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Putting Science to Work

Immunogenicity Wanted: Differences between assays for biologics and vaccines

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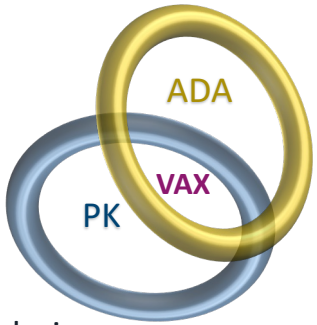
Topics for discussion

1. Immunogenicity Wanted- Why?
2. Repurposing assay formats- Biologics to Vaccines
3. Our learnings about critical differences between immunogenicity assays for Biologics and Vaccines
    - Differences in critical reagents
    - Different parameters studied
    - How data calculation and interpretation differs
    - Documents that can be used to guide assay development, validation and sample analysis



Immunogenicity: Unwanted and Wanted

Unwanted

- Biologics induced antibodies bind to therapeutic proteins
- Eliminate or reduce efficacy
- These antibodies may be life-threatening

Biologics Immunogenicity (ADA)

Wanted

- Vaccine induced antibodies generate a protective effect against the antigen
- Predict vaccine efficacy
- Derivation ICPs*

Vaccine Immunogenicity (AVA)

The final immunogenicity output is **Seroconversion:** *Defined as ≥ x-fold increase in antibody titer compared with baseline antibody titer*

* ICP= Immune correlates of protection

References: Immunogenicity of Protein-based Therapeutics, USFDA and Guidelines on clinical evaluation of vaccines: regulatory expectations, WHO

Repurposing assay formats- Biologics to Vaccines

The LBA is a widely adapted assay platform for biologics' immunogenicity

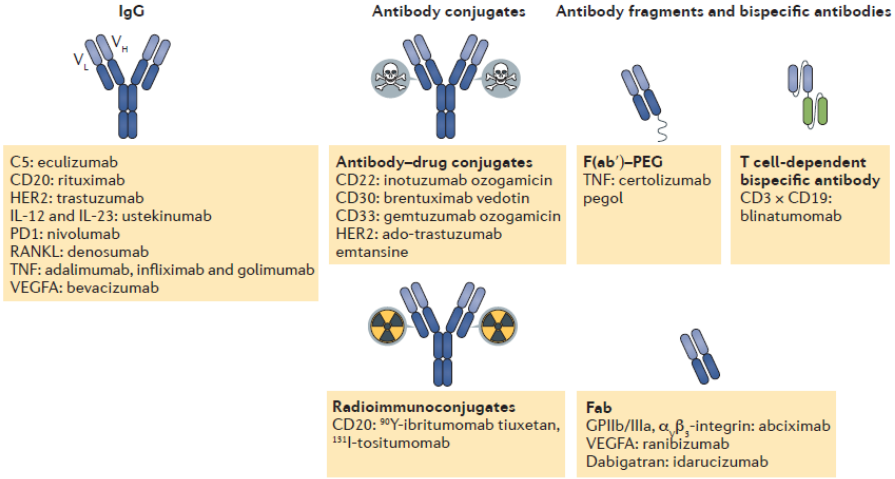
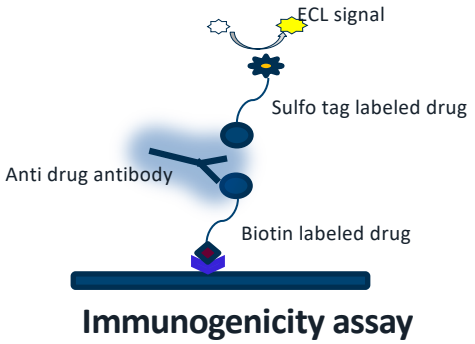


Figure 1 | **Formats of approved antibody drugs.** The majority of approved antibody drugs are immunoglobulin G (IgG) molecules, including the examples shown, which were the top-ten selling IgGs in 2016 (TABLE 1). All currently marketed drugs are shown for antibody conjugates, fragments and bispecific antibodies. Not shown is the bispecific

