates were washed as above and a solution of 4 mg of o-Phenylenediamine dihydrochloride (OPDA, Sigma P8787) and 5 µl of H_2O_2 in 10 ml of citrate 0.1M PH (pH) 4.5) was added to each well (100 µl/well) for 15 minutes in darkness. The reaction was stopped by addition of 50 µl of HCl 1N and the absorbance was read at 490 nm (620 nm for the reference filter). The titers were calculated by the 4-parameters method using the SOFTMAX Pro software.

Bactericidal assay

Bactericidal titres were measured in pooled sera (5 pools/ group) collected at day 42 using the following protocol: *Moraxella catarrhalis* was cultivated overnight on Petri dish at 37°C + 5% CO2. Bacteria were transferred in 10 ml BHi (broth heart infusion) medium in order to get an OD 620 of 0.650. Serum samples were heated for 45 minutes at 56 °C to inactivate the endogenous complement. Serial two-fold dilutions of sera in SBA buffer (HBSS–BSA 0.1%) were added on a 96-well round bottom microtitre plate (25 µl/well). Subsequently, 50

µl of SBA buffer was added to each well. Then 25 µl of Moraxella catarrhalis strain 25238 at 4x10^3 cfu/ml was added to the wells containing sera and incubated for 15 minutes at room temperature. Finally, 25 µl of freshly thawed baby rabbit complement diluted 1/8 in HBSS-BSA 0.1% was added to reach a final volume of 125 µl. Plates were incubated for 1 hour at 37 °C with orbital shaking (210 rpm). The reaction was stopped by laying the microplate on ice for at least 5 minutes. A 20 µl aliquot from each well of the plate was transferred into the corresponding well of a 96-well flat bottom microplate and 50 µl of Mueller Hinton Broth–0.9% agar was added to each well. 50 µl of PBS 0.9% agar was added as a second layer. After 3 hours at 37°C with 5% CO_2, plates were incubated overnight at 25°C. Moraxella colonies were counted using an automated image analysis system (KS 400, Zeiss, Oberkochen, Germany). Eight wells without serum sample were used as bacterial controls to determine the number of Moraxella per well. The mean number of colony forming units (CFU) of the control wells was determined and used for the calculation of the killing activity for each serum sample. Anti-*Moraxella catarrhalis* bactericidal titres were measured in pooled sera (5 pools/ group post-III) collected at day 42. The bactericidal titers were expressed at the reciprocal dilution of serum inducing 50% of killing. The bactericidal assay was performed against *Moraxella catarrhalis* strain ATCC 25238™, expressing a homologous UspA2or a heterologous (F10) UspA2. UspA2 induced high bactericidal titers against both strains as (Figure 7).

The IgG responses induced against UspA2, PD, PE and PilA in mice by PE-PEPilA-UspA2 vaccine are shown in Figures 6A, 6B, 6C, 6D and 6E respectively. No major impact of the addition of UspA2 on PD and PEPilA immunogenicity in AS01E was observed.

Example 3 – Phase II Study of investigational NTHi-Mcat Vaccine

An $AS01_E$-adjuvanted formulation containing 10 µg of PD, 10 µg of the PE-PilA fusion protein and 3.3 µg of UspA2 is evaluated. The antigens and formulation may be prepared and tested as described in WO2015/125118.

Table 2: Study vaccines

Treatment name	Vaccine/ product name	Formulation	Presentation	Volume to be administered	Number of doses
10-10-3/AS01$_E$	NTHi-Mcat/ 10-10-3.3	PD=10 µg; PE-PilA=10 µg; UspA2= 3.3 µg	Freeze-dried antigens in monodose vial	0.5 ml	3
	AS01E	MPL=25µg; QS21=25µg; Liposomes	Liquid in monodose vial		
Placebo	Formulation buffer S9b	Na_2HPO_4=0.4mg; KH_2PO_4=56µg; NaCl=1,16mg; KCl=30µg; $MgCl_2$=15µg	Liquid in monodose vial	0.5 ml	1

MPL = 3-O-desacyl-4′-monophosphoryl lipid A; **QS-21** = Quillaja saponaria Molina, fraction 21 (Licensed by GSK from Antigenics Inc, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation).

Experimental design

Phase II, observer-blind, randomised, multi-centric study with two parallel groups.

Study groups

Adults aged 40 to 80 years with a smoking history of at least 10 pack-years, will receive 2 doses of the NTHi-Mcat investigational vaccine, at 0 and 2 months, followed by either a third, booster, dose of the investigational NTHi-Mcat vaccine at 6 or 12 months or placebo control at 6 or 12 months. The study evaluates the safety and reactogenicity profile of the NTHi-Mcat vaccine administered according to two schedules and provides additional data relating to the humoral and cellular immunogenicity of the NTHi-Mcat investigational vaccine.

Schedule 1: Approximately 100 subjects receive three doses of the AS01E-adjuvanted NTHi-Mcat investigational vaccine containing 10 µg of PD, 10 µg of PE-PilA, and 3.3 µg of UspA2 at Day 1, Day 61, Day 181 and one dose of placebo at Day 361.

Schedule 2: Approximately 100 subjects receive three doses of the AS01E-adjuvanted NTHi-Mcat investigational vaccine containing 10 µg of PD, 10 µg of PE-PilA, and 3.3 µg of UspA2 at Day 1, Day 61, Day 361 and one dose of placebo at Day 181.

Subjects are allocated to a study group using a centralised randomisation system on internet (SBIR). The randomisation algorithm uses a minimisation procedure accounting for age (40 59 years or 60 80 years), for smoking status (current or former smokers), for centre and for forced expiratory volume in 1 second (FEV1) / forced vital capacity (FVC) (≥ 0.7 or < 0.7).

Table 3: Dosage and administration

Type of contact and timepoint	Study group	Treatment name	Volume to be administered	Route [1]	Site		
					Location	Directionality[2]	Laterality [3]
Visit 1 (Day 1)	Schedule 1	10-10-3/AS01E	0.5 ml	IM	Deltoid	Upper	Non-dominant
	Schedule 2						
Visit 3 (Day 61)	Schedule 1	10-10-3/AS01E	0.5 ml	IM	Deltoid	Upper	Non-dominant
	Schedule 2						
Visit 6 (Day 181)	Schedule 1	10-10-3/AS01E	0.5 ml	IM	Deltoid	Upper	Non-dominant
	Schedule 2	Placebo					
Visit 8 (Day 361)	Schedule 1	Placebo	0.5 ml	IM	Deltoid	Upper	Non-dominant
	Schedule 2	10-10-3/AS01E					

[1]Intramuscular (IM)

[2]Directionality is a qualifier for further detailing the location of the vaccine administration (e.g. Upper, Lower)

[3]The non-dominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the non-dominant arm, an injection in the dominant arm may be performed.

Sampling schedule

Blood samples for assessment of humoral immunogenicity are collected from all subjects at Visit 1 (Day 1), Visit 2 (Day 31), Visit 3 (Day 61), Visit 4 (Day 91), Visit 5 (Day 181), Visit 6 (Day 211), Visit 7 (Day 361), Visit 8 (Day 391) and Visit 9 (Day 451).

Blood samples for assessment of cell-mediated immunogenicity (CMI) are collected from a sub-cohort at Visit 1 (Day 1), Visit 6 (Day 211) and at Visit 8 (Day 391).

Laboratory Assays

Total IgG concentrations are measured by ELISA using qualified procedures.

Table 4: Humoral Immunity (Antibody determination)

System	Component	Method	Unit	Cut-off
SERUM	anti-PD antibody	ELISA	EU/ml	153
	anti-PE antibody			8
	anti-PilA antibody			7
	anti-UspA2 IgG antibody			18

EU/ml = ELISA unit per millilitre

Cell-mediated immune assays are performed using qualified procedures including ELISpot and Flow Cytometry.

Note: assay cut-off may be updated after qualification.

Table 5: Cell-mediated Immunity (CMI) using flow cytometry

System	Component Family	Scale	Method	Unit
PBMCs	Specific $CD4^+/CD8^+$ T-cells	Quantitative	Flow cytometry ICS	Number of specific $CD4^+/CD8^+$ T-cells $/10^6$

Additional testing on peripheral blood mononuclear cells (PBMCs), such as, but not limited to, evaluation of NTHi and/or Mcat-specific memory B-cells, intracellular cytokine staining (ICS) testing using other bacterial antigens, may be performed.

Table 6: Immunological read-outs

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 1 (Day 1)	Pre-Vacc I	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
		CMI sub-cohort*	~40	Specific $CD4^+/CD8^+$ T-cells
Visit 2 (Day 31)	Post-Vacc I	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
Visit 3 (Day 61)	Pre-Vacc II	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
Visit 4 (Day 91)	Post-Vacc II	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
Visit 5 (Day 181)	Pre-Vacc III	All subjects	~120	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
Visit 6 (Day 211)	Post-Vacc III	All subjects	~120	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
		CMI sub-cohort*	~40	Specific $CD4^+/CD8^+$ T-cells
Visit 7 (Day 361)	Pre-Vacc IV	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
Visit 8 (Day 391)	Post-Vacc IV	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2
		CMI sub-cohort*	~40	Specific $CD4^+/CD8^+$ T-cells
Day 9 (Day 451)	Post-Vacc IV	All subjects	~200	Anti-PD, Anti-PE, Anti-PilA and Anti-UspA2

* Approximately 20% of the subjects in each group will be part of a sub-cohort for CMI analysis.

Immunogenicity

- Humoral immune response to the components of the NTHi-Mcat investigational vaccine formulations, on Day 1, Day 31, Day 61, Day 91, Day 181, Day 211, Day 361, Day 391 and Day 451 in all subjects, is measured in all groups:
  - Anti-PD, anti-PE, anti-PilA and anti-UspA2 antibody concentrations.
- Cell-mediated immune response to components of the NTHi-Mcat investigational vaccine formulations on Day 1, Day 211 and Day 391, in a sub-cohort of subjects, is measured in all groups:
  - Frequency of specific CD4+/CD8+ T-cells measured on cryopreserved PBMCs and identified by ICS expressing two or more markers (such as IL-2, IL-13, IL-17, IFN-γ, TNF-α and CD40L).

Example 4 – Phase I Study of investigational NTHi-Mcat Vaccine in healthy adults aged 18-40 years and in current and former smokers aged 50-70 years

Three investigational NTHi-Mcat vaccine formulations were evaluated according to a 0, 2 months schedule administered in a staggered design in a Phase 1 study (NTHi-MCAT-001). Firstly (Step 1), healthy adults aged 18 - 40 years were enrolled and vaccinated with non-adjuvanted (plain) vaccine containing 10 µg of PD, 10 µg of PE-PilA and 10 µg of UspA2 or a placebo control, and secondly (Step 2), current/former smokers aged 50-70 years were vaccinated with either a placebo control, or one of two AS01E-adjuvanted formulations, i.e. 10 µg of PD, 10 µg of PE-PilA and 10 µg of UspA2 (Group 10-10-10-AS) or 10 µg of PD, 10 µg of PE-PilA and 3.3 µg of UspA2 (Group 10-10-3-AS). The placebo used as control was an isotonic saline solution. A total of 76 subjects received at least 1 dose of any NTHi-Mcat formulation and 44 received the placebo.

The vaccine formulations were delivered by intramuscular injection of 0.5 ml volume into the deltoid of the non-dominant arm. If it was not possible to inject in the non-dominant arm, an injection into the dominant arm was performed.

Duration of the study: For each subject, the study lasted approximately 15 months, from screening to study end.
Epoch 001: Primary started at Screening Visit and ended at, and including Visit 6 (Day 90).
Epoch 002: Follow-up started at Visit 7 (Day 210) and ended at Visit 8 (Day 420).

Study groups:
(F1 Group) 10-10-10: Subjects received two doses at Day 0 and Day 60 of the non-adjuvanted GSK Biologicals' NTHi-Mcat investigational vaccine containing 10 µg of PD, PE-PilA and UspA2 during Step 1 of the study.
PLACEBO 1: Subjects received two doses at Day 0 and Day 60 of placebo (saline solution) during Step 1 of the study.
(F2 Group) 10-10-10-AS: Subjects received two doses at Day 0 and Day 60 of the AS01E-adjuvanted GSK Biologicals' NTHi-Mcat investigational vaccine containing 10 µg of PD, PE-PilA and UspA2 during Step 2 of the study.
(F3 Group) 10-10-3-AS: Subjects received two doses at Day 0 and Day 60 of the AS01E-adjuvanted GSK Biologicals' NTHi-Mcat investigational vaccine containing 10 µg of PD, 10 µg of PE-PilA, and 3.3 µg of UspA2 during Step 2 of the study.
PLACEBO 2: Subjects received two doses at Day 0 and Day 60 of placebo (saline solution) during Step 2 of the study.

Sampling schedule:

Blood samples for safety (haematology/ biochemistry) were collected from all subjects at Screening Visit (pre-Day 0), at Visit 1 (Day 0), Visit 2 (Day 7), Visit 4(Day 60), Visit 5 (Day 67), Visit 7 (Day 210) and at Visit 8 (Day 420).

