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Phase 1 Randomized, Placebo-Controlled, Dose-Escalating Study to Evaluate OVX836, a Nucleoprotein-Based Influenza Vaccine: Intramuscular Results

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Background. OVX836 is a recombinant protein vaccine targeting the highly conserved influenza nucleoprotein (NP), which could confer broad-spectrum protection against this disease.

Methods. A randomized, placebo-controlled, double-blind, dose-escalating, single- center, first-in-human study was conducted in 36 healthy adults aged 18–49 years. Twelve subjects per cohort (9 vaccine and 3 placebo) received 2 OVX836 intramuscular administrations on days 1 and 28 at the dose level of 30 µg, 90 µg, or 180 µg. Safety and immunogenicity were assessed after each vaccination and for 150 days in total.

Results. OVX836 was safe and well tolerated at all dose levels, with no difference in solicited local and systemic symptoms, and unsolicited adverse events between the first and second administration, or between dose levels. All subjects presented pre-existing NP-specific immunity at baseline. OVX836 induced a significant increase in NP-specific interferon-gamma T cells and anti-NP immunoglobulin G at all dose levels after the first vaccination. The second vaccination did not further increase the response. There was a trend for a dose effect in the immune response.

Conclusions. The safety and reactogenicity profile, as well as the humoral and cellular immune responses, encourage further evaluation of OVX836 in a larger Phase 2a study.

Keywords. influenza; nucleoprotein; OVX836; Phase 1; vaccine.

Seasonal influenza is estimated to result in approximately 3 to 5 million cases of severe illness worldwide every year, with approximately 290 000 to 650 000 deaths, mostly among people aged 65 years or older [1].

Annual vaccination is considered the most effective way to prevent influenza. Among the general population in the United States, seasonal trivalent or quadrivalent influenza vaccine effectiveness has been limited to 42% on average over the last 10 years (32% in the elderly) [2]. Similar data are available in Europe [3]. When circulating viruses did not match the vaccine viruses, effectiveness may drop to 10%–20% only, as in the season 2014–2015 [2]. There is therefore a medical need for improving influenza vaccines efficacy.

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Although antibody threshold values for viral surface hemagglutinin and neuraminidase are recognized as surrogates/correlates for efficacy in clinical trials on most current vaccines [4], cellular responses, in particular CD4- and CD8-mediated responses, are very likely to contribute to protection, in particular in the elderly population [5–8].

In humans, there is growing evidence on the role of T-cell immunity against conserved internal antigens (nucleoprotein [NP], matrix protein 1 [M1], and polymerase B1 [PB1]) in the protection against influenza. A prospective cohort study conducted during the H1N1 pandemic of 2009 showed that higher frequencies of pre-existing T cells specific to conserved CD8 epitopes from NP, M1, and PB1 were found in individuals who developed less severe illness [9]. In a challenge study, pre-existing $CD4^+$, but not $CD8^+$, T cells responding to influenza NP and matrix protein were associated with lower virus shedding and less severe illness [10]. The Flu Watch Cohort Study has suggested that pre-existing T-cell responses targeting NP provide protective immunity against pandemic and seasonal influenza. The presence of NP-specific T cells before exposure to virus correlated with fewer cases of symptomatic, polymerase chain reaction (PCR)-positive influenza A, during both pandemic and seasonal influenza periods [11]. In addition, NP is highly conserved across A strains [12], as well as

partially conserved between A and B strains [13, 14]. These results provide the rationale to develop NP-based vaccines against influenza.

OVX836 (OSIVAX, Lyon, France) is a recombinant protein developed as a universal vaccine against influenza A strains [15], with cross-protection against B strains (J.D., F.N. and M.C., unpublished data, 2018). The antigenic part corresponds to the NP sequence of the A/WSN/1933(H1N1) influenza virus. OVX836 protein contains 7 copies of NP, each fused to OVX313 (Osivax's proprietary pro-immunogenic tag, named Oligodom). The OVX313 sequence is derived from the C-terminal oligomerization domain of the human C4b binding protein (hC4BP) [16], but it is modified to minimize homology with the human sequence (hybrid chicken sequence; homology less than 20%). When fused by deoxyribonucleic acid (DNA) engineering to an antigen, and after protein expression, OVX313 has the unique property to heptamerize antigens, thus improving the antigen's accessibility to the immune system and increasing their humoral and cellular immune responses [15]. Because NP is less subject to antigenic variation than influenza surface antigens, OVX836 will not have to be adapted annually, as required for current seasonal influenza vaccines. Animal studies have demonstrated OVX836's ability to elicit humoral and cellular immunity—including $CD8^+$ T cells in the lungs—as well as protection in mice [15] and ferrets [17] against influenza challenges. More importantly, OVX836 administered by the intramuscular (IM) route protected mice against lethal viral challenge using 3 different influenza A subtypes isolated several decades apart (100% survival in all cases), demonstrating the cross-protective properties of the vaccine [15]. This was accompanied by approximately 1 log reduction of viral load in lungs 4 days postchallenge. Noticeably, OVX836 did not provide sterilizing immunity since the infected mice lost some weight before recovering. Recent adoptive transfer experiments demonstrated that protection is mediated by NP-specific $CD8^+$ T-cells isolated from the lung and spleen of mice vaccinated with OVX836 [18]. This study presents the first clinical safety and immunogenicity results obtained in healthy volunteers with OVX836 administered by the IM route.

