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Correlation of protection against varicella in a randomized Phase III varicella-containing vaccine efficacy trial in healthy infants

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ABSTRACT

Background: Varicella vaccination confers high and long-lasting protection against chickenpox and induces robust immune responses, but an absolute correlate of protection (CoP) against varicella has not been established. This study models the relationship between varicella humoral response and protection against varicella.

Methods: This was a *post-hoc* analysis of data from a Phase IIIb, multicenter, randomized trial (NCT00226499) conducted in ten varicella-endemic European countries. Healthy children aged 12–22 months were randomized 3:3:1 to receive one dose of measles-mumps-rubella and one dose of varicella vaccine (one-dose group) or two doses of measles-mumps-rubella-varicella vaccine (two-dose group) or two doses of measles-mumps-rubella vaccine (control group) six weeks apart. The study remained observer-blind until completion, except in countries with obligatory additional immunizations. The objective was to correlate varicella-specific antibody concentrations with protection against varicella and probability of varicella breakthrough, using Cox proportional hazards and Dunning and accelerated failure time statistical models. The analysis was guided by the Prentice framework to explore a CoP against varicella.

Results: The trial included 5803 participants, 5289 in the efficacy (2266: one-dose group, 2279: two-dose group and 744: control group) and 5235 (2248, 2245 and 742 in the same groups) in the immunogenicity cohort. The trial ended in 2016 with a median follow-up time of 9.8 years. Six weeks after vaccination with one- or two-dose varicella-containing vaccine, more than 93.0% of vaccinees were seropositive for varicella-specific antibodies. Estimated vaccine efficacy correlated positively with antibody concentrations. The fourth Prentice CoP criterion was not met, due to predicted positive vaccine efficacy in seronegative participants. Further modelling showed decreased probability of moderate to severe varicella breakthrough with increasing varicella-specific antibody concentrations (ten-year probability <0.1 for antibody concentrations ≥2-fold above the seropositivity cut-off).

Conclusions: Varicella-specific antibody concentrations are a good predictor of protection, given their inverse correlation with varicella occurrence.

Clinical trial: NCT00226499.

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1. Introduction

Varicella is a common childhood disease, transmitted by the highly contagious varicella zoster virus (VZV), but preventable through vaccination [1]. The currently available varicella vaccine formulations include monovalent vaccines containing the live attenuated Oka strain (V), and tetravalent vaccines combining antigens against measles, mumps, rubella, and varicella (MMRV) [1,2]. These vaccines are highly efficacious, preventing >89.5% of moderate to severe varicella cases in children, even after one-dose vaccination [3–6]. Despite their high efficacy and the induction of anti-VZV antibodies in the recipients, there is no universally accepted absolute correlate of protection (CoP) for any of the listed varicella vaccines [1].

A CoP is commonly defined as an immunological endpoint (e.g. pathogen-specific serum antibody level, pathogen neutralization or elimination activity of serum antibodies, or an antigen-specific cellular response) fully indicative of how protected a vaccinated individual is against the disease [7]. Having a CoP for a given vaccine significantly improves the logistical aspects of clinical trials, by simplifying their design and reducing the overall costs [8]. Furthermore, defining a CoP leads to a better understanding of the disease pathology and its interaction with the immune system [7].

A commonly used definition of CoP was proposed by Prentice [9], leading to the following four criteria necessary to establish that an immunological endpoint is a full surrogate for a disease endpoint [10]: (1) vaccination should influence the disease endpoint; (2) vaccination should be correlated with the immunological endpoint; (3) the immunological endpoint should be correlated with the disease endpoint; (4) considering the immunological endpoint, the probability of disease should be independent of the vaccination status – i.e. the full effect on the disease endpoint should be captured by the immunological endpoint. An immune marker that meets all four of these criteria can be considered as a CoP for a given disease [9].

Despite its convenience, the Prentice framework received some criticism for its lack of wide applicability to different vaccine strategies [8]. Additionally, new terminologies have emerged to more precisely define the correlation between immune markers and protection against the corresponding disease [11]. For instance, Qin and colleagues distinguish between the following: i) correlate of risk (CoR), which is an immunological endpoint predictive of a clinical endpoint, and ii) surrogate of protection (SoP), which is a CoR able to predict vaccine efficacy (VE) [11]. Following on this work, Plotkin and Gilbert proposed a unifying nomenclature for immunological endpoints, where CoP would be a blanket term for any immunological endpoint significantly correlated with protection against the corresponding disease [12]. They suggested to distinguish between the following terms: a mechanistic CoP (mCoP; marker both mechanistically and causally linked to protection) and a non-mechanistic CoP (nCoP; marker only predictive of protection through its connection with the protective immune response) [12].

Until now, only an approximate immunological CoP against varicella was obtained [13,14]. These data originate from immunogenicity studies on the monovalent Oka-based varicella vaccine, using an enzyme-linked immunosorbent assay (ELISA) to measure levels of antibodies against the VZV glycoproteins (gP). One of these studies identified a cut-off value for anti-gP antibody titers of $\geq$5 gP ELISA units/mL as an approximate CoP in children aged