Paul J. Carter & **Greg A. Lazar**
Next generation antibody drugs: pursuit of the 'high-hanging fruit'-Nature Reviews Drug Discovery volume17, pages197–223 (2018)

Frequently used immunogenicity detection assay format

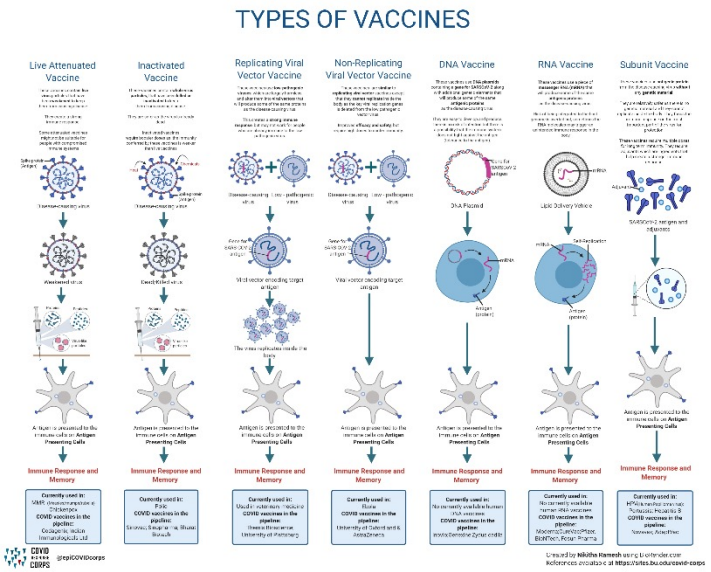
- High sensitivity
- High drug tolerance
- Master-mix advantage



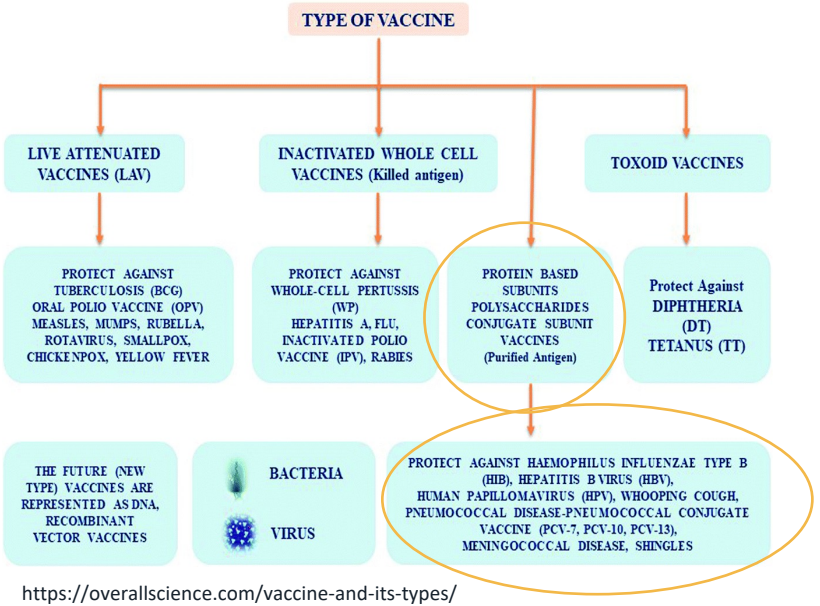
* LBA= Ligand binding assay

Repurposing assay formats- Biologics to Vaccines

We decided to deploy the LBA to study the immunogenicity of protein based vaccines



https://sites.bu.edu/covid-corps/projects/science-communication/types-of-vaccines-infographics/