METHODS

This randomized, placebo-controlled, double-blind, dose-escalation Phase 1 study was conducted at the University of Antwerp (Antwerpen, Belgium), in accordance with Good Clinical Practice. It was approved by the Ethics Committee of the Antwerp University Hospital and the University of Antwerp and by the Belgian Federal Agency for Medicines and Health Products (FAMHP). An independent data and safety monitoring board regularly reviewed the data to allow progression of the study. Written informed consent was obtained from all participating subjects. The EudraCT number was 2018-000341-39 and the Clinicaltrials.gov number was NCT03594890.

Healthy adults aged 18–49 years, with a body mass index between 18 and 25 kg/m², were eligible for the study. The main exclusion criteria were previous influenza vaccination within 6 months before screening, pregnancy or unwillingness to practice birth control, positive test for the human immunodeficiency virus or hepatitis B/C viruses, presence of an acute febrile illness on the day of vaccination, treatments that could affect the immune response such as systemic corticosteroids, cytotoxic drugs, anti-inflammatory drugs and other immunomodulatory drugs, and history of significant medical illness such as autoimmune disorders, uncontrolled diabetes or hypertension, heart, renal, or hepatic diseases.

Twelve subjects were included into each of the 3 sequential cohorts (low dose 30 µg, medium dose 90 µg, high dose 180 µg) (Supplementary Figure I). Each cohort was randomized at a 3:1 ratio between OVX836 vaccine (N = 9) and placebo (N = 3).

The vaccine (300 µg/mL active substance) or the placebo (consisting of sodium chloride 0.9%) was administered in the deltoid muscle of the nondominant arm at low (30 µg in 0.1 mL), medium (90 µg in 0.3 mL), or high (180 µg in 0.6 mL) dose. The study was divided in 2 phases: (1) an active treatment phase from day 1 to day 57, consisting of 2 IM vaccinations, each followed by 28 days of follow-up, and (2) a follow-up phase from day 58 to day 150 (month 5) after first administration.

A diary card was used to collect solicited local (administration site pain, redness, swelling, and induration) and systemic (fever [measured through oral temperature], cough, headache, arthralgia, myalgia, malaise/tiredness, and vomiting) symptoms that occurred within 7 days after each administration. Unsolicited adverse events (AEs) were recorded using open questions for 28 days after each administration. Intensities of AEs were graded as mild, moderate, severe, or potentially life-threatening, and these were monitored throughout the active phase. Serious AEs (SAEs) were monitored throughout the study up to month 5. A predefined set of safety laboratory analyses (hematology and clinical chemistry including coagulation parameters and evaluation of C-reactive protein) was performed at screening and then on days 8, 29, 36, and 57.

Whole blood samples were collected on days 1, 8, 29, 36, 57, and 150 for isolation of peripheral blood mononuclear cells (PBMCs) and determination of the NP-specific interferon-gamma (IFN-γ) T-cell response using an enzyme-linked immunospot assay (ELISPOT). Serum samples were collected on days 1, 29, 57, and 150 for the determination of anti-NP, anti-OVX313, and anti-hC4BP immunoglobulin G (IgG) using enzyme-linked immunosorbent assays (ELISAs). Immunoassays are described in the Supplementary Methods.

The study was not powered for any statistical hypothesis testing. With a sample size of 27 subjects exposed to OVX836, the probability of observing at least 1 AE would be

75% when the probability of the event is 5% (at least 1 subject of 27). Descriptive statistics were used to summarize all relevant parameters: number and percentage for discrete variables and mean (arithmetic or geometric), median, standard deviation (SD), interquartile range (IQR), 95% confidence interval (CI), minimum (min), and maximum (max) for continuous variables.