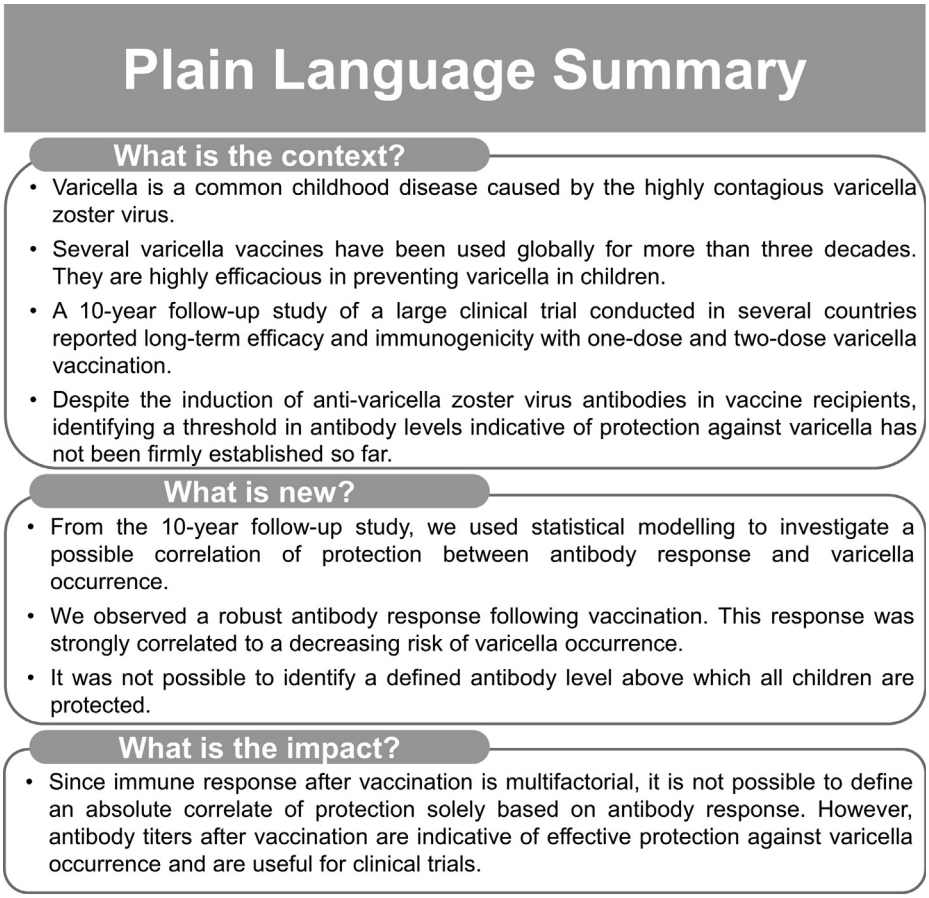

Fig. 1. Plain Language Summary.

between one and 12 years [14]. The same study found that breakthrough varicella was 3.5-fold more likely to develop in participants with anti-gP antibody titers <5 units/mL compared to those with titers ≥5 units/mL. While the data fitted well with the statistical predictions of protection, an absolute CoP for varicella was not identified. This was due to discrepancies between varicella occurrence and the level of seropositivity for anti-gP antibodies: some participants with the lowest anti-gP antibody titers were protected against the disease, while varicella breakthrough occurred in some participants with high antibody levels [14]. A further study, using elaborate statistical modelling, found that the probability of contracting varicella was influenced by both anti-gP antibody titer and vaccination status [13]. Referring to the Prentice criteria described above, these data thus failed to fulfill the fourth criterion. These studies indicated that anti-gP humoral response alone was insufficient as predictor of protection and that further, controlled long-term studies were needed to establish a CoP against varicella [13,14].

Previous findings on immunization with one-dose and two-dose varicella-containing vaccines were obtained in a ten-year follow-up of a Phase IIIb clinical trial in children aged 12–22 months, who were vaccinated with: one dose of measles-mumps-rubella (MMR) and one dose of V (MMR + V; one-dose varicella-containing vaccine), two doses of MMRV (two-dose varicella-containing vaccine), and two doses of MMR (control vaccine). The ten-year VE was 67.2% and 95.4% in the one-dose and two-dose varicella-containing vaccine groups, respectively, compared to the control group [5]. The sharp increase in anti-VZV antibody concentrations after immunization was observed in recipients of varicella-containing vaccines, but not in the control group [5]. Furthermore, the anti-VZV antibody concentrations after both types of varicella vaccination were persistent during the ten-year follow-up and were at least five-fold above the seropositivity threshold.

The present analysis investigates a possible CoP for varicella after vaccination of children with one or two varicella-containing vaccine doses, using post-immunization samples. As an indication of protection, both anti-VZV and anti-glycoprotein E (anti-gE) antibody levels were assessed. The anti-gE ELISA was developed in-house, using the recombinant VZV gE protein as antigen and can be used to assess both varicella and zoster vaccine immunogenicity thus facilitating comparisons between clinical studies. In future clinical trials, the anti-gE ELISA assay could be used as an alternative assay to measure the antibody response to varicella vaccines. This anti-gE ELISA yielded 99.0% overall agreement with the anti-VZV ELISA in terms of seropositivity prevalence in the study participants [15].

The present analysis assessed if varicella occurrence decreased with increasing anti-VZV and anti-gE antibody concentrations and if the varicella-specific antibody titer is the only parameter explaining the probability of decreasing varicella case incidence. Statistical modelling was applied to data on varicella breakthrough and vaccine immunogenicity to compare the observed and expected probabilities of breakthrough varicella disease [16,17]. A summary contextualizing the outcomes of this analysis is displayed in the Plain Language Summary (Fig. 1) for the convenience of health care professionals.

2. Methods

2.1. Study design and participants

This was a *post-hoc* analysis of a Phase IIIb, randomized, multicenter, controlled clinical trial conducted in healthy children in their second year of life, and for whom written informed consent was obtained [3,5,6]. The trial was conducted in ten varicella-endemic European countries, in accordance with the Declaration of Helsinki and International Council for Harmonization Good Clinical Practice guidelines. Ethics committees or independent review boards of all countries approved the study. A summary of the approved study protocol is available at www.gsk-clinicalstudyregister.com (study IDs: 100388, 103494, 104105, 104106).

The participants were randomized (3:3:1) by an internet randomization system to receive the following vaccines six weeks (42 days) apart: one dose of MMR and one dose of V (one-dose varicella-containing vaccine group, also named one-dose group), two doses of MMRV (two-dose varicella-containing vaccine group, also named two-dose group), and two doses of MMR vaccine (control group) (Supplementary Fig. S1). The vaccines were administered subcutaneously into the deltoid region of the participant's left arm. The study remained observer-blind until its completion for all participants, except for most children in the one-dose group (but none of the other groups), who were obliged to receive a second dose of MMR vaccine according to local regulations of six participating countries [5].

2.2. Objectives

All primary and most secondary objectives of the clinical trial, concerning VE, immunogenicity and safety, were previously published [3,5,6,18]. The objectives of the current analysis were secondary and descriptive. This exploratory analysis aimed to: 1) assess the impact of VZV-specific antibody concentrations on the occurrence of varicella; 2) investigate if a defined VZV-specific antibody threshold, measured six weeks after the second vaccine dose, can be correlated with protection against the varicella disease through the ten years of follow-up. For the latter objective, the analysis was conducted for all occurrences and for moderate to severe varicella occurrences.

