Hand-in-hand work between the Sponsor and the BA lab : a 5-year journey feedback toward the successful use of an IFN-γ ELISpot method supporting the clinical development of a new vaccine candidate

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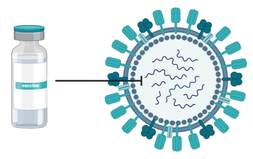
ELISpot for monitoring cellular responses to a flu vaccine

Context

OVX836 is a recombinant protein-based **universal flu vaccine -** developed by Osivax - targeting the highly conserved influenza **nucleoprotein (NP).**

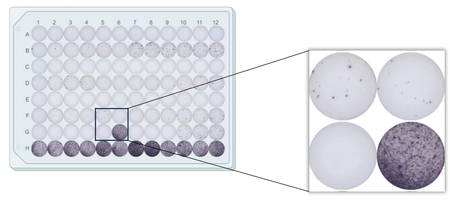
Osivax, as the Sponsor, and Active Biomarkers, as the bioanalytical lab, have worked hand-in-hand to develop a method for evaluating the OVX836 **immunogenicity**.



Approach

IFN-γ ELISpot method developed to monitor T-cell responses to OVX836.

Performance of the method adapted to the **Context of Use** of the biomarker throughout clinical development of OVX836.



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1. Establishing pre-requisites for documenting immunogenicity of OVX836 vaccine

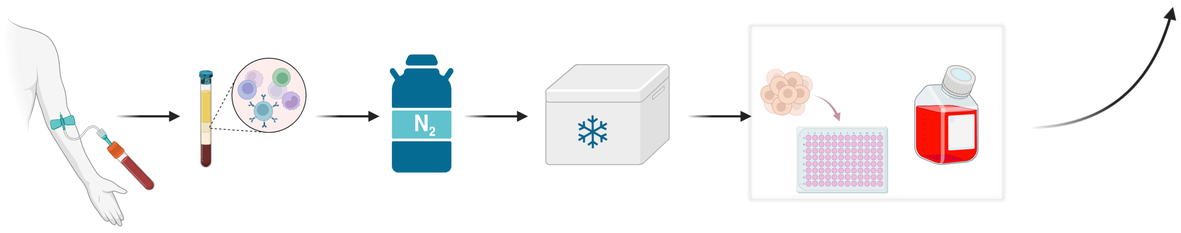
A. Ensuring sample integrity

Study needs

High-quality samples for securing sample analysis and reducing variability

Solutions

- **Standardization** of preparation, cryopreservation & shipping of clinical samples
- SOPs for thawing and ELISpot



Sample processing **within 8 hours** after venipuncture

Standardization of sample processing/cryopreservation

Guidelines for sample handling prior to/ and during shipping

Use of **serum-free medium**

Pre-analytical phase

Clinical lab

Bioanalytical lab

B. Fit-for-purpose development & validation of an IFN-γ ELISpot method

Study needs

Method development for assessing NP-specific cellular immune responses – in reference samples

Solutions

- **Optimal conditions** for: antigen, viral strain, incubation time and peptide concentration
- **4 reference samples** with low, medium, high response range

Antigen	Viral strain	Incubation time	Peptide conc.
Recombinant NP	H1N1 ✓	24h ✓	Conc A ✓
Peptide pool ✓	H2N2	48h	Conc B

✓ : retained condition for clinical runs

+ **Method precision**: intra-run CV<30% and inter-run/ inter-operator <50%,
+ **QC suitability samples** for monitoring clinical analytical runs

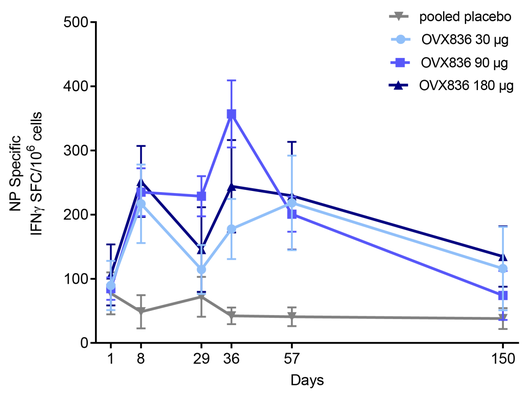
2. Evaluating cellular response in vaccinated subjects

