Vaccine 37 (2019) 6102–6111

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Non-typeable *Haemophilus influenzae* protein vaccine in adults with COPD: A phase 2 clinical trial

Tom M.A. Wilkinson [a,b,c], Stuart Schembri [d], Christopher Brightling [e], Nawar D. Bakerly [f], Keir Lewis [g], William MacNee [h], Lars Rombo [i], Jan Hedner [j], Martin Allen [k], Paul P. Walker [l], Iris De Ryck [m], Annaelisa Tasciotti [m], Daniela Casula [m], Philippe Moris [n], Marco Testa [m,*], Ashwani K. Arora [m]

[a] *Clinical and Experimental Sciences, University of Southampton, Faculty of Medicine, Southampton General Hospital, Southampton, UK*
[b] *Southampton NIHR Respiratory Biomedical Research Unit, Southampton General Hospital, Southampton, UK*
[c] *Wessex Investigational Sciences Hub, University of Southampton, Faculty of Medicine, Southampton General Hospital, Southampton, UK*
[d] *Scottish Centre for Respiratory Research, University of Dundee, Dundee, UK*
[e] *Institute for Lung Health, Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK*
[f] *Salford Royal NHS Foundation Trust, Salford, UK*
[g] *School of Medicine, University of Swansea, Wales, UK*
[h] *MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK*
[i] *Clinical Research Centre, Sormland County Council, Uppsala University, Uppsala, Sweden*
[j] *Pulmonary Medicine, Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden*
[k] *Department of Respiratory Medicine, Royal Stoke Hospital, University Hospitals of North Midlands, Stoke-on-Trent, UK*
[l] *Department of Respiratory Medicine, Aintree University Hospital, Liverpool, UK*
[m] *GSK, Siena, Italy*
[n] *GSK, Rixensart, Belgium*

ARTICLE INFO

Article history:
Received 29 March 2019
Received in revised form 30 July 2019
Accepted 31 July 2019
Available online 22 August 2019

Keywords:
Non-typeable *Haemophilus influenzae*
COPD
Exacerbation
Vaccine
Safety
Immunogenicity

ABSTRACT

Loss of airway microbial diversity is associated with non-typeable *Haemophilus influenzae* (NTHi) infection and increased risk of exacerbation in chronic obstructive pulmonary disease (COPD). We assessed the safety and immunogenicity of an investigational vaccine containing NTHi antigens, recombinant protein D (PD) and combined protein E and Pilin A (PE-PilA), and AS01 adjuvant in adults with moderate/-severe COPD and prior exacerbations.

In this phase 2, observer-blind, controlled trial (NCT02075541), 145 COPD patients aged 40–80 years randomly (1:1) received two doses of NTHi vaccine or placebo 60 days apart, on top of standard care.

Reactogenicity in the 7-day post-vaccination period was higher following NTHi vaccine than placebo. Most solicited adverse events (AEs) were mild/moderate. At least one unsolicited AE was reported during the 30-day post-vaccination period by 54.8% of NTHi vaccine and 51.4% of placebo recipients. One serious AE (placebo group) was assessed by the investigator as vaccine-related. Anti-PD, anti-PE and anti-PilA geometric mean antibody concentrations increased up to 30 days after each NTHi vaccine dose, waned thereafter, but remained higher than baseline (non-overlapping confidence intervals) up to 13 months post-dose 2. The frequency of specific $CD4^+$ T cells increased following two doses of NTHi vaccine and remained higher than baseline. Exploratory analysis showed a statistically non-significant lower yearly rate of moderate/severe exacerbations in the NTHi vaccine group than following placebo (1.49 versus 1.73) in the one-year period post-dose 2, with estimated vaccine efficacy of 13.3% (95% confidence interval −24.2 to 39.5; p = 0.44).

The NTHi vaccine had an acceptable safety and reactogenicity profile and good immunogenicity in adults with COPD.

1. Introduction

Acute exacerbations contribute substantially to the morbidity and mortality of chronic obstructive pulmonary disease (COPD) [1]. Changes in the composition of the lung microbiome are associated with COPD severity and exacerbation occurrence [2–5]. In the

Abbreviations: AECOPD, acute exacerbations of COPD; AERIS, Acute Exacerbation and Respiratory InfectionS in COPD; COPD, chronic obstructive pulmonary disease; ELISA, enzyme-linked immunosorbent assay; GOLD, Global Initiative for Chronic Obstructive Lung Disease; NTHi, non-typeable *Haemophilus influenzae*; PD, protein D; PE, protein E; PilA, Pilin A.

* Corresponding author at: Clinical Research and Development, GSK, via Fiorentina 1, 53100 Siena, Italy.
E-mail address: marco.x.testa@gsk.com (M. Testa).

https://doi.org/10.1016/j.vaccine.2019.07.100
0264-410X/

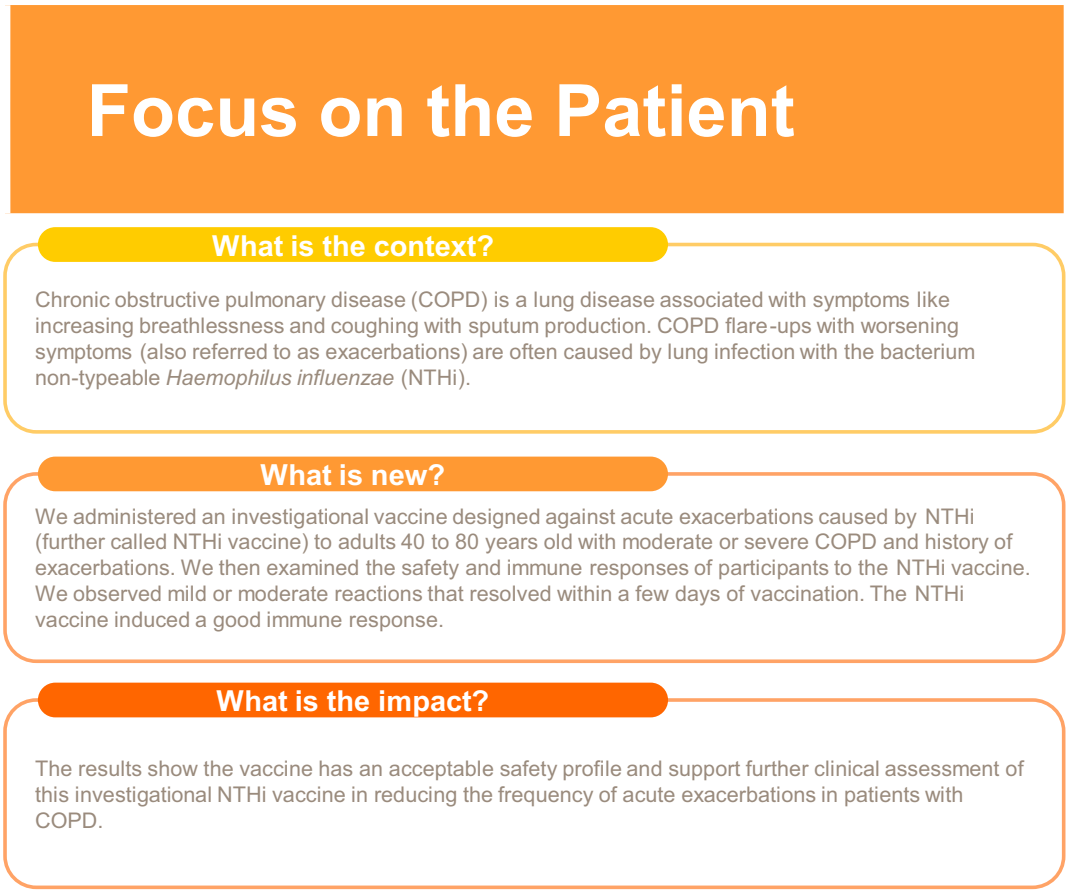

Fig. 1. Focus on the patient section.

prospective, observational cohort study, Acute Exacerbation and Respiratory InfectionS in COPD (AERIS), loss of microbial diversity was associated with non-typeable *Haemophilus influenzae* (NTHi) infection and increased risk of exacerbation [6]. AERIS and other studies also showed changes in the prevalence of airway bacteria at exacerbation compared with the stable state [7–11]. Global Initiative for Chronic Obstructive Lung Disease (GOLD) currently recommends influenza and pneumococcal vaccines for COPD patients [12] but no vaccine is specifically indicated for the prevention of acute exacerbations of COPD (AECOPD).

