Immunogenicity, safety and preliminary efficacy evaluation of OVX836, a nucleoprotein-based universal influenza A vaccine candidate: randomised, double-blind placebo-controlled, Phase 2a trial

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Summary

Background:

OVX836, a recombinant vaccine containing the nucleoprotein (NP) of the A/WSN/1933(H1N1) influenza virus and the oligomerization domain OVX313 has shown promising results in preclinical studies (cross-protection in mice against several influenza A strains). In previous clinical studies, OVX836 displayed an excellent safety profile, and elicited humoral and cellular immune responses when administered as an intramuscular (IM) dose of 90 µg or 180 µg.

Methods:

This Phase 2a, randomised, double-blind, placebo-controlled study was performed to evaluate the immunogenicity and safety of one single IM administration of OVX836 influenza vaccine at three dose levels (180 µg, 300 µg and 480 µg) in healthy subjects aged 18-55 years.

Findings:

OVX836 had a favourable safety profile up to 480 µg without major reactogenicity and without reaching the maximum tolerated dose. Eight days after vaccination, it increased the frequency of NP-specific interferon gamma (IFNγ) spot forming cells (SFCs) per million peripheral blood mononuclear cells (PBMCs): +124 SFC/10^6 PMBC [95%CI=67-180] p=0·002 at 180 µg; +202 SFC/10^6 PMBC [95%CI=138-267] p<0·0001 at 300 µg; +223 SFC/10^6 PMBC [95%CI=147-299] p<0·0001 at 480 µg; -1 SFC/10^6 PMBC [95% CI=-24-22] in the placebo. Dose-dependent and polyfunctional NP-specific $CD4^+$ T-cell responses were observed, and a $CD8^+$ T-cell response was elicited at 300 µg and 480 µg. A level of protection of 84% (95% CI=17%-97%) was observed in an epidemiological context of H3N2 circulation (season 2021-2022).

Interpretation:

OVX836 appears to be a safe and well-tolerated candidate vaccine, that elicits humoral and cellular NP-specific immune responses (including $CD8^+$ T-cells at the highest dose levels) and exhibited a preliminary signal of protection against influenza. Further work is needed to confirm the potential of

OVX836 as a universal vaccine that might protect against all influenza A strains, to evaluate the duration of protection and to measure the potential protective efficacy against B-strains.

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Research in context

Evidence before the study

The FluWATCH study, a prospective cohort trial, has shown that T-cell immunity to the influenza nucleoprotein (NP) was associated with protection against symptomatic influenza (Fragaszy et al., 2017, Int. J. Epidemiol, 46(2): e18). In mice experiments, administration of OVX836, a candidate influenza vaccine containing NP of the A/WSN/1933(H1N1) influenza virus, was able to prevent mortality following a lethal challenge with influenza A (Del Campo et al., 2019, npj Vaccines, 4: 4) and B strains (unpublished data). Clinical Phase 1 and Phase 2a trials have demonstrated the safety, tolerability and dose-dependent immune response of OVX836 up to 180 µg (Leroux-Roels et al., 2022, Front Immunol, 13: 852904).

Added value of this study

A dose-dependent immune response was observed with OVX836 in the 180 µg to 480 µg dose range for NP-specific interferon gamma-spot forming cells and NP-specific $CD4^+$ T-cells. In addition, $CD8^+$ T-cell responses were elicited with OVX836 at the 300 µg and 480 µg dose levels. The good tolerability and safety profile of OVX836, observed in previous studies, was confirmed up to 480 µg, without reaching the maximum tolerated dose. The present study showed a protection level of OVX836 in preventing reverse transcriptase-polymerase chain reaction-confirmed influenza A cases of 84% (95% confidence interval = 17%-97%), in an epidemiological context of H3N2 circulation (season 2021-2022). This is in line with the trend observed in a previous study for the prevention of influenza-like illness during the influenza season 2020-2021 (level of protection = 75% [-15%-95%]) (Leroux-Roels et al., 2022, Front Immunol, 13: 852904).

Implications of all the available evidence

This study paves the way for late-stage development of this candidate universal influenza vaccine that could be highly effective against both pandemic and seasonal influenza without the need for an annual update of its antigenic composition.