2.3. Immunogenicity assessment

Vaccine immunogenicity was measured by anti-VZV and anti-gE ELISA assays to cover both the broad response to the whole VZV (anti-VZV) and the gE-specific immune response (anti-gE) [3,5,6,15]. Anti-gE ELISA was originally designed during development of an adjuvanted recombinant protein herpes zoster vaccine (RZV) in 2018 [19–21]. For the present analysis, the anti-gE assay was carried out after clinical trial completion, since it was recently reported as a valid alternative to anti-VZV ELISA for assessment of immune response against VZV [15]. Hence, anti-gE ELISA results have not been disclosed in previous publications from the same trial.

Blood samples were collected six weeks after the second vaccination, a timepoint denominated Day 84, at which 4 mL of blood was drawn from each participant and then assayed by the two ELISA types [6,15]. The anti-VZV ELISA assay was performed as previously published [6]. The anti-gE ELISA assay used microplates pre-coated with purified, recombinant gE produced in-house and was performed as previously published [15]. Measurements were expressed in milli-international units per mL (mIU/mL) and seropositivity cut-off values were set to 25 mIU/mL for anti-VZV and 97 mIU/mL for anti-gE ELISA. The antibody levels of seronegative participants (antibody levels below the pre-defined cut-off) were arbitrarily set to half the cut-off value for each assay for calculating the geometric mean concentrations (GMCs). GMCs were the anti-log value of the mean logarithmic concentration. For each GMC, 95% confidence intervals (CIs) were plotted as described previously [3,5,6]. Vaccine immunogenicity was evaluated on the according-to-protocol (ATP) cohort for immunogenicity. This cohort included all participants who: 1) fulfilled protocol require-

ments; 2) had anti-VZV antibody concentrations below 25 mIU/mL preceding the first study vaccination; 3) had no varicella before the blood draw planned six weeks after the second study vaccination; and 4) provided a valid blood sample at the indicated timepoints.

2.4. Varicella incidence determination

Varicella case reporting and classification were conducted as previously published [3,5,6]. All varicella-like rashes were initially reported by the participants' parents or legal guardians and were then evaluated by the study investigators. The investigator-confirmed cases were sent for review by the Independent Data Monitoring Committee (IDMC) and to laboratory analyses in parallel. The IDMC was blinded to the laboratory test results (anti-VZV and anti-gE antibody ELISA results and VZV DNA presence/absence) and established if the varicella case met the clinical definition. A confirmed varicella case always entailed the fulfilled clinical case definition together with one of the following two criteria: a positive PCR test or a clear epidemiological link with a valid index case [3,5,6].

2.5. Assessment of vaccine efficacy

VE was calculated as described previously [3,5,6]. The Cox proportional hazards regression model was used to estimate the hazard ratios (HRs) for one- and two-dose varicella-containing vaccine recipients versus the control group. The model was chosen as it accounts for individual follow-up time of each participant and post-infection censoring of data. The latter feature was important, since varicella case follow-up time was censored at rash onset date. VE was calculated as $100 \times (1–HR)$, with a two-sided 95% CI. All VE analyses were performed using the ATP cohort for efficacy, which included children with completed vaccinations, with a follow-up visit six weeks after the second study vaccination and fulfilled protocol requirements. The follow-up time was censored at the time a non-study varicella vaccine was administered, or varicella disease was detected.

2.6. Statistical modelling

The present analysis included participants with valid ELISA results obtained after the second dose of vaccination (Day 84). Descriptive analysis of anti-VZV and anti-gE antibody concentrations was performed on ATP cohort for immunogenicity. All other analyses used ATP cohort for efficacy and used pooled data from all study groups (one-dose, two-dose, and control group).

Percentages of seropositive participants at Day 84 were tabulated with their 95% CIs by study group.

Participants were grouped according to post-vaccination anti-VZV and anti-gE concentration ranges. The number (N) and proportion of participants with confirmed (n_1 and $100 \times n_1/N$) and moderate to severe (n_2 and $100 \times n_2/N$) varicella cases was computed for each concentration range.

2.6.1. The Prentice framework

The Prentice framework [9] was used as the point of reference in attempting to identify a CoP against varicella using the data of this efficacy study.

The first and second criterion (vaccination with one or two doses of varicella-containing vaccine has an effect on varicella disease and correlates with Day 84 anti-VZV and anti-gE antibody concentrations) will not be discussed in detail here. The first criterion is fulfilled by the previously published ten-year VE of 67.2% and 95.4% for the one-dose and two-dose groups, respectively [5]. The second criterion is met by the significant differences in humoral immune response between the groups (see also Table 1) [5]. This analysis therefore focuses on the third and fourth Prentice criteria.

Prentice criterion 3: The following model was used to show that the Day 84 anti-VZV or anti-gE antibody concentrations (surrogate endpoint X) correlate with the occurrence of confirmed varicella cases (Y).

$$h(t|X_j) = \lambda(t)\exp(\beta_0 + \beta_1 X_j)$$

where X_j is the logarithm of the Day 84 antibody concentration and $h(t|X_j)$ is the hazard function for the time to a varicella case conditional on X_j. The immunogenicity endpoint is considered as being significantly correlated with occurrence of confirmed varicella cases if the p-value associated with H_0: $\beta_1 = 0$ is below 0.05.

Prentice criterion 4: The following model was used to show that the probability of a confirmed varicella case (Y) is independent of treatment status (Z), given the immunogenicity endpoint (X):

$$h(t|X_j, Z_j) = \lambda(t)\exp(\beta_0 + \beta_1 X_j + \beta_{2A} Z_{jA} + \beta_{2B} Z_{jB} + \beta_{12A} X_j Z_{jA} + \beta_{12B} X_j Z_{jB})$$

where $h(t|X_j, Z_j)$ is the hazard function for the time to a varicella case conditional on X_j and Z_j, X_j is the logarithm of the Day 84 anti-VZV or anti-gE antibody concentration, and Z_{jA} and Z_{jB} are dummy variables for two of the three treatment groups.

An ideal CoP would be one where the p-value associated with the global H_0 on the five parameters H_{01}: $\beta_1 = \beta_{2A} = \beta_{12A} = \beta_{2B} = \beta_{12B} = 0$ is lower than 0.05, and the p-value for the H_{02}: $\beta_{2A} = \beta_{12A} = \beta_{2B} = \beta_{12B} = 0$ is greater than 0.05.

For both Prentice criteria 3 and 4, a Cox regression model was used to account for the variability in the follow-up time between participants.

Table 1
Number and percentage of seropositive participants and the respective GMC values, as measured by anti-VZV and anti-gE ELISA at Day 84 (ATP cohort for immunogenicity).