An oral whole cell NTHi vaccine did not provide convincing protection against exacerbations of COPD or bronchitis [13,14]. Consequently, the use of conserved surface-exposed proteins as vaccine antigens is being investigated as an alternative approach [15,16]. A multi-component investigational NTHi vaccine has been developed consisting of three conserved surface proteins in the form of two vaccine antigens: a free recombinant protein D (PD) and a recombinant fusion protein combining protein E and Pilin A (PE-PilA). PD is a highly conserved lipoprotein among encapsulated and non-encapsulated *H. influenzae* strains [17] that is also used as carrier in the licensed 10-valent pneumococcal conjugate vaccine (*Synflorix*, GSK) [18]. PE has a role in NTHi adhesion to host tissues [19] and human complement resistance [20,21], while PilA plays a key role in biofilm formation, adherence to human epithelial cells and colonisation of the upper respiratory tract [22]. Investigational NTHi vaccine formulations that included the Adjuvant System $AS01_E$ produced higher humoral and cellular immune responses in current or former smokers aged 50–70 years than those that included alum or non-adjuvanted formulations [23]. In that phase 1 study, and another conducted in healthy 18–40 year-olds [23], the NTHi vaccine formulations had an acceptable safety and reactogenicity profile and induced antigen-specific immune responses.

Here, we report the results of a phase 2 placebo-controlled study in which the safety and reactogenicity of the investigational NTHi vaccine was evaluated, for the first time, in adults with moderate or severe COPD. The vaccine's humoral and cellular immunogenicity was assessed as a secondary objective and an exploratory tertiary analysis evaluated efficacy against AECOPD.

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 patients

This randomised, observer-blind, placebo-controlled study was conducted in four centres in Sweden and 11 in the UK between 8 July 2014 and 19 April 2017 (ClinicalTrials.gov identifier: NCT02075541). Patients, enrolled between 6 August 2014 and 30 October 2015, were aged 40–80 years and had a confirmed diagnosis of COPD, with stage 2/3 (moderate to severe) airflow limitation, according to GOLD staging [12]. All patients were current or former smokers (smoking history $\geq$ 10 pack-years). Current smokers were defined as people who were smoking or had stopped smoking less than six months before entry into the study and former smokers as people who had stopped smoking for at least six months. A pack-year is defined as 20 cigarettes smoked every day for one year and the number of pack-years was calculated as: (average number of cigarettes smoked per day times number of years smoked)

divided by 20. Enrolled patients had stable COPD (last exacerbation was resolved for at least 30 days), with a documented history of at least one moderate or severe AECOPD within the last 12 months. Each participant was, in the opinion of the investigator, capable of complying with the requirements of the protocol, including regular sputum production. Women of non-childbearing potential or on adequate contraception could be enrolled. Exclusion criteria for the study are described in the supplementary methods.

An AECOPD was defined as worsening of at least two major symptoms (dyspnoea, sputum volume and sputum purulence) or worsening of at least one major symptom and one minor symptom (sore throat, colds [nasal discharge and/or nasal congestion], fever [oral temperature $\geq$ 37.5 °C] without other cause, increased cough and increased wheeze) for at least two consecutive days. An exacerbation was considered mild if self-managed by the patient using inhaled therapy, moderate if it required treatment with oral corticosteroids, antibiotics or both, and severe if the patient required hospitalisation or an equivalent home care intervention [24].

Patients were randomised (1:1) to receive, on top of standard care, two doses by intramuscular injection 60 days apart of the investigational NTHi vaccine or saline placebo using a centralised randomisation system (SBIR), with a minimisation procedure accounting for age category (40–59 or 60–80 years), number of moderate or severe exacerbations in the previous year (<2 or $\geq$2) and GOLD stage (2 or 3). 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/placebo recipients and those responsible for the evaluation of any study endpoint were blinded to the administered formulation and formulations were prepared and administered by authorised clinical personnel who did not participate in any of the study clinical evaluations or assays.

The NTHi vaccine contained 10 μg PD and 10 μg PE-PilA per dose with $AS01_E$ (containing immunostimulants, 3-O-desacyl-4′-monophosphoryl lipid A and saponin QS-21, 25 μg each, and liposomes), as described previously [23]. Patients were followed for approximately 15 months after the first vaccination. The primary objective was to assess safety and reactogenicity and the secondary objective was to describe the vaccine's humoral and cellular immunogenicity.

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 the Southampton and South West Hampshire Research Ethics Committee, UK, and Regional Research Ethics Committee, Gothenburg, Sweden. All participants provided written informed consent before study entry. The study protocol is available at https://www.gsk-studyregister.com (study identifier, 200157). Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

2.2. Safety and reactogenicity

Solicited local (pain, redness and swelling at the injection site) and general (fatigue, fever, gastrointestinal symptoms and headache) adverse events (AEs) were recorded for 7 days after each dose on diary cards. Unsolicited AEs with onset during the 30 days after each dose were recorded. AE intensity was graded on a 0–3 scale. Grade 3 redness or swelling was defined as diameter > 100 mm and grade 3 fever as temperature > 39.5 °C. Other grade 3 AEs were defined as preventing normal activities.

Haematological and biochemical parameters were measured before, at 7 and 30 days after each vaccination and at Days 270 and 450 (7 and 13 months post-dose 2). Abnormal laboratory findings that were judged by the investigator to be clinically significant were recorded as an AE or serious AE (SAE) and graded 1–4 [25]. Data on potential immune-mediated diseases (pIMDs) [26] and SAEs were collected for the duration of the study.

2.3. Humoral and cellular immunogenicity

Immunoglobulin G antibody geometric mean concentrations (GMCs) to each vaccine antigen were measured by enzyme-linked immunosorbent assay (ELISA), developed and optimised by GSK Biologicals, of blood samples taken before and 30 days after each vaccination, and at Days 270 and 450. Sera were stored at −20 °C until assayed. Standardised procedures and in-house-made serum were used for each assay. The cut-off of the assays was 153 ELISA units (EU)/mL, 8 EU/mL and 7 EU/mL for anti-PD, anti-PE and anti-PilA antibodies, respectively.

NTHi-specific cell-mediated immune (CMI) responses (antigen-specific $CD4^+$ and $CD8^+$ T cells) were measured by flow cytometry using intracellular cytokine staining (ICS) on peripheral blood mononuclear cells, following an adaptation of previously described methods in which the T cells are re-stimulated by incubation with antigen in the presence of costimulatory antibodies to CD28 and CD49d [27]. Blood samples for CMI response analysis were taken before vaccination, 30 days after the second vaccination and at Days 270 and 450 in a sub-cohort of patients (15 per group) selected randomly using a centralised randomisation system (SBIR). The frequency of antigen-specific $CD4^+$ and $CD8^+$ T cells expressing selected cytokine markers among CD40L, IL-2, TNF-α, IFN-γ, IL-13 and IL-17 and a combination of at least two of the above markers was calculated.

