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Safety and immunogenicity of non-typeable *Haemophilus influenzae-Moraxella catarrhalis* vaccine

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ABSTRACT

Non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat) are frequent pathogens in acute exacerbations of COPD. We assessed the safety, reactogenicity and immunogenicity of different investigational vaccine formulations containing surface proteins of NTHi (PD and PE-PilA) and Mcat (UspA2) in adults with smoking history ≥10 pack-years, to immunologically represent the COPD population.

Participants received two doses 60 days apart in a randomised, observer-blind, placebo-controlled study (NCT02547974). In step 1, 30 healthy adults aged 18–40 years were randomised (1:1) to receive a non-adjuvanted formulation (10-10-PLAIN) or placebo. In step 2, 90 smokers/ex-smokers aged 50–70 years randomly (1:1:1) received an AS01-adjuvanted formulation containing either 10 μg of each antigen (10-10-AS01) or 10 μg of each NTHi antigen and 3.3 μg of Mcat antigen (10-3-AS01), or placebo.

Incidences of solicited local adverse events (AEs) tended to be highest in the AS01-adjuvanted vaccine groups. Most solicited AEs had mild/moderate intensity. No vaccine-related serious AEs were reported. The 10-3-AS01 formulation induced the best humoral immune response against the NTHi antigens. Responses against the Mcat antigen were similar across groups, with waning immunogenicity after 30 days post-dose 2.

The investigational NTHi-Mcat vaccine had an acceptable safety and reactogenicity profile and good immunogenicity in older adults with a smoking history.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a debilitating, progressive disease with a global prevalence around 10–12% among older adults [1,2], which is likely to increase because of population aging and continued exposure to the main risk factors, cigarette smoke and air pollution [3]. Its course is characterised by exacerbations of respiratory symptoms, which increase the morbidity and mortality associated with the disease [4] and account for a significant proportion (40–60%) of medical costs for COPD [5].

Acquisition of new bacterial strains is believed to be an important cause of acute exacerbations of COPD (AECOPD) [6]. Although estimates vary widely, non-typeable *Haemophilus influenzae* (NTHi) appears to be the main bacterial pathogen associated with exacerbations, followed by *Moraxella catarrhalis* (Mcat) and *Streptococcus pneumoniae* [7–9]. Vaccination to reduce the frequency of AECOPD caused by bacteria may therefore be beneficial. No vaccine is indicated for the prevention of AECOPD, although influenza and pneumococcal vaccines, which are routinely recommended to COPD patients [3], may have some effect on their frequency [10].

There is evidence that NTHi and Mcat are co-pathogens in respiratory tract infections and COPD [11]. Complement resistance factors present on outer membrane vesicles produced by Mcat appear to protect NTHi from complement-mediated killing *in vitro* [12]. Co-infection with NTHi and Mcat also promotes the increased resistance of biofilms to antibiotics and host clearance [13,14].

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An investigational adjuvanted multi-component vaccine has been developed to potentially reduce the frequency of moderate and severe AECOPD associated with NTHi and Mcat, containing four surface proteins involved in the virulence mechanisms of both bacterial pathogens. Three conserved proteins were selected from NTHi, a free recombinant protein D (PD) and a recombinant fusion protein combining protein E and Pilin A (PE-PilA), and the fourth from Mcat, ubiquitous surface protein A2 (UspA2). PD is a highly conserved surface lipoprotein [15], while PE is an adhesin involved in direct interactions with lung epithelial cells and host proteins [16] and human complement resistance [17,18]. PilA plays a key role in biofilm formation, adherence to human epithelial cells and colonisation of the upper respiratory tract [19]. UspA2 is a putative autotransporter macromolecule and vitronectin-binding protein involved in mediating Mcat serum resistance [20,21] as well as epithelial cell adherence [22]. Anti-UspA2 antibodies significantly reduced the lung bacterial load in mice challenged with homologous or heterologous Mcat strains [23], suggesting UspA2 can induce broad cross protection.

The NTHi proteins had an acceptable safety and reactogenicity profile and induced antigen-specific immune responses in phase I studies in current and former smokers aged 50–70 years and healthy 18–40 year-olds [24]. Formulations that included the Adjuvant System AS01, a liposome-based vaccine adjuvant system containing two immunostimulants (3-O-desacyl-4′-monophosphoryl lipid A and the saponin QS-21 [25]) produced the highest humoral and cellular immune responses in older adults.

We conducted a phase I study to evaluate the safety, reactogenicity and immunogenicity of a two-dose schedule of different formulations of the investigational NTHi-Mcat vaccine in adults. Cigarette smoking is the most commonly encountered risk factor for COPD and evidence suggests it can alter the immune system before COPD is recognised [26–28]. Therefore, as in one of the previous phase I studies of the NTHi protein vaccine [24], we recruited adults with a smoking history of at least 10 pack-years to immunologically represent the COPD population.

A Focus on the Patient section (Fig. 1) summarises the clinical relevance and impact of this study on the patient population.

2. Methods

2.1. Study design and subjects

This phase I, randomised, observer-blind, placebo-controlled study was conducted in three centres in Belgium between August 2015 and March 2017 (ClinicalTrials.gov identifier: NCT02547974). Participants were randomised, using a blocking scheme (1:1 ratio at step 1 and 1:1:1 ratio at step 2), to receive two vaccine formulation doses 60 days apart (Fig. 2). The two randomisation lists were generated at GSK using MATerial Excellence (MATEX), a program developed by GSK for use with Statistical Analysis Systems (SAS) software. Treatment was allocated at each site via a central randomisation system (SBIR), using a minimisation algorithm accounting for centre at step 1 and age category (50–59 or 60–70 years), smoking status (current or former smoker) and forced expiratory volume in 1 s/forced vital capacity ratio ($\geq$0.7 or <0.7) at step 2. Due to differences in the appearance of the study vaccine and placebo formulation, the study was conducted in an observer-blind manner, i.e. vaccine recipients and those responsible for the evaluation of any study endpoint were blinded to the administered vaccines, although cell-mediated immune (CMI) response analyses could be unblinded. Vaccines were prepared and administered by authorised medical personnel who did not participate in any of the study's clinical evaluations or assays.