Introduction

A systematic review and meta-analysis of aggregated (un)published data from surveillance platforms has estimated that more than 32 million cases and 5·7 million hospitalizations occur yearly from influenza-associated disease in adults worldwide, with the highest hospitalization rates in elderly.[1]

Quadrivalent influenza vaccines eliciting antibody responses against virus surface glycoproteins (haemagglutinin [HA] and neuraminidase [NA]) are the mainstay of influenza prevention. However, there is a definite need for improved influenza vaccines since current vaccine efficacy (VE) varies from season to season and can be low. During the 2019/2020 influenza season in Europe, VE against any laboratory-confirmed influenza, in primary care and hospital settings, ranged between 29% and 61%.[2]

In addition to the effects of antibodies, cell-mediated immunity (CMI) very likely also contributes to vaccine-induced protection.[3–5] More and more studies are suggesting to analyse potential cellular correlates of protection to complement serological parameters [6,7] and measurement of vaccine-induced CMI is recommended by major regulatory agencies (EMA/CHMP/VWP/457259/2014). The increase of interferon gamma (IFNγ) secretion by peripheral blood mononuclear cells (PBMCs) stimulated *in vitro* with purified, live influenza virus, was significantly associated with protection.[8] During the 2009 H1N1 pandemic, persons with higher levels of pre-existing T-cells to conserved $CD8^+$ epitopes developed less severe illness.[9] A human challenge study has demonstrated that pre-existing $CD4^+$ T-cells responding to internal influenza proteins were associated with lower virus shedding and less severe illness.[9,10]

The influenza nucleoprotein (NP) provides structural and functional support to the viral replication machinery,[11] is expressed early-on in the virus replication cycle,[12] has a low mutation rate resulting in conserved epitopes,[13–15] and is considered as an interesting target to develop a broad-spectrum (universal) vaccine against influenza. In a prospective observational study, participants with higher levels of pre-existing T-cells to NP had a lower incidence of symptomatic reverse transcriptase-polymerase chain reaction (RT-PCR)-confirmed influenza.[16]

OVX836 vaccine candidate (Osivax, Lyon, France) is a recombinant protein containing the full-length NP of the A/WSN/1933(H1N1) influenza virus and OVX313 (oligoDOM®), OSIVAX's proprietary

self-assembling nanoparticle technology. This recombinant protein spontaneously assembles to form positively charged nanoparticles composed of 7 copies of the NP fused to OVX313.[17,18]

In mice, OVX836 elicited NP-specific humoral and cellular immune responses, including tissue-resident and long-lasting CD8+ T-cells in the lungs, and protected mice against lethal challenges with diverse influenza A subtypes.[17,19] In OVX836 vaccinated ferrets, a decrease of viral load in lungs was observed after H1N1pdm09 challenge (unpublished data; Primard C. and Nicolas F.).

A first-in-human study [20] has demonstrated that OVX836 was safe at 30 µg, 90 µg and 180 µg, administered as a two-dose schedule with one month interval. A single injection of 90 µg or 180 µg was able to significantly increase the number of circulating NP-specific IFNγ Spot Forming Cells (SFCs) at Day 8 and anti-NP immunoglobulin G (IgG) titres at Day 29. The second vaccination (28 days after the first) did not amplify the immune response. The dose of 30 µg was unable to induce a strong immune response.

The 90 µg and 180 µg dose levels were further evaluated in a randomised, reference-controlled (Influvac Tetra™ [Mylan]; quadrivalent influenza subunit vaccine), parallel group, double-blind, Phase 2a study in 300 healthy volunteers, aged 18-65 years, during the 2019/2020 influenza season.[21] OVX836 was safe and presented a reactogenicity profile similar to Influvac Tetra. The maximum tolerated dose was not reached. Both dose levels induced a significant increase in terms of total NP-specific IFNγ SFCs, NP-specific $CD4^+$ T-cells and anti-NP IgG responses. OVX836 induced strong cellular responses, i.e. SFCs measured by IFNγ ELISpot and $CD4^+$ T-cells expressing IFNγ measured by flow cytometry. The influenza-like illness (ILI) cumulative hazard as a function of time between study and ILI start dates, during the influenza season and from 14 days post-vaccination onwards, reached higher values in the OVX836 90 µg group compared to the OVX836 180 µg and Influvac Tetra groups with 8, 2 and 3 ILIs respectively in the OVX836 90 µg, OVX836 180 µg and Influvac Tetra groups. This was interpreted as a sign of potential protection by OVX836 at the dose of 180 µg.