2.4. Vaccine efficacy

An exploratory analysis was performed of vaccine efficacy (VE) against AECOPD, which was a tertiary endpoint of the study. Exacerbations, detected using daily electronic diary cards, were recorded as mild, moderate or severe (see supplementary methods).

2.5. Statistical analyses

The study's primary objective was to evaluate safety. Thus, sample size was based on estimates of the percentage of subjects in each group with AEs following vaccination. With approximately 70 eligible COPD patients enrolled in each study group, the chance of detecting an AE with true incidence of 5% was >97%. The safety analysis was performed on the total vaccinated cohort, which included all vaccinated participants. The incidence of AEs per study group was calculated with exact 95% confidence intervals (CIs) after each vaccine dose. Since the number of patients enrolled was limited, no formal statistical comparisons of reactogenicity were performed between groups.

The immunogenicity analysis was performed on the according-to-protocol (ATP) cohort for immunogenicity, i.e. eligible patients who received the study vaccine correctly and complied with study procedures; patients who received immune-modifying treatment for > 14 days were eliminated. Antibody GMCs and their 95% CIs were determined. The between groups GMC ratio at one month post-dose 2 and its 95% CI for each vaccine antigen was computed with an analysis of covariance model that considers heterogeneity of variances (via Proc Mixed) on the $\log_{10}$ transformation of concentrations. The model included country, age category, number of moderate or severe exacerbations in the previous year (<2 or $\geq$2), GOLD stage and baseline concentration (as covariate) as fixed effects. The NTHi vaccine group and placebo group were considered significantly different if the 95% CI for the GMC ratio (adjusted for baseline concentration) between the two groups did not

contain 1. No adjustment for multiplicity was performed and the clinical relevance of any difference is unknown.

Cellular immunity responses were expressed as the frequency of specific $CD4^+$ or $CD8^+$ T cells identified as expressing at least two markers upon *in vitro* stimulation with the relevant pool of peptides, after subtraction of the corresponding frequency of $CD4^+$ or $CD8^+$ T cells similarly "stimulated" with medium instead of the peptide pool (background frequency). The frequency of specific $CD4^+$ and $CD8^+$ T cells was summarised for a subset of subjects using descriptive statistics.

To assess clinical efficacy, VE was estimated from the yearly rate of AECOPD calculated from one month post-dose 2 in the total vaccinated cohort, using a negative binomial model with treatment group, country, GOLD stage, history of exacerbations and age category as factors. A complementary post-hoc analysis was performed to evaluate time to first exacerbation event (any or moderate/severe) from one month post-dose 2. Further details are provided in the supplement.

Statistical analyses were performed using Statistical Analysis Systems (SAS) version 9.2.

3. Results

3.1. Study population

Of 268 COPD patients who were screened, 145 met the inclusion criteria; 73 were enrolled and vaccinated in the NTHi vaccine group and 72 were enrolled in the placebo control group (Fig. 2). Thirty-three patients were enrolled in Sweden (16 in vaccine group, 17 in control group) and 112 in the UK (57 and 55, respec-

Table 1
Demographic characteristics of the study participants (total vaccinated cohort).

Characteristic	NTHi vaccine (N = 73)	Placebo (N = 72)
Age (years) at dose 1, mean (SD)	67.0 (8.41)	66.8 (7.21)
Age range (years)	45–78	46–81
Male gender, n (%)	39 (53.4)	36 (50.0)
Smoking status, n (%)		
Current smoker	23 (31.5)	24 (33.3)
Former smoker	50 (68.5)	48 (66.7)
Number of exacerbations in preceding 12 months, according to severity, mean (SD)		
Mild	0.3 (0.9)	0.3 (0.8)
Moderate	2.3 (1.6)	2.7 (2.3)
Severe	0.2 (0.5)	0.2 (0.5)
FEV_1 after bronchodilator use (L), mean (SD)	1.5 (0.5)	1.5 (0.5)
FEV_1 after bronchodilator use (% predicted), mean (SD)	55.3 (14.4)	56.0 (13.9)
FEV_1/FVC, n (%)		
<0.70	71 (97.3)	67 (93.1)
≥0.70	0	2 (2.8)
Missing data	2 (2.7)	3 (4.2)
COPD assessment test score, mean (SD)	18.2 (7.4)	17.6 (8.7)
mMRC score, mean (SD)	2.4 (1.0)	2.3 (1.0)
Sputum positive for *H. influenzae**, n (%)	18 (24.7)	19 (26.4)

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; mMRC, modified Medical Research Council dyspnoea scale; N, number of participants; n, number of participants in a specific category; SD, standard deviation.
**Haemophilus influenzae* was identified, using conventional culture techniques, from sputum samples obtained by spontaneous expectoration or induced, processed according to standard methods [11].

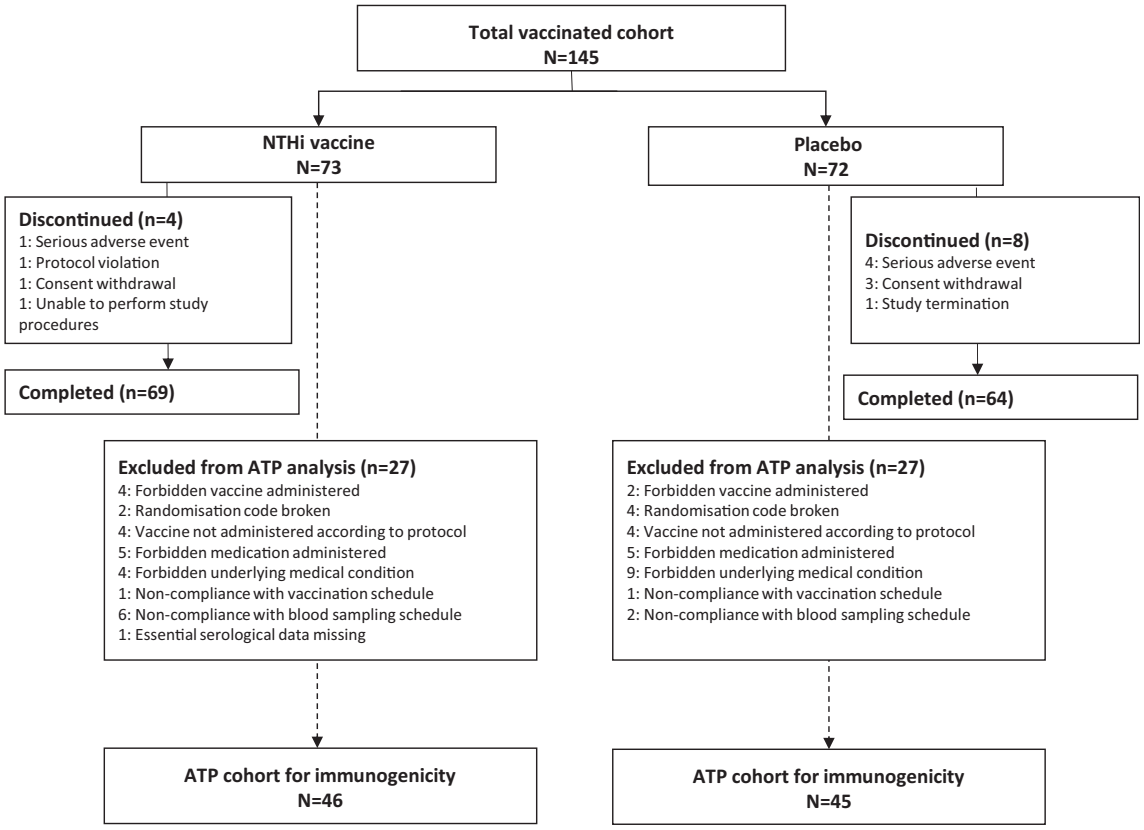

Fig. 2. Disposition of the study participants and reasons for exclusion from the according-to-protocol cohort for immunogenicity. ATP, according-to-protocol; N, number of participants; n, number of participants in a specific category.

tively). Sixty-nine participants in the NTHi vaccine group and 64 in the placebo group completed the study; reasons for discontinuation are shown in Fig. 2. Twenty-seven participants in each group were excluded from the ATP cohort for immunogenicity because of protocol deviations (Fig. 2).