Blood samples for immunogenicity were collected from all subjects for humoral immunity at Visit 1(Day 0), Visit 3 (Day 30), Visit 4 (Day 60), Visit 6(Day 90), Visit 7 (Day 210) and at Visit 8 (Day 420), and from a sub-cohort of subjects for cell-mediated immunity (CMI) at Visit 1 (Day 0), Visit 4 (Day 60), Visit 6 (Day 90), Visit 7 (Day 210) and at Visit 8 (Day 420).

Laboratory assays

Humoral immunogenicity:

Humoral immunogenicity was assessed. Serological assays for the quantification of antibodies were performed by ELISA.

Table 7: Humoral Immunogenicity

System	Component	Method	Unit	Cut-off
NTHi specific				
Serum	Anti-PD	ELISA	EL.U/mL	153
Serum	Anti-PE	ELISA	EL.U/mL	25
Serum	Anti-PilA	ELISA	EL.U/mL	7 (Visit , Day 0; Visit 3, Day 30; Visit 4, Day 60; Visit 6, Day 90) 16 (Visit 7, Day 210; Visit 8, Day 420)
NTHi-Mcat specific				
Serum	Anti-UspA2	ELISA	EL.U/mL	38

EL.U/mL = ELISA unit per millilitre

Antibody concentrations are measured by enzyme -linked immunosorbent assay (ELISA) and expressed as geometric mean concentrations (GMCs) in ELISA units per millilitre (EL.U/mL).

Table 8: Cell-mediated immunogenicity

System	Component	Scale	Method	Unit
CMI	Specific $CD4^+/CD8^+$ T-cells	Quantitative	Flow cytometry	Number of specific $CD4^+/CD8^+$ T cells $/10^6$

CMI = cell-mediated immunogenicity;

Frequency of specific CD4+ T-cells were measured by flow cytometry intracellular cytokine staining (ICS) expressing two or more markers (such as Interleukin [IL]-2, IL-13, IL-17, Interferon gamma [FN-γ], Tumour necrosis factor alpha [TNF-α] and CD40L). The frequency of specific CD4+ T-cells are summarised [descriptive statistics: Mean and standard deviation (SD)] against each antigen (PD, PE, PilA and UspA2), by group in Step 2 at each time point during which blood samples were collected for CMI.

Frequency of specific CD8+ T-cells were measured by flow cytometry intracellular cytokine staining (ICS) expressing two or more markers (such as IL-2, IL-13, IL-17, IFN-γ, TNF-α and CD40L). The frequency of specific CD8+ T-cells are summarised [descriptive statistics: Mean and standard deviation (SD)] against each antigen (PD, PE, PilA and UspA2), by group in Step 2 at each time point during which blood samples were collected for CMI.

Results

Results are provided in Table 9 and in Figure 8.

Table 9: Measured Values

	F1 Group	F2 Group	F3 Group	PLACEBO Group
Concentration of antibodies against the NTHi-Mcat anti-PD (protein D of Haemophilus influenzae) vaccine component				
Number of Participants Analyzed	14	31	29	43
Units:EL.U/mL *Geometric Mean (95% Confidence Interval)*				
Anti-PD antibody, Day 0				
	109.7 (71.2 to 169.1)	102.8 (77.5 to 136.4)	88.1 (72.4 to 107.1)	89 (75 to 105.7)
Number of Participants Analyzed	14	30	29	43
Anti-PD antibody, Day 30				
	239.8 (123 to 467.6)	569.4 (335.9 to 965.3)	818 (532 to 1257.8)	90.8 (76.5 to 107.8)
Number of Participants Analyzed	14	31	29	43
Anti-PD antibody, Day 60				
	220.9 (121.1 to 402.9)	327.1 (207.4 to 515.6)	495 (307.2 to 797.5)	88.6 (75.2 to 104.3)
Number of Participants Analyzed	14	30	29	43
Anti-PD antibody, Day 90				
	289.2 (144.6 to 578.5)	984.4 (662.3 to 1463.2)	1538.5 (1134.6 to 2086.2)	91.8 (77.8 to 108.3)
Number of Participants Analyzed	14	31	29	43
Anti-PD antibody, Day 210				
	179.5 (96.7 to 333.5)	471.8 (310.2 to 717.5)	806.1 (560.2 to 1159.9)	92.9 (79 to 109.2)

Number of Participants Analyzed	14	31	29	43
Anti-PD antibody, Day 420				
	165.1 (89.7 to 303.8)	370.2 (238.2 to 575.3)	538.1 (369.1 to 784.5)	88.8 (77.3 to 102.1)
Number of Participants Analyzed	14	31	29	43

	F1 Group	F2 Group	F3 Group	PLACEBO Group
Concentration of antibodies against the NTHi-Mcat anti-PE (protein E of Haemophilus influenzae) vaccine component				
Number of Participants Analyzed	14	31	29	43
Units:EL.U/mL *Geometric Mean (95% Confidence Interval)*				
Anti-PE antibody, Day 0				
	31.3 (16.4 to 59.7)	20.6 (15.7 to 27)	19.7 (14.8 to 26.2)	21.9 (17 to 28.1)
Number of Participants Analyzed	14	31	29	43
Anti-PE antibody, Day 30				
	178.3 (64.6 to 491.9)	627.2 (401 to 980.9)	1287.8 (816.2 to 2032)	21.3 (16.7 to 27.1)
Number of Participants Analyzed	13	31	29	43
Anti-PE antibody, Day 60				
	151.9 (58 to 397.6)	573.1 (360.1 to 912.1)	1207.1 (753.8 to 1932.9)	20.3 (15.9 to 25.9)

Number of Participants Analyzed	14	31	29	43
Anti-PE antibody, Day 90				
	719.1 (357.4 to 1446.8)	5945.2 (4069.5 to 8685.5)	8983.9 (7150.4 to 11287.5)	21.8 (17.1 to 27.8)
Number of Participants Analyzed	14	31	29	43
Anti-PE antibody, Day 210				
	385.8 (191.2 to 778.4)	2390.9 (1655.4 to 3453.1)	3247.6 (2285.2 to 4615.3)	20.7 (16.5 to 26)
Number of Participants Analyzed	14	31	29	43
Anti-PE antibody, Day 420				
	244.1 (112.4 to 529.9)	1206.6 (817.5 to 1781)	1701 (1192.1 to 2427.1)	22.8 (17.5 to 29.7)
Number of Participants Analyzed	14	31	29	43

	F1 Group	F2 Group	F3 Group	PLACEBO Group
Concentration of antibodies against the NTHi-Mcat anti-PilA (type IV pili subunit of non-typeable Haemophilus influenzae) vaccine component				
Number of Participants Analyzed	14	31	29	43
Units:EL.U/mL *Geometric Mean (95% Confidence Interval)*				
Anti-PilA antibody, Day 0				
	11.6 (5.3 to 25.2)	13.5 (8 to 22.6)	17.1 (11 to 26.5)	8.8 (6.1 to 12.6)

Number of Participants Analyzed	14	31	29	43
Anti-PilA antibody, Day 30				
	37.2 (13.6 to 101.5)	238.6 (123.1 to 462.5)	330.9 (211.7 to 517.2)	9.2 (6.4 to 13.3)
Number of Participants Analyzed	14	29	29	42
Anti-PilA antibody, Day 60				
	33.2 (11.3 to 97.9)	177 (83.4 to 375.7)	321.2 (210.1 to 491.2)	9 (6.3 to 13)
Number of Participants Analyzed	14	30	29	43
Anti-PilA antibody, Day 90				
	165.8 (76.7 to 358.4)	1127.5 (751 to 1692.8)	1722.3 (1383.4 to 2144.2)	8.6 (5.8 to 12.6)
Number of Participants Analyzed	14	31	29	39
Anti-PilA antibody, Day 210				
	52 (18.4 to 146.8)	367.2 (220.2 to 612.3)	671 (508.6 to 885.2)	11.5 (9 to 14.8)
Number of Participants Analyzed	14	31	29	43
Anti-PilA antibody, Day 420				
	40.8 (16.5 to 100.7)	181.8 (108 to 305.9)	322.2 (233.4 to 444.9)	11.9 (9.2 to 15.5)
Number of Participants Analyzed	14	31	29	43

	F1 Group	F2 Group	F3 Group	PLACEBO Group
Concentration of antibodies against the NTHi-Mcat anti-UspA2 (ubiquitous surface protein A2 of Moraxella catarrhalis) vaccine component				
Number of Participants Analyzed	14	31	29	43
Units:EL.U/mL *Geometric Mean (95% Confidence Interval)*				
Anti-UspA2 IgG antibody, Day 0				
	572.5 (254.4 to 1288.6)	384.1 (253 to 583.2)	468.1 (330.6 to 662.8)	548.3 (399.4 to 752.8)
Number of Participants Analyzed	14	31	29	43
Anti-UspA2 IgG antibody, Day 30				
	879.9 (396.6 to 1952.4)	1006.7 (732.5 to 1383.6)	913.5 (693.3 to 1203.7)	571.5 (416.9 to 783.4)
Number of Participants Analyzed	14	31	29	43
Anti-UspA2 IgG antibody, Day 60				
	780.7 (365.4 to 1668.3)	754.4 (533.1 to 1067.6)	714.7 (538.9 to 947.8)	567 (418 to 769.1)
Number of Participants Analyzed	14	31	29	43
Anti-UspA2 IgG antibody, Day 90				
	1172 (654.7 to 2097.9)	1440.7 (1065.8 to 1947.5)	1279 (1026.1 to 1594.4)	621.5 (449.1 to 860)
Number of Participants Analyzed	14	31	29	43
Anti-UspA2 IgG antibody, Day 210				

	775.1 (361.3 to 1662.8)	882.5 (629.8 to 1236.7)	767.2 (584.8 to 1006.5)	525.7 (386.8 to 714.5)
Number of Participants Analyzed	14	31	29	43
Anti-UspA2 IgG antibody, Day 420				
	732 (339.2 to 1579.5)	703.6 (501.8 to 986.5)	673.4 (504.3 to 899.2)	552.9 (399.7 to 764.9)
Number of Participants Analyzed	14	31	29	43

	F2 Group	**F3 Group**	**PLACEBO Group**
Frequency of specific Cluster of differentiation (CD)4+ T-cells against NTHi-Mcat antigens collected for the evaluation of cell-mediated immune response			
Number of Participants Analyzed	16	12	15
Units:CD4+ T-cells/million cells Mean ± Standard Deviation			
CD4+.PD, Day 0			
	28.3 ± 42.1	81.8 ± 92.29	37.4 ± 46.29
Number of Participants Analyzed	12	12	13
CD4+.PD, Day 60			
	105.4 ± 123.95	107.8 ± 117.92	55 ± 91.94
Number of Participants Analyzed	16	12	15
CD4+.PD, Day 90			
	283.3 ± 236.93	349.9 ± 216.5	90.5 ± 117.41
Number of Participants Analyzed	16	11	13

CD4+.PD, Day 210			
	120.4 ± 99.23	176.9 ± 101.39	78.7 ± 112.16
Number of Participants Analyzed	11	11	13
CD4+.PD, Day 420			
	154.3 ± 165.98	164.8 ± 123.33	60.3 ± 82.01
Number of Participants Analyzed	13	9	14
CD4+.PE, Day 0			
	89.2 ± 199.87	41.4 ± 48.55	47.1 ± 70.65
Number of Participants Analyzed	12	12	13
CD4+.PE, Day 60			
	370.7 ± 372.5	176.9 ± 172.74	50.9 ± 47.91
Number of Participants Analyzed	16	12	15
CD4+.PE, Day 90			
	1182.2 ± 1507.29	732.7 ± 804.67	52.4 ± 56.2
Number of Participants Analyzed	16	11	13
CD4+.PE, Day 210			
	469.1 ± 381.87	337.4 ± 228.12	39.1 ± 65.14
Number of Participants Analyzed	12	11	14
CD4+.PE, Day 420			
	545 ± 735.89	215.8 ± 137.79	26.6 ± 51.74
Number of Participants Analyzed	14	10	14

CD4+.PilA, Day 0			
	32.8 ± 43.6	60.5 ± 92.87	32.6 ± 48.69
Number of Participants Analyzed	12	12	13
CD4+.PilA, Day 60			
	169.1 ± 150.22	110.2 ± 67.81	57.1 ± 99.26
Number of Participants Analyzed	16	11	15
CD4+.PilA, Day 90			
	508.6 ± 474.31	330.5 ± 412.4	99 ± 152.45
Number of Participants Analyzed	16	11	13
CD4+.PilA, Day 210			
	326 ± 208.31	220.8 ± 148.59	43.7 ± 69.03
Number of Participants Analyzed	7	9	11
CD4+.PilA, Day 420			
	348.8 ± 376.87	131.1 ± 75.89	59.1 ± 53.4
Number of Participants Analyzed	10	8	12
CD4+.UspA2, Day 0			
	115.4 ± 154.96	126.3 ± 97.92	131.1 ± 182.97
Number of Participants Analyzed	11	12	13
CD4+.UspA2, Day 60			
	383.9 ± 326.2	253 ± 202.76	278.7 ± 672.84
Number of Participants Analyzed	16	12	15