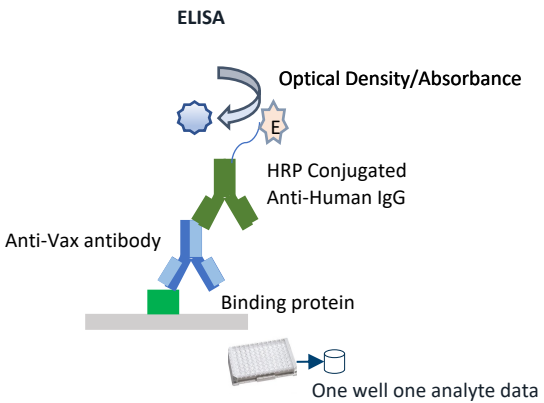
https://overallscience.com/vaccine-and-its-types/

Repurposing assay formats- Biologics to Vaccines

The LBA was deployed for vaccine immunogenicity

Case study 1: Vaccine against Malaria

- Duffy binding protein against Malaria
- Deployed ELISA for all 4 tiers of the study
  - Binding, Confirmation (binding inhibition), Titer, Characterization



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www.nature.com/npjvaccines

ARTICLE OPEN

Malaria vaccine candidate based on Duffy-binding protein elicits strain transcending functional antibodies in a Phase I trial

Kavita Singh[1], Paushali Mukherjee[1], Ahmad Rushdi Shakri[2], Ankita Singh[1], Gaurav Pandey[1,3], Meenakshi Bakshi[1], Geetanjali Uppal[1], Rajender Jena[1], Ankita Rawat[1], Purnima Kumar[2], Ruknnini Bhardwaj[2], Syed Shams Yazdani[2], Dhiraj Hans[1], Shantanu Mehta[1], Ajay Srinivasan[1], K. Anil[4], R. L. Madhusudhan[4], Jaya Patel[4], Amit Singh[4], Rajeshwar Rao[4], Santosh Gangireddy[4], Rudrappa Patil[4], Swarnendu Kaviraj[5], Sanjay Singh[5], Darrick Carter[6], Steve Reed[6], David C. Kaslow[7], Ashley Birkett[7], Virander S. Chauhan[2] and Chetan E. Chitnis[2,8]

Reticulocyte invasion by *Plasmodium vivax* requires interaction of the Duffy-binding protein (PvDBP) with host Duffy antigen receptor for chemokines (DARCs). The binding domain of PvDBP maps to a cysteine-rich region referred to as region II (PvDBPII). Blocking this interaction offers a potential path to prevent *P. vivax* blood-stage growth and *P. vivax* malaria. This forms the rationale for development of a vaccine based on PvDBPII. Here we report results of a Phase I randomized trial to evaluate the safety and immunogenicity of recombinant PvDBPII formulated with glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE). Thirty-six malaria-naive, healthy Indian male subjects aged 18–45 years were assigned into three cohorts corresponding to doses of 10, 25 and 50 µg of PvDBPII formulated with 5 µg of GLA-SE. Each cohort included nine PvDBPII/GLA-SE vaccinees and three hepatitis B control vaccine recipients. Each subject received the assigned vaccine intramuscularly on days 0, 28 and 56, and was followed up till day 180. No serious AE was reported and PvDBPII/GLA-SE was well-tolerated and safe. Analysis by ELISA showed that all three doses of PvDBPII elicited antigen-specific binding-inhibitory antibodies. The 50 µg dose elicited antibodies against PvDBPII that had the highest binding-inhibitory titres and were most persistent. Importantly, the antibody responses were strain transcending and blocked receptor binding of diverse PvDBP alleles. These results support further clinical development of PvDBPII/GLA-SE to evaluate efficacy against sporozoite or blood-stage challenge in controlled human malaria infection (CHMI) models and against natural *P. vivax* challenge in malaria endemic areas.

npj Vaccines (2018)3:48 ; doi:10.1038/s41541-018-0083-3

Study objectives

The primary objectives of the study were to assess the safety and reactogenicity of three different doses of malaria vaccine candidate, PvDBPII, formulated with GLA-SE in malaria naïve healthy male adults. The secondary objectives were to assess the immunogenicity of PvDBPII/GLA-SE by evaluating the humoral immune response against PvDBPII by ELISA, BIA and IFA assays.