As this was the first time the Mcat UspA2 antigen was to be administered to humans, participants were enrolled in a staggered manner in two steps, with advancement to step 2 or second dose administration dependent on acceptable safety data during the

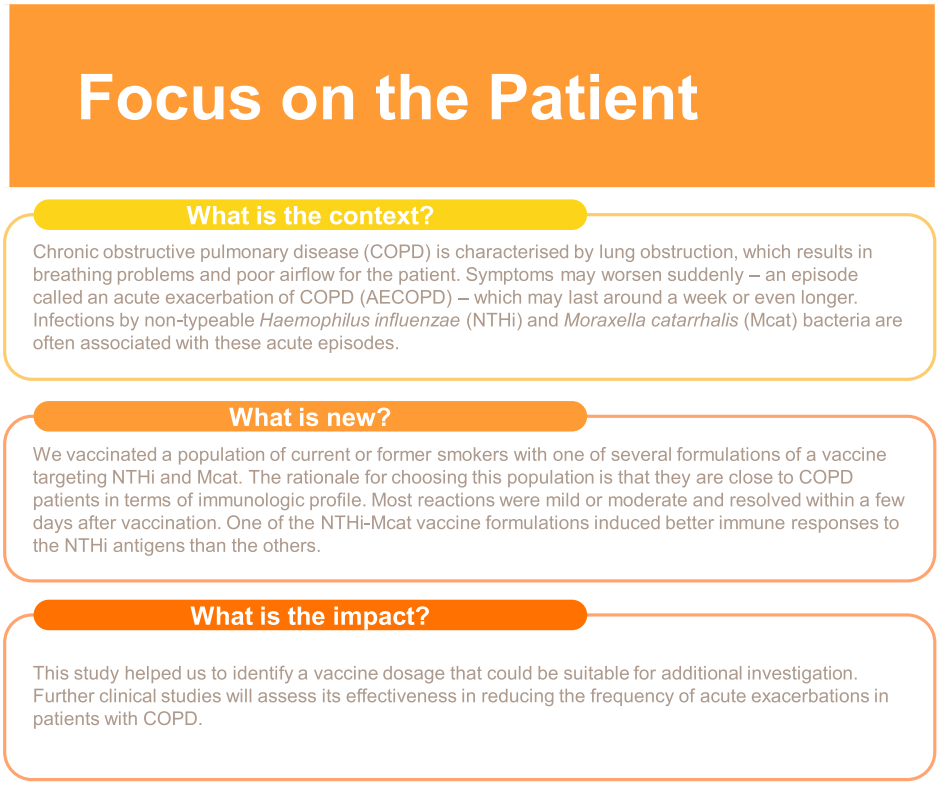

Fig. 1. Focus on the patient section.

week after administration of the previous dose, as determined by an internal safety review committee. In step 1, 30 healthy adults aged 18–40 years received a non-adjuvanted vaccine formulation containing 10 μg of each NTHi antigen and 10 μg of UspA2 antigen per dose (10-10-PLAIN) or placebo (Fig. 2). In step 2, 90 adults aged 50–70 years with a smoking history of at least 10 pack-years received an AS01-adjuvanted formulation, containing either 10 μg of each antigen per dose (10-10-AS01) or 10 μg of each NTHi antigen and 3.3 μg of Mcat UspA2 (10-3-AS01), or placebo. All study inclusion and exclusion criteria are provided in the online supplementary methods.

The primary objective was to assess the safety and reactogenicity of the investigational NTHi-Mcat vaccine formulations and the secondary objective was to describe their humoral and cellular immunogenicity after vaccination and persistence of immune responses up to 12 months after the second dose (Day 420). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The protocols and associated documents were reviewed and approved by an independent ethics committee. All participants provided written informed consent before study entry.

2.2. Study vaccines

Three formulations of investigational NTHi-Mcat vaccine were assessed:

- *10-10-Plain*: containing 10 μg PD, 10 μg PE-PilA (fusion protein) and 10 μg UspA2
- *10-10-AS01*: containing 10 μg PD, 10 μg PE-PilA (fusion protein) and 10 μg UspA2, with adjuvantation ($AS01_E$)
- *10-3-AS01*: containing 10 μg PD, 10 μg PE-PilA (fusion protein) and 3.3 μg UspA2, with adjuvantation ($AS01_E$).

The NTHi antigens and $AS01_E$ were described previously [24]. The recombinant *Escherichia coli* BLR (DE3) B2690-Rix4517 strain was used to express the Mcat UspA2 protein. The UspA2 protein was produced by fermentation of the recombinant *E. coli*, followed by protein purification and sterile filtration. In animal models, inclusion of UspA2 in the investigational vaccine did not have any relevant impact on the immune response to the NTHi antigens (unpublished data). The 10 μg UspA2 dose used in the 10-10-Plain and 10-10-AS01 formulations of the study vaccine was selected as it was the same dose selected for the NTHi antigens based on the results of phase I clinical trials, where no antigen-dose effect was observed for the NTHi antigens [29,30].

The 3.3 μg UspA2 dose used in the 10-3-AS01 formulation of the study vaccine was selected due to the detection of natural anti-UspA2 antibodies in a high proportion of adults, including COPD patients [29].

The vaccines were administered intramuscularly.

2.3. Safety and reactogenicity

Solicited local (pain, redness and swelling at the injection site) and general (fatigue, gastrointestinal symptoms, headache, myalgia and fever) adverse events (AEs) were recorded for 7 days after each dose and unsolicited AEs for 30 days after each dose on diary cards. AE intensity was graded on a 0–3 scale. Grade 3 intensity was defined as redness or swelling of diameter >100 mm, temperature >39.5 °C and, for all other AEs, prevention of normal activities. Data on potential immune-mediated diseases (pIMDs; including autoimmune and other inflammatory or neurologic disorders of interest) [31] and serious AEs (SAEs) were collected throughout the study.

Haematological and biochemical parameters, measured at screening, before and 7 days after each vaccination, and 5 and 12 months after the second dose, were graded 0–4 [32]. Abnormal laboratory findings that were judged by the investigator to be clinically significant were recorded as an AE or SAE.