Group	Anti-VZV[a]						Anti-gE					
	N	≥25 mIU/mL			GMC		N	≥97 mIU/mL			GMC	
		n	%	95% CI	Value	95% CI		n	%	95% CI	Value	95% CI
One-dose	2191	2081	95.0	94.0; 95.9	95.4	91.6; 99.3	2228	2087	93.7	92.6; 94.6	385.4	370.5; 401.0
Two-dose	2209	2204	99.8	99.5; 99.9	1840.1	1773.9; 1908.8	2225	2218	99.7	99.4; 99.9	5107.2	4907.6; 5314.8
Control	727	33	4.5	3.1; 6.3	14.2	13.5; 14.9	738	51	6.9	5.2; 9.0	57.0	54.0; 60.1

Day 84, blood sampling timepoint at six weeks after the second vaccination; ELISA, enzyme-linked immunosorbent assay; ATP, according-to-protocol; VZV, varicella zoster virus; gE, glycoprotein E; mIU/mL, milli-international units per mL; One-dose, recipients of the one-dose varicella-containing vaccine (one dose of monovalent varicella vaccine administered at the second vaccination); Two-dose, recipients of two doses of varicella-containing vaccine (two doses of tetravalent measles-mumps-rubella-varicella vaccine at the first and second vaccination); Control, recipients of two doses of trivalent measles-mumps-rubella vaccine at the first and second vaccination (no varicella-containing vaccine administered); GMC, geometric mean antibody concentration; N, number of participants with available results; n/%, number/percentage of participants with concentration equal to or above specified value; 95% CI, 95% confidence interval.

[a] Anti-VZV results were published previously [5] and were included here to allow comparison with anti-gE results, which were not yet published.

2.6.2. Dunning and accelerated failure time (AFT) statistical models

The relationship between the Day 84 anti-VZV or anti-gE antibody concentration and the occurrence of varicella breakthrough was investigated by two methods: the Dunning method and an AFT model with a Weibull exponential hazard, in order to take the timing of varicella events into account [13,14,16,17]. These models were then used to fit the observed varicella breakthrough rates to the rates predicted from Day 84 anti-VZV and anti-gE antibody levels.

The models allow estimation of VE using Day 84 anti-VZV and anti-gE antibody concentrations. The probability of a confirmed varicella case in the control group was computed by fitting each model with seronegative antibody concentration estimates (half of the assay cut-off). VE was estimated based on this probability.

2.6.2.1. The Dunning model. This model is a logistic regression approach which considers the force of infection in the population (via the λ parameter). The probability that an individual, i, develops varicella disease can be expressed as follows:

$$P(Y_i = 1) = \lambda(1 - \pi(X_i))$$

where $\pi(X_i)$ represents the probability that an individual is protected and can be derived from:

$$\pi(X_i) = \frac{\exp(\beta_0 + \beta_1 X_i)}{1 + \exp(\beta_0 + \beta_1 X_i)}$$

The parameters β_0 and β_1 can be estimated by modelling the probability that an individual develops the disease:

$$P(Y_i = 1) = \frac{\lambda}{1 + \exp(\beta_0 + \beta_1 X_i)}$$

where λ is the probability that a susceptible individual develops the disease.

In case the model did not converge, $\pi(X_i)$ was replaced by the following square root sigmoid function [22]:

$$\pi(X_i) = \frac{(\beta_0 + \beta_1 X_i)}{\sqrt{1 + (\beta_0 + \beta_1 X_i)^2}}$$

2.6.2.2. The AFT model. If T_i is a random variable denoting the failure time (i.e. time to a varicella case) for the i^{th} participant, and if X_{i1} is the logarithm (base 10) of the antibody concentration at Day 84, then:

$$\log T_i = \beta_0 + \beta_1 X_{i1} + \sigma\varepsilon_i$$

where ε_i is a random disturbance term, and β_0, β_1, and σ are parameters to be estimated.

To estimate these parameters, we used the maximum likelihood estimation with an extreme value (two-parameter) distribution assumption on ε, corresponding to a Weibull distribution for *T*.

3. Results

3.1. Study population

Demographic characteristics of participants have been previously published [3,5,6]. Briefly, the mean age of enrolled children was 14.2 months (standard deviation: 2.5 months), 98.1% were of European origin and 47.9% were female. Allocation of participants to each of the study cohorts and reasons for exclusion are outlined in Supplementary Fig. S1. A total of 5803 participants were enrolled and vaccinated between September 2005 and May 2006. The last study visit occurred in December 2016 and the median follow-up time was 9.8 years. The ATP cohort for immunogenicity included 2248 recipients of one-dose varicella-containing, 2245 recipients of two-dose varicella-containing, and 742 recipients of the control vaccine. The frequency of varicella occurrence was determined in the ATP cohort for efficacy, which included 5289 participants (2266 in the one-dose group, 2279 in the two-dose group, and 744 in the control group) (Supplementary Fig. S1).

3.2. Vaccine immunogenicity

Immune responses induced by vaccination with either one or two doses of varicella-containing vaccine are shown in Table 1. The anti-VZV results were published previously [5] and were included in the Table 1 to allow comparison with anti-gE results, which were not yet published. The percentage of seropositive participants were more than 95.0% (anti-VZV) and 93.7% (anti-gE) in the one-dose group and more than 99.0% (seropositive for both anti-gE and anti-VZV antibodies) in the two-dose varicella-