Study needs

Exploratory assessment of cellular NP-specific immune responses in **subjects vaccinated with OVX836**

Solutions

- PBMC prepared & cryopreserved in serum-free medium according to Sponsor's SOP
- T cell responses evaluated with validated ELISpot method (see 1B)
- Control charts for monitoring suitability of ELISpot method during clinical sample analysis



Withanage K, et al., J Infect Dis. 2022
*SFC/10^6 cells: spot-forming cells per million

Phase 1

- **Result: Expansion of NP-specific T cells**, 1 week after each administration of OVX836 followed by a contraction phase 3 weeks later
- T cell responses dramatically impacted by PBMC quality ⇨ **ACTion :** set a **viability** threshold: flagging samples at 80% viability – setting 70% as the acceptance limit

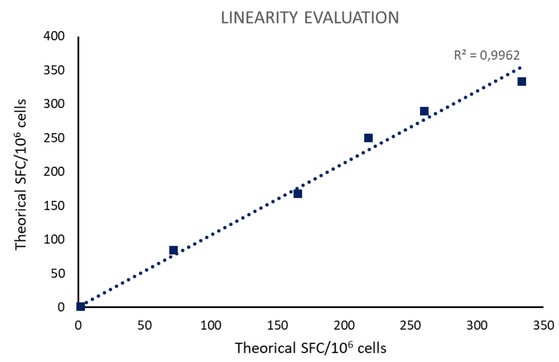
3. Method performance adjusted to change in biomarker status and long-term use in clinical development

Study needs

Immunogenicity at day 8 as a primary endpoint in phase 2a
⇨ **Upgrading** method validation

Solutions

- Fine-tuning precision, establishing method sensitivity and linearity
- Checking consistency across lots of critical reagents



- **Precision** of the method confirmed
- **Linearity** of the method (see above) demonstrated from LLOQ
- **Consistency** of the method across different lots of critical reagents

Pre-phase 2a

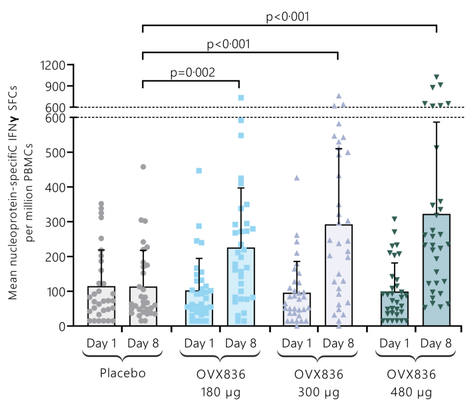
4. Exploring vaccine immunogenicity at high doses

Study needs

NP-specific immune responses at day 8 as a primary endpoint to explore **high doses of OVX836**

Solutions

- Streamlined process for sample preparation and analysis
- Flagging samples with low viability
- Monitoring suitability of validated ELISpot method (see 3)



Leroux-Roels I, Lancet Infect Dis. 2023

Increase in NP-specific T cells by OVX836 at day 8

Phase 2a

5. Robustness of the method proven across clinical studies

- ✓ Close communication with the Sponsor
- ✓ Well-trained & committed analysts
- ✓ Sample integrity (viability >70% after overnight resting)
- ✓ Strict control of the critical reagents (bridging studies)
- ✓ Standardization of the analytical workflow: from sample preparation, through cryopreservation and shipping, to sample analysis by ELISpot

The method proved to be robust throughout the clinical development of OVX836

Reach out today to start a conversation about ELISpot-based assays and other functional tests for your vaccine/drug development.