Monitoring immunogenicity of your candidate vaccine by ELISpot
How to reduce assay variability?

Alain Poyau, Claire Serraz, Audrey Pabiou, Aurélie Chene, Lucie Levast, Marc Auvergnon, Luc Boban, Florian Brajon, Marie-Ambre Monet, Romain Roth, Caroline Seinera, Julie Silva, Cécile Valverde, Cendrine Josson, Christine Bain-Wendlinger

1. KCAS Bio www.kcasbio.com | +33 (0) 4 37 70 87 00 | 60F Rockefeller Av., Bioserra II – 69008 – Lyon – France

UNDERSTANDING THE CONTEXT OF USE OF THE ASSAY

Understanding Science

- What's the indication?
- What is the expected magnitude of responses?
- What's the target of your product?
- What is the expected impact of your product on the immune system?

Organizational constraints of the clinical study

- Starting date & duration of the study
- How many clinical sites & where are they located?
- What is the injection & sampling schedule?
- Who are the other stakeholders? Is there a central laboratory, a CRO, a logistical partner....?

and what will the data be used for?

- Monitor changes from baseline?
- Compare cohorts of patients?
- Is it a primary, secondary or exploratory endpoint?
- Make decisions on route, schedule, formulation, dose of injection...?
- Regulatory submission?

HIGH QUALITY SAMPLES

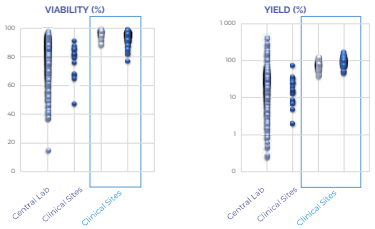
SHIFTING THE PARADIGM FROM CENTRALIZATION TO MULTI-SITE LOCAL LABORATORIES

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**



Central Lab
- Standard SOP
- Samples from 28 sites in US & EU
- Samples shipped overnight

Clinical sites (n=3)
- Non-standardized protocol
- PBMC prepared and frozen within 8h from venipuncture

PBMC network (ACTIVE)
- Laboratory audited, trained and qualified
- Standardized protocol
- PBMC prepared and frozen within 8h from venipuncture

Clinical site / Courier — **Bioanalytical lab**

METHOD PERFORMANCE

I. IFN-γ ELISPOT METHOD THAT FITS YOUR NEEDS

A. Antigen	B. Peptide conc.	C. Standardization	D. Assay format / target responding cells
Recombinant NP	Conc A ✅	Human plasma	Ex-vivo (circulating effector T cells)
Peptide pool ✅	Conc B	Serum-free medium ✅	After 5-12-days *In vitro* expansion (circulating central memory T cells

✅ : retained condition for clinical runs

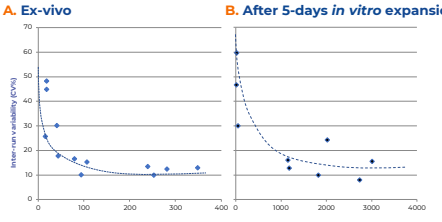
PBMC
- From 3 healthy donors (A, D)
- From 6 patients (B)

Stimulation
- HCMV pp65 peptide pool (PP) (D); Test PP1-2 (A, D) ; Individual peptides from test PP (P1-3) ; CEFT peptide pool (C) Negative control (Med)
- Recombinant target antigen (Rec Ag)

IFN-γ ELISpot
- Ex vivo (A, D)
- 5-days expansion (B, C, D)
- 10-days expansion (D)

II. VARIABILITY OF THE METHOD DEPENDS ON ELISPOT DESIGN AND LEVELS OF RESPONSES

A. Ex-vivo — **B. After 5-days *in vitro* expansion**



Samples : In-study samples with different levels of response

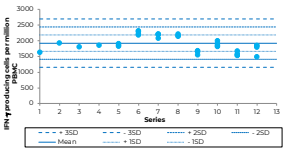
Antigen : Vaccine-derived peptide pool

ELISpot :
- Ex-vivo
- After 5-days expansion

Design :
- 3 independent runs
- At 3 different days
- By 3 operators

III. CONTROL CHART & QUALITY MONITORING

A. Controlling suitability of the method along clinical testing



Samples : Reference sample (QC)

Antigen : CEFT peptide pool

Design :
- Acceptance limits = mean +/- 3SD of first 10 analytical runs
- Over an 8-month period
- 3 operators

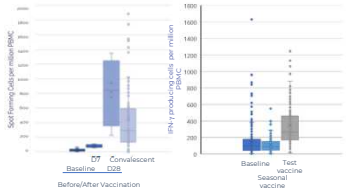
B. External Quality Assessment
- Proficiency testing with EQAPOL (Duke University)
- CIMT-CIP

C. Quality Management System
- GLP Certificate
- ISO 9001:2015

MONITORING VACCINE-SPECIFIC IMMUNE RESPONSES

I. ACUTE INFECTIONS / PROPHYLACTIC VACCINATION

A. SARS-CoV2 — **B. Influenza virus**



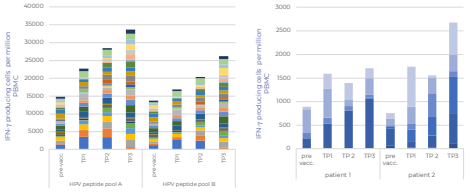
IFN-γex-vivo ELISpot
- O/N Resting
- 24h stimulation

Volunteers before/after SARS-CoV-2 or Flu vaccination

COVID-19 convalescent patients from, not vaccinated

II. CHRONIC INFECTIONS / THERAPEUTIC VACCINATION

A. HPV16 cervical cancer — **B. Chronic Hepatitis B**



IFN-γELISpot with expansion
- 5 days in vitro expansion

31 HPV+ patients before (pre-vacc) & after vaccination (TP1-3)

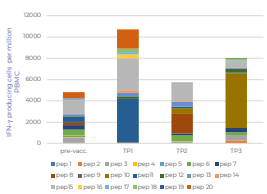
2 vaccine-derived peptide pools

IFN-γELISpot after 10-days in vitro expansion
- O/N Resting
- 10 days in vitro expansion

2 patients HBV+ before (pre-vacc.) & after (TP1-3) vaccination

5 vaccine-derived peptide pools

III. Solid tumors / personalized neoantigen-based vaccine



IFN-γELISpot with 10-days in vitro expansion
- O/N Resting
- 10 days expansion
- 20 peptide pools from neoAg

Patient with solid tumor before/after personalized neoantigen vaccine

RESPONSE CRITERIA – A NEVER ENDING DEBATE

How to define **positivity threshold** *(Moodie Z. et al, Cancer Immunol Immunother. 2010, 59: 1489)*:
- Empirical rules or statistical tests (need analysis in quadruplicate)
- **Our recommendation** = empirical rule based on both
  1. Specific / mock spots ratio > 3-4
  2. Specific minus mock spots > 50 spots per million PBMC