CD4+.UspA2, Day 90			
	1392.6 ± 1145.48	979.2 ± 807.03	143.5 ± 237.29
Number of Participants Analyzed	16	11	13
CD4+.UspA2, Day 210			
	582.9 ± 376.54	322.8 ± 273.5	143.9 ± 242.72
Number of Participants Analyzed	13	11	15
CD4+.UspA2, Day 420			
	723.2 ± 763.51	385.4 ± 174.08	126.6 ± 187.52
Number of Participants Analyzed	14	10	14

	F2 Group	**F3 Group**	**PLACEBO Group**
Frequency of specific CD8+ T-cells against NTHi-Mcat antigens collected for the evaluation of cell-mediated immune response			
Number of Participants Analyzed	15	12	13
Units:CD8+ T-cells/million cells Mean ± Standard Deviation			
CD8+.PD, Day 0			
	66 ± 88.69	27.9 ± 80.68	46.9 ± 73.28
Number of Participants Analyzed	11	12	13
CD8+.PD, Day 60			
	29.4 ± 49.67	1 ± 0	20.5 ± 55.1
Number of Participants Analyzed	15	11	12
CD8+.PD, Day 90			
	66.5 ± 97.16	7.7 ± 21.19	49 ± 64.89

Number of Participants Analyzed	14	10	12
CD8+.PD, Day 210			
	66.4 ± 101.79	16.8 ± 42.84	17.7 ± 33.01
Number of Participants Analyzed	11	12	11
CD8+.PD, Day 420			
	43.2 ± 51.32	10.6 ± 17.98	37.3 ± 70.58
Number of Participants Analyzed	13	8	12
CD8+.PE, Day 0			
	42.4 ± 52.5	33.3 ± 59.32	74.4 ± 175.42
Number of Participants Analyzed	11	12	12
CD8+.PE, Day 60			
	27.8 ± 46.77	26.1 ± 40.81	42.7 ± 107.03
Number of Participants Analyzed	15	11	12
CD8+.PE, Day 90			
	30.4 ± 52.48	19.8 ± 43.69	45.4 ± 82.76
Number of Participants Analyzed	14	10	12
CD8+.PE, Day 210			
	31.8 ± 52.08	30.6 ± 62.25	44.2 ± 85.37
Number of Participants Analyzed	12	12	12
CD8+.PE, Day 420			
	5.7 ± 10.89	27 ± 58.46	19.4 ± 44.74

Number of Participants Analyzed	15	10	12
CD8+.PilA, Day 0			
	34.6 ± 57.02	16 ± 40.27	44.7 ± 58.58
Number of Participants Analyzed	11	12	13
CD8+.PilA, Day 60			
	26 ± 82.07	35.1 ± 39.81	28.7 ± 72.65
Number of Participants Analyzed	15	11	12
CD8+.PilA, Day 90			
	50.8 ± 82.01	27.9 ± 43.03	28.3 ± 45.69
Number of Participants Analyzed	14	10	11
CD8+.PilA, Day 210			
	11.3 ± 19.17	30.9 ± 70.04	21.4 ± 35.86
Number of Participants Analyzed	8	9	9
CD8+.PilA, Day 420			
	28 ± 50.45	23 ± 37.7	28 ± 39.21
Number of Participants Analyzed	10	7	10
CD8+.UspA2, Day 0			
	22.5 ± 35.07	34.9 ± 50.24	85.3 ± 140.42
Number of Participants Analyzed	10	12	13
CD8+.UspA2, Day 60			
	29.3 ± 67.7	27.7 ± 50.12	21 ± 36.81

Number of Participants Analyzed	15	11	12
CD8+.UspA2, Day 90			
	74.1 ± 99.92	20.6 ± 26.17	37.4 ± 76.73
Number of Participants Analyzed	14	10	11
CD8+.UspA2, Day 210			
	61.5 ± 89.07	17.3 ± 26.54	48.1 ± 53.97
Number of Participants Analyzed	13	11	13
CD8+.UspA2, Day 420			
	16.7 ± 24.45	25.3 ± 70.42	48.8 ± 86.71
Number of Participants Analyzed	15	10	12

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CLAIMS

1. A method of boosting a pre-existing immune response against non-typeable *Haemophilus influenzae* and *Moraxella catarrhalis* in a subject, the method comprising the step of administering an immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof to the subject in an amount sufficient to elicit a further or additional, immune response relative to the pre-existing immune response.

2. The method according to claim 1 wherein the pre-existing immune response has been elicited by prior administration of at least two doses of the immunogenic composition comprising (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

3. The method according to claim 1 or 2, wherein the subject has a previous history of Chronic Obstructive Pulmonary Disease (COPD).

4. The method according to claim 3, wherein the subject has a previous history of moderate and severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease (AECOPD).

5. The method according to claim 2, 3 or 4 wherein the immunogenic composition is administered six to 13 months (e.g. administered between six and 12 months; administered at six months; or administered at 12 months) after administration of the first of the at least two doses of vaccine.

6. The method according to claim 5 wherein the immunogenic composition is subsequently administered every 12 months on the anniversary of administration of the first of the at least two doses of vaccine.

7. The method according to any preceding claim, wherein the further or additional immune response is sufficient to induce protective or therapeutic immunity against non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat).

8. The method according to any preceding claim, wherein the immune response is against PD, PE, PilA and UspA2 and is sufficient to reduce the frequency of AECOPD.

9. The method according to claim 7 or 8, wherein the subject is a human.

10. The method according to claim 9, wherein the subject is an adult human aged between 18 and 40 or between 50 and 70 or between 40 and 80 years of age.

11. The method according to claim 9, wherein the subject has a smoking history of at least ten pack years.

12. The method according to any preceding claim wherein the UspA2 is at least 63%, 66%, 70%, 72%, 74%, 75%, 77%, 80%, 84%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical, over the entire length, to SEQ ID NO: 13.

13. The method according to any preceding claim wherein the UspA2 consists essentially of an immunogenic fragment of UspA2 selected from the group consisting of amino acids 30-540 of SEQ ID NO. 13 (SEQ ID NO: 61, 62, 63 or 64), amino acids 31-540 of SEQ ID NO: 13 (SEQ ID NO: 71), amino acids 30-519 of SEQ ID NO: 13 (SEQ ID NO: 65 or 66), amino acids 30-564 of SEQ ID NO: 13 (SEQ ID NO: 67 or 68) and amino acids 31-564 of SEQ ID NO: 13 (SEQ ID NO: 69 or 70).

14. The method according to any preceding claim wherein PE and PilA are present as a fusion protein, particularly SEQ ID NO:72 or SEQ ID NO:73.

15. The method according to any preceding claim wherein the immunogenic composition comprises UspA2 (SEQ ID NO: 69), Protein D (SEQ ID NO:1) and a PE-PilA fusion protein (SEQ ID NO: 72).

16. The method according to any preceding claim further comprising an adjuvant, particularly the adjuvant AS01E.

17. The method according to claim 15 wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 10 µg of UspA2 and (4) adjuvant AS01E.

18. The method according to claim 15 wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 3.3 µg of UspA2 and (4) adjuvant AS01E.

19. A vaccination protocol comprising administering a first, a second and a third immunologically effective dose of an immunogenic composition to a subject, wherein the third dose of the immunogenic composition is administered at least 6 months after administration of the first dose of the immunogenic composition, wherein the immunogenic composition comprises (i) protein D from *Haemophilus influenzae* (PD) or a fragment thereof, (ii) Protein E from *Haemophilus influenzae* (PE) or a fragment thereof, (iii) pilin A from *Haemophilus influenza* (PilA) or a fragment thereof and (iv) Ubiquitous surface protein A2 from *Moraxella catarrhalis* (UspA2) or a fragment thereof.

20. The vaccination protocol according to claim 19 wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 10 µg of UspA2 and (4) adjuvant AS01E.

21. The vaccination protocol according to claim 19 wherein the immunogenic composition comprises (1) 10 µg of PD, (2) 10 µg of a PE-PilA fusion protein, (3) 3.3 µg of UspA2 and (4) adjuvant AS01E.

22. The vaccination protocol according to claim 20 or 21 wherein the immune response against PD, PE, PilA and UspA2 is sufficient to induce protective or therapeutic immunity against non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat).

23. The vaccination protocol according to claim 20, 21 or 22 wherein the immune response against PD, PE, PilA and UspA2 is sufficient to reduce the frequency of AECOPD.

24. The vaccination protocol according to claim 23 wherein the subject is a human.

25. The vaccination protocol according to claim 24 wherein the subject is an adult human aged between 18 and 40 or between 50 and 70 or between 40 and 80 years of age.

26. The vaccination protocol according to claim 25 wherein the subject has a smoking history of at least ten pack years.

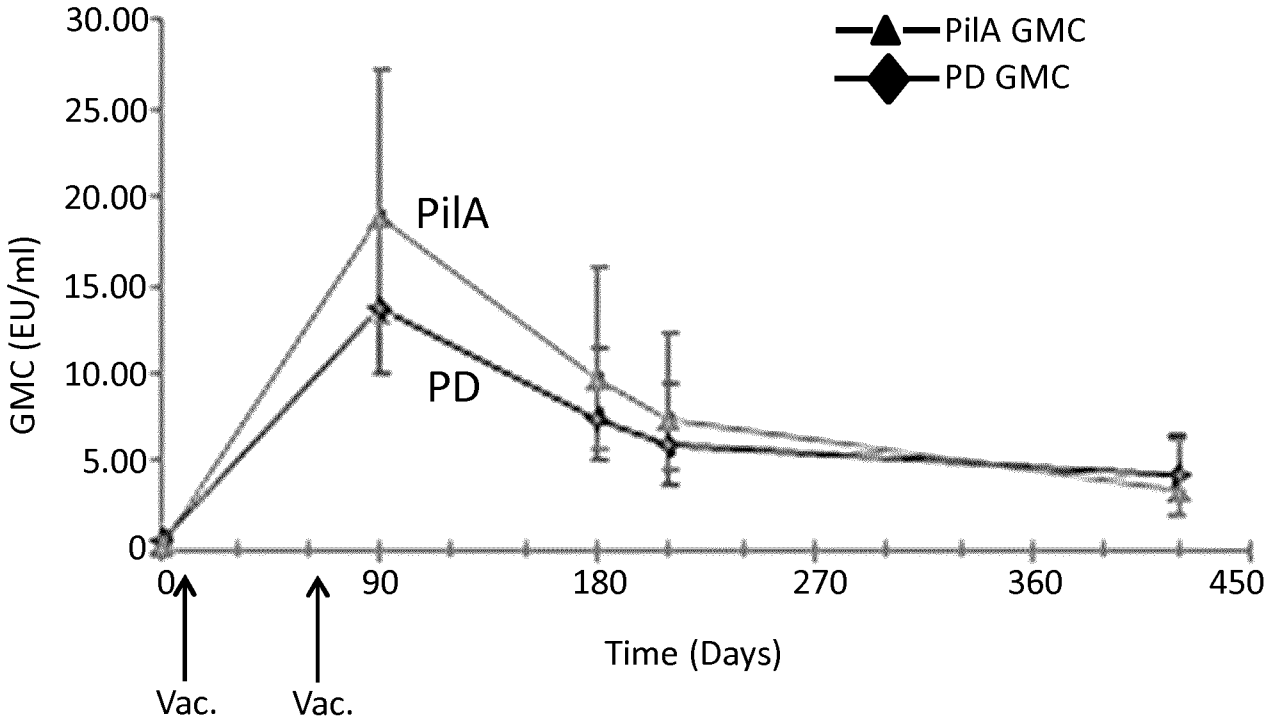

Figure 1(a)

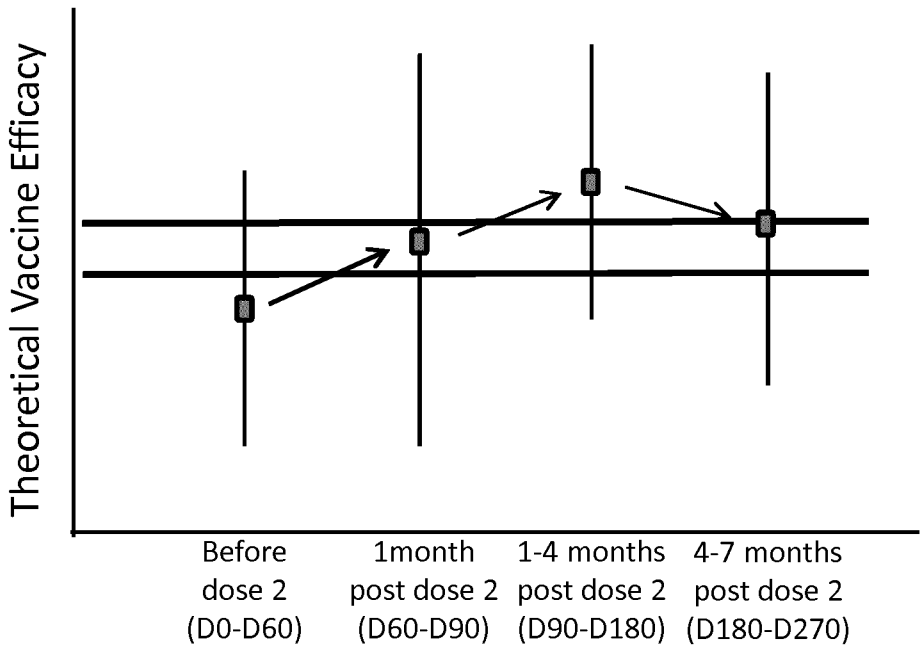

Figure 1(b)