With these results in mind, the present study aimed to evaluate the immunogenicity and safety of higher dose levels of OVX836, but also to explore the effect of OVX836 on the occurrence of ILIs and/or RT-PCR-confirmed influenza cases during the influenza season 2021/2022.

Methods

This study was reported in accordance with CONSORT guidelines.[22]

Study design

This randomised, placebo-controlled, double-blind Phase 2a study was performed in a single centre (Center for Vaccinology (CEVAC), Ghent University and University Hospital, Ghent, Belgium), in accordance with Good Clinical Practice. It was approved by the Ethics Committee of the Ghent University Hospital and by the Belgian Federal Agency for Medicines and Health Products (FAMHP). All participants gave their written informed consent.

Two higher dose levels (300 µg and 480 µg) of OVX836 were compared to the 180 µg dose level used in earlier studies (OVX836-001 [NCT03594890] and OVX836-002 [NCT04192500]). The placebo group received saline (negative control). Four equally sized groups of 33 healthy adults (18 to 55 years old) each, enrolled after open-label vaccination of 6 sentinel subjects who received the 300 µg (N=3) and 480 µg (N=3) dose levels, were randomised in a 1:1:1:1 ratio. OVX836 or placebo were administered in the deltoid muscle (non-dominant arm). A separate cohort of 100 older adults (65 years old and older) was vaccinated (same doses and randomisation ratio as younger subjects) and will be reported separately.

An epidemiological study (OVX-FLU-001) was conducted in parallel to the present study in 66 untreated healthy subjects to follow the occurrence of ILIs and/or RT-PCR-confirmed influenza cases with the aim to pool them with the placebo subjects from the present study (see Methods supplements, Appendix page 4).

Participants

A total of 137 male and female subjects (based on subject's reporting gender), aged 18-55 years were identified from the CEVAC's volunteers database and included in the study. Subjects had to be fully vaccinated with a licensed SARS-CoV-2 (COVID-19) vaccine according to the national recommendations. This allowed to minimise and homogenise within the cohort the risk for intercurrent ILI resulting from COVID-19. The main exclusion criteria were body mass index ≥35 kg/m², active smoking (more than 10 cigarettes/day), and pregnancy or unwillingness to practice effective birth control. Any known or suspected immunodeficient conditions, autoimmune disorders or chronic diseases, or presence of an acute febrile illness on the day of vaccination led to exclusion, as well as previous influenza vaccination within 6 months before screening, vaccination within three months or one month prior to the day of study vaccination for live attenuated or inactivated vaccines, respectively, and administration of a European Union-authorized COVID-19 vaccine within two weeks prior to the day of study vaccination. Subjects taking medication that could affect the immune response (systemic corticosteroids, cytotoxic drugs, anti-inflammatory drugs and immunomodulatory drugs) were also excluded.

Randomisation and masking

The randomisation list (allocation ratio 1:1:1:1; block size=4) was prepared by an unblinded statistician of the Statistical CRO Staburo (Munich, Germany) using SAS (Version 9.4). It was communicated to the unblinded staff at the Data Management CRO Clinfidence (Rosmalen, The Netherlands) for integration into the eCRF, and to the unblinded study nurse from the investigational centre in charge of the injection of vaccines.

The study was double-blind for treatment allocation, except for the 6 sentinel subjects who were vaccinated in an open-label manner before enrolling the randomised cohort. Because placebo and OVX836 had a different appearance, preparation and administration of study vaccines was performed by an unblinded nurse (independent from study staff members). The Investigator and the staff (co-Investigators, study nurses, study coordinators) involved in the observation of the subjects after vaccination were kept blinded to the treatment allocation up to the end of the study. The unblinded staff was responsible for vaccine accountability, vaccine storage, vaccine preparation and vaccine

administration, and was not involved in the observation of the subject after vaccination on Day 1, or any further evaluation of safety and immunogenicity.