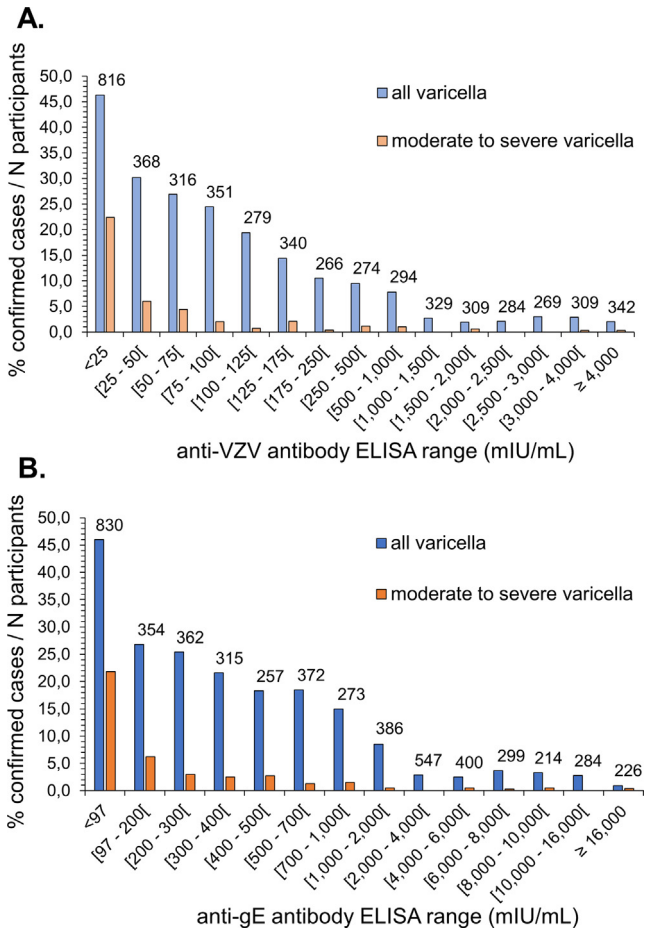

Fig. 2. Frequency of observed varicella occurrence, for participants with Day 84 anti-VZV (panel A) and anti-gE (panel B) antibody concentrations within the specified ranges (ATP cohort for efficacy)*. * The antibody concentrations were measured at Day 84 (blood sampling timepoint six weeks after the second vaccination). The control group received no varicella vaccine (two doses of trivalent measles-mumps-rubella vaccine at the first and second vaccination), the one-dose group received one dose of monovalent varicella vaccine at the second vaccination, and the two-dose group received two doses of tetravalent measles-mumps-rubella-varicella vaccine at the first and second vaccination. Data are presented for all groups pooled together. ATP, according-to-protocol; ELISA, enzyme-linked immunosorbent assay; VZV, varicella zoster virus; gE, glycoprotein E; mIU/mL, milli-international units per mL; N participants, number of participants in whom the level of antibodies within each given range was measured by ELISA (values displayed above bars corresponding to each measured antibody concentration range); all varicella, frequency of all confirmed varicella cases; moderate to severe varicella, frequency of moderate to severe varicella cases.

containing vaccine group at Day 84. At this timepoint, the one-dose group had received one dose of V six weeks before, in contrast to the two-dose group, who had received two doses of MMRV 12 and six weeks before. In the control group, 4.5% and 6.9% of participants were seropositive for anti-VZV and anti-gE antibodies at Day 84, respectively. A second dose of varicella-containing vaccine resulted in a 13- to 19-fold increase in anti-VZV and anti-gE antibody GMCs when compared to one dose (Table 1).

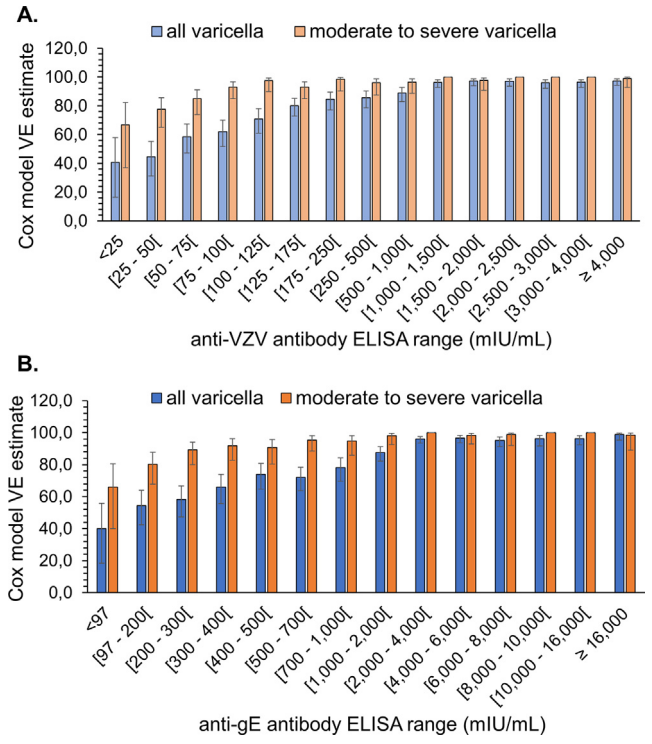

Fig. 3. Cox model estimates of varicella VE for participants with the Day 84 anti-VZV (panel A) and anti-gE antibodies (panel B) antibody concentrations within the indicated ranges* (ATP cohort for efficacy). * The ten-year vaccine efficacy was calculated for recipients of one and two doses of varicella-containing vaccine, compared to the control group participants. The control group received no varicella vaccine (two doses of trivalent measles-mumps-rubella vaccine at the first and second vaccination), the one-dose group received one dose of monovalent varicella vaccine at the second vaccination, and the two-dose group received two doses of tetravalent measles-mumps-rubella-varicella vaccine at the first and second vaccination. Data are presented for all groups pooled together. The error bars represent the upper and lower limits of the 95% confidence interval. ATP, according-to-protocol; VE, vaccine efficacy; ELISA, enzyme-linked immunosorbent assay; VZV, varicella zoster virus; gE, glycoprotein E; mIU/mL, milli-international units per mL; Day 84, blood sampling timepoint at six weeks after the second vaccination; all varicella, all confirmed varicella cases; moderate to severe varicella, moderate to severe confirmed varicella cases.

3.3. Descriptive analysis of the observed varicella disease cases during the ten-year follow-up

We plotted the occurrence of confirmed varicella cases from the three pooled study groups against the anti-VZV and anti-gE antibody concentrations measured by ELISA (Fig. 2). The control group participants contributed to the majority of the seronegative bar (<25 mIU/mL for anti-VZV and <97 mIU/mL for anti-gE ELISA). The values were comparable between anti-VZV and anti-gE ELISA. The increase in anti-VZV and anti-gE antibody concentrations led to a progressive decrease in varicella incidence. The percentage of confirmed varicella cases was highest in seronegative participants (46% for all varicella and 22% for moderate to severe varicella cases). In contrast, between 1.0% and 3.0% of all varicella breakthrough cases were detected in participants with antibody concentrations more than 100 times above the seropositivity cut-off value. The incidence of all varicella decreased approximately ten-fold between participants with antibody concentrations slightly above the cut-off value and those with the highest concentrations (Fig. 2). The largest changes in varicella incidence occurred in the lower halves of the antibody concentration ranges (Fig. 2), which largely correspond to those observed in the one-dose group.