Mean age in both groups was 67 years and around half of patients were men (Table 1). *H. influenzae* was detected in approximately one-quarter of cultured sputum samples at baseline. All participants were white (European heritage).

3.2. Safety and reactogenicity

Pain and fatigue were the most frequently reported solicited local and general AEs, respectively, during the 7-day post-vaccination period (Fig. 3). The incidence of each solicited local AE was higher in the NTHi vaccine group than in the placebo group after each dose (Fig. 3). There were three reports of grade 3 pain after the first NTHi vaccine dose and five after the second dose, all of which lasted no longer than 3 days and resolved spontaneously.

Incidences of the solicited general symptoms in the NTHi vaccine group tended to be higher after the second dose, although 95% CIs overlapped (Fig. 3). There were four reports of grade 3 solicited general events related to vaccination (two fatigue, two headache) after the first NTHi vaccine dose and six (three fatigue, two headache, one gastrointestinal symptoms) after the second dose, all of which resolved spontaneously and lasted no longer than 3 days, apart from one report of headache post-dose 2 that lasted 7 days. In the placebo group, there were five reports of grade 3 vaccine-related general events (one fatigue, one gastrointestinal symptoms, two headache, one fever) after the first dose and three after the second dose (two fatigue, one headache) that lasted no longer than 2 days.

During the 30-day post-vaccination period, at least one unsolicited AE was reported by 54.8% of participants in the NTHi vaccine group and 51.4% of participants in the placebo group (Table 2). Grade 3 vaccine-related unsolicited AEs were reported

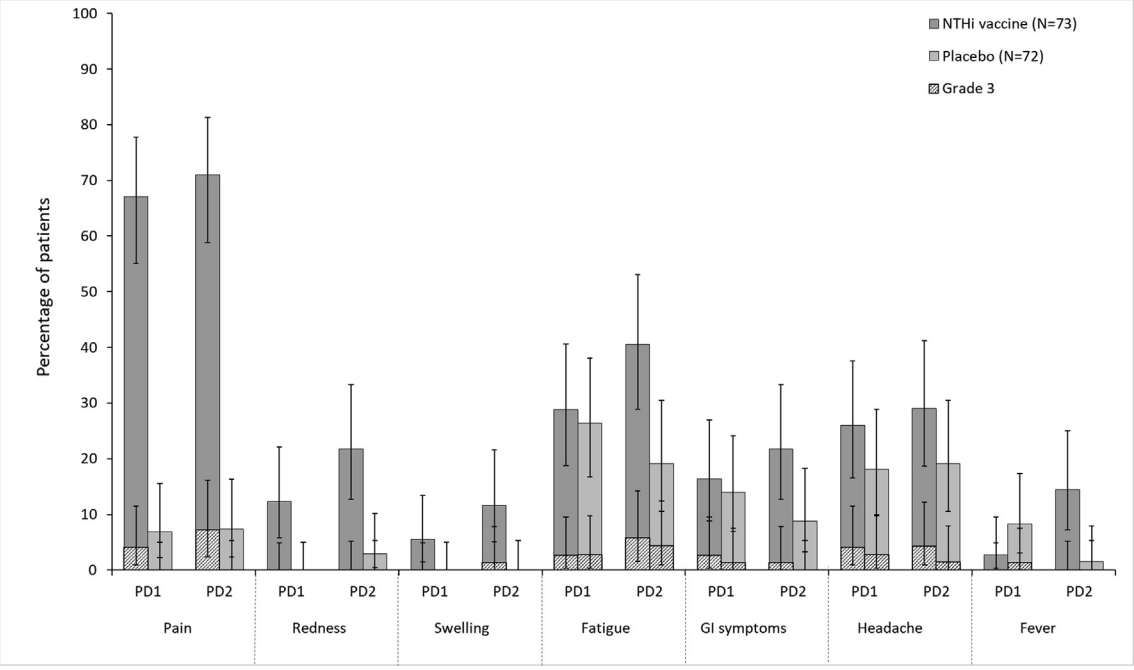

Fig. 3. Percentages of participants (with exact 95% confidence intervals) reporting solicited local (pain redness and swelling) and general (fatigue, gastrointestinal symptoms, headache and fever) adverse events during 7-day post-vaccination period after each dose of NTHi vaccine or placebo control (total vaccinated cohort). PD1, 7-day period post-dose 1; PD2, 7-day period post-dose 2. GI (gastrointestinal) symptoms defined as nausea, vomiting, diarrhoea and/or abdominal pain. Fever defined as temperature ≥ 37.5 °C. Grade 3 defined as diameter > 100 mm (redness and swelling), temperature > 39.5 °C (fever), preventing normal activity (pain, fatigue, gastrointestinal symptoms and headache). N, number of participants.

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

	Percentage of patients (95% CI)	
	NTHi vaccine (N = 73)	Placebo (N = 72)
At least one unsolicited AE	54.8 (42.7–66.5)	51.4 (39.3–63.3)
Related to vaccination	24.7 (15.3–36.1)	11.1 (4.9–20.7)
Grade 3 intensity	6.8 (2.3–15.3)	9.7 (4.0–19.0)
Grade 3 intensity related to vaccination	5.5 (1.5–13.4)	2.8 (0.3–9.7)
Unsolicited AEs reported in > 5.0% of subjects in at least one group		
Nasopharyngitis	9.6 (3.9–18.8)	5.6 (1.5–13.6)
Oropharyngeal pain	6.8 (2.3–15.3)	6.9 (2.3–15.5)
Urinary tract infection	1.4 (0.0–7.4)	5.6 (1.5–13.6)
Cough	6.8 (2.3–15.3)	1.4 (0.0–7.5)

95% CI, 95% confidence interval; N, number of participants.