Procedures

After the vaccination on Day 1, the subjects were kept under control in the investigational centre for 60 minutes, before being discharged. This was followed by a 7-day period wherein solicited local (injection site pain, redness and swelling) and systemic (fatigue, headache, arthralgia, malaise, myalgia and fever) signs and symptoms were collected using an eDiary, a 29-day period for reporting of unsolicited adverse events (AEs), and a 180-day period (end of study) for reporting serious adverse events (SAEs). The severity AEs was assessed according to the FDA document: "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials". All subjects visited the Investigator's site on Day 1, Day 8, Day 29 and Day 180 post-injection. Three sentinel subjects received either the 300 µg or the 480 µg dose and attended an additional follow-up phone call from the study centre on Day 3 (48 hours post-vaccination). The absence of any safety concern in sentinel subjects triggered the vaccination of the other subjects.

ILI (definition in the Appendix page 4) episodes were recorded throughout the duration of the study up to Day 180 (Month 6) post-vaccination. Subjects presenting with ILI were requested to monitor severity of the disease until resolution using a standardized questionnaire for patient-reported outcomes (Flu-PRO®) provided electronically (via the eDiary), and to return to the investigational centre for an additional visit. Nasopharyngeal and nasal swabs were collected for RT-PCR confirmation of influenza infection. Material for nasal self-swabbing was provided to subjects and had to be used for repeat sampling according to instructions given by the Investigator upon the follow-up contact for the ILI notification. Whenever possible, at least one nasopharyngeal swab performed by the Investigator was obtained.

Blood samples (50 mL of heparinized whole blood to isolate PBMCs and 10 mL of blood to harvest serum) for determination of cell-mediated and humoral immunity were drawn in all subjects on Day 1,

Day 8, Day 29 (serum only) and Day 180. Assays are described in the Appendix (pages 1 to 4). In females of childbearing potential, a urine sample was collected on Day 1 to perform a pregnancy test.

An electronic Case Report Form (eCRF) was used for data collection.

Outcome measures

The two primary endpoints were the cell-mediated immune response to OVX836 at the three dose levels in terms of change of NP-specific IFNγ SFC frequencies in the PBMC population measured by IFNγ ELISpot, at Day 8 versus pre-injection baseline (Day 1), and the safety evaluation of OVX836 in comparison to placebo (number and percentage of subjects reporting solicited symptoms and unsolicited [S]AEs, number and severity of ILI episodes during the whole study duration to follow the potential risk of vaccine-associated enhanced respiratory disease [VAERD]).

The secondary endpoints were the percentage of NP-specific $CD4^+$ and $CD8^+$ T-cells expressing interleukin 2 (IL2), tumour necrosis factor alpha (TNFα) and/or IFNγ (and all possible combinations between these three cytokines) as measured by flow cytometry, and the geometric mean titres (GMTs) of anti-NP IgG (ELISA in serum) at each timepoint.

The main exploratory endpoint was the level of protection induced by OVX836 in terms of decrease in number of RT-PCR-confirmed influenza cases during the influenza season and during the whole study period in the vaccinated versus placebo groups.

Other exploratory endpoints were the anti-OVX313 tag IgG levels, OVX313-specific $CD4^+$ and $CD8^+$ T-cell percentages, expressing IL2, TNFα and/or IFNγ at the different timepoints, and potential cross-reactivity with the human C4 binding protein (hC4BP) oligomerization domain.

Statistical analysis

A sample size of approximately 30 evaluable subjects in each group would have 80% power to detect a difference in mean ratios of NP T-cell activity of 1·782 (the difference between a Group 1 mean ratio [new dose level], μ1, of 4·412 and a Group 2 mean ratio [OVX836 180 μg], μ2, of 2·630) assuming that the common SD was 2·190 using a two-group t-test with a 0·025 (Bonferroni's correction for two

comparisons) two-sided significance level (nQuery Advisor, Version 7). Considering a 10% dropout rate, a total of 33 subjects was randomised in each of the four groups.

When the one-way ANOVA comparing the IFNγ SFCs Day 8/Day 1 ratios in the four treatment groups was statistically significant ($p<0{\cdot}05$), post-hoc tests would be authorized between groups considered in pairs.