RESULTS

Study Population, Demographics, and Baseline Characteristics

The study was performed between June 12, 2018 and March 27, 2019. A total of 36 subjects were included and 33 subjects (91.7%) completed the whole study. Three subjects (1 in each vaccine group) did not receive the second administration: 1 (30-µg group) for Salmonellosis (not considered related to the vaccine), 1 (90-µg group) for alanine aminotransferase increased (not considered related to the vaccine), and 1 (180-µg group) for malaise (tiredness) and severe fever (considered possibly related to the vaccine) after the first administration (Supplementary Figure II).

The 36 subjects, all white-Caucasians, were 29.7 ± 8.6 years old on average (mean ± SD) (min-max = 18–49 years). Mean body mass index was 21.9 ± 1.8 kg/m² (min-max = 18.0–24.7 kg/m²). There were 22 females (61%) and 14 males (39%) (Table 1).

Reactogenicity and Safety

The percentages of subjects reporting solicited local signs and systemic symptoms after the 2 vaccinations are shown in Figure 1A and B, respectively. No solicited local signs were reported in the placebo subjects, whereas most subjects vaccinated with OVX836 presented transient mild to moderate pain at the injection site. There was neither clear OVX836 dose-effect relationship in the number of solicited local signs nor in the number of affected subjects: 13 symptoms in 8 subjects at 30 µg, 22 symptoms in 7 subjects at 90 µg, and 16 symptoms in 7 subjects at 180 µg. There was also no apparent increase in solicited local signs after the second vaccination compared with the first one (Supplementary Table I). None of the solicited local symptoms was severe (grade 3) in cohorts 1 (30 µg) and 3 (180 µg). Two solicited local signs (induration and edema) were severe in 1 subject (11.1%) in cohort 2 (90 µg) (Figure 1A). None of the solicited local signs, except 1 induration (lasting 14 days in the 30-µg cohort), was ongoing at the end of the observation period after either vaccination, and the vast majority of signs lasted less than 4 days.

There was neither dose-effect relationship in the number of solicited systemic symptoms nor in the number of affected subjects: 12 symptoms in 4 subjects at 30 µg, 13 symptoms in 6 subjects at 90 µg, and 15 symptoms in 6 subjects at 180 µg (Supplementary Table II). In comparison, 12 solicited systemic symptoms were reported by 5 of the 9 placebo subjects. Two severe solicited systemic symptoms were reported in 2 subjects vaccinated with OVX836 (the 2 subjects mentioned above who did not receive the second vaccination). They consisted of 1 severe malaise (tiredness, concomitant with Salmonellosis) lasting 11 days in cohort 1 (30 µg) and 1 severe fever (≥39°C, probably related to a respiratory infection with concomitant reporting of cough, headache, and malaise) lasting 2 days in cohort 3 (180 µg) (Figure 1B). All the other systemic symptoms lasted less than 4 days, except in 1 subject of the 90-µg cohort: headache and malaise lasting 8 days.

The percentages of subjects reporting unsolicited AEs during the 28-day period after each vaccination (all, severe [grade 3], causally related to the study vaccine according to the investigator) and SAEs are shown in Supplementary Figure III. Overall, 23 unsolicited AEs were reported in 8 subjects in cohort 1 (30 µg), 21 AEs in 8 subjects in cohort 2 (90 µg), and 21 AEs in 8 subjects in cohort 3 (180 µg), versus 25 AEs in 6 subjects in the pooled placebo groups. Severe unsolicited AEs were reported in 3 (33.3%), 1 (11.1%), 3 (33.3%), and 2 (22.2%) subjects in the pooled placebo, OVX836 30-µg, 90-µg, and 180-µg groups, respectively (Supplementary