Moderate to severe varicella case incidence followed a similar profile, but the differences were less marked due to the fewer cases reported. The frequency of these cases was also highest in the seronegative participants, for both anti-VZV and anti-gE ELISA.

The Cox proportional hazards regression model was used to estimate the ten-year VE against all varicella and moderate to severe varicella in the recipients of one- and two-dose varicella-containing vaccines (Fig. 3). Based on this model, the estimated VE against all varicella increased from approximately 50.0% (44.6% for anti-VZV and 54.4% for anti-gE ELISA) in mildly seropositive participants to above 96.0% (96.1% for anti-VZV and 96.2% for anti-gE ELISA) in participants with antibody concentrations more than 100-fold above threshold (Fig. 3). Therefore, increase in VE correlated positively with increase in anti-VZV and anti-gE antibody concentrations.

3.4. Prentice criteria

Prentice criteria 1 and 2 were met for both anti-VZV and anti-gE ELISA, as shown by the previously published VE [3,5,6] and by the immunogenicity data presented in Table 1.

Prentice criterion 3 was met, as a clear decrease in varicella incidence rates with increasing antibody concentrations was observed for both assays (Fig. 2). The calculated p-values (Table 2) were <0.0001 for both anti-VZV and anti-gE ELISA.

Prentice criterion 4 was not met for either of the ELISA assays or the type of confirmed varicella (all or moderate to severe) investigated (Table 2).

Table 2
Prentice criteria parameter estimates and p-values (ATP cohort for efficacy).

Endpoint	Prentice criterion	Null hypothesis	p-value	
			Anti-VZV	Anti-gE
All confirmed varicella cases	Criterion 3	$\beta_1 = 0$	<0.0001	<0.0001
	Criterion 4	H_{01}: $\beta_1 = \beta_{2A} = \beta_{12A} = \beta_{2B} = \beta_{12B} = 0$	<0.0001	<0.0001
		H_{02}: $\beta_{2A} = \beta_{12A} = \beta_{2B} = \beta_{12B} = 0$	0.0163	<0.0001
Moderate to severe confirmed varicella cases	Criterion 3	$\beta_1 = 0$	<0.0001	<0.0001
	Criterion 4	H_{01}: $\beta_1 = \beta_{2A} = \beta_{12A} = \beta_{2B} = \beta_{12B} = 0$	<0.0001	<0.0001
		H_{02}: $\beta_{2A} = \beta_{12A} = \beta_{2B} = \beta_{12B} = 0$	<0.0001	<0.0001

ATP, according-to-protocol; VZV, varicella zoster virus; gE, glycoprotein E; Criterion 3, third Prentice criterion; Criterion 4, fourth Prentice criterion; H_{01} and H_{02}, the first and second null hypotheses for evaluating Prentice criterion 4 (acceptance p-values are $p < 0.05$ for H_{01} and $p > 0.05$ for H_{02}); β_1, β_{2A}, β_{12A}, β_{2B} - β_{12B}, regression parameters.

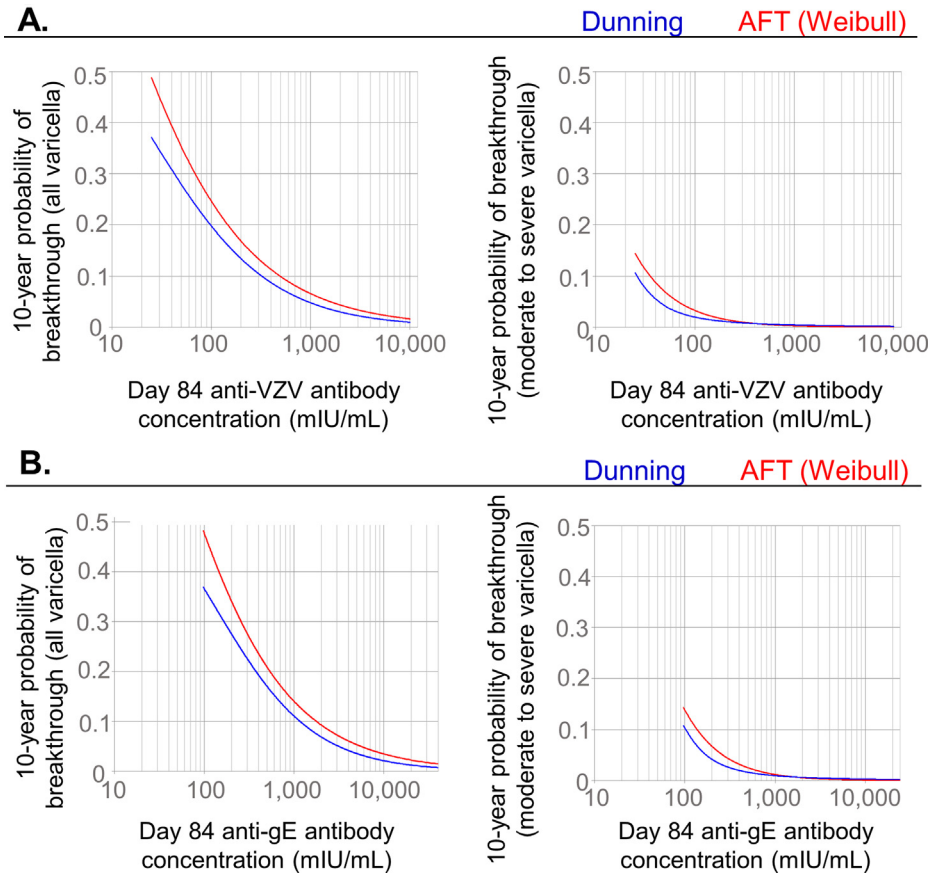

Fig. 4. Probability of all (left) and moderate to severe* (right) confirmed varicella breakthrough up to ten years post-vaccination versus Day 84 anti-VZV (panel A) and anti-gE (panel B) concentrations using the Dunning and AFT models (ATP cohort for efficacy). ATP, according-to-protocol; Dunning, Dunning statistical model used to estimate varicella breakthrough probability based on antibody concentrations; *, for probability calculations of moderate to severe varicella breakthrough, Dunning square root sigmoid function was used; AFT (Weibull), accelerated failure time model with Weibull hazard used to estimate varicella breakthrough probability based on antibody concentrations; VZV, varicella zoster virus; gE, glycoprotein E; mIU/mL, milli-international units per mL; Day 84, blood sampling timepoint at six weeks after the second vaccination; all varicella, all confirmed varicella cases; moderate to severe varicella, moderate to severe confirmed varicella cases.