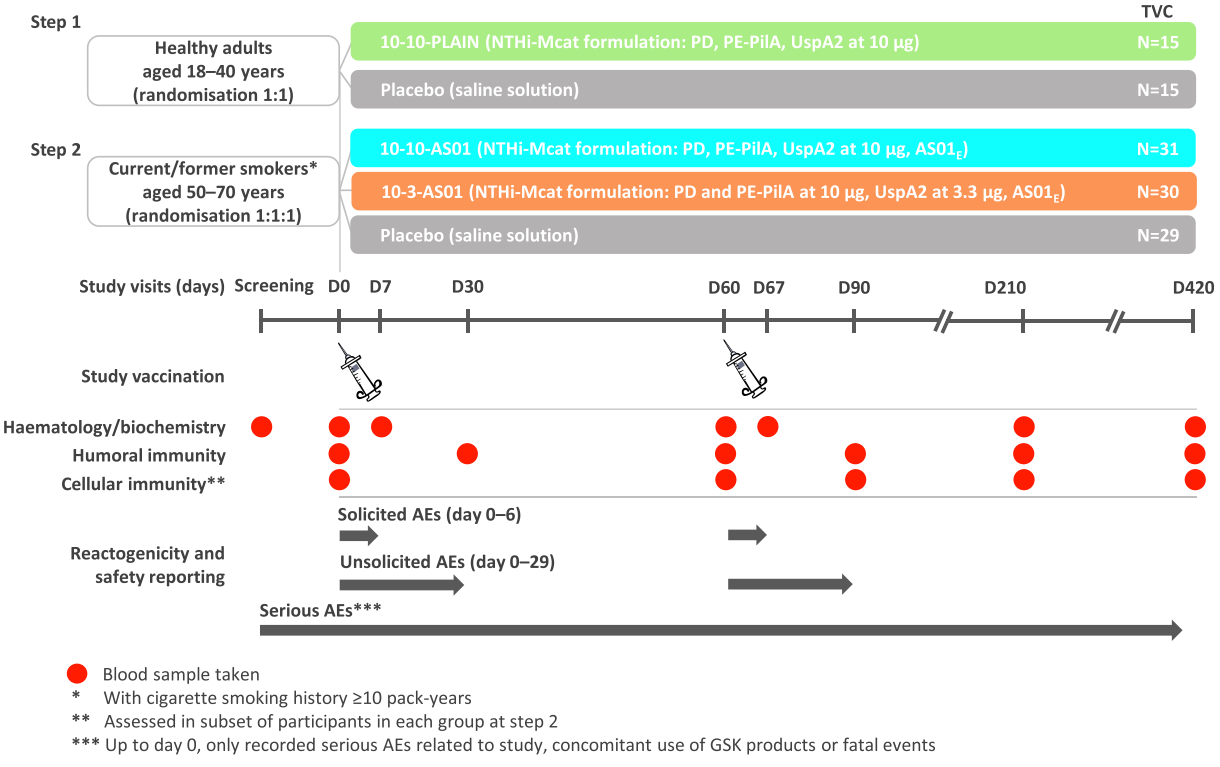

Fig. 2. Study design. AE, adverse event; D, days; N, number of participants in total vaccinated cohort (TVC). NTHi, non-typeable *Haemophilus influenzae*; Mcat, *Moraxella catarrhalis*; PD, protein D; PE, protein E; PilA, Pilin A; UspA2, ubiquitous surface protein A2.

2.4. Humoral and cellular immunogenicity

Immunoglobulin G antibody geometric mean concentrations (GMCs) to each vaccine antigen were measured by enzyme-linked immunosorbent assay of blood samples taken before and 30 days after each vaccination, and 5 and 12 months after the second dose. Sera were stored at −20 °C until assayed. Blood samples for CMI response analysis were taken before vaccination, 30 days and 5 and 12 months post-dose 2 in a sub-cohort of subjects at step 2 (50% of subjects per group). CMI responses (antigen-specific $CD4^+$ and $CD8^+$ T cells) were measured by flow cytometry using intracellular cytokine staining on peripheral blood mononuclear cells, following an adaptation of previously described methods [33]. The frequency of antigen-specific $CD4^+$ and $CD8^+$ T cells expressing at least two cytokine markers among CD40L, IL-2, TNF-α, IFN-ɣ, IL-13 or IL-17 was calculated.

2.5. Statistical analysis

As this was a first-time-in-humans study for the Mcat UspA2 antigen and the primary objective was safety, sample size was not powered to demonstrate any hypothesis. The probability of detecting an AE, assuming a Poisson model and true incidence of 5%, was 78.5% with 30 participants per group in step 2. Data were pooled from the groups that received placebo in step 1 and step 2.

Since this phase I study enrolled a limited number of subjects, no formal statistical comparisons between groups were conducted. The safety analysis was performed on the total vaccinated cohort, including all vaccinated participants. The incidence of AEs per study group was calculated with exact 95% confidence intervals (CIs) after each vaccine dose. The immunogenicity analysis was performed on the according-to-protocol (ATP) cohort for immunogenicity, including eligible adults who received the study vaccine as specified in the protocol and complied with study procedures. Antibody GMCs were determined with 95% CIs. The two adjuvanted vaccine groups were considered significantly different if the 95% CI for the antibody GMC ratio (adjusted for baseline concentration) between the groups did not contain the value 1. These results should be interpreted with caution as there was no adjustment for multiplicity and any clinical relevance is unknown. The frequency of specific $CD4^+$ and $CD8^+$ T cells expressing at least two cytokine markers was summarised by study group using descriptive statistics. Intracellular cytokine staining data were expressed as frequency of $CD4^+$ and $CD8^+$ T cells expressing cytokines (number/10^6 $CD4^+$/$CD8^+$ T cells).

Statistical analyses were performed using SAS version 9.3 on SAS Drug Development 4.3.