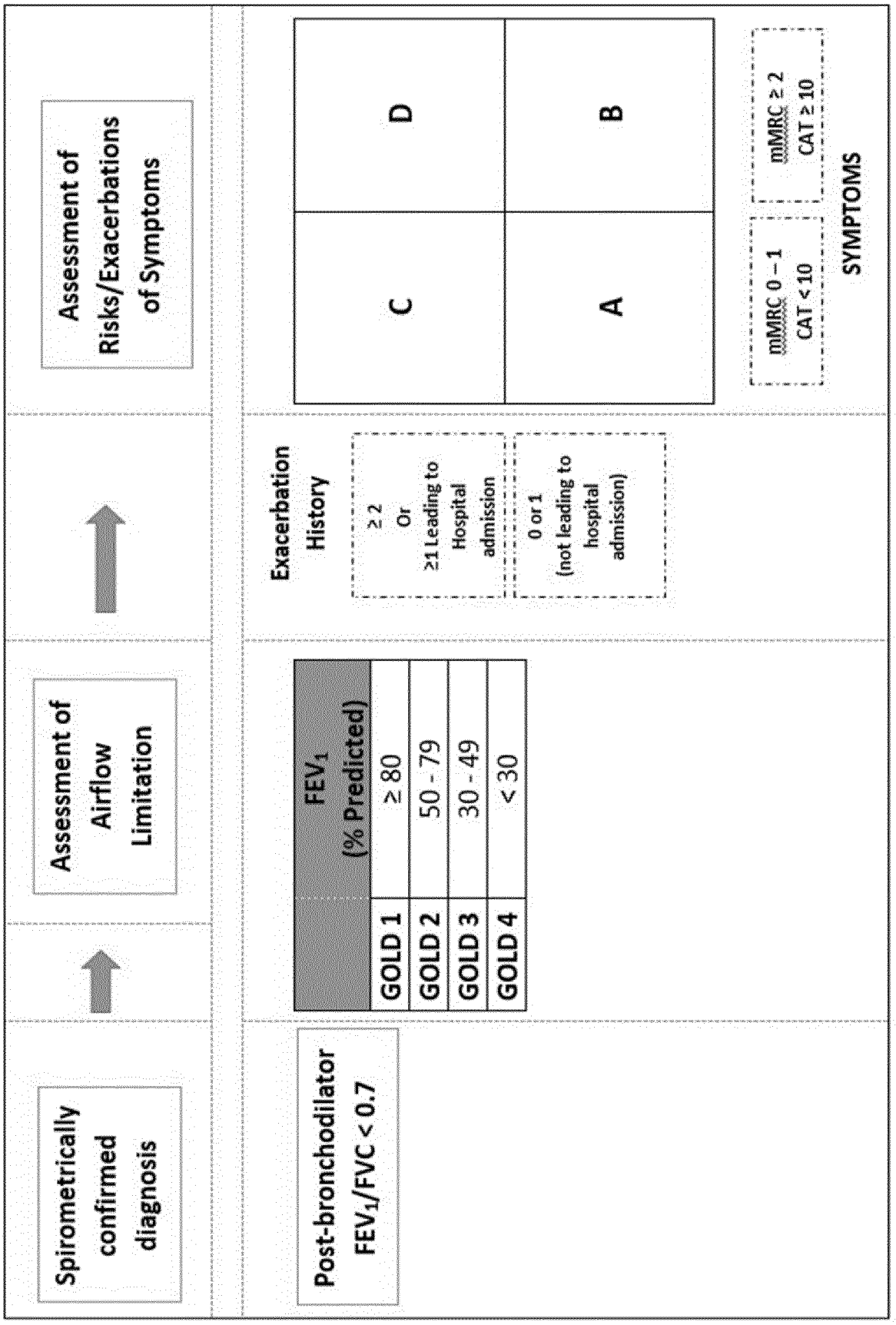
Spirometrically confirmed diagnosis
Post-bronchodilator FEV1/FVC < 0.7
Assessment of Airflow Limitation
FEV1 (% Predicted)
GOLD 1 ≥ 80
GOLD 2 50 - 79
GOLD 3 30 - 49
GOLD 4 < 30
Exacerbation History
≥ 2 Or ≥1 Leading to Hospital admission
0 or 1 (not leading to hospital admission)
Assessment of Risks/Exacerbations of Symptoms
C
D
A
B
mMRC 0 – 1 CAT < 10
mMRC ≥ 2 CAT ≥ 10
SYMPTOMS

Figure 2

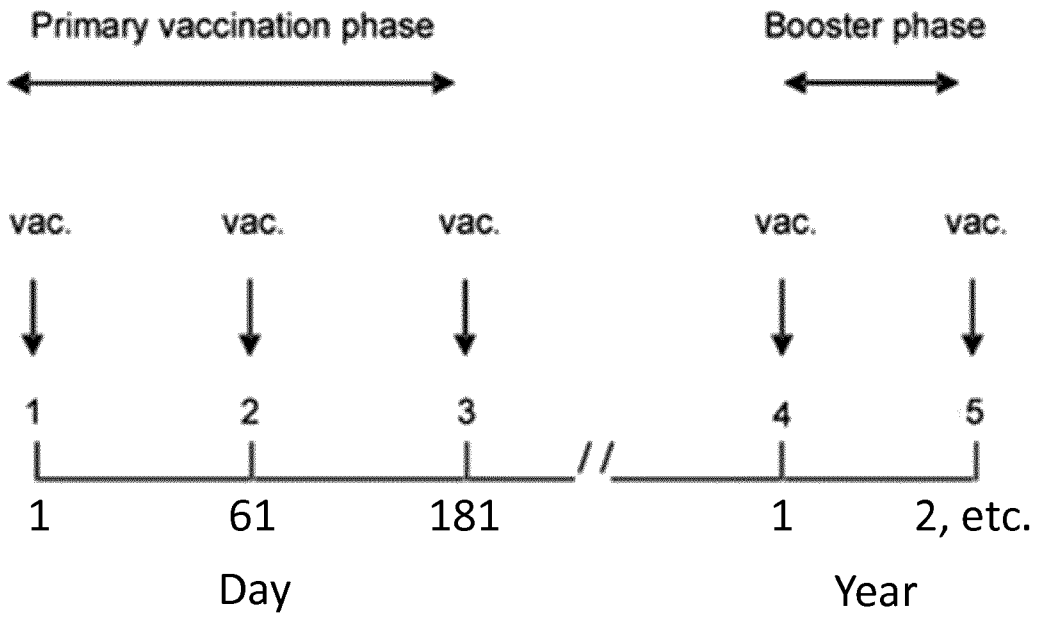

Figure 3(a)

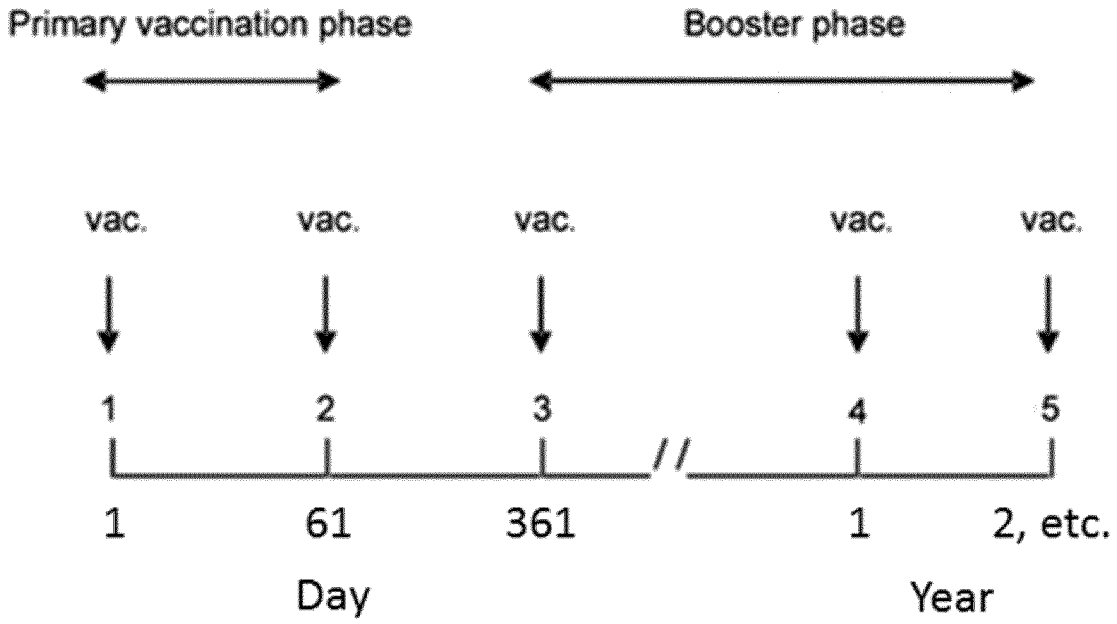

Figure 3(b)

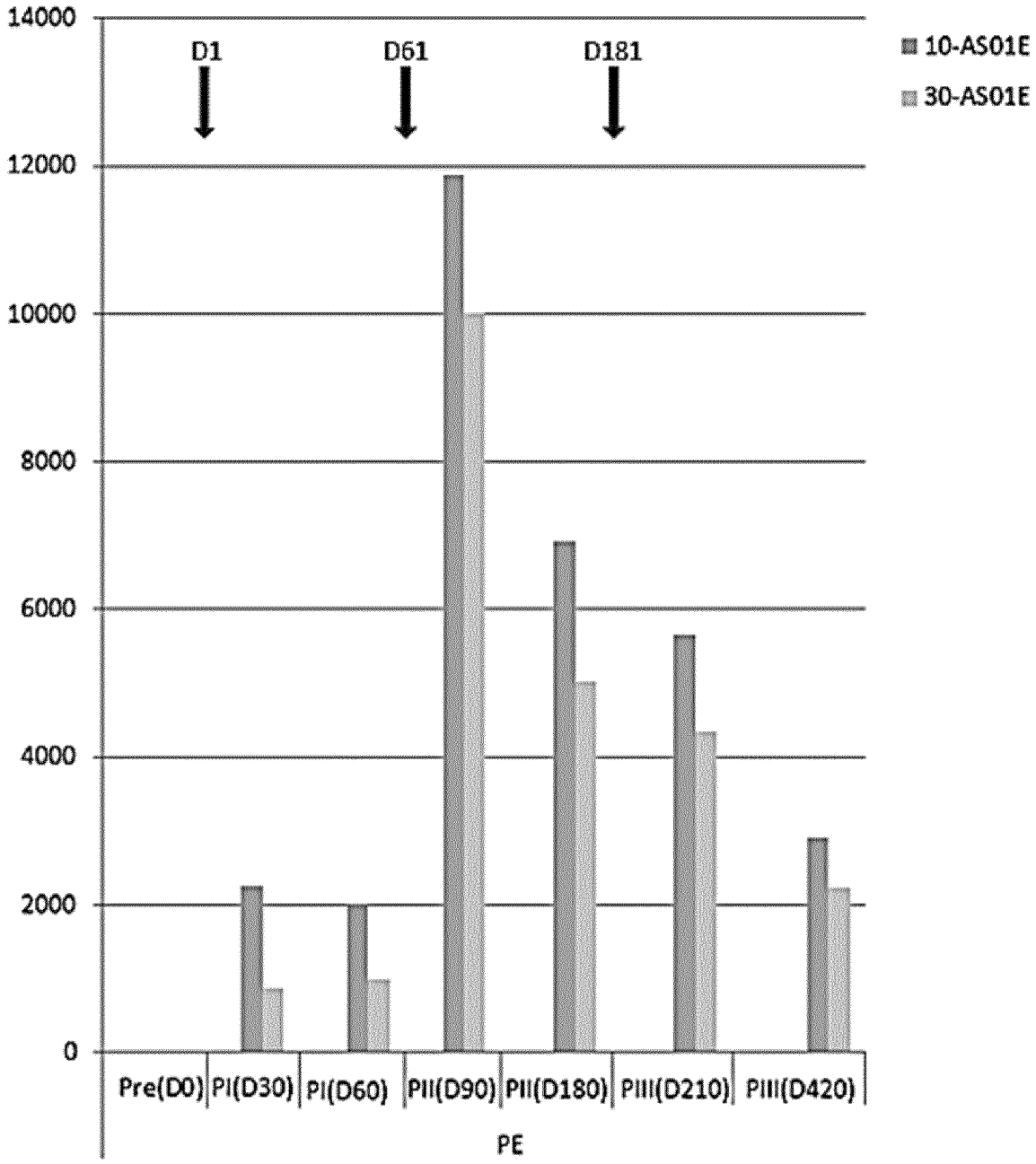
14000
12000
10000
8000
6000
4000
2000
0
D1
D61
D181
10-AS01E
30-AS01E
Pre(D0)
PI(D30)
PI(D60)
PII(D90)
PII(D180)
PIII(D210)
PIII(D420)
PE