3.5. Dunning and AFT statistical modelling

Probability of breakthrough varicella during the entire follow-up time of ten years was computed using both Dunning and AFT model with the exponential Weibull hazard (Fig. 4). The parameters calculated based on each model are shown in Supplementary Tables 1 and 2. The model-based probabilities of experiencing any confirmed or a moderate to severe varicella case over the follow-up period were plotted based on Day 84 anti-VZV and anti-gE antibody concentrations. We used these values to obtain modelled estimates of VE for each Day 84 antibody concentration, with the denominator for VE obtained using predictions for seronegative participants (Fig. 5).

Both Dunning and AFT models showed similar decreasing probabilities of varicella breakthrough with increasing antibody concentrations (ten-year probability of all varicella and moderate to severe varicella was <0.1 for antibody concentrations ≥30-fold and ≥2-fold above the seropositivity cut-off). The results were consistent between the anti-VZV and anti-gE ELISA. The varicella breakthrough rate estimated by the AFT model with Weibull exponential hazard was marginally higher at lower antibody concentrations (Fig. 4). Additionally, the predicted VE against all and moderate to severe varicella was positively correlated with increasing anti-VZV and anti-gE antibody concentrations (Fig. 5).

4. Discussion

This analysis expands on the previous findings concerning VE and immunogenicity in children receiving one or two doses of varicella-containing vaccines [3,5,6]. Guided by the Prentice framework and statistical modelling, the present study describes VZV-specific humoral responses as a reliable predictor of (breakthrough) varicella occurrence. Unlike previous publications from the same ten-year trial, the present analysis used anti-gE ELISA in parallel with the anti-VZV ELISA to assess vaccine immunogenicity [15].

Following one- and two-dose varicella vaccination, between 93.7% and 99.8% of vaccinated participants were seropositive for anti-VZV and anti-gE antibodies at Day 84. Seropositivity rates were in similar ranges when assessed with anti-VZV and anti-gE ELISA assays. The frequency of varicella occurrence decreased with the increase in antibody concentrations in the vaccinated participants. However, not all seronegative participants developed (breakthrough) varicella and some varicella breakthrough occurred in participants with high anti-VZV and anti-gE antibody concentrations.

As estimated by the Cox model, anti-VZV and anti-gE antibody concentrations were found to be a reliable predictor of VE. Increasing antibody concentrations were associated with the predicted

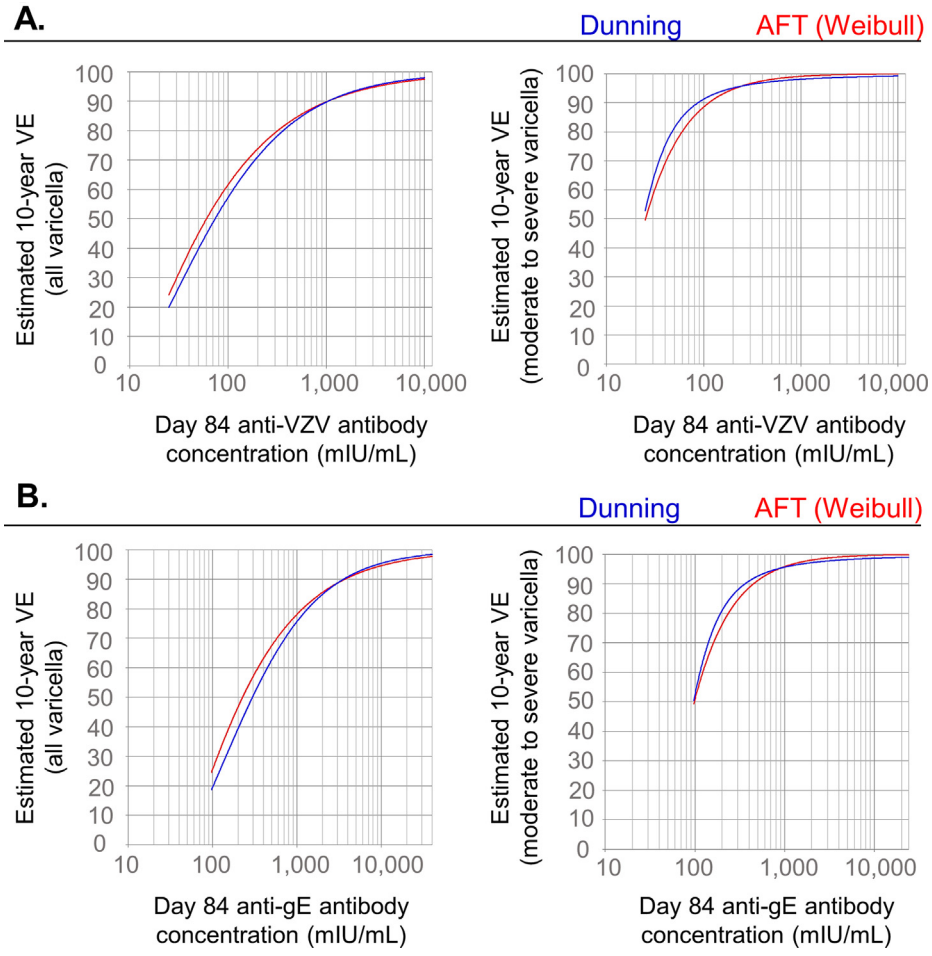

Fig. 5. Estimated ten-year vaccine efficacy (VE) against all (left) and moderate to severe (right) confirmed varicella cases versus Day 84 anti-VZV (panel A) and anti-gE (panel B) concentrations using the Dunning and AFT models (ATP cohort for efficacy). ATP, according-to-protocol; Dunning, Dunning statistical model used to estimate ten-year varicella-containing vaccine efficacy based on antibody concentrations; *, for probability calculations of vaccine efficacy against moderate to severe varicella occurrence, Dunning square root sigmoid function was used; AFT (Weibull), accelerated failure time model with Weibull hazard used to estimate varicella-containing vaccine efficacy based on antibody concentrations; VZV, varicella zoster virus; gE, glycoprotein E; mIU/mL, milli-international units per mL; Day 84, blood sampling timepoint at six weeks after the second vaccination; all varicella, all confirmed varicella cases; moderate to severe varicella, moderate to severe confirmed varicella cases.

increasing VE. Together with the above findings, these results indicate that anti-VZV and anti-gE antibody concentrations correlate well with the protection level against varicella.