3. Results

3.1. Study population

One hundred and twenty adults were enrolled and vaccinated; 15 healthy adults aged 20–40 years (mean 29 years) were vaccinated with non-adjuvanted NTHi-Mcat vaccine at step 1 and 61 current or former smokers aged 50–71 years (mean 59 years) with an adjuvanted NTHi-Mcat vaccine formulation at step 2 (Table 1). Forty-four adults (15 at step 1 and 29 at step 2) were enrolled in the placebo control group. Three participants were excluded from the ATP cohort for immunogenicity for reasons shown in Fig. 3 and 119 completed the study (one consent withdrawal not due to AE in 10-10-PLAIN group; also excluded from ATP cohort).

Most participants (73%) were female in the 10-10-PLAIN group, while 42% and 43% were female in the 10-10-AS01 and 10-3-AS01 groups, respectively (Table 1). All participants were white (European heritage).

Table 1
Characteristics of the study participants at enrolment (total vaccinated cohort).

Characteristic	10-10-PLAIN (N = 15)	10-10-AS01 (N = 31)	10-3-AS01 (N = 30)	Placebo (N = 44)*
Age (years) at dose 1, mean (SD)	29.1 (5.91)	58.9 (6.49)	58.5 (5.82)	47.2 (16.72)
Age range (years)	20–40	50–71	51–70	19–69
Male gender, n (%)	4 (26.7)	18 (58.1)	17 (56.7)	23 (52.3)
Smoking status, n (%)				
Current smoker	0	13 (41.9)	10 (33.3)	10 (34.5)
Former smoker	0	18 (58.1)	20 (66.7)	19 (65.5)

N, number of participants; n, number of participants in a specific category; SD, standard deviation.
* 15 healthy participants aged 18–40 years, 29 current or former smokers aged 50–70 years.

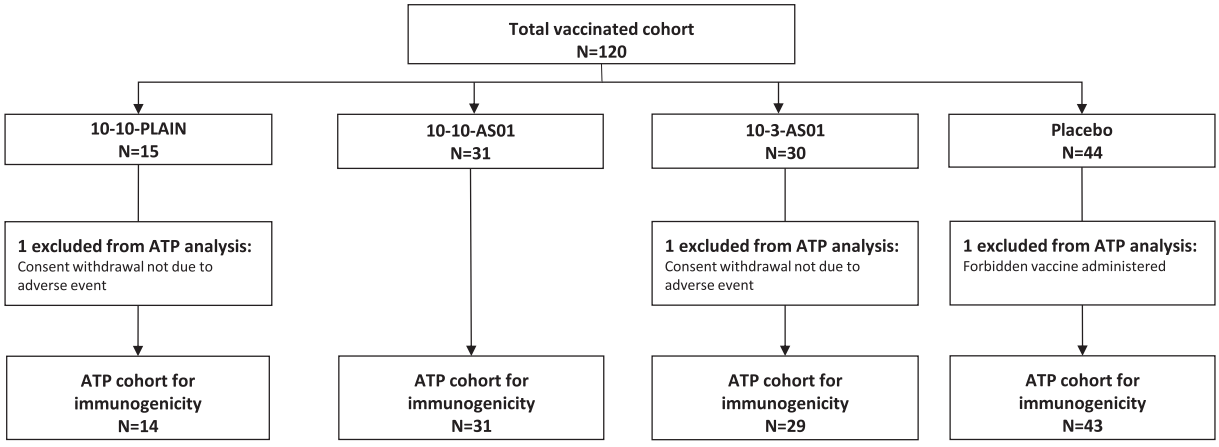

Fig. 3. Disposition of the study participants and reasons for exclusion from the according-to-protocol cohort for immunogenicity. N, number of participants; ATP, according-to-protocol.

3.2. Safety and reactogenicity

Pain was the most frequent solicited local AE during the 7-day post-vaccination period, with overall incidences per subject of 46.7%, 80.6%, 93.3% and 25.0% in the 10-10-PLAIN, 10-10-AS01, 10-3-AS01 and placebo groups, respectively (Fig. 4). The most frequent solicited general AEs were headache in the 10-10-PLAIN group (overall incidence per subject 40.0%), myalgia in the 10-10-AS01 group (35.5%) and fatigue in the 10-3-AS01 (60.0%) and placebo groups (27.3%) (Fig. 4).

After each dose and overall per subject, incidences of individual solicited local AEs were higher in the groups who received adjuvanted NTHi-Mcat vaccine than those who received unadjuvanted vaccine or placebo (Fig. 4). There was one report of grade 3 pain after the first dose in the 10-10-AS01 group and one after the second dose in the 10-3-AS01 group. There were seven reports of grade 3 general solicited AEs, two of which were in the 10-10-PLAIN group (fatigue and headache after the first dose). The remaining five reports were in the adjuvanted NTHi-Mcat vaccine groups (one report of headache after first 10-3-AS01 dose and one headache [10-10-AS01], one fatigue [10-3-AS01] and two myalgia cases [10-10-AS01 and 10-3-AS01] after second dose). All grade 3 solicited AEs were transient, lasting no longer than 2 days, and resolved spontaneously.

During the 30-day post-vaccination period, at least one unsolicited AE was reported by 53.3%, 64.5%, 60.0% and 65.9% of par-