All analyses were done with SAS (Version 9.4). The primary cohort for the analysis of safety and efficacy included all subjects that received the vaccine or placebo (safety cohort [SC]). The primary cohort for the analysis of immunogenicity was the per protocol (PP) cohort including all subjects that received the vaccine or placebo, and had baseline and complete post-administration blood samples up to Day 8 for immunogenicity analyses, and without significant protocol deviations.

The statistical methods are exhaustively described in the Appendix (pages 4 and 5). For the analysis of secondary and exploratory endpoints no correction was applied for multiplicity, and all p-values lower than 5% should be considered cautiously.

This trial is registered with ClinicalTrials.gov NCT05060887 and EudraCT 2021-002535-39.

Role of the funding source

The sponsor (Osivax) was responsible for study design, data management, data analysis, data interpretation and writing of the report. The corresponding author (Jacques Bruhwyler), Alexandre Le Vert, Florence Nicolas and Geert Leroux-Roels (academic) had full access to all the data and final responsibility for the decision to submit for publication. Osivax has received funding from Bpifrance (grant nr DOS0105407/00) and from the European Union's Horizon 2020 Research and Innovation Program under grant agreement Nr 961112.

Results

Vaccinations were performed between 15th November 2021 and 1st February 2022. The last subject's visit 3 (Day 29) took place on 1st March 2022. For the observation of ILI and the exploratory analysis

of the protection level, all data collected from the first subject's first visit until the end of the influenza season (15th April 2022 according to Sciensano, the Belgian Institute of Public Health) were taken into account.

A total number of 137 subjects received the study vaccine or placebo as follows: 33 received OVX836 180 µg, 35 OVX836 300 µg, 36 OVX836 480 µg and 33 placebo. The PP cohort included 130 subjects: 33 in the OVX836 180 µg group, 32 in the OVX836 300 µg group, 33 in the OVX836 480 µg group and 32 in the placebo group. The 3 sentinel subjects from the OVX836 300 µg and 480 µg groups were excluded because they had been treated in an open manner and 1 subject was excluded in the placebo group because that person's Day 8 visit was outside the authorized window. All 137 subjects completed the Day 29 visit and were followed-up at least until the end of the influenza season (analysis of the immune response at Day 180 is still ongoing) (Supplementary Figure 1).

Subjects were 34·5 ± 11·1 years old (mean ± SD; range=18-55 years). The majority of subjects were females (71%) and White-Caucasians (96%). The baseline characteristics were similar between the four treatment groups (Table 1).

In terms of NP-specific IFNγ SFCs in PBMCs as measured by ELISpot, there were no differences between groups at baseline (Day 1) and no responses on Day 8 in the placebo group (-1 SFC/10^6 PMBC [95% CI = -24-22]). OVX836 vaccination induced a dose-dependent (R^2=0·912; slope p-value=0·045) response on this marker. At Day 8, statistically significant increases were observed with the three OVX836 doses versus placebo: +124 SFC/10^6 PMBC [95% CI = 67-180] p=0·002 for OVX836 180 µg; +202 SFC/10^6 PMBC [95% CI = 138-267] p<0·0001 for OVX836 300 µg; +223 SFC/10^6 PMBC [95% CI = 147-299] p<0·0001 for OVX836 480 µg (Figure 1 and Supplementary Figure 2), while the differences between dose levels were not statistically significant (p=0·194 to 0·849).

In terms of safety (primary endpoint), the frequency of solicited local AEs (mainly mild to moderate injection site pain) in the three OVX836 groups was higher than in the placebo group, without major differences between the three dose levels. The percentage of subjects reporting systemic solicited symptoms (mainly mild to moderate fatigue, headache and myalgia), as well as unsolicited AEs, was

similar in the three OVX836 groups (Table 2). For the solicited systemic symptoms higher frequencies were seen in the three OVX836 groups as compared to placebo for myalgia, whereas no difference was seen for headache and fatigue. One subject reported a severe arthralgia in the 180 µg group (associated with an unsolicited AE of lower back pain that was considered unrelated to study vaccination). In the 480 µg group one subject reported a severe headache and another subject a severe fatigue. Three severe unsolicited AEs were reported (back pain in one subject of the 180 µg group and COVID-19 in two subjects of the 480 µg group) but none was considered related to OVX836. No SAEs related to study vaccination were reported. There was no clear dose-effect relationship and dose-limiting toxicity was not reached at 480 µg. The exhaustive list of unsolicited AEs can be found in Supplementary Table 1.