Figure 4(A)

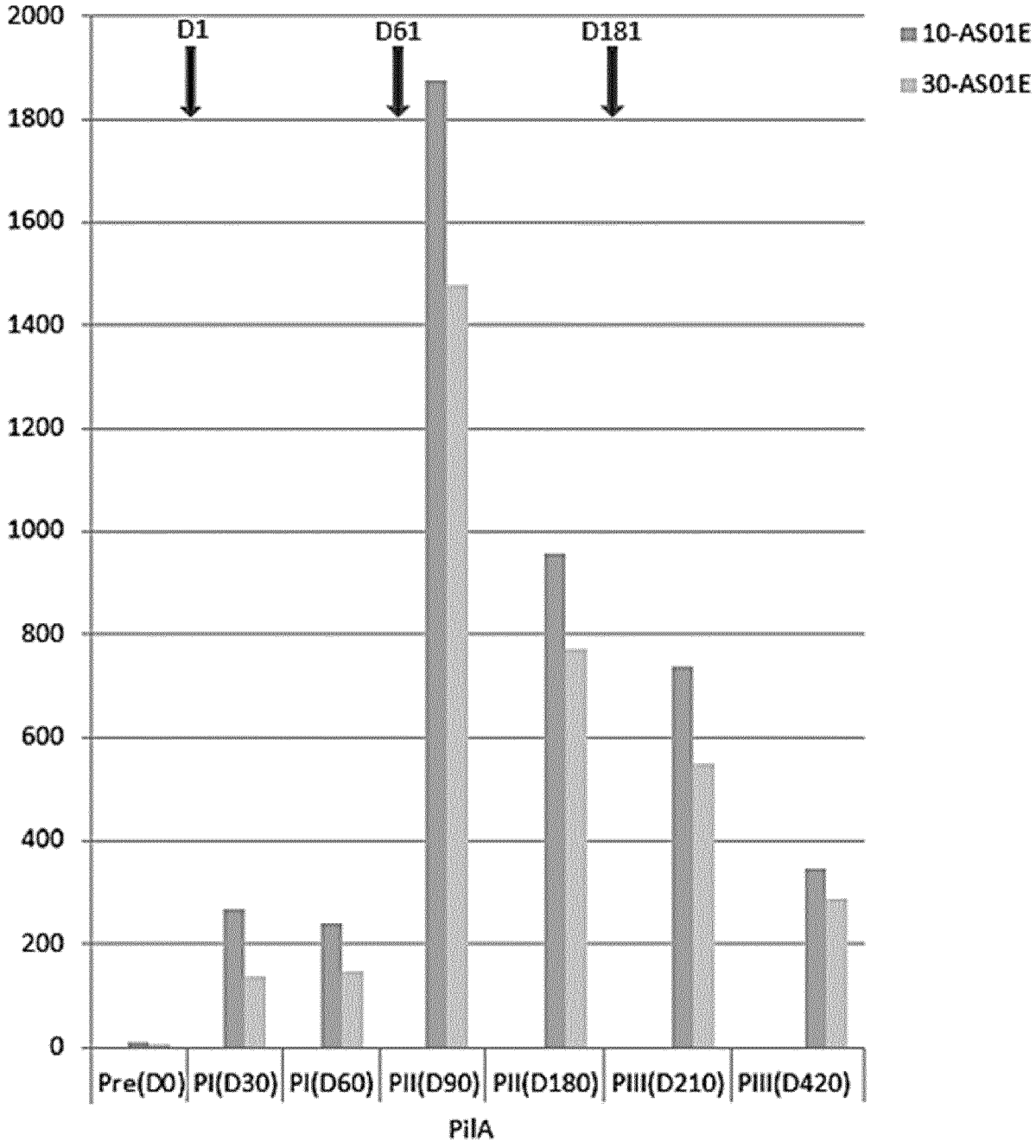
2000
1800
1600
1400
1200
1000
800
600
400
200
0
D1
D61
D181
10-AS01E
30-AS01E
Pre(D0)
PI(D30)
PI(D60)
PII(D90)
PII(D180)
PIII(D210)
PIII(D420)
PilA

Figure 4(B)

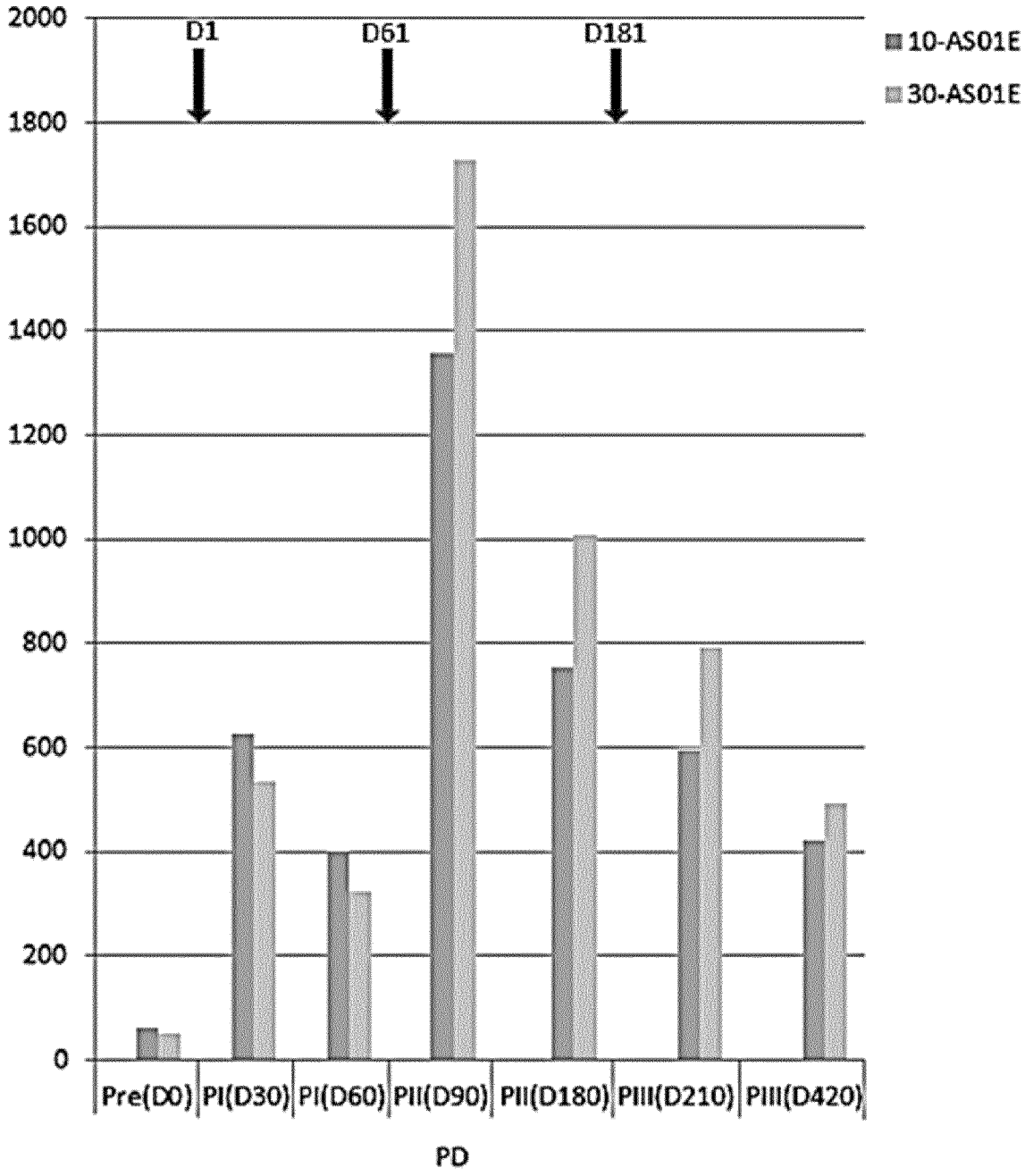
2000
1800
1600
1400
1200
1000
800
600
400
200
0
D1
D61
D181
10-AS01E
30-AS01E
Pre(D0)
PI(D30)
PI(D60)
PII(D90)
PII(D180)
PIII(D210)
PIII(D420)
PD

Figure 4(C)

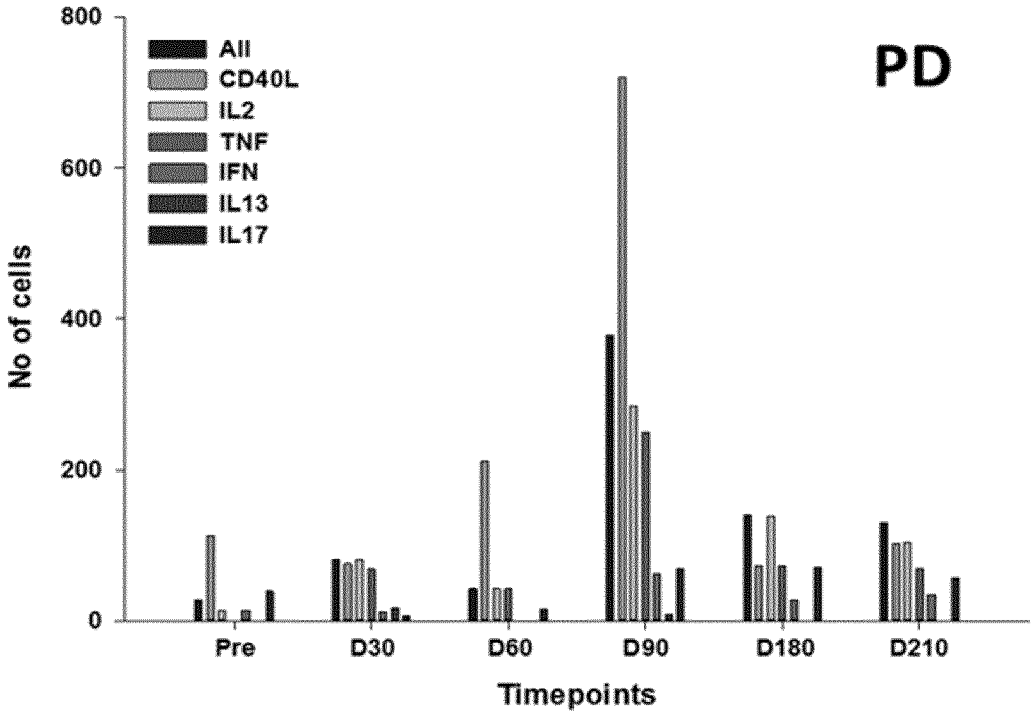

Figure 5(A)

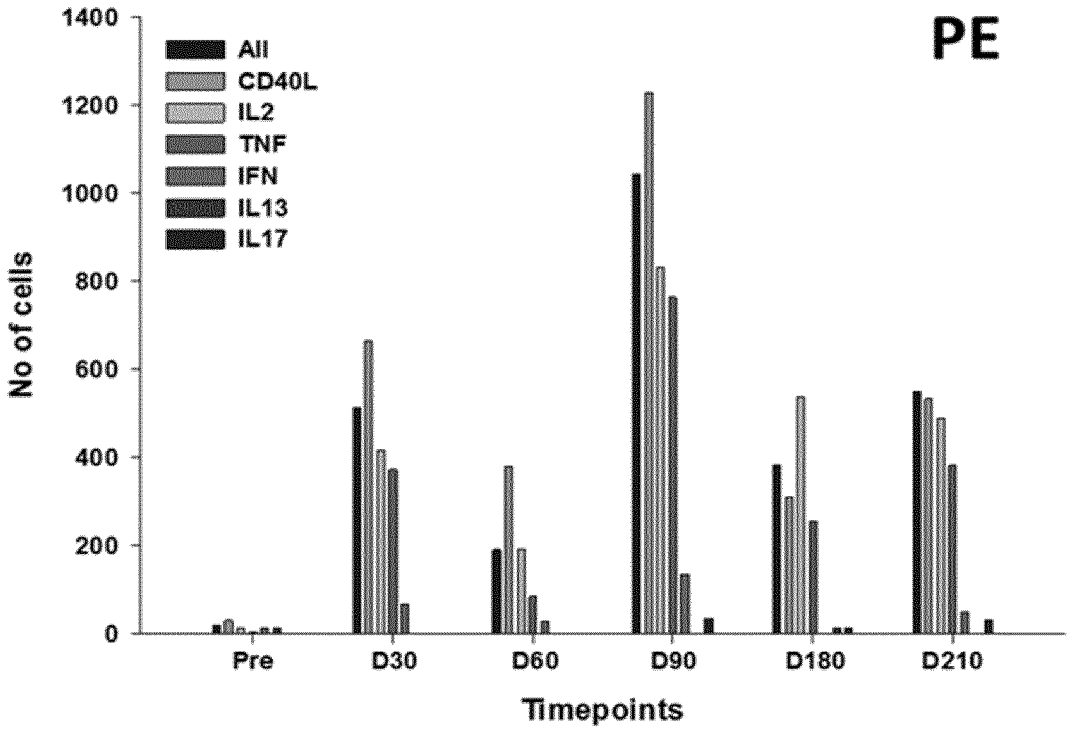

Figure 5(B)