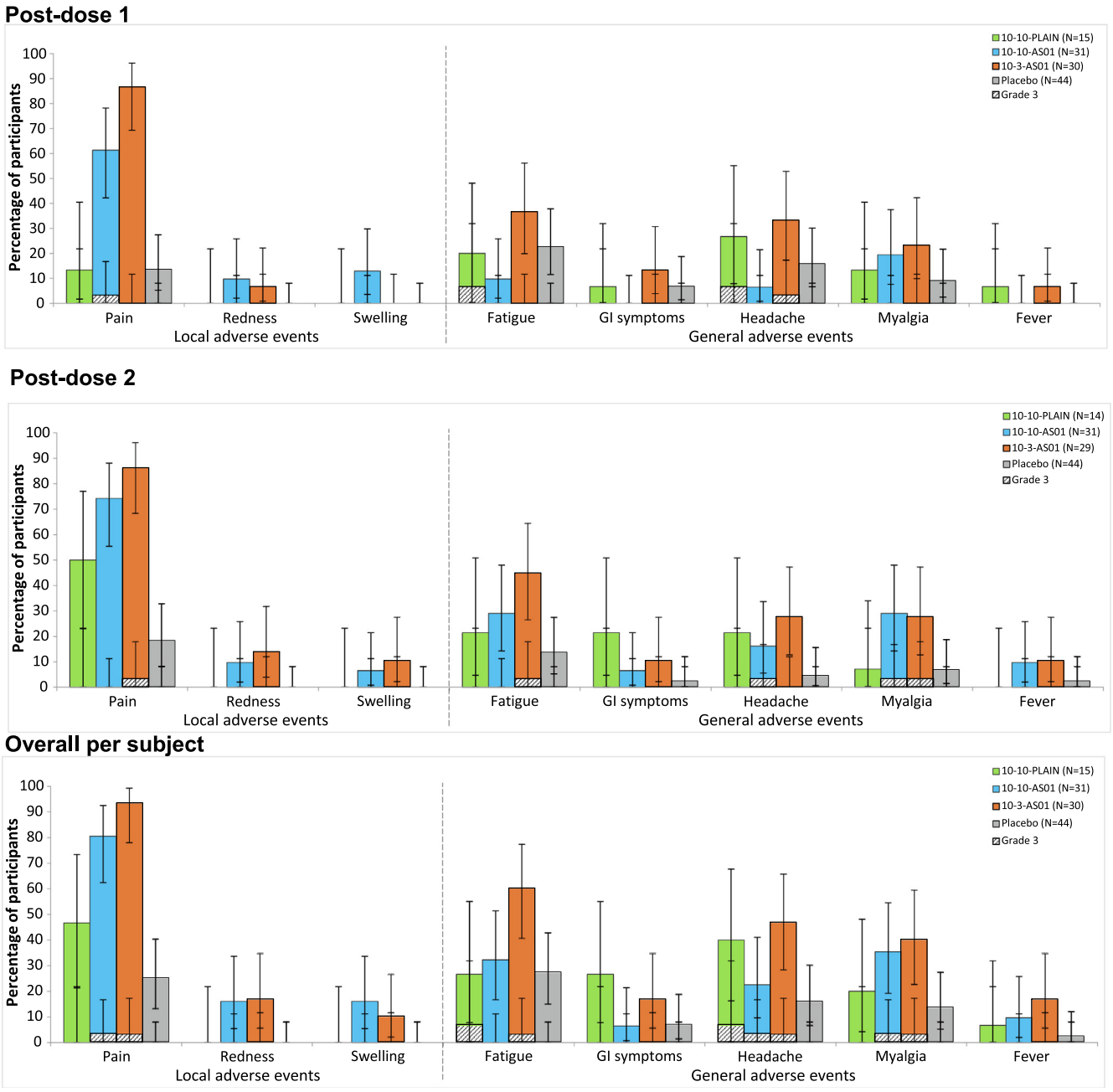

Fig. 4. Percentages of participants (with exact 95% confidence intervals) reporting solicited local (pain, redness and swelling) and general (fatigue, gastrointestinal symptoms, headache, myalgia and fever) adverse events during the 7-day post-vaccination period after each dose and overall per subject (total vaccinated cohort). GI (gastrointestinal) symptoms defined as nausea, vomiting, diarrhoea and/or abdominal pain. Fever defined as temperature ≥37.5 °C. Grade 3 intensity defined as redness or swelling of diameter >100 mm, temperature >39.5 °C and, for all other AEs, prevention of normal activities. N, number of participants.

ticipants in the 10-10-PLAIN, 10-10-AS01, 10-3-AS01 and placebo groups, respectively, most commonly nasopharyngitis and headache (Table 2). During the entire study, two participants in the active vaccine groups reported four SAEs (gastroenteritis in one subject in 10-10-PLAIN group; pneumonia, rib fracture and nasal septum deviation in one subject in 10-3-AS01 group) and two in the placebo group reported two SAEs (inguinal hernia and ankle fracture), none of which were considered as causally related to vaccination. Two pIMDs were reported. Dermatomyositis, diagnosed by skin biopsy, was reported in a 28-year-old subject in the 10-10-PLAIN group, with onset at Day 177 post-dose 2, that did not resolve by the end of the study. Trigeminal neuralgia (left upper cheek) was reported in a 60-year-old subject in the 10-3-AS01 group, with symptom onset 63 days after the first dose, before receiving the second dose, which resolved by the end of the study. The subject had a medical history of left-sided facial nerve paralysis of unknown cause approximately 10 years before this event. Both pIMDs were considered by the investigator as non-serious and causally related to vaccination.

No grade 3 or 4 laboratory abnormalities were observed during the study, apart from one grade 3 change in haemoglobin level in the placebo group.

3.3. Immunogenicity

GMCs for anti-PD, anti-PE and anti-PilA antibodies increased one month after each adjuvanted vaccine dose and waned 5 and 12 months after the second dose but remained higher than baseline (Fig. 5). The 10-3-AS01 formulation tended to induce higher or similar humoral responses in comparison to the 10-10-AS01 formulation against each NTHi antigen at all time points. Antibody GMC ratios adjusted for baseline concentration showed significantly higher GMCs in the 10-3-AS01 group compared to the 10-

Table 2
Percentage of participants reporting unsolicited adverse events (AEs) during the 30-day period after each vaccine dose (total vaccinated cohort).

	Percentage of participants (95% CI)			
	10-10-PLAIN (N = 15)	10-10-AS01 (N = 31)	10-3-AS01 (N = 30)	Placebo (N = 44)
At least one unsolicited AE	53.3 (26.6–78.7)	64.5 (45.4–80.8)	60.0 (40.6–77.3)	65.9 (50.1–79.5)
Related to vaccination	6.7 (0.2–31.9)	16.1 (5.5–33.7)	10.0 (2.1–26.5)	6.8 (1.4–18.7)
Grade 3 intensity	6.7 (0.2–31.9)	3.2 (0.1–16.7)	13.3 (3.8–30.7)	4.5 (0.6–15.5)
Grade 3 intensity related to vaccination	0	0	3.3 (0.1–17.2)*	0
Unsolicited AEs reported in >10.0% of participants in at least one group				
Nasopharyngitis	20.0 (4.3–48.1)	16.1 (5.5–33.7)	23.3 (9.9–42.3)	25.0 (13.2–40.3)
Headache	13.3 (1.7–40.5)	3.2 (0.1–16.7)	10.0 (2.1–26.5)	9.1 (2.5–21.7)
Diarrhoea	13.3 (1.7–40.5)	3.2 (0.1–16.7)	0	2.3 (0.1–12.0)

N, number of participants; 95% CI, 95% confidence interval.
* Trigeminal neuralgia in one subject also reported as a non-serious potential immune-mediated disease.