As a secondary endpoint, the effect of vaccine administration on the NP-specific $CD4^+$ and $CD8^+$ T-cell responses was analysed. There were no differences between the four groups at baseline nor any changes between Day 8 and Day 1 in the placebo group.

In the three OVX836 groups statistically significant ($p<0{\cdot}0001$) increases from the pre-vaccination values for IFNγ+IL2 and IFNγ+IL2+ $CD4^+$ T-cells (without pairwise statistically significant differences between dose levels; $p=0{\cdot}063$ to $0{\cdot}793$) were observed (Figure 2). A trend towards dose-dependency ($R^2>0{\cdot}86$; slopes p values=$0{\cdot}058$ to $0{\cdot}071$) was noted.

A trend towards an increase of the mean percentage of NP-specific IFNγ+IL2- and IFNγ+IL2+ $CD8^+$ T-cells was noted on Day 8 after administration of 300 µg or 480 µg OVX836 (Figure 3A). The delta values (i.e., value on Day 8 minus value on Day 1) of IFNγ+IL2+ $CD8^+$ T-cells were significantly higher in the OVX836 300µg ($p=0{\cdot}021$) and OVX836 480 µg ($p<0{\cdot}0001$) groups as compared to placebo. In IFNγ+IL2- and IFNγ+IL2+ $CD8^+$ T-cells, there was a positive dose-effect relationship ($R^2>0{\cdot}93$; slopes p values=$0{\cdot}010$ to $0{\cdot}032$; without pairwise statistically significant differences between dose levels) (Figure 3B). The percentage of responders, defined as subjects presenting a change (difference) between Day 1 and Day 8 superior to the 95^{th} percentile of the same change in the placebo group, was higher in the three OVX836 groups ($p=0{\cdot}013$ to $p<0{\cdot}0001$) compared to placebo for the IFNγ+IL2+ $CD8^+$ T-cells. Here also a dose-effect relationship ($R^2=0{\cdot}935$; slope p-value=$0{\cdot}033$) was observed (Figure 3C).

The expression of TNFα has also been systematically studied, without detecting any statistically significant response (Supplementary Figures 3-4).

No positive correlation was found between the NP-specific $CD4^+$ and $CD8^+$ immune responses in the placebo, OVX836 180 µg and 300 µg groups, but a positive correlation was observed in the OVX836 480 µg group ($p<0{\cdot}0001$) (Supplementary Figure 5). No correlation was found between the anti-NP IgG, and $CD4^+$ or $CD8^+$ immune responses (data not shown).

As observed in previous studies, OVX836 also induced a strong humoral immune response. In the three OVX836 groups, there was a statistically significant increase ($p<0{\cdot}0001$) in GMTs on Day 8 and Day 29 versus the pre-vaccination titre (Day 1). There was a trend for a dose-effect relationship and the difference between the OVX836 480 µg and 180 µg groups reached statistical significance ($p=0.012$) on Day 8, but not on Day 29. No changes in anti-NP IgG geometric mean titres (GMTs were observed in the placebo recipients (Supplementary Figure 6).

The three OVX836 dose levels induced a moderate increase in anti-OVX313 IgG titres in some subjects (Supplementary Figure 7). As OVX313 is derived from an avian sequence of the C4BP, results confirmed the absence of any cross-reaction with human C4BP oligomerization domain. No OVX313-specific nor hC4BP oligomerization domain-specific $CD4^+$ and $CD8^+$ T-cells responses were observed following OVX836 vaccination (Supplementary Figure 8).