Table 1. Demographics and Baseline Subject Characteristics

Characteristics		OVX836 30 µg (N = 9)	OVX836 90 µg (N = 9)	OVX836 180 µg (N = 9)	Pooled Placebo (N = 9)	All (N = 36)
Age (years)	Mean (SD)	29.89 (10.30)	29.00 (8.77)	30.22 (9.74)	29.78 (6.92)	29.72 (8.64)
	Median	25.0	28.0	27.0	29.0	27.5
	Min; Max	18.0; 47.0	20.0; 49.0	20.0; 48.0	22.0; 41.0	18.0; 49.0
Sex	Female, n (%)	6 (66.7)	6 (66.7)	3 (33.3)	7 (77.8)	22 (61.1)
	Male, n (%)	3 (33.3)	3 (33.3)	6 (66.7)	2 (22.2)	14 (38.9)
Body mass index (kg/m^2)	Mean (SD)	22.03 (1.39)	22.13 (2.00)	21.43 (1.33)	21.82 (2.36)	21.85 (1.76)
	Median	22.10	21.99	21.07	22.06	21.84
	Min; Max	19.7; 24.0	19.3; 24.7	19.9; 24.2	18.0; 24.5	18.0; 24.7
Alcohol use	Current, n (%)	7 (77.8)	8 (88.9)	7 (77.8)	9 (100.0)	31 (86.1)
	Never, n (%)	2 (22.2)	1 (11.1)	2 (22.2)	0 (0.0)	5 (13.9)
Tobacco use	Current, n (%)	1 (11.1)	0 (0.0)	1 (11.1)	1 (11.1)	3 (8.3)
	Never, n (%)	8 (88.9)	9 (100.0)	8 (88.9)	8 (88.9)	33 (91.7)

Abbreviations: Max, maximum; Min, minimum; SD, standard deviation.