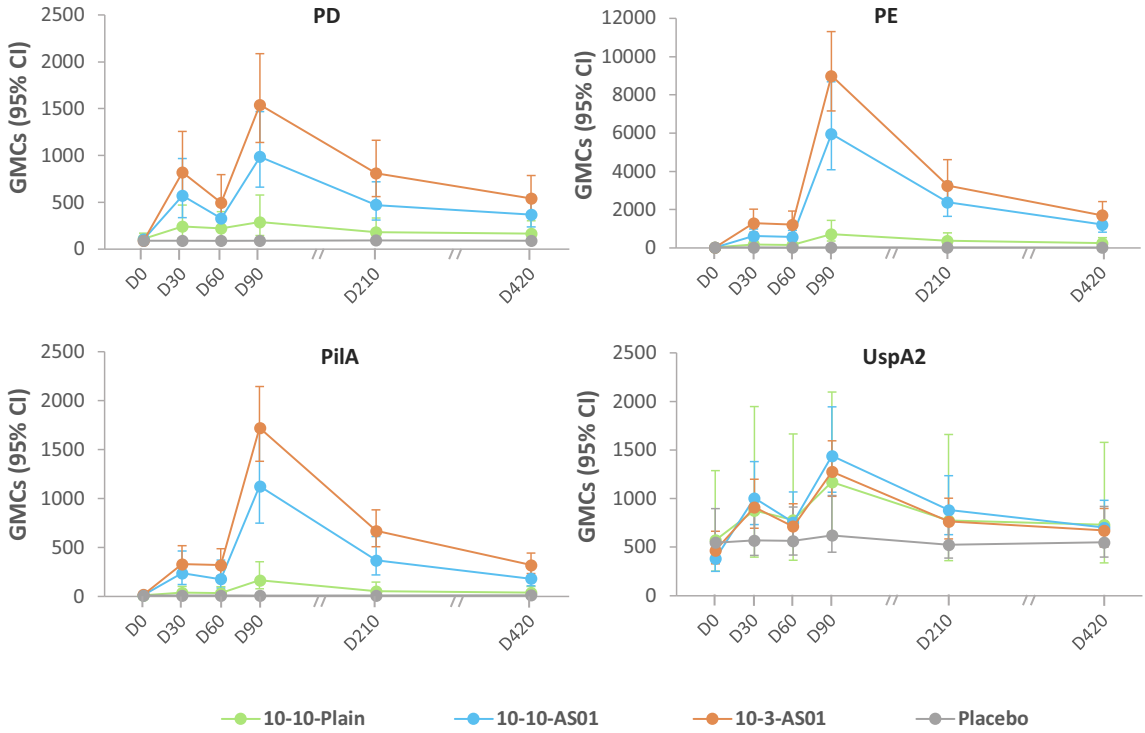

Fig. 5. Geometric mean concentrations of anti-PD, anti-PE, anti-PilA and anti-UspA2 antibodies (ATP cohort for immunogenicity). GMC, geometric mean concentration; 95% CI, 95% confidence interval. PD, protein D; PE, protein E; PilA, Pilin A; UspA2, ubiquitous surface protein A2. D0, pre-dose 1; D30, 30 days post-dose 1; D60, pre-dose 2; D90, 30 days post-dose 2; D210, 5 months post-dose 2; D420, 12 months post-dose 2. 10-10-Plain contained 10 μg PD, 10 μg PE-PilA, 10 μg UspA2; 10-10-AS01 contained 10 μg PD, 10 μg PE-PilA, 10 μg UspA2, with $AS01_E$; 10-3-AS01 contained 10 μg PD, 10 μg PE-PilA, 3.3 μg UspA2, with $AS01_E$. Number of subjects with available results at each time point: 13 or 14 in 10-10-PLAIN group, between 29 and 31 in 10-10-AS01 and 10-3-AS01 groups, between 39 and 43 in placebo control group.

10-AS01 group in terms of anti-PD antibody response one month post-dose 2 and anti-PilA antibody response 12 months post-dose 2 (online supplementary Table S1).

Anti-UspA2 antibody GMCs at baseline were relatively high in all groups (384.1–572.5 EU/mL). There was a transient increase in anti-UspA2 antibody GMCs after each vaccination, which peaked 30 days after dose 2, with comparable responses among active dose groups at each time point (Fig. 5).

Evaluation of the frequency of antigen-specific CD4+ T cells expressing at least two markers among CD40L, IL-2, TNF-α, IFN-γ, IL-13 and IL-17 showed increases from baseline up to one month after the second adjuvanted NTHi-Mcat vaccine dose (Fig. 6). There was high variability in the number of specific $CD4^+$ T cells for each vaccine antigen at each time point and $CD4^+$ T cell responses were low. No antigen-specific CD8+ T cell responses were detected in any group (data not shown).