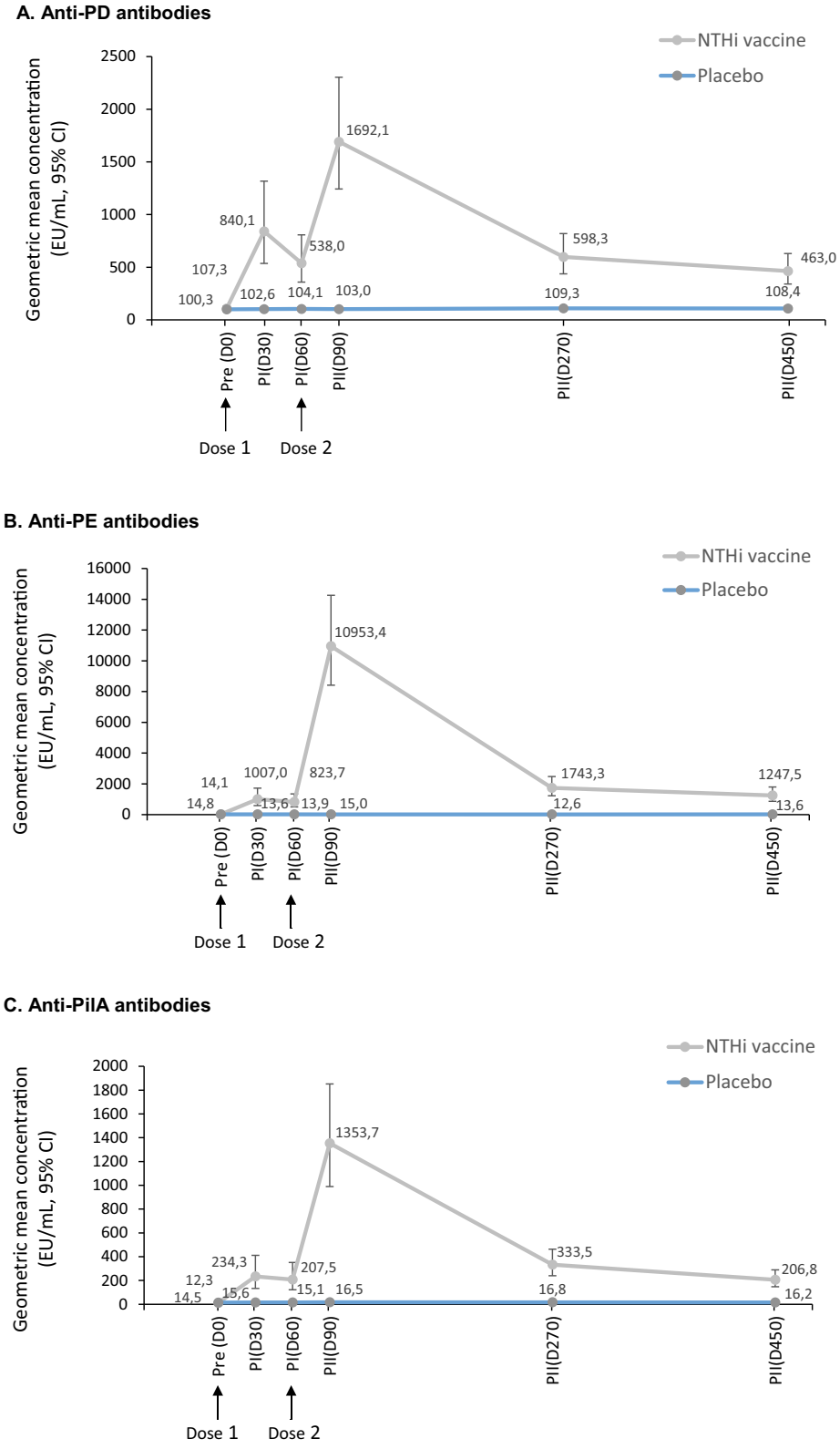

Fig. 4. Geometric mean concentrations of (A) anti-PD, (B) anti-PE and (C) anti-PilA antibodies measured by enzyme-linked immunosorbent assay (ATP cohort for immunogenicity). Number of subjects with available results at each time point: between 39 and 46 in NTHi vaccine group, between 38 and 45 in placebo control group. A. Anti-PD antibodies. B. Anti-PE antibodies. C. Anti-PilA antibodies. EU, ELISA (enzyme-linked immunosorbent assay) units; 95% CI, 95% confidence interval. Pre (D0), pre-dose 1; PI(D30), 30 days post-dose 1; PI(D60), pre-dose 2; PII(D90), 30 days post-dose 2; PII(D270), 7 months post-dose 2; PII(D450), 13 months post-dose 2.

Table 3
Comparison of anti-PD, anti-PE and anti-PilA antibody responses to NTHi vaccine and placebo in terms of GMC ratio adjusted for baseline GMC one month after the second vaccine dose (according-to-protocol cohort for immunogenicity). An analysis of covariance model was used, considering heterogeneity of variances (via Proc Mixed) on the $\log_{10}$ transformation of concentrations, and including country, age category, number of moderate or severe exacerbations in the previous year (<2 or ≥2), GOLD stage and baseline concentration of antigen (as covariate) as fixed effects. Statistical significance was indicated if the 95% CI for the GMC ratio excluded 1.

	NTHi		Placebo		Adjusted GMC ratio NTHi:Placebo (95% CI)
	n	Adjusted GMC (EU/mL)	n	Adjusted GMC (EU/mL)	
Anti-PD	44	1259.9	45	80.7	15.61 (11.30–21.56)
Anti-PE	41	9094.9	44	12.0	760.46 (519.78–1112.6)
Anti-PilA	39	1293.4	41	14.6	88.30 (60.78–128.28)

GMC, geometric mean concentration; 95% CI, 95% confidence interval; n, number of participants with available results; PD, protein D; PE, protein E; PilA, Pilin A.

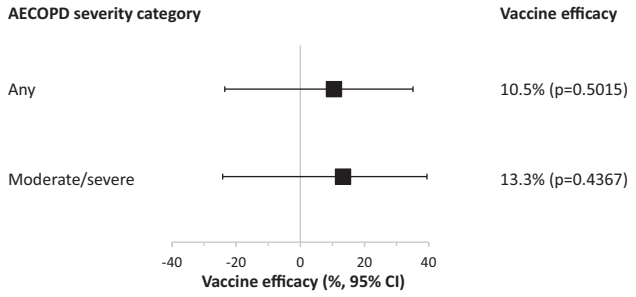

Fig. 5. Estimated efficacy of the investigational NTHi vaccine against AECOPD of any severity and moderate/severe AECOPD in the one-year period after one month post-dose 2 (total vaccinated cohort). In this exploratory analysis, efficacy was defined as 1 minus the yearly rate of AECOPD in the NTHi vaccine group versus the yearly rate in the placebo group. 95% CI, 95% confidence interval; AECOPD, acute exacerbations of chronic obstructive pulmonary disease.

by two (2.8%) participants in the placebo group (nasopharyngitis and migraine, lasting 15 days and 1 day, respectively) and four participants (5.5%) in the NTHi vaccine group: fatigue (two participants; duration 2 and 4 days), headache (one participant; duration 7 days) and dizziness, cough, oropharyngeal pain and rhinorrhoea (one participant; oropharyngeal pain lasting 45 days, duration unknown for other AEs).

Fifteen participants in the NTHi vaccine group and 17 in the placebo group reported a total of 62 SAEs (28 and 34, respectively). One SAE (infective exacerbation of COPD that lasted 37 days, reported by a 63-year-old patient at Day 200 after the second placebo dose) was considered by the investigator as causally related to vaccination. There were three deaths; one in the NTHi group (68-year-old participant who died due to COPD, pneumonia and respiratory failure) and two in the placebo group (68-year-old participant who died due to metastatic bronchial cancer and 68-year-old patient with fatal myocardial infarction). None were considered related to vaccination. Two pIMDs were reported in the NTHi vaccine group, neither of which was considered related to vaccination. Facial paralysis (Bell's palsy) was reported at Day 128 after the second vaccine dose by a patient aged 54 years, which was considered by the investigator as serious, and non-serious gout was reported at Day 63 after the second dose by a 72-year-old participant.

Three grade 3 abnormal haematology or biochemistry results were reported in three participants (4.1%) in the NTHi vaccine group and eight were reported by six participants (8.3%) in the placebo group. A grade 4 change in haemoglobin from baseline was reported in one participant (1.4%) in the placebo group. All grade 3 or 4 abnormal haemoglobin results were reported at least one month after vaccination.