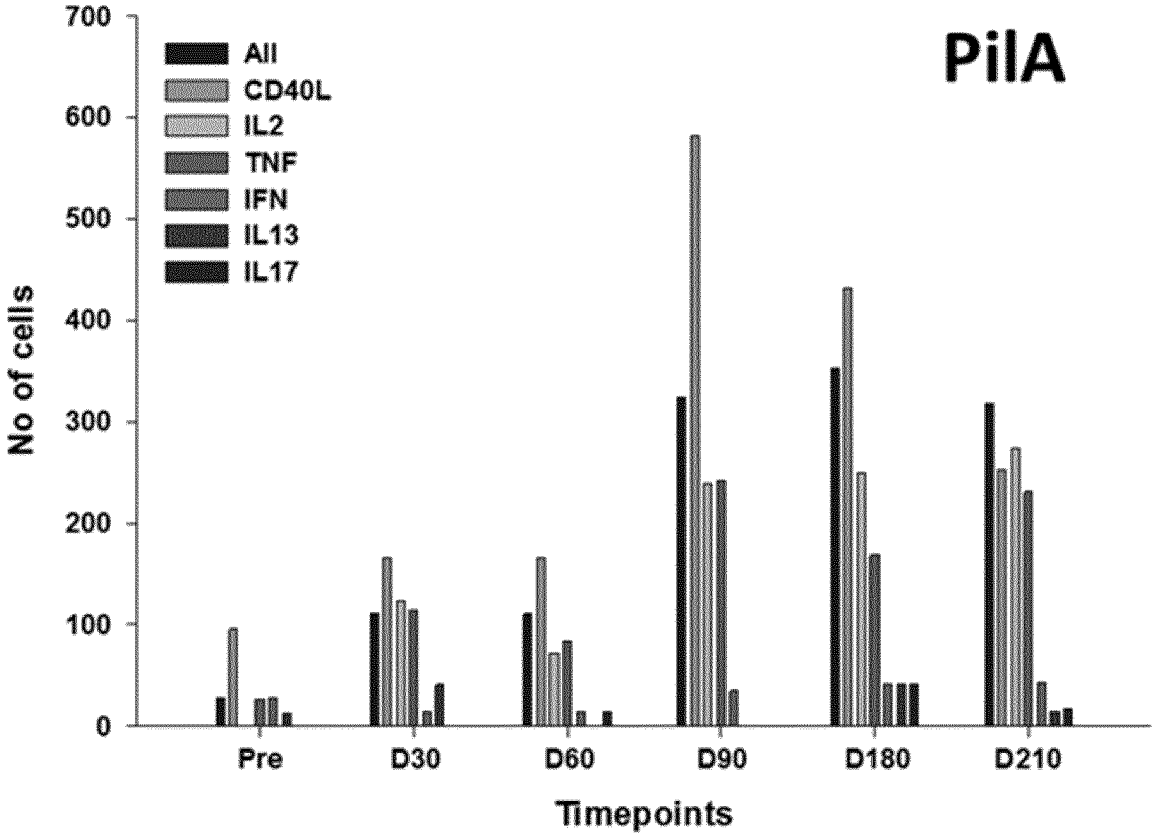
PilA
All
CD40L
IL2
TNF
IFN
IL13
IL17
No of cells
700
600
500
400
300
200
100
0
Pre
D30
D60
D90
D180
D210
Timepoints

Figure 5(C)

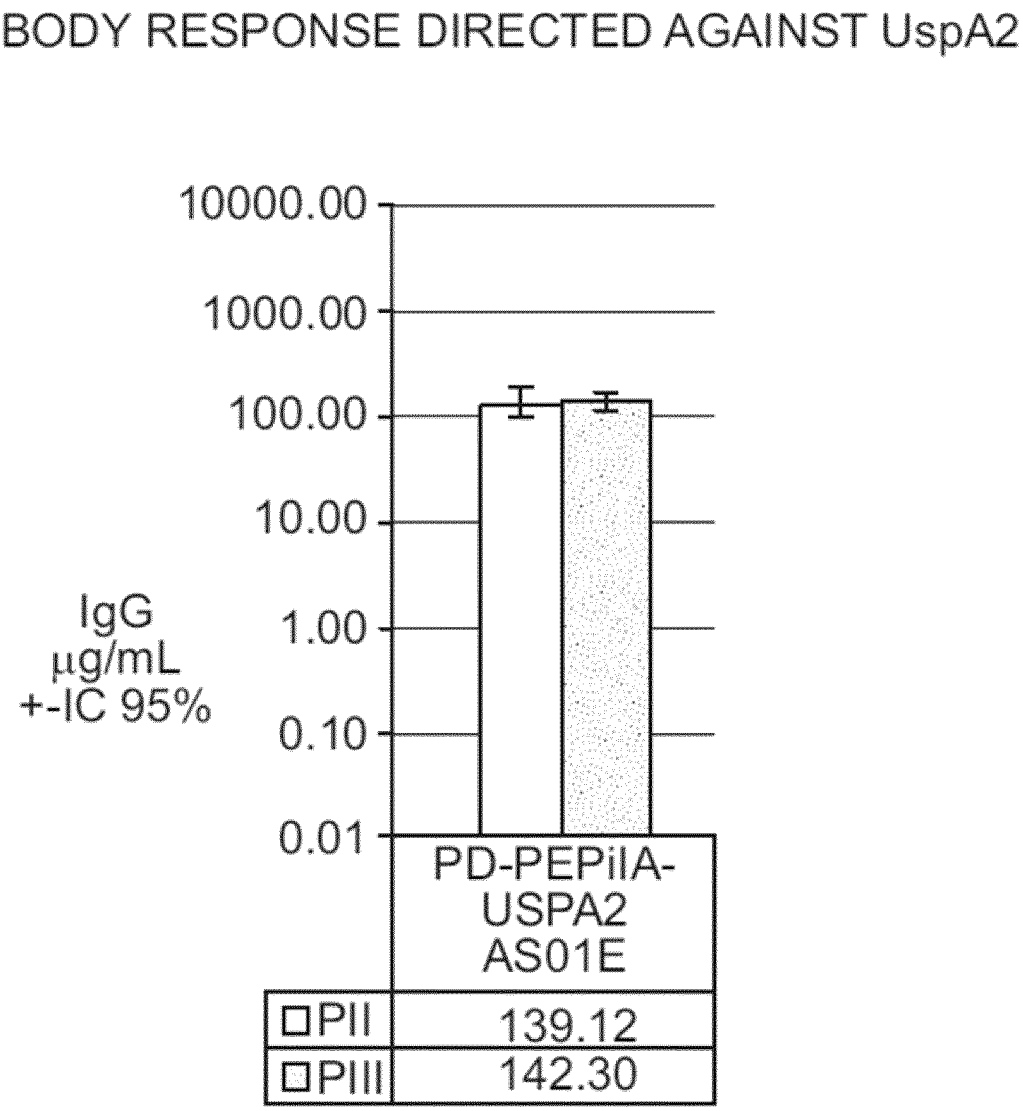
ANTIBODY RESPONSE DIRECTED AGAINST UspA2
10000.00
1000.00
100.00
10.00
1.00
0.10
0.01
IgG
µg/mL
+-IC 95%
PD-PEPilA-USPA2 AS01E
PII
139.12
PIII
142.30

Figure 6(a)

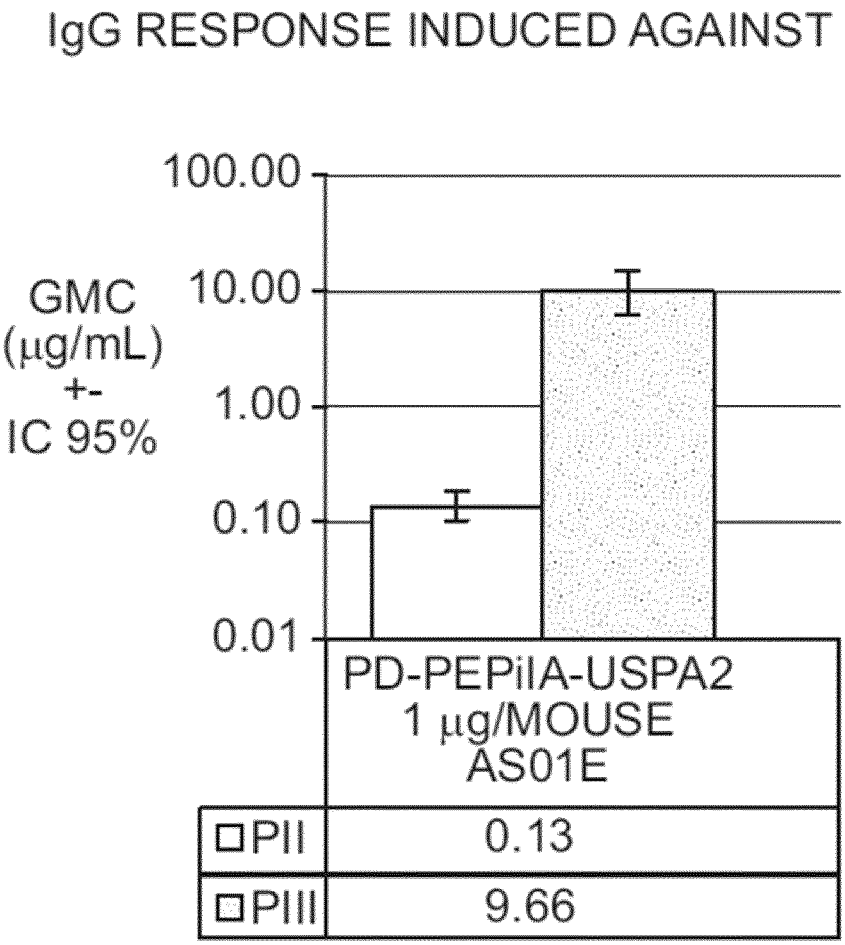
IgG RESPONSE INDUCED AGAINST PD
GMC (µg/mL) +- IC 95%
100.00
10.00
1.00
0.10
0.01
PD-PEPilA-USPA2
1 µg/MOUSE
AS01E
PII
0.13
PIII
9.66

Figure 6(B)

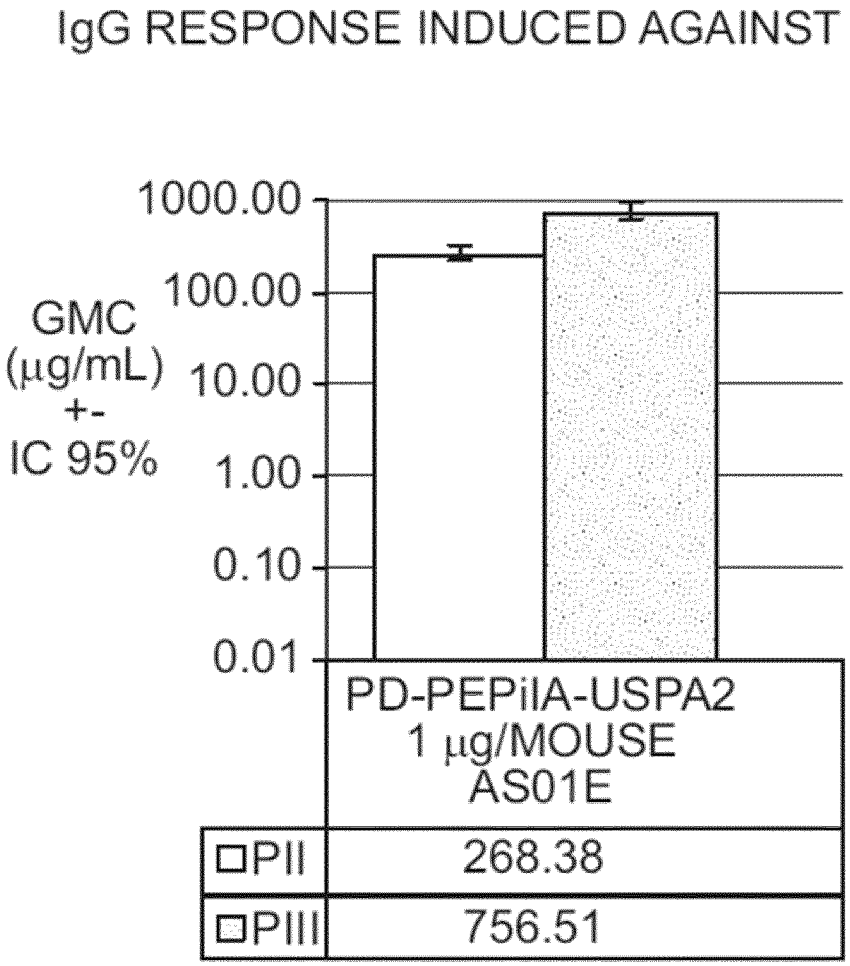
IgG RESPONSE INDUCED AGAINST PE
GMC (µg/mL) +- IC 95%
1000.00
100.00
10.00
1.00
0.10
0.01
PD-PEPilA-USPA2
1 µg/MOUSE
AS01E
PII
268.38
PIII
756.51