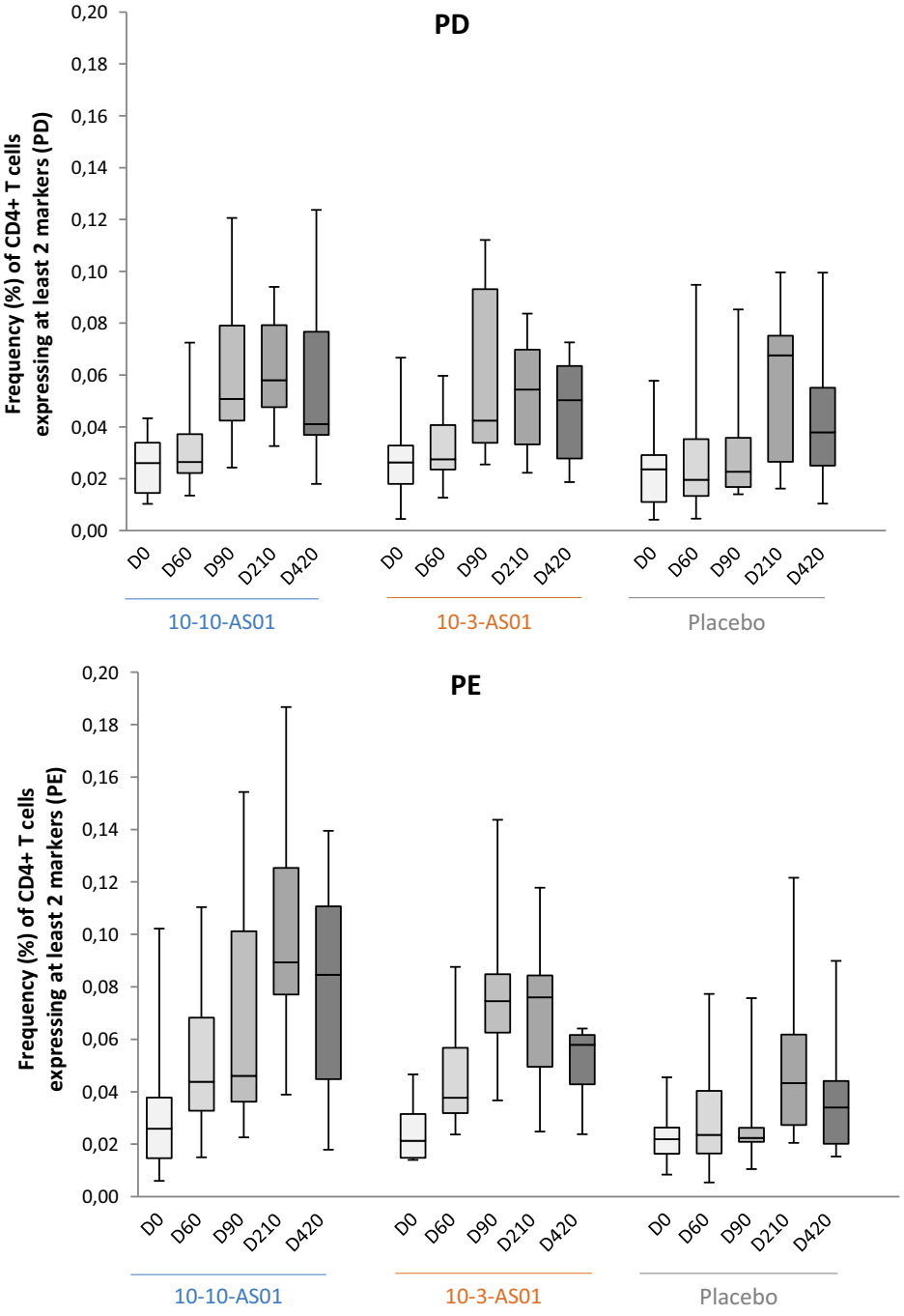

Fig. 6. Frequency (%) of PD, PE, PilA and UspA2 specific $CD4^+$ T cells expressing at least two markers amongst CD40L, IL-2, TNF-α, IFN-γ, IL-13 and IL-17 before and after vaccination (ATP cohort for immunogenicity). Median, first/third quartile and maximum/minimum percentages shown. PD, protein D; PE, protein E; PilA, Pilin A; UspA2, ubiquitous surface protein A2. 10-10-Plain contained 10 μg PD, 10 μg PE-PilA, 10 μg UspA2; 10-10-AS01 contained 10 μg PD, 10 μg PE-PilA, 10 μg UspA2, with $AS01_E$; 10-3-AS01 contained 10 μg PD, 10 μg PE-PilA, 3.3 μg UspA2, with $AS01_E$. D0, pre-dose 1, D60, pre-dose 2; (D90, 30 days post-dose 2; D210, 5 months post-dose 2; D420, 12 months post-dose 2. Frequency, number/10^6 $CD4^+$ T cells, expressed as percentage. Number of subjects with available results at each time point: between 7 and 16 in 10-10-AS01 group, between 8 and 12 in 10-3-AS01 group, between 11 and 15 in placebo control group.