3.3. Immunogenicity

In the NTHi vaccine group, antibody GMCs against all three antigens increased after each vaccine dose, while no increases were observed in the placebo group (Fig. 4). GMCs waned in the NTHi vaccine group after Day 90 but remained higher than baseline at subsequent time points. One month after the second dose, antibody responses were significantly higher in the NTHi vaccine group than with placebo for each antigen, with lower limits of the 95% CIs of adjusted GMC ratios above 10 (Table 3).

The frequency of NTHi 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 to 30 days after the second vaccine dose (supplementary Fig. S1). This was followed by a reduction in response up to the last time point, although responses remained higher than baseline. Frequencies overlapped between groups but tended to be higher in the NTHi group.

Exploring the T helper functionality profile of specific $CD4^+$ T cells, no vaccine-induced IL-13 expression (associated with a type 2 T helper [Th2] profile) was detected (supplementary Fig. S2), although for all markers there was high variability in the number of specific T cells for all vaccine antigens. There were no detectable specific $CD8^+$ T cell responses (data not shown).

3.4. Efficacy

The study was not powered to find differences in efficacy and this was a tertiary analysis. In the one-year period starting one month post-dose 2, the number of reports of moderate/severe AECOPD was lower in the NTHi group (107 in 73 patients) than following placebo (120 in 71 patients), albeit the difference between groups was not statistically significant, with estimated VE of 13.3% (−24.2, 39.5; p = 0.44) (Fig. 5). For AECOPD of any severity, there were 130 reports by 73 patients in the NTHi group and 140 by 71 patients following placebo, with estimated VE of 10.5% (95% CI: −23.6, 35.1; p = 0.50). Seventeen patients (23.3%) in the NTHi group and 20 (28.2%) receiving placebo reported no exacerbations (any cause: infection-related or other). The observed yearly rate of moderate/severe AECOPD was 1.49 and 1.73 in the NTHi group and placebo group, respectively, and 1.80 and 2.02, respectively, for AECOPD of any severity. Examination of the model covariates showed a significant effect (p < 0.05) of history of AECOPD on the yearly rate of any and moderate/severe AECOPD, and of country on any AECOPD (supplementary Table S1). Time to first AECOPD tended to be longer in the NTHi group than in the placebo group (Fig. 6), although the difference between groups was not statistically significant (hazard ratio p = 0.23 for moderate/severe AECOPD, p = 0.44 for any AECOPD).

4. Discussion

This is the first study to assess the safety, reactogenicity and immunogenicity of a two-dose schedule of an investigational NTHi vaccine, based on conserved NTHi surface proteins (PD and PE-PilA), in adults aged 40–80 years with moderate or severe COPD. The NTHi vaccine had an acceptable safety and reactogenicity profile in this group, which was consistent with findings from phase 1

studies of the investigational vaccine in healthy adults aged 18–40 years and current or former smokers aged 50–70 years without COPD [23].

Since the number of patients enrolled was limited, no formal statistical comparisons of reactogenicity between groups were performed. The most commonly reported solicited local AE was pain, with overall per subject incidences of 80.8% (95% CI: 69.9–89.1) in the NTHi vaccine group and 12.5% (95% CI: 5.9–22.4) in the placebo group, while the most common solicited general AE was fatigue, with overall per subject incidences of 50.7% (95% CI: 38.7–62.6) and 31.9% (95% CI: 21.4–44.0), respectively. Higher frequencies of solicited AEs in adjuvanted vaccine groups versus groups who received non-adjuvanted vaccine formulations or placebo were reported in previous studies, including the phase 1 NTHi vaccine study [23,28–30]. This may be due to enhanced activation of the innate immune response induced by the adjuvant at the injection site and increased production of cytokines and chemokines [23,31,32]. Overall, however, the reported solicited AEs were transient and mild to moderate in intensity. The frequency of unsolicited AEs was similar between groups and there was only one reported vaccine-related SAE (infective exacerbation of COPD), which was in the placebo group.

The NTHi investigational vaccine induced substantial humoral immune responses and elicited specific $CD4^+$ T cell responses in patients with COPD. Antibody GMCs increased after the first vaccine dose followed by a further increase after the second dose. Thereafter, antibody GMCs decreased gradually but remained above baseline levels 13 months post-dose 2. The NTHi-specific CMI response increased but was highly variable in terms of $CD4^+$ T cell expression, which was similar to that observed in the phase 1 study of healthy smokers/ex-smokers in a similar age range [23]. There was no $CD8^+$ T cell induction detected, as observed in other studies of AS01-adjuvanted vaccines in adults [23,33–36].

Exploratory analysis showed a VE point estimate of 13% (95% CI: −24, 39) for moderate/severe AECOPD and 10% (−24, 35) for any AECOPD albeit this was not statistically significant and the study was not powered for this outcome. When looking at covariates in the negative binomial model, only history of AECOPD (any and moderate/severe AECOPD) and country (any AECOPD only) had a significant effect on AECOPD yearly rate.

The study had limitations. All participants were white Europeans, limiting the generalisability of the results to other ethnic groups, and the sample size was small. A high number of participants were excluded from the ATP cohort for immunogenicity, most commonly because of non-compliance with the blood sam-

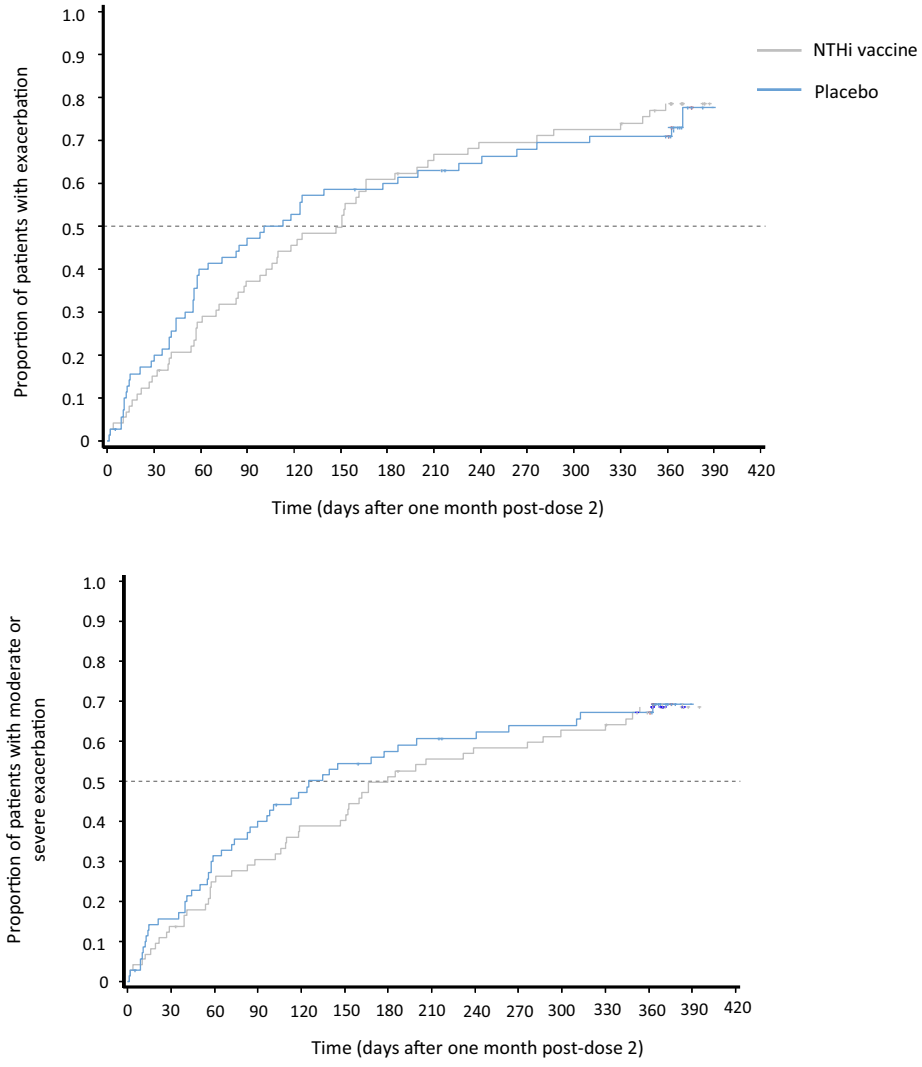

Fig. 6. Time to first AECOPD event after one month post-dose 2 for AECOPD of any severity and moderate/severe AECOPD (total vaccinated cohort). Hazard ratio for NTHi group versus control group was 0.86 (95% CI: 0.58, 1.26; p = 0.44) for any AECOPD and 0.78 (0.52, 1.17; p = 0.23) for moderate/severe AECOPD.