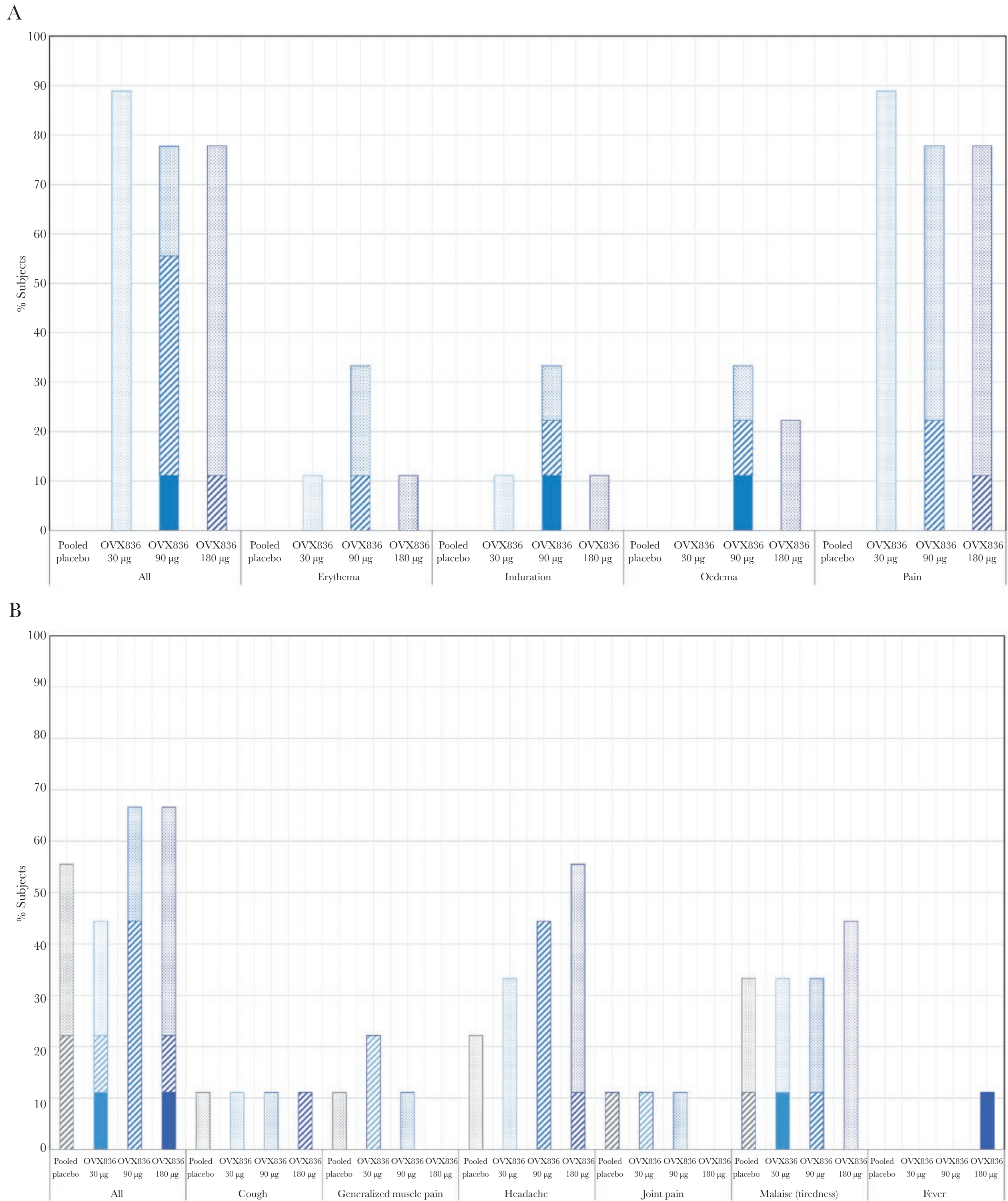

Figure 1. Reactogenicity of OVX836. (A) Percentage of subjects reporting solicited local signs (mild in dotted pattern, moderate in diagonal stripped pattern and severe in plain pattern) in pooled placebo subjects (N = 9) and in the 3 vaccine cohorts (30 µg, 90 µg, and 180 µg; N = 9 in each cohort) during 7 days after the 2 vaccinations (days 1 and 29). (B) Percentage of subjects reporting solicited systemic symptoms (mild in dotted pattern, moderate in diagonal stripped pattern, and severe in plain pattern) in pooled placebo subjects (N = 9) and in the 3 vaccine cohorts (30 µg, 90 µg, and 180 µg; N = 9 in each cohort) during 7 days after the 2 vaccinations (days 1 and 29).

Figure III). No clear OVX836 dose-effect relationship could be observed. There was also no increase in unsolicited AEs after the second versus the first vaccination (Supplementary Table III). The most frequent AEs (more than 1 occurrence in any group), reported after the 2 administrations in the placebo group and in the 3 vaccine groups, are listed in Table 2.

One SAE was reported in 1 OVX836 90-µg recipient consisting of a urinary tract infection occurring approximately

Table 2. **List of the Most Frequent (More Than 1 Occurrence in Any Group) Cumulated Unsolicited AEs Reported During 28 Days After Each of the 2 Vaccine/Placebo Administrations, in the Placebo Group and in the 3 Pooled Vaccine Groups**[a]

	OVX836 30, 90, 180 µg (N = 51)			Pooled Placebo (N = 18)		
MedDRA Preferred Term	n	%	E	n	%	E
Any unsolicited adverse event	11	64.7	23	10	66.7	21
Diarrhea	2	3.9	3	1	5.6	1
Nausea	2	3.9	2	1	5.6	1
Injection site hemorrhage[b]	2	3.9	2	1	5.6	1
Nasopharyngitis	5	9.8	5	1	5.6	1
Sinusitis	2	3.9	2	0	0.0	0
C-reactive protein increased	4	7.8	4	2	11.1	2
Headache	1	2.0	1	3	16.7	3
Presyncope	4	7.8	4	0	0.0	0
Nasal congestion	2	3.9	2	0	0.0	0
Oropharyngeal pain	4	7.8	5	0	0.0	0

Abbreviations: AE, adverse event; E, total number of events; MedDRA, Medical Dictionary for Regulatory Activities; n, number of administered doses followed by an AE; N, total number of administered doses; %, percentage of doses followed by an AE.

[a]Frequencies are shown per dose administered.

[b]Corresponding to injection site ecchymosis (MedDRA lower level term).

40 days after the second vaccination. The SAE lasted for 11 days and was considered unrelated to the vaccine by the investigator.

Nucleoprotein-Specific T-Cell Immune Response

The over-time evolution of NP-specific IFN-γ-producing T cells in the 3 OVX836 vaccine and placebo groups is shown in Figure 2A. All subjects had a pre-existing signal at baseline (mean: 89 [median, 48] NP-specific IFN-γ spot-forming cells (SFCs)/10^6 PBMCs, min-max = 5–478) with no difference between groups. After OVX836 vaccination, the kinetic of the response overall demonstrated an expansion phase, 1 week after each administration (days 8 and 36), followed by a contraction phase 3 weeks after (days 29 and 57). On day 8, the median SFCs/10^6 PBMCs values were 123 (IQR = 87–304) for the OVX836 30-µg group, 245 (IQR = 127–323) for the OVX836 90-µg group, and 308 (IQR = 78–397) for the OVX836 180-µg group versus 32 (IQR = 6–42) for the placebo (Figure 2B). There was a trend for an increase of the median NP T-cell response as a function of the OVX836 dose level at this specific