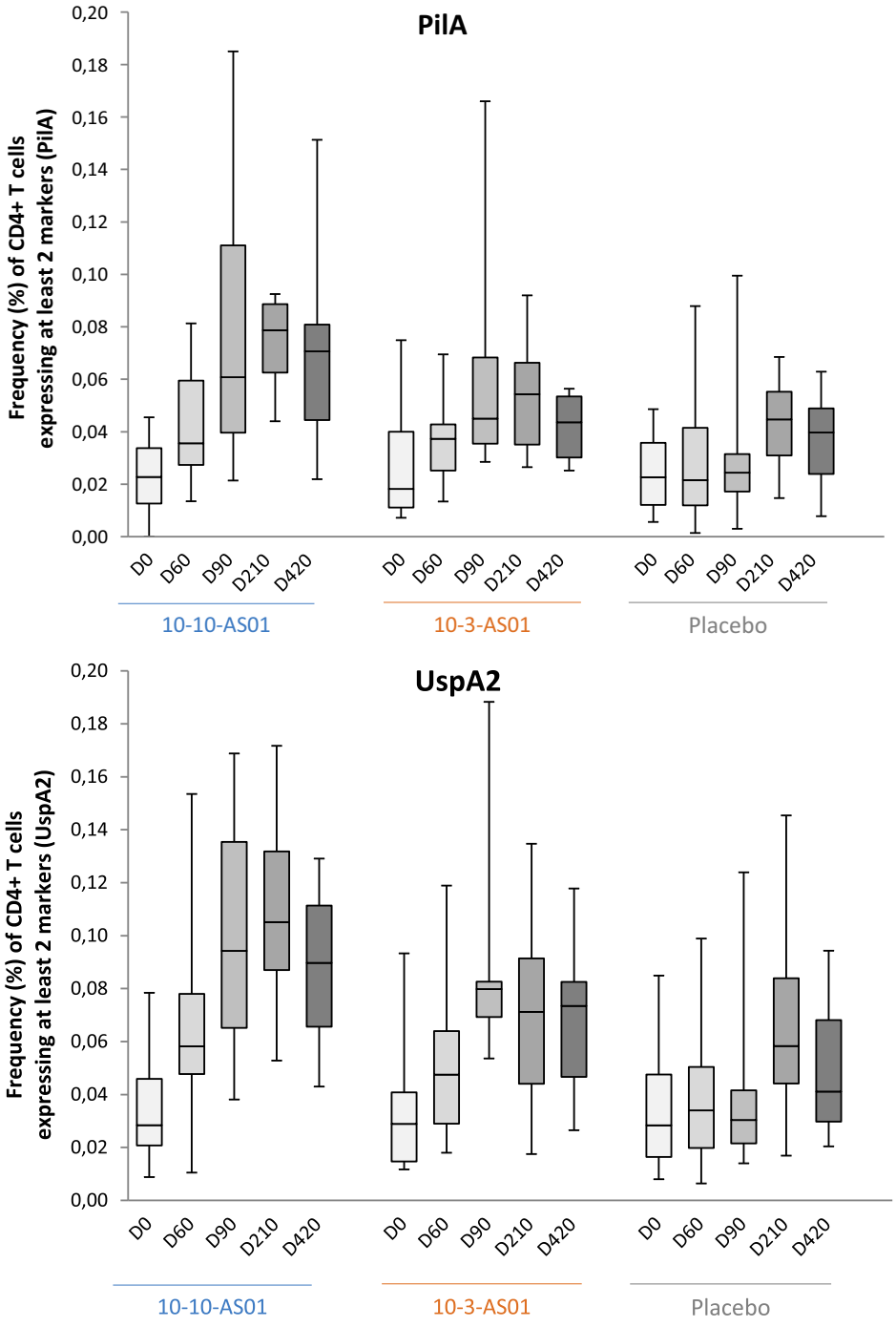

Fig. 6 (*continued*)

4. Discussion

This is the first report of the safety, reactogenicity and immunogenicity of an investigational vaccine containing both NTHi and Mcat surface proteins administered in a two-dose schedule to adults. Since a limited number of participants was enrolled, no formal statistical comparisons between groups were performed. However, the results show all NTHi-Mcat vaccine formulations had acceptable safety and reactogenicity profiles, including the adjuvanted formulations administered to current or former smokers. This was in line with observations with an NTHi investigational vaccine containing the same NTHi antigen doses and $AS01_E$, when administered to older smokers/ex-smokers of similar age [24]. Additionally, incidences of solicited local or general AEs following the first dose were similar to those following the second dose across all vaccine formulations.

As expected from other studies that compared adjuvanted vaccines to non-adjuvanted formulations or placebo [24,34–36], reactogenicity was highest in the adjuvanted NTHi-Mcat vaccine groups, which may be related to more intense activation of the innate immune response by AS-adjuvanted vaccines [37]. Most reported solicited AEs were mild to moderate in intensity and any grade 3 events were transient.

Humoral immune responses to the NTHi antigens were comparable to those observed in the previous study of the NTHi vaccine in smokers/ex-smokers [24]. The adjuvanted vaccine formulations

induced persistent specific immune responses against the NTHi antigens up to 12 months after the second vaccine dose. There was a moderate and transient specific response against the Mcat antigen, which may have been due to the relatively high concentration of anti-UspA2 antibodies before vaccination. It has been reported previously that anti-UspA2 antibody concentrations tend to increase with age [38].

The CMI response results must be interpreted with caution due to the small number of subjects included in the investigation and high individual variability in number of specific T cells for all antigens and time points. Antigen-specific CMI responses one month after the second dose, in terms of $CD4^+$ T cells expressing at least two markers, tended to be higher in the adjuvanted vaccine groups than with placebo, although responses were low. A low CMI response was also noted for NTHi antigens in the previous study of smokers/ex-smokers [24] and may be due to age-related immunosenescence among the older adults [39] as well as smoking status, since cigarette smoking can stimulate dysregulation of the immune response [26–28]. $CD8^+$ T cell induction was not detected, which was consistent with other studies of AS01-adjuvanted recombinant protein vaccines in adults [24,37,40–42].

In conclusion, this phase I study demonstrated that the investigational NTHi-Mcat vaccine formulations have acceptable safety, reactogenicity and immunogenicity in older adults with a smoking history of at least 10 pack-years. The formulation containing 10 μg NTHi and 3.3 μg Mcat antigen doses has been selected for further investigation, taking into consideration summary scores derived from the immune response data (see online supplementary methods) and adjusted GMC ratios from the present study, together with results with the investigational NTHi vaccine [24]. Clinical studies will assess the effectiveness of the NTHi-Mcat vaccine in reducing the frequency of acute exacerbations in patients with COPD.

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Author contributions

AKA and MT were involved in the study conception and design. GLR, PVD, CV, IRD, MD, AKA, AT, LM and MT were involved in acquisition and generation of data and/or performed the study. GLR, PVD, CV, IRD, MD, AKA, AT, LM and MT were involved in data analysis and data interpretation. All authors contributed substantially to the development of the manuscript and approved the final version.

Support statement

The study funder, GlaxoSmithKline Biologicals SA, designed the study in collaboration with the investigators, and coordinated collection, analysis and interpretation of data. The investigators obtained data and cared for the study participants. The authors had full access to all data in the study, contributed to the writing of the report and had final responsibility for the decision to submit for publication.

Data sharing statement

Anonymised individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Conflict of interest

PVD acts as principal investigator for vaccine trials conducted on behalf of the University of Antwerp, for which the University obtains research grants from vaccine manufacturers. GLR reports his institutions, Ghent University Hospital and Ghent University, have received financial compensations from GSK Vaccines for the conduct of the study. CV reports grants from GSK. PVD, GLR and CV received no personal remuneration for this work. IDR, AT, MD, LM, MT and AKA are employees of the GSK group of companies and IDR, AT, MD, LM, MT and AKA hold shares/restricted shares in the GSK group of companies.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.04.041.

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