Figure 6(C)

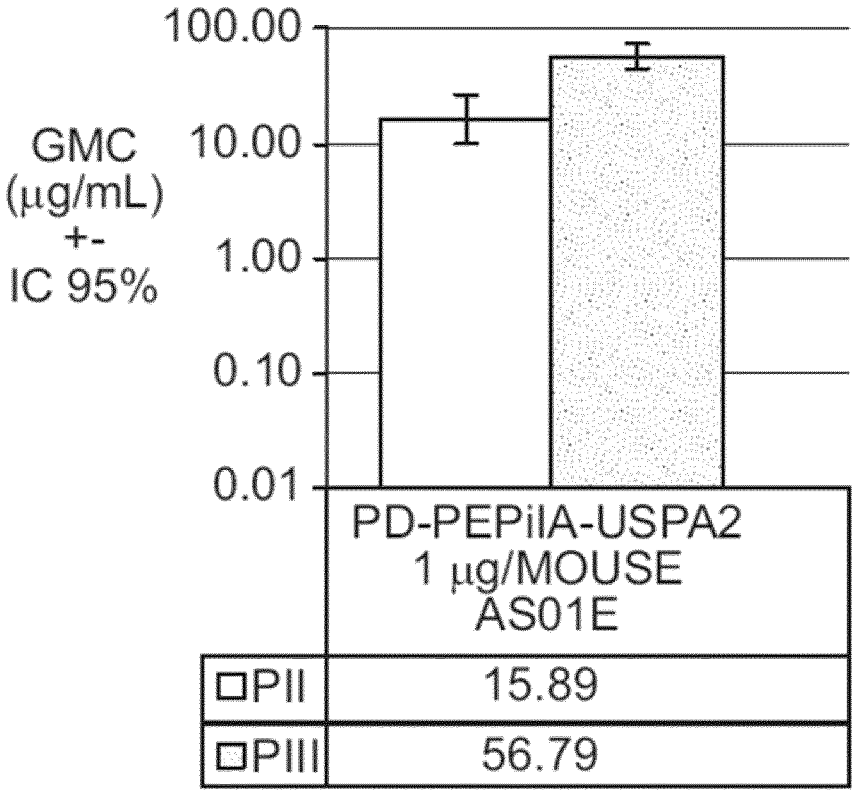
IgG RESPONSE INDUCED AGAINST PilA
GMC (µg/mL) +- IC 95%
100.00
10.00
1.00
0.10
0.01
PD-PEPilA-USPA2
1 µg/MOUSE
AS01E
PII
15.89
PIII
56.79

Figure 6(D)

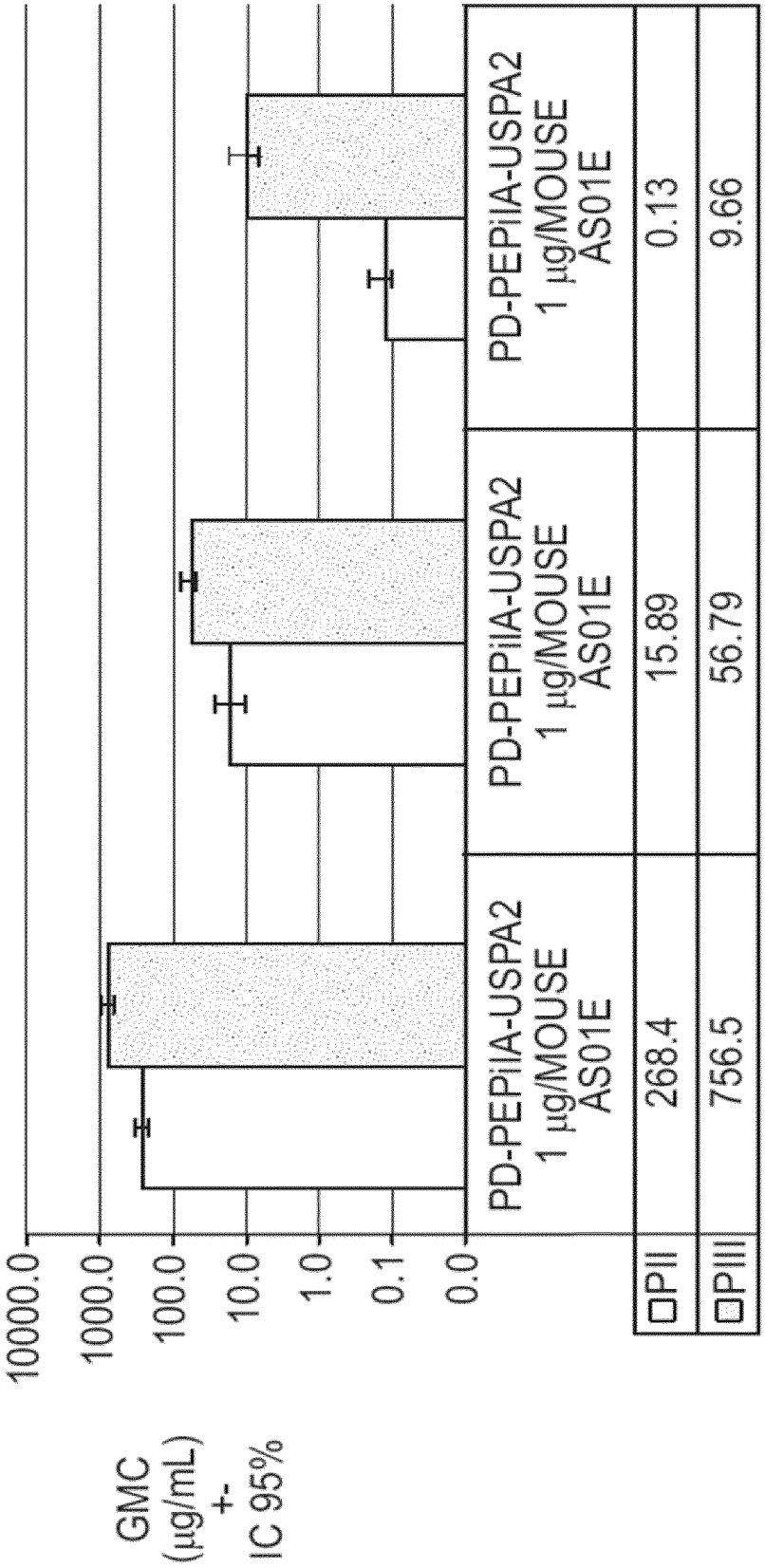
GMC (µg/mL) + - IC 95%
10000.0
1000.0
100.0
10.0
1.0
0.1
0.0
PD-PEPiIA-USPA2 1 µg/MOUSE AS01E
PD-PEPiIA-USPA2 1 µg/MOUSE AS01E
PD-PEPiIA-USPA2 1 µg/MOUSE AS01E
PII
PIII
268.4
15.89
0.13
756.5
56.79
9.66

Figure 6(E)

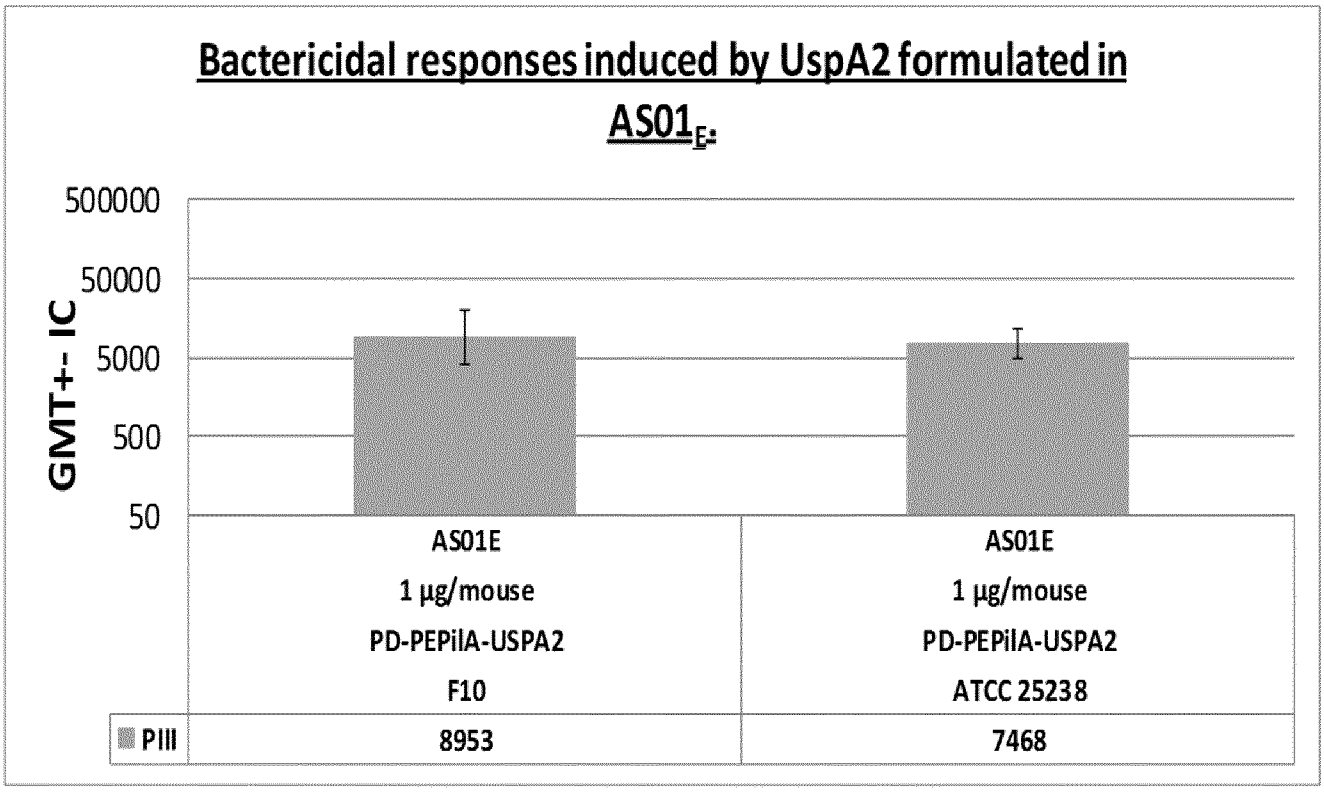
Bactericidal responses induced by UspA2 formulated in AS01E.
GMT+- IC
500000
50000
5000
500
50
AS01E 1 µg/mouse PD-PEPilA-USPA2 F10
AS01E 1 µg/mouse PD-PEPilA-USPA2 ATCC 25238
PIII
8953
7468

Figure 7

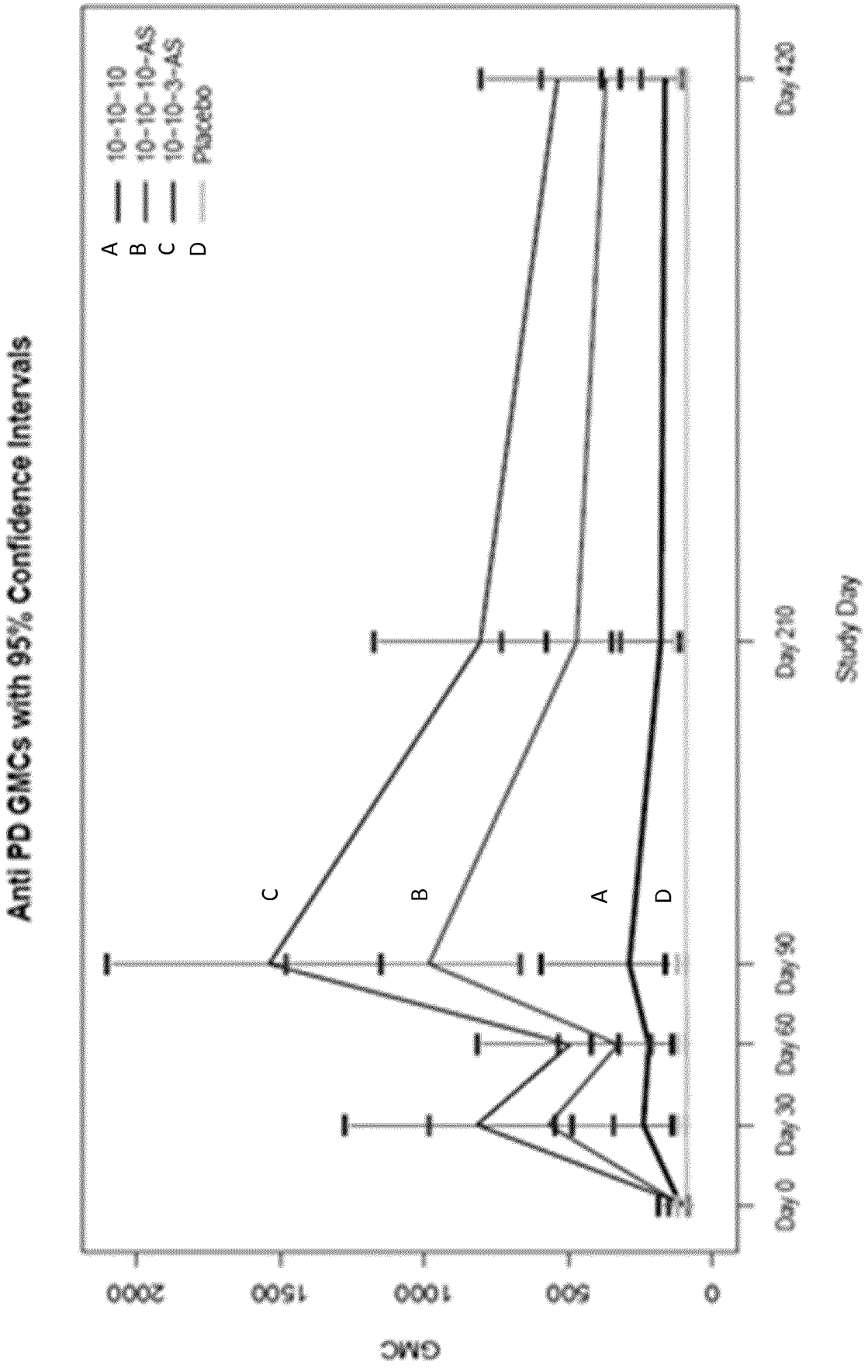
Anti PD GMCs with 95% Confidence Intervals
GMC
0
500
1000
1500
2000
Study Day
Day 0
Day 30
Day 60
Day 90
Day 210
Day 420
A
B
C
D
10-10-10
10-10-10-AS
10-10-3-AS
Placebo