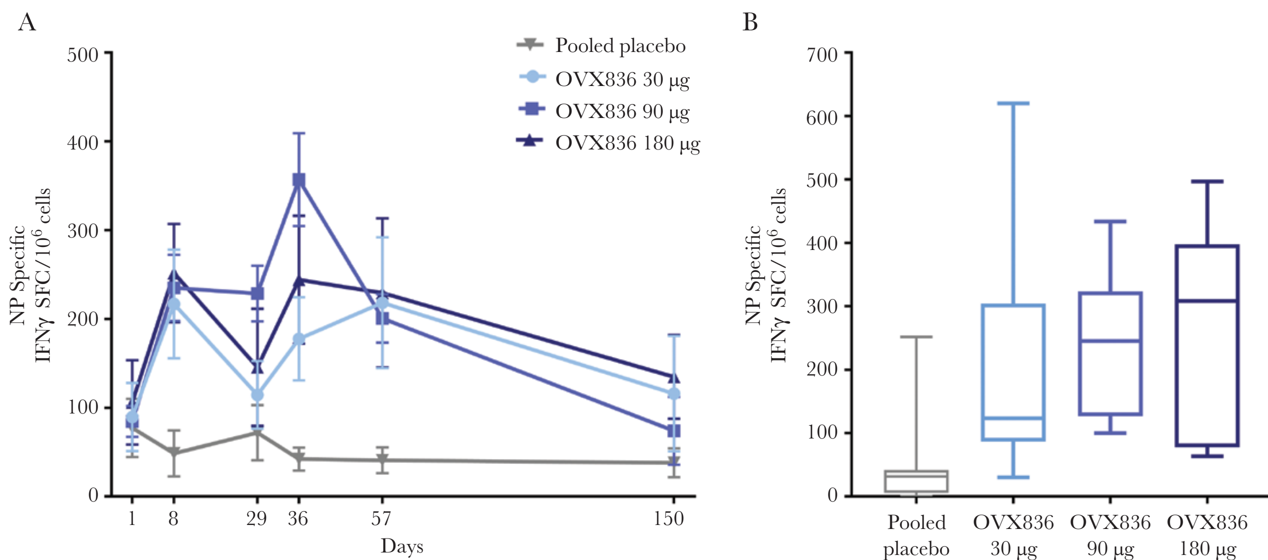

Figure 2. Cell-mediated immunity: nucleoprotein (NP)-specific interferon (IFN)-γ response. (A) Over-time evolution of the number of NP-specific IFN-γ spot forming T cells (SFC)/10^6 cells in the pooled placebo and the 3 OVX836-vaccinated groups (30 µg, 90 µg, and 180 µg) from baseline (day 1, prevaccination) to day 150 (4 months after second administration). Results are presented as arithmetic mean ± standard error of the mean. (B) Number of NP-specific IFN-γ SFC/10^6 cells in the different groups on day 8. Results are presented as box plots showing the median (horizontal bar in the box), the interquartile range (extremities of the box), and the minimum and maximum values (lower and upper error bars).

time point. The percentage of subjects with a 2-fold increase of NP-specific IFN-γ-producing T cells at day 8 versus baseline was 67%, 78%, and 56% in the OVX836 30-µg, 90-µg, and 180-µg groups, respectively, versus 0% in the placebo. The second vaccination did not allow to further increase the response on day 36 (1 week after second vaccination), except to some extent in the OVX836 90-µg group. On day 150 (4 months after second vaccination), the number of NP-specific IFN-γ-producing T cells were still slightly above the placebo in the OVX836 groups.

Nucleoprotein-Specific Humoral Immune Response

The over-time evolution of anti-NP IgG geometric mean titers (GMTs) in the 3 OVX836 vaccine and placebo groups is shown in Figure 3A. All subjects presented pre-existing anti-NP IgG at baseline (GMT = 5593; min-max = 1600–25 600), with no difference between groups. On day 29, after the first vaccination, the GMT remain unchanged in the placebo group, whereas it increased to 16 127 (95% CI = 9466–27 476) for the OVX836 30-µg group, 20 319 (95% CI = 13 940–29 615) for the OVX836 90-µg group, and 23 702 (95% CI = 13 517–41 562) for the OVX836 180-µg group. The second vaccination did not allow a further increase of the anti-NP IgG GMTs on day 57 (28 days after second vaccination), and GMTs remained high at day 150 (4 months after second vaccination). There was a trend for an increase of the anti-NP IgG GMTs as a function of the OVX836 dose level.

The percentage of subjects with a 4-fold increase in anti-NP IgG titers at the different time points postvaccination versus baseline is shown in Figure 3B. On day 29 (after first vaccination) and day 57 (28 days after second vaccination), between 44.4% and 87.5% of OVX836-vaccinated subjects presented a 4-fold increase of their baseline titer, versus 0% in the placebo group. On day 150 (4 months after second vaccination), between 37.5% and 50.0% of OVX836-vaccinated subjects still presented a 4-fold increase of their baseline titer, versus 0% in the placebo group.