pling schedule, administration of a forbidden medication, or the presence of a prohibited underlying medical condition. Also, the low number of subjects included in the CMI response analysis and high variability in the results limited the ability to interpret these data.

In conclusion, the data from this phase 2 study demonstrate that the investigational NTHi vaccine has an acceptable safety and reactogenicity profile, induces substantial humoral responses and elicits NTHi-specific $CD4^+$ T cell responses in adults aged 40–80 years with moderate or severe COPD. Definition of a protective antibody response was beyond the scope of this study since efficacy was a tertiary exploratory endpoint and thus, no specific antibody protective level was determined. These results and those of previous studies support further clinical assessment of the vaccine in reducing the frequency of AECOPD, as well as its effect on COPD symptoms and long-term outcomes. Future studies will also aim to define antibody correlates of protection and will explore the relationship over time between VE and NTHi-specific antibody responses and $CD4^+$ T cell expression.

Acknowledgements

The authors thank the study participants and their families. The authors would like to thank Laura Taddei, Simona Rondini and Ruth Tal Singer for their critical review of the paper, and Ruth Moss, Jo Brown, Michael Martin and Andrea Pammolli for their contributions to the study.

The authors would like to also thank Regis Azizieh and Salomé Murinello (XPE Pharma & Science, Wavre, Belgium), on behalf of GSK (Rixensart, Belgium), for coordination and editorial support, and Joanne Knowles (independent writer, on behalf of GSK) for writing assistance.

Author contributions

TMAW, SS, CB, NB, DC, PM and AKA were involved in the study conception and design. TMAW, SS, CB, NB, KL, WM, LR, JH, MA, PPW, IDR, AT, DC, PM, MT and AKA were involved in acquisition and generation of data. TMAW, SS, CB, NB, KL, WM, LR, JH, MA, PPW, AT, DC, MT and AKA performed the study. TMAW, LR, IDR, JH, AT, DC, PM, MT and AKA 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.

Declaration of Competing Interest

Over the last 5 years, SS has received funding from GSK group of companies, Chiesi, Napp and Boehringer Ingelheim to attend scientific meetings. He has been a consultant to Chiesi, Novartis, GSK group of companies and Bayer. SS is or has been the principal investigator on trials funded by GSK group of companies, Novartis, Oncimmune and Nycomed. KL's institution received funding from GSK group of companies and KL received personal fees from Chiesi, AstraZeneca and TEVA and is a non-executive director of Bond Digital Health. NB declares fee from GSK group of companies, grants from AstraZeneca, personal fees from Novartis, other from BI, outside the submitted work. WM reports grants from GSK group of companies, during the conduct of the study; grants and personal fees from GSK group of companies, grants and personal fees from AstraZeneca, personal fees from Chiesi, personal fees from Boehringer Ingelheim, personal fees from Janssen, grants and personal fees from Pfizer, grants from Philips, grants from Kamada, outside the submitted work. LR reports personal fees from GSK group of companies, outside the submitted work. JH reports grants and personal fees from AstraZeneca, ResMed, Desitin and Philips outside the submitted work. PPW reports personal fees from Chiesi and AstraZeneca Pharmaceuticals outside the submitted work. TMAW reports grants and non-financial support from GSK group of companies during the conduct of the study; and grants and other from Boehringer Ingelheim and other from Astra Zeneca, outside the submitted work. CB and MA report no conflict of interest.

IDR, AT, DC, PM, MT and AKA are employees of the GSK group of companies and hold shares/restricted shares in the GSK group of companies.

Trademark statement

Synflorix is a trademark owned by 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.2019.07.100.

References

[1] Mackay AJ, Hurst JR. COPD exacerbations: causes, prevention, and treatment. Immunol Allergy Clin North Am 2013;33:95–115. https://doi.org/10.1016/j.iac.2012.10.006.

[2] Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The microbiome and the respiratory tract. Annu Rev Physiol 2016;78:481–504. https://doi.org/10.1146/annurev-physiol-021115-105238.

[3] Wang Z, Bafadhel M, Haldar K, Spivak A, Mayhew D, Miller BE, et al. Lung microbiome dynamics in COPD exacerbations. Eur Respir J 2016;47:1082–92. https://doi.org/10.1183/13993003.01406-2015.

[4] Sze MA, Hogg JC, Sin DD. Bacterial microbiome of lungs in COPD. Int J Chron Obstruct Pulmon Dis 2014;9:229–38. https://doi.org/10.2147/COPD.S38932 [doi];copd-9-229 [pii].

[5] Sethi S, Evans N, Grant BJ, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med 2002;347:465–71. https://doi.org/10.1056/NEJMoa012561 [doi];347/7/465 [pii].

[6] Mayhew D, Devos N, Lambert C, Brown JR, Clarke SC, Kim VL, et al. Longitudinal profiling of the lung microbiome in the AERIS study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations. Thorax 2018;73:422–30. https://doi.org/10.1136/thoraxjnl-2017-210408.

[7] Garcha DS, Thurston SJ, Patel AR, Mackay AJ, Goldring JJ, Donaldson GC, et al. Changes in prevalence and load of airway bacteria using quantitative PCR in stable and exacerbated COPD. Thorax 2012;67:1075–80. doi: thoraxjnl-2012-201924 [pii];10.1136/thoraxjnl-2012-201924 [doi].

[8] Huang YJ, Sethi S, Murphy T, Nariya S, Boushey HA, Lynch SV. Airway microbiome dynamics in exacerbations of chronic obstructive pulmonary disease. J Clin Microbiol 2014;52:2813–23. doi: JCM.00035-14 [pii];10.1128/JCM.00035-14 [doi].

[9] Millares L, Ferrari R, Gallego M, Garcia-Nuñez M, Pérez-Brocal V, Espasa M, et al. Bronchial microbiome of severe COPD patients colonised by *Pseudomonas aeruginosa*. Eur J Clin Microbiol Infect Dis 2014;33:1101–11. https://doi.org/10.1007/s10096-013-2044-0.

[10] Wang H, Gu X, Weng Y, Xu T, Fu Z, Peng W, et al. Quantitative analysis of pathogens in the lower respiratory tract of patients with chronic obstructive pulmonary disease. BMC Pulm Med 2015;15:94. https://doi.org/10.1186/s12890-015-0094-z.

[11] Wilkinson TMA, Aris E, Bourne S, Clarke SC, Peeters M, Pascal TG, et al. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. Thorax 2017;72:919–27.