Figure 8(A)

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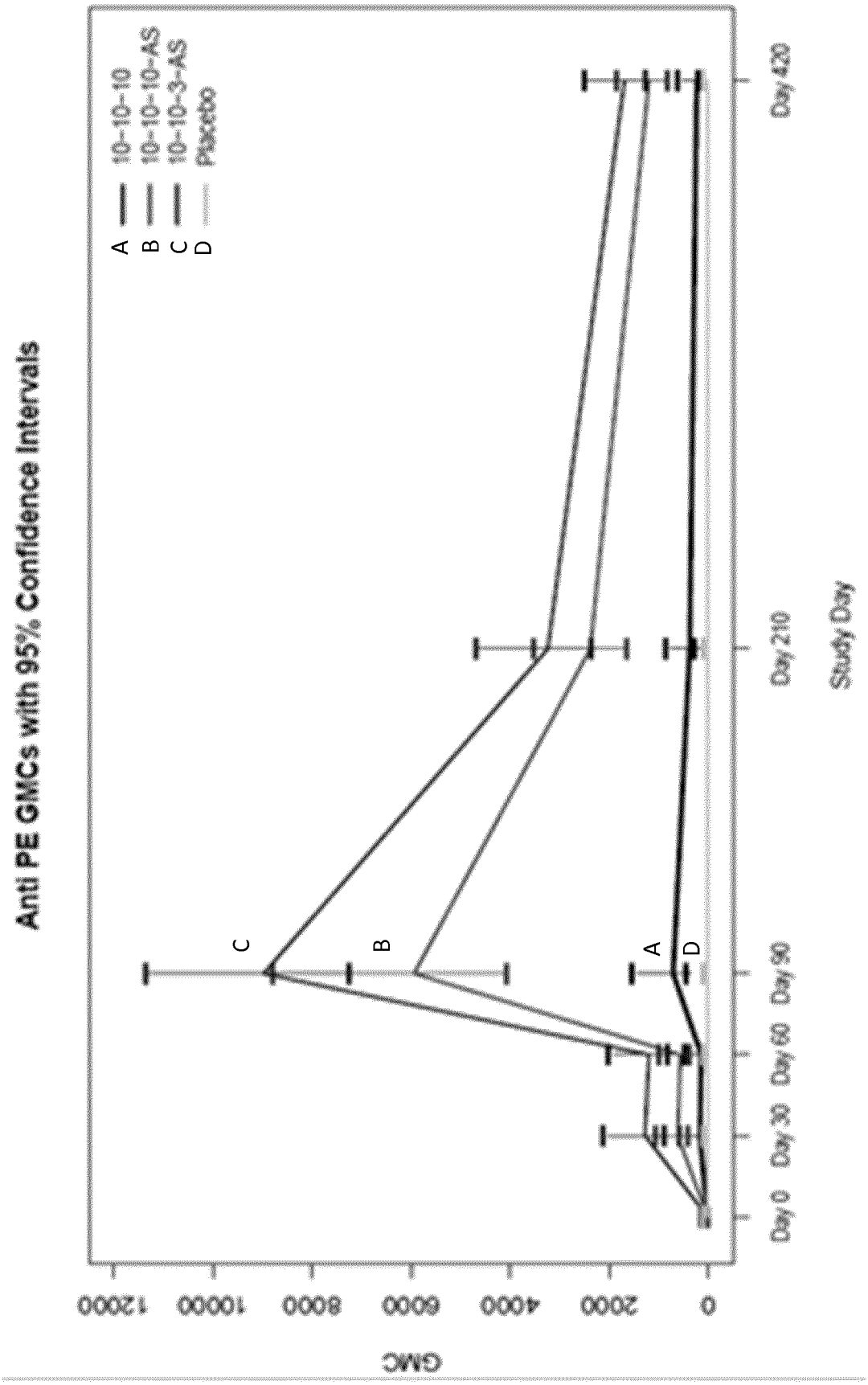

Figure 8(B)

17/18

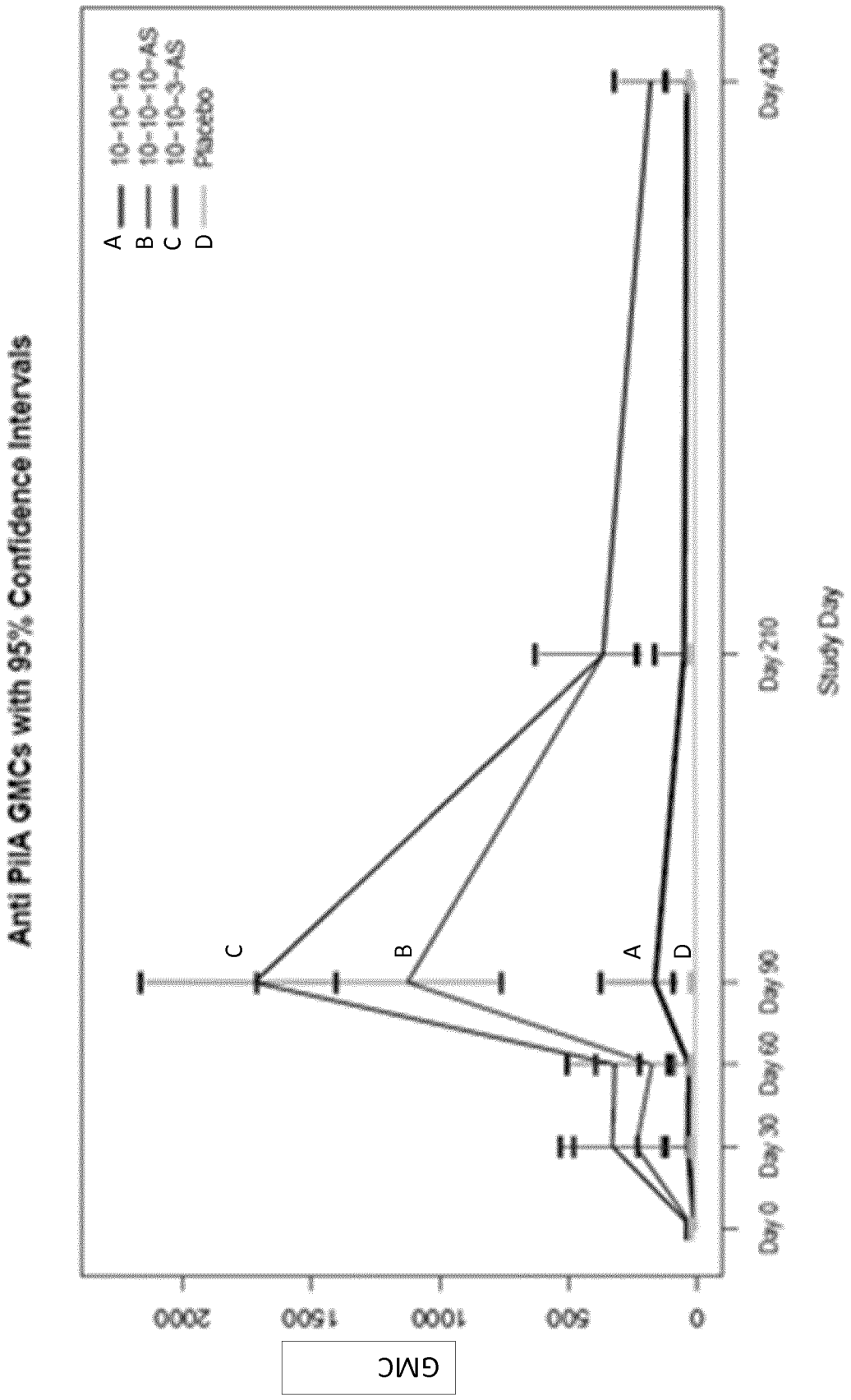

Figure 8(C)

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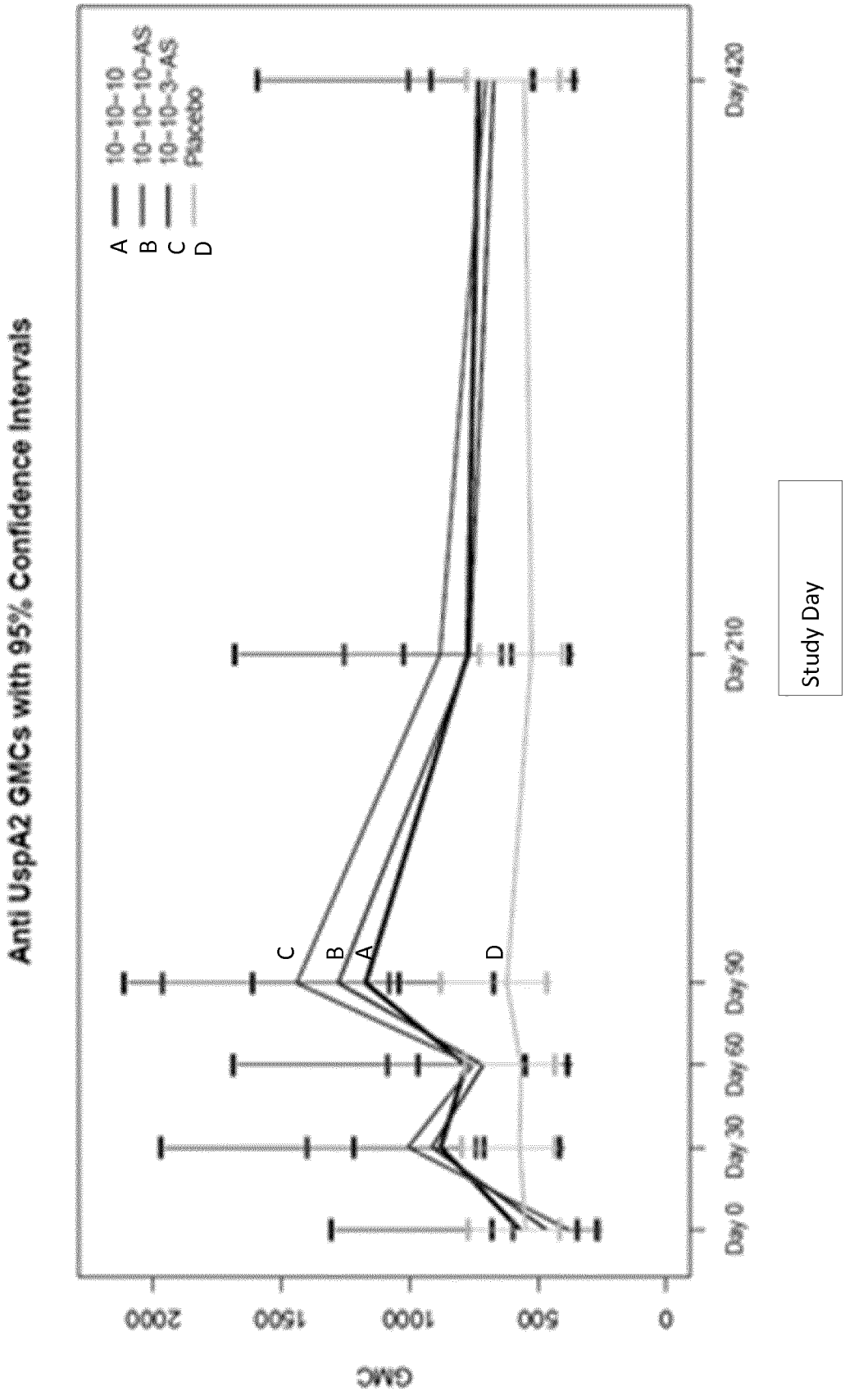

Figure 8(D)