Anti-OVX313 and Anti-hC4bp Humoral Immune Response

A moderate anti-OVX313 IgG response was noted in the 3 OVX836 vaccine groups from day 29 (28 days after first vaccination), reaching a maximum on day 57 (28 days after second vaccination), and decreasing at day 150 (4 months after second vaccination). At day 57, 50% to 63% in the OVX836-treated groups presented a detectable anti-OVX313 IgG titer (min-max = 12.5–1600). There was a trend for an OVX836 dose-level effect on the anti-OVX313 GMTs and on the number of subjects presenting a response (Supplementary Figure 4A). None of the anti-OVX313 IgG generated after OVX836 vaccination cross-reacted with the human C4bp oligomerization domain (Supplementary Figure 4B).

DISCUSSION

In this first-in-human, randomized, placebo-controlled trial, OVX836 candidate vaccine appears to be safe and well tolerated between 30 µg and 180 µg (IM), as a 2-dose schedule at 1-month interval. No significant dose-effect relationship was observed in terms of reactogenicity or safety up to the 180-µg dose.

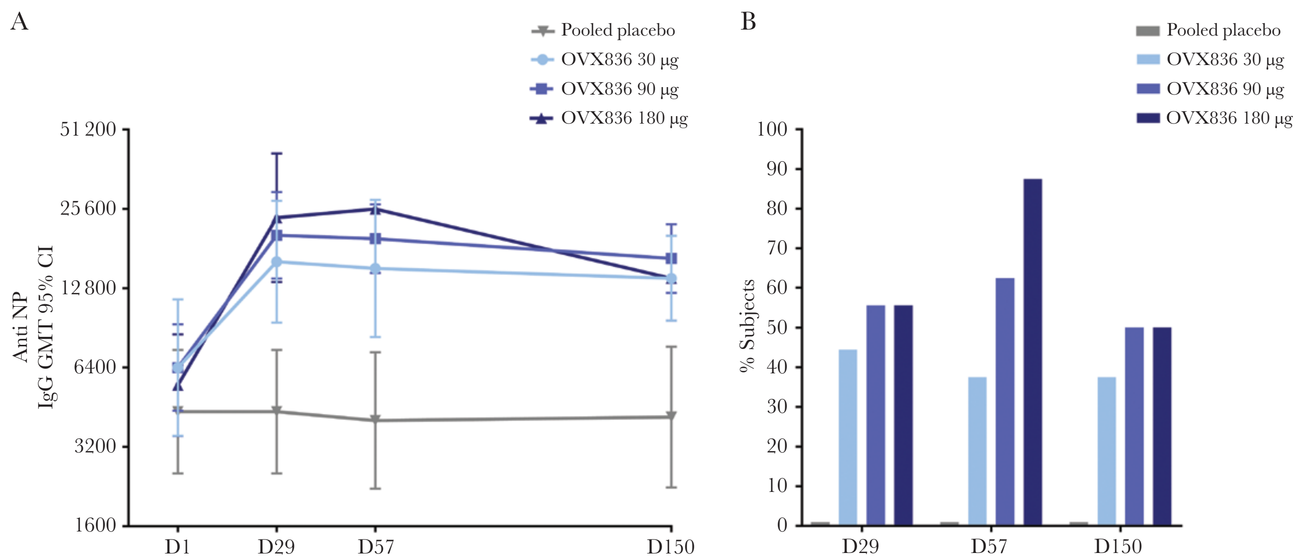

Figure 3. Humoral immunity: nucleoprotein (NP)-specific immunoglobulin G (IgG). (A) Over-time evolution of NP-specific IgG geometric mean titers (GMTs ± 95% confidence interval [CI]) from baseline (day 1 prevaccination) up to day 150 (4 months after second administration) in the pooled placebo and 3 OVX836 vaccine groups (30 µg, 90 µg, and 180 µg). (B) Percentage of subjects presenting a 4-fold increase of the NP-specific IgG titer between baseline (day 1 prevaccination) and day 29 (28 days after first administration), day 57 (28 days after second administration), and day 150 (4 months after second administration) in the pooled placebo and 3 OVX836 vaccine groups (30 µg, 90 µg, and 180 µg).

At baseline, all subjects included in the study presented pre-existing NP immunity, consistent with previous findings showing that NP-specific T cells [8, 11] and antibodies against NP [19, 20] are present in the blood of healthy individuals. This is assumed to be associated with periodical exposure to influenza virus over life course. In fact, approximately 5%–20% of unvaccinated adults are estimated to be infected by seasonal influenza annually, with rates of symptomatic influenza being approximately half of these estimates [21, 22]. High variability observed at baseline between subjects, especially for NP-specific T cells, could be related to the time elapsed between the last influenza infection and day 1 sampling in the current study. Since split seasonal vaccines contain residual NP [23], one may envisage that pre-existing NP immunity might also be related to annual vaccination. However, there is no clear evidence that seasonal vaccination can increase NP responses [24, 25].