As a planned exploratory endpoint, the study also evaluated the protection level of the vaccine against RT-PCR-confirmed influenza A cases. During the influenza season a total of 4 RT-PCR-confirmed influenza A cases were observed in the placebo group (N=33) whereas 2 cases were diagnosed in the OVX836 treated subjects (N=104; both in the 300 µg group) ($p=0{\cdot}030$). This resulted in an observed level of protection of 84% (95% CI=17%-97%) for OVX836 at the time of maximum exposure to influenza. These results were plotted as cumulative period prevalence between study vaccination and RT-PCR-confirmed influenza date (or end of study) and analysed using a Kaplan-Meier approach (log rank $p= 0{\cdot}014$) (Figure 4). Additional analyses of the ILIs can be found in the Supplementary Tables 2 and 3.

Additional information on the level of protection achieved with OVX836 was also obtained from a post-hoc analysis after pooling of the placebo subjects from the present study and the untreated subjects from the OVX-FLU-001 study. This pooled analysis was legitimized by the similar patient characteristics (Table 1) as well as observed incidence of RT-PCR-confirmed-influenza and COVID-19 cases (Supplementary Figure 9) between the placebo group of the current study and the participants (unvaccinated) of the epidemiological study (OVX-FLU-001). During the influenza season a total of 9 RT-PCR-confirmed influenza A cases were observed in non-OVX836 treated subjects (4 in the placebo group and 5 in the untreated OVX-FLU-001 cohort) whereas 2 cases were diagnosed in the OVX836 treated subjects (both in the 300 µg group). This resulted in an observed protective level of 79% (95% CI=5%-95%) (see Supplementary Results [Appendix page 6], Supplementary Table 4 [Appendix page 13], and Supplementary Figures 9 and 10 [Appendix pages 22-23]).

Discussion

This study evaluated OVX836, a broad-spectrum influenza vaccine candidate with a novel mechanism of action targeting the influenza NP, at 3 dose levels (180 µg, 300 µg and 480 µg) covering a larger range than tested in previous clinical trials (up to 180 µg).[20,21] The vaccine was safe and immunogenic at all dose levels. OVX836 showed a good safety profile with a low local and systemic reactogenicity. The immunogenicity results obtained at 180 µg were consistent with those obtained in previous studies while all immunological markers (anti-NP IgG, NP-specific IFNγ SFCs, NP-specific $CD4^+$ T-cells) were higher when the dose level was increased from 180 µg to 480 µg. Induction of a measurable $CD8^+$ response against a non-adjuvanted recombinant protein vaccine is not easy in humans and rarely reported in the literature. In previous studies,[20,21] at 90 µg and 180 µg, OVX836 was not able to induce such a response, which was however measured in the present study at 300 µg and 480 µg. This confirms our findings in mice, in which we observed NP-specific $CD8^+$ T-cell response after OVX836 vaccination, and more specifically tissue-resident memory $CD8^+$ T-cells in the lungs.[19]

In the present study, we report that OVX836 vaccination provided 84% [95% CI=17%-97%] level of protection against PCR-confirmed symptomatic influenza, in an epidemiological context of H3N2 circulation (season 2021-22). After pooling the present study with the epidemiological cohort of 66 untreated subjects (OVX-FLU-001, post-hoc analysis), considering all biases this merge could involve, the level of protection was 79% (95% CI = 5%-95%; 9 cases in the placebo/untreated groups versus 2 cases in the OVX836 pooled groups), thus corroborating the previous value. If the observed vaccine efficacy is confirmed in an adequate and well-controlled trial, the result would be in line with the universal influenza vaccine target product profile set by the US National Institutes of Health for a universal vaccine (>75% efficacy against symptomatic influenza infection).[23,24] These results are particularly encouraging in the context of recent attempts of universal influenza vaccine development. Indeed, up to now, the few vaccine candidates in clinical stage pursuing a T-cell mechanism of action targeting NP have been relatively disappointing with no proven or only partial VE in Phase 2b and Phase 3. Neither the M-001 vaccine candidate (recombinant protein containing nine epitopes of various influenza antigens HA, NP and M1; BiondVax Pharmaceuticals; evaluated in a Phase 3 trial - NCT03450915) nor the modified vaccinia Ankara (MVA)-NP+M1; Vaccitech; evaluated in two placebo-controlled Phase 2b trials (the FLU009 field study (NCT03880474)[25] and a challenge study (NCT03883113) reported any significant effect on the primary efficacy endpoint of each study. FLU-v (mix of 4 synthetic peptides that originate from conserved regions of M1, M2 and NP; ConserV Bio) provided partial protection following a single-dose of adjuvanted-FLU-v after challenge vs placebo (33% vs 55% developed mild to moderate influenza disease, p = 0.035); however, efficacy was not confirmed in subjects who had received two vaccinations [26], nor in another Phase 2b field study.[24] The observed protection level of OVX836 may stem from the different technologies and approaches involved: full-length NP covering all HLA groups for OVX836 instead of selected epitopes for FLU-v and M-001; and recombinant multimeric protein for OVX836 versus viral vector for MVA-NP+M1. It may result in broader NP-specific immunogenicity, with a capacity to induce different pathways of the immune system including NP specific T-cells ($CD4^+$ and $CD8^+$) together with strong anti-NP humoral responses.