Safety analysis

Data on AEs were collected throughout the study at regular intervals. The subjects were closely monitored for immediate reactogenicity on the day of each dosing. Other safety data assessed included specified solicited symptoms collected by diary card during Days 0–7; unsolicited AEs collected on Days 0–28 after each dose and serious AEs collected Days 0–180. Laboratory assessments were done on Day 7 post each dose and then at the end-of-study visit by performing predefined laboratory parameter testing as listed in the Supplementary Clinical Trial Protocol. Solicited events, unsolicited events and changes in laboratory parameters are graded as per the standard US FDA Toxicity Grading Scale for Preventive Vaccine Clinical Trials.[24] An independent data safety monitoring committee reviewed 7 days of safety data for each cohort escalation.

Determination of anti-PvDBPII IgG antibody titres by ELISA assay

Immunogenicity analyses were conducted on the PP Population. Sera collected on Days 0, 28, 56, 84 and 180 were assessed for recognition of PvDBPII by ELISA assay. The humoral immune response to PvDBPII was assessed in reference to Day 0 samples collected before the first dose. In brief, Nunc-ImmunoMaxisorp™ 96-well plates were coated overnight at 4 °C with recombinant PvDBPII (2 µg/ml) in 100 µl of carbonate-bicarbonate coating buffer per well (Carbonate-Bicarbonate Buffer capsule, Sigma). The plates were washed three times using wash buffer and blocked with Starting Block T20 solution (Thermo) for 2 h at 37 °C. Antigen-coated wells were incubated in duplicate with test and reference standard immersion using a confocal scanning laser microscope (× 100).

Expression and purification of DARC-Fc

A plasmid encoding DARC-Fc was generated by ligating the first 60 codons of human DARC (Fy8) to sequences encoding Fc region of human IgG (DARC-Fc) in a mammalian expression vector.[25,26] Recombinant DARC-Fc was produced by co-transfecting 293 T cells with plasmids encoding DARC-Fc fusion protein and human sulfotransferase. Recombinant DARC-Fc protein was purified from cell culture supernatants by affinity chromatography using Protein A. The concentration of purified DARC-Fc was determined by bicinchoninic acid.

ELISA-based binding-inhibition assay

The ability of anti-PvDBPII sera to inhibit PvDBPII-DARC interaction was measured using an ELISA-based BIA assay as described previously.[10] Recombinant DARC-Fc (1 µg/well) was coated on to a 96-well plate and the plate was blocked using blocking buffer T20 (Thermo). Recombinant PvDBPII in a range of 0.8–25 ng/ml was used to generate a PvDBPII standard curve using a four-parameter logistic curve. CT serum samples were analysed at different dilutions (1:10 to 1:5000) and each of the diluted sera was then incubated with 25 ng/ml PvDBPII test antigen for 1 h at room temperature. In addition, combinations of PvDBPII test antigen (fixed conc. of 25 ng/ml) and fixed dilution (1:10) of positive control human sera was used as positive control and naive human reference sera (1:10) was used as negative control. The reaction mixture and standard dilution of PvDBPII antigen were then added to the wells of DARC-coated plate. PvDBPII antigen bound to DARC was probed with anti-PvDBPII polyclonal rabbit sera and detected with anti-Rabbit IgG HRP-conjugated secondary antibody. The assay was developed using the chromogenic substrate TMB (3,3′,5,5′-tetramethylbenzidine) and colour development was stopped with sulfuric acid (H_2SO_4). Absorbance was measured at a wavelength of 450 nm, with background subtraction at 630 nm. The amount of bound PvDBPII was estimated by converting OD values to protein concentrations using the PvDBPII standard/calibration curve. The obtained protein