A single injection of OVX836 was able to increase the number of NP-specific T cells at day 8 and anti-NP IgG titers at day 29. The second vaccination 28 days later did not confer additional benefit over the first one, with anti-NP IgG or NP-specific T-cell levels showing either no or only a marginal increase compared with the levels observed after first vaccination. Taken together, these findings are consistent with a recall of the NP pre-existing immune memory after OVX836 vaccination. For both the humoral and cellular NP-specific responses, there was a trend for an OVX836 dose effect, without correlations between these 2 types of responses (data not shown).

The response kinetics were different for anti-NP IgG levels (humoral response) and NP-specific effector T-cell response (IFN-γ-mediated cellular response). Whereas the IgG level remained high from day 57 up to day 150 (GMTs remaining above placebo at this late time point), T cells peaked 1 week after each vaccination and decreased afterwards while tending to remain above placebo at day 150. This NP-T cell kinetic after OVX836 vaccination is consistent with the kinetic described after influenza infection: peak of $CD4^+$ and $CD8^+$ T cells within 1 week then return to baseline 3 or 4 weeks after [10, 26], corresponding to the expansion and contraction phases described for T-cell responses [27, 28]. The disappearance of T cells from the blood compartment coincides with the conversion of some of them into functional memory cells localized in secondary organs or nonlymphoid organs, and these cells play a critical role in the fight against subsequent infections [29].

The levels of NP-specific T cells measured in this study were above the threshold of 20 SFCs/10^6 PBMCs, found by Hayward et al [11], as predictive of an efficacy against PCR-confirmed symptomatic influenza. The levels achieved 1 week after OVX836 vaccination were consistent with the threshold of 100 SFCs/10^6 PBMCs in IFN-γ ELISPOT analyses identified by Forrest et al [30] as correlating with protection against symptomatic influenza infection in young children vaccinated with a live-attenuated influenza vaccine. However, it remains difficult to compare results between studies due to ELISPOT assay technical specificities and lack of method standardization [4, 29].

OVX836 also induced an increase of anti-NP IgG levels. Because NP is included inside the virus, anti-NP IgG cannot exhibit any direct action on the virus, such as neutralization. However, anti-NP IgG may play a role in the destruction of infected cells [31] either through antibody-dependent cellular cytotoxicity [32], through immune complex natural killer cell priming [33] or other mechanisms [34]. The relevance of these mechanisms in humans remains unclear.

One vaccine targeting the full-length NP has been evaluated in clinical trials: a viral vector vaccine (Modified Vaccinia Ankara) targeting NP + M1. This vaccine candidate recently failed to reach the primary endpoints in both a field efficacy trial (ClinicalTrials.gov Identifier: NCT03880474) and in a challenge trial (ClinicalTrials.gov Identifier: NCT03883113). OVX836 is a new attempt to develop a broad-spectrum influenza vaccine based on full-length NP only and using a different technology (recombinant protein).

One limitation in this study is that the T-cell phenotype was not evaluated ($CD4^+$ and $CD8^+$ T cells) and that only IFN-γ was studied as functional marker. As already mentioned, the respective role of $CD4^+$ and $CD8^+$ in the protection against influenza remains to be clarified and should be evaluated in future studies. Another limitation of the study is the low number of subjects per group (N = 9) and the high interindividual variability in the pre-existing immunity at baseline.

CONCLUSIONS

It can be concluded from this Phase 1 study that OVX836 induced NP-specific immune responses and showed an acceptable safety and reactogenicity profile. The dose levels of 90 µg and 180 µg have been selected for further evaluation in a larger Phase 2 study (ClinicalTrials.gov Identifier: NCT04192500).

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. P. V. D. is leading the Centre for the Evaluation of Vaccination in charge of this study; K. W. was the Principal Investigator; I. D. C. is senior medical doctor in the team; and N. C. was in charge of the peripheral blood mononuclear cell processing of all samples of the study. In this context, the University of Antwerp was funded by Osivax.

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Potential conflicts of interest. A. L. V. and Florence Nicolas are shareholders and executive members of Osivax. M. C., M. L., and J. T. are employed by Osivax. S. V. and P. W. are consultants who received fees from Osivax. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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