In humans, there is a growing body of evidence showing the importance of NP-specific $CD8^+$ as well as $CD4^+$ T-cells in the protection against the influenza virus.[4-11] Anti-NP IgG antibodies may contribute to the elimination of infected cells, although they are not neutralizing as NP is an internal antigen of the influenza virus. In mice, it has been observed that extracellular NP (released by infected cells) exacerbated influenza pathogenesis. [27] As a consequence, NP humoral response might have the potential to protect against this pathogenic mechanism of influenza. It has recently been observed that antibodies against the SARS-CoV-2 nucleocapsid improve protection against this virus in mice. Nucleocapsid-specific antibodies supported natural killer-mediated antibody-dependent cellular cytotoxicity (ADCC) against infected cells and protected mice against SARS-CoV-2 challenge.[28] A similar mechanism may be active in influenza as demonstrated in mice. Although ADCC associated to anti-NP IgG has been described, [29,30] the relevance of this mechanism in the protection against influenza in humans has not yet been demonstrated.

A limitation of this study is the small sample size to estimate the protection level against influenza. The results of the present study warrant further evaluation of OVX836 in Phase 2b/3 clinical trials, involving large participant numbers and properly designed to demonstrate the VE against seasonal influenza A and B strains, to determine the duration of protection, to better characterise the OVX836 mechanism of action, ideally to find a potential correlate of protection, and further confirm vaccine safety.

In older adult populations where the efficacy of current seasonal vaccines is known to decrease, it may be tempting to complement the NP-specific T-cell response triggered by OVX836 with an HA-specific antibody response elicited by an inactivated influenza vaccine (IIV), to provide a superior protection. In a mouse challenge model, synergistic efficacy has been observed when combining both vaccines.[17] A first clinical trial is ongoing to study safety and immunogenicity of concomitant administration of OVX836 and IIV in healthy adults (NCT05284799).

In summary, high dose levels (up to 480 µg) of OXVX836, a universal influenza candidate vaccine, were well-tolerated and induced strong humoral and dose-dependent cellular immune responses. The observed protection level against RT-PCR-confirmed influenza warrants further investigation of this vaccine in larger trials.

Contributors

Every author contributed to the study: ILR was the Principal Investigator at CEVAC, BJ and AA were her Sub-Investigators, FDB was the Study Coordinator at CEVAC, GW and YJ were responsible for flow cytometry analyses, JT was the Sponsor's Clinical Development Director, PW, as Chief Medical Officer was in charge of overall design of the study, medical monitoring and pharmacovigilance, FN was responsible for clinical immunology, JB was the medical writer of this manuscript together with ALV and FN. JB was also supervising the biostatistics. GLR was consultant for Osivax and contributed to the interpretation of the clinical and immunological data.

All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

ALV, GLR (Academic author) and JB had direct access to the data and verified them in the manuscript.

Declaration of interests

JT, FN and ALV are employees and shareholders of OSIVAX.

PW, JB and GLR received consulting honoraria from OSIVAX.

ILR, GW, YJ, FDB, AA and BJ, all working at CEVAC Clinical Unit or Laboratory have been sponsored by OSIVAX.

Data sharing

The data sharing plan has been communicated into the Clinical Study Protocol (§ 10.8.3.1). The results will be published on the EudraCT website as well as on the ClinicalTrials.gov website within 12 months after the end of the study follow-up phase. The Clinical Study Protocol will be made available with publication.

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