The Prentice criteria were used in this analysis as the guiding postulates to determine a CoP against varicella. Prentice criteria 1, 2, and 3 were met for all confirmed cases and moderate to severe cases indicating a clear inverse relationship between Day 84 antibody concentrations and the probability of breakthrough varicella disease. Prentice criterion 4, however, was not met for either the anti-VZV or anti-gE ELISA, and for any varicella and moderate to severe varicella cases. This implies that the reduced probability of varicella disease cannot be fully explained solely by the increasing Day 84 VZV-specific antibody concentrations. Furthermore, the number of varicella-containing vaccine doses (one or two) has a non-negligible impact on varicella disease rate. This is also apparent from Cox model estimates, where VE among seronegative participants in varicella vaccinees is clearly positive (≥40.0%). This positive VE in seronegative participants in return caused both Dunning and AFT models to slightly underestimate the VE over the whole range of Day 84 anti-VZV and anti-gE antibody concentrations compared to the Cox model. The probability of varicella breakthrough during the ten-year follow-up based on Day 84 anti-VZV and anti-gE antibody concentrations was relatively similar when estimated by the Dunning and the AFT models. Neither model could however identify a clear threshold, or narrow range of thresholds, at which protection increases sharply.

Data on protection against varicella disease in vaccinated but seronegative children were previously reported, suggesting that vaccination effectively primes the immune system to counteract varicella [23]. There may be an underlying protection mechanism provided by vaccination that cannot be inferred from only the humoral response measurements. As reviewed previously, innate and adaptive cellular immune responses are promptly activated by VZV infection and defend against varicella [24–27]. Long-lived CD4 memory T-cells persist for decades after primary VZV infection and CD4-mediated immunity can be boosted after re-exposure to VZV and subclinical virus reactivation [26,27]. A complete view on the CoP against varicella will therefore likely involve a combination of humoral and cellular immunity factors.

Also, modelling varicella incidence rates based on half the assay cut-off value in the control group may be limiting, since these rates are reduced by the presence of seronegative yet partially protected participants.

Determining a Prentice-defined serological CoP threshold against varicella is challenging, as it requires explaining the protective effect of any single varicella vaccine solely based on one

immune marker [10,13]. Since post-vaccination immune responses are multifactorial, the Prentice framework may require adaptations. This is also apparent from the abundant newly-coined terms to describe different levels of vaccine-induced protection. These terms introduced confusion concerning CoP definitions and prompted new classification of immune markers [7,11]. Therefore, following Prentice criteria and lacking a more suitable model for a CoP against varicella was likely a limitation of this analysis.

Furthermore, clinical trial designs and serological methods to assess antibody responses may introduce significant heterogeneity when assessing CoP against varicella. The previously defined approximate CoP threshold for the one-dose varicella-containing vaccine was set at 5 ELISA units/mL for anti-gP antibodies [14], obtained by a non-commercial assay. This threshold was determined in a heterogeneous group of vaccine recipients, where age was a relevant covariate in the statistical analyses [13,14]. In contrast, the present analysis was based on a homogeneous participant group, without significant variability in anti-VZV or anti-gE antibody levels in the control group. Homogeneity in participant demographic characteristics may be perceived as both a strength and a limitation of this study. While it ensured a well-controlled design setting and comparability between the study groups, it eliminated a variability factor beneficial for statistical CoP modelling. However, an important strength of the current analysis was inclusion and long follow-up time of the control group, which served as a comparator for recipients of varicella-containing vaccines.

Despite the lack of an absolute CoP, varicella vaccination clearly induces a robust immune response, as measured by anti-VZV and anti-gE antibodies, with antibody levels inversely correlated with varicella disease occurrence. Given this inverse correlation, which was also previously observed [14], the anti-VZV and anti-gE antibody concentrations satisfy the CoR definition by Qin and colleagues and the nCoP definition by Plotkin and Gilbert [11,12].

The present analysis sheds further light on humoral immune protection against (breakthrough) varicella in vaccinated children. In addition, the modelling statistical approaches presented here can be applied to other vaccination strategies. It will be important to define a unifying terminology for correlates and surrogates of protection in future trials, to facilitate data interpretation. Despite its clarity and traditional use, the Prentice framework may need to be adapted, to account for different levels of protection induced by vaccination and measured by different assays.

5. Conclusion

Vaccination with one or two doses of varicella-containing vaccine induced robust immune responses against varicella, as measured by both anti-VZV and anti-gE ELISA assays. Still, neither assay could define a correlate (or surrogate) of protection according to the Prentice framework, suggesting that cellular immunity may need to be considered when defining a CoP. Nevertheless, varicella-specific antibody levels measured six weeks after vaccination are a reliable proxy to estimate protection against varicella disease.

Declaration of Competing Interest

Md Ahsan Habib, Stephane Carryn, Ouzama Henry, Stéphanie Ravault, Paul Gillard and Michael Povey are employees of the GSK groups of companies. Md Ahsan Habib, Stephane Carryn, Ouzama Henry, Stéphanie Ravault and Paul Gillard hold shares in the GSK group of companies as part of their employee remuneration. Roman Prymula received grant support from the GSK group of companies. Susanna Esposito received grant support from Abbott and DMG companies; personal fees from MSD company; and grant support and personal fees from the GSK group of companies, Sanofi, Vifor Pharma and Janssen Pharmaceutica. Jacek Wysocki received personal fees from the GSK group of companies during the conduct of the study. Vytautas Usonis received grant from the GSK group of companies during the conduct of the study and as educational grant. All authors have no non-financial interest to declare.

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Authors' contribution

All authors attest they meet the ICMJE criteria for authorship. Roman Prymula, Stephane Carryn and Susanna Esposito were involved in the conception or the design of the study. Roman Prymula, Susanna Esposito, Stéphanie Ravault, Vytautas Usonis and Jacek Wysocki participated in the collection or generation of the study data. Stéphanie Ravault also contributed to the study with materials/analysis tools. Roman Prymula, Susanna Esposito, Vytautas Usonis and Jacek Wysocki performed the study. All authors were involved in the analyses and interpretation of the data. All authors revised the manuscript critically for important intellectual content and gave final approval to submit for publication.

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Data sharing statement

To request access to patient-level data and documents for this study, please submit an enquiry via www.clinicalstudydatarequest.com.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.02.074.

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