[12] Global Initiative for Chronic Obstructive Lung Disease (GOLD), Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2019 report; 2019. https://goldcopd.org/wp-content/uploads/2018/11/GOLD-2019-v1.5-FINAL-04Nov2018_WMS.pdf. [accessed 25 February 2019].

[13] Clancy RL, Dunkley ML, Sockler J, McDonald CF. Multi-site placebo-controlled randomised clinical trial to assess protection following oral immunisation with inactivated non-typeable *Haemophilus influenzae* in chronic obstructive pulmonary disease. Intern Med J 2016;46:684–93. https://doi.org/10.1111/imj.13072.

[14] Teo E, Lockhart K, Purchuri SN, Pushparajah J, Cripps AW. van Driel ML. *Haemophilus influenzae* oral vaccination for preventing acute exacerbations of chronic bronchitis and chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2017;6:CD010010. https://doi.org/10.1002/14651858.CD010010.pub3.

[15] Poolman JT, Bakaletz L, Cripps A, Denoel PA, Forsgren A, Kyd J, et al. Developing a nontypeable *Haemophilus influenzae* (NTHi) vaccine. Vaccine 2000;19(Suppl 1):S108–15.

[16] Murphy TF. Vaccines for nontypeable *Haemophilus influenzae*: the future is now. Clin Vaccine Immunol 2015;22:459–66. https://doi.org/10.1128/cvi.00089-15.

[17] Forsgren A, Riesbeck K, Janson H. Protein D of *Haemophilus influenzae*: a protective nontypeable *H. influenzae* antigen and a carrier for pneumococcal conjugate vaccines. Clin Infect Dis 2008;46:726–31. https://doi.org/10.1086/527396.

[18] Prymula R, Schuerman L. 10-valent pneumococcal nontypeable *Haemophilus influenzae* PD conjugate vaccine: Synflorix™. Expert Rev Vaccines 2009;8:1479–500. https://doi.org/10.1586/erv.09.113.

[19] Singh B, Al-Jubair T, Mörgelin M, Thunnissen MM, Riesbeck K. The unique structure of *Haemophilus influenzae* protein E reveals multiple binding sites for host factors. Infect Immun 2013;81:801–14. https://doi.org/10.1128/iai.01111-12.

[20] Hallström T, Blom AM, Zipfel PF, Riesbeck K. Nontypeable *Haemophilus influenzae* protein E binds vitronectin and is important for serum resistance. J Immunol 2009;183:2593–601. https://doi.org/10.4049/jimmunol.0803226.

[21] Singh B, Jalalvand F, Mörgelin M, Zipfel P, Blom AM, Riesbeck K. *Haemophilus influenzae* protein E recognizes the C-terminal domain of vitronectin and modulates the membrane attack complex. Mol Microbiol 2011;81:80–98. https://doi.org/10.1111/j.1365-2958.2011.07678.x.

[22] Jurcisek JA, Bookwalter JE, Baker BD, Fernandez S, Novotny LA, Munson Jr RS, et al. The PilA protein of non-typeable *Haemophilus influenzae* plays a role in biofilm formation, adherence to epithelial cells and colonization of the mammalian upper respiratory tract. Mol Microbiol 2007;65:1288–99. https://doi.org/10.1111/j.1365-2958.2007.05864.x.

[23] Leroux-Roels G, Van Damme P, Haazen W, Shakib S, Caubet M, Aris E, et al. Phase I, randomized, observer-blind, placebo-controlled studies to evaluate the safety, reactogenicity and immunogenicity of an investigational non-typeable *Haemophilus influenzae* (NTHi) protein vaccine in adults. Vaccine 2016;34:3156–63. https://doi.org/10.1016/j.vaccine.2016.04.051.

[24] Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med 1987;106:196–204.

[25] Food and drug administration, guidance for industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials; 2007. https://www.fda.gov/downloads/BiologicsBloodVaccines/ucm091977. [accessed 25 February 2019].

[26] Tavares Da Silva F, De Keyser F, Lambert PH, Robinson WH, Westhovens R, Sindic C. Optimal approaches to data collection and analysis of potential immune mediated disorders in clinical trials of new vaccines. Vaccine 2013;31:1870–6. https://doi.org/10.1016/j.vaccine.2013.01.042.

[27] Moris P, van der Most R, Leroux-Roels I, Clement F, Dramé 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. https://doi.org/10.1007/s10875-010-9490-6.

[28] Leroux-Roels I, Devaster JM, Leroux-Roels G, Verlant V, Henckaerts I, Moris P, et al. Adjuvant system $AS02_V$ enhances humoral and cellular immune responses to pneumococcal protein PhtD vaccine in healthy young and older adults: randomised, controlled trials. Vaccine 2015;33:577–84. https://doi.org/10.1016/j.vaccine.2013.10.052.

[29] Berglund J, Vink P, Tavares Da Silva F, Lestrate P, Boutriau D. Safety, immunogenicity, and antibody persistence following an investigational *Streptococcus pneumoniae* and *Haemophilus influenzae* triple-protein vaccine in a phase 1 randomized controlled study in healthy adults. Clin Vaccine Immunol 2014;21:56–65. https://doi.org/10.1128/cvi.00430-13.

[30] Pauksens K, Nilsson AC, Caubet M, Pascal TG, Van Belle P, Poolman JT, et al. Randomized controlled study of the safety and immunogenicity of pneumococcal vaccine formulations containing PhtD and detoxified pneumolysin with alum or adjuvant system $AS02_V$ in elderly adults. Clin Vaccine Immunol 2014;21:651–60. https://doi.org/10.1128/cvi.00807-13.

[31] Awate S, Babiuk LA, Mutwiri G. Mechanisms of action of adjuvants. Front Immunol 2013;4:114. https://doi.org/10.3389/fimmu.2013.00114.

[32] Didierlaurent AM, Collignon C, Bourguignon P, Wouters S, Fierens K, Fochesato M, et al. Enhancement of adaptive immunity by the human vaccine adjuvant AS01 depends on activated dendritic cells. J Immunol 2014;193:1920–30. https://doi.org/10.4049/jimmunol.1400948.

[33] Kester KE, Cummings JF, Ofori-Anyinam O, Ockenhouse CF, Krzych U, Moris P, et al. Randomized, double-blind, phase 2a trial of falciparum malaria vaccines RTS, S/AS01B and RTS, S/AS02A in malaria-naive adults: safety, efficacy, and immunologic associates of protection. J Infect Dis 2009;200:337–46. https://doi.org/10.1086/600120.

[34] Vandepapelière P, Horsmans Y, Moris P, Van Mechelen M, Janssens M, Koutsoukos M, et al. Vaccine adjuvant systems containing monophosphoryl lipid A and QS21 induce strong and persistent humoral and T cell responses against hepatitis B surface antigen in healthy adult volunteers. Vaccine 2008;26:1375–86. https://doi.org/10.1016/j.vaccine.2007.12.038.

[35] Van Braeckel E, Bourguignon P, Koutsoukos M, Clement F, Janssens M, Carletti I, et al. An adjuvanted polyprotein HIV-1 vaccine induces polyfunctional cross-reactive $CD4^+$ T cell responses in seronegative volunteers. Clin Infect Dis 2011;52:522–31. https://doi.org/10.1093/cid/ciq160.

[36] Leroux-Roels G, Marchant A, Levy J, Van Damme P, Schwarz TF, Horsmans Y, et al. Impact of adjuvants on $CD4^+$ T cell and B cell responses to a protein antigen vaccine: Results from a phase II, randomized, multicenter trial. Clin Immunol 2016;169:16–27. https://doi.org/10.1016/j.clim.2016.05.007.