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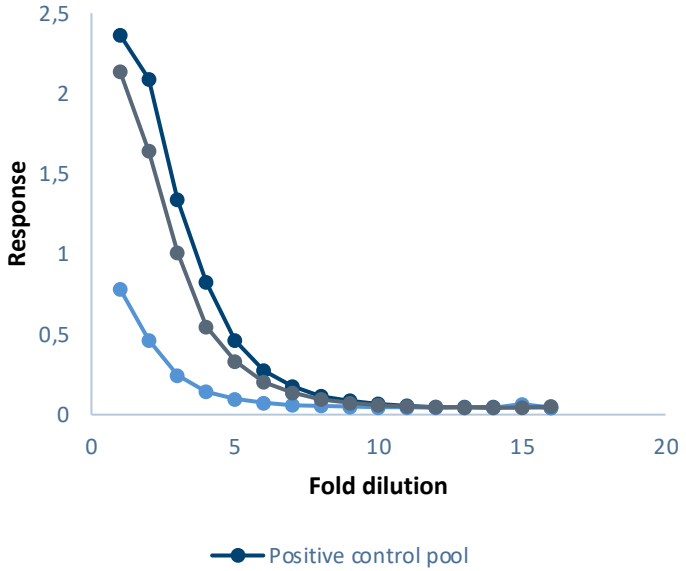
Our learnings about critical differences between immunogenicity assays for Biologics and Vaccines was full of surprises!

Vaccine immunogenicity assays are a combination of PK + ADA assays for biologics

Parameter	Biologics immunogenicity assays	Vaccine immunogenicity assays
Purpose	Qualitative	Quantitative
Assay platform	Singleplex	Singleplex and multiplex
Critical reagents	Drug/Ligand	Antigen
Reference standard	Affinity-purified positive control antibody Monoclonal or polyclonal	Polyclonal serum pool from vaccine-positive individuals (unpurified)
Assay calibrations	Not required	With International Reference Standards
Assay cut points	Based on response	Based on concentrations
Confirmatory formats	Drug	Antigen/Ligand
Titration	The magnitude of response- inverse of dilution factor	Numerical values of concentrations
Data calculation	Tabulation	Regression
Acceptance criteria	>NC, S/N, % CV	Slope, S/N, % CV
Guidelines	Immunogenicity- EMA/ USFDA	ICH Q2 (R1)

Vaccine LBAs need additional sets of critical reagents, dilution pattern and automation when compared to ADA assays

- **Primary reference standard- International Standard**
  - If available with WHO/NIBSC
- **Secondary standard**
  - Pooled sera from vaccinated individuals calibrated against international standards
- **Types of QCs**
  - 2 positive control sera (medium and low)
  - 8 point standard curve
  - 1 negative control serum
  - 1 blank (no serum)
- **Sample analysis (partially automated)**
  - Serum or plasma – X, Y, Z, … dilution series
  - Tested in singlets at 3-8 dilutions
  - Starting dilution-1:100 unvaccinated samples; minimum at least 1:999 for vaccinated samples
  - **ISR!**



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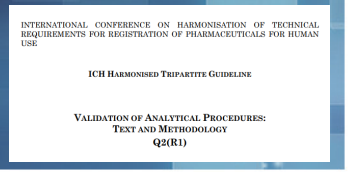
Vaccine LBAs followed method validation as per ICH Q2 R1


INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

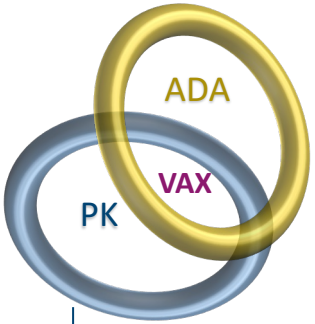
ICH HARMONISED TRIPARTITE GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1)

Parameter/Activities	Number of Batches/Assays	No. of Analysts
Calibration of reference standard	4	Minimum two analysts on two different days
Linearity and Assay range , LOQ and LOD	6	Minimum two analysts on three different days
Precision	3 (intra assay) + 6 (inter assay)	Minimum two analysts on four different days
Accuracy	6	Minimum two analysts on three different days
Robustness	9	One/two analysts on different days
Specificity	4	One/two analysts
Matrix comparability	1	One analyst in a day
Stability Room temperature, 2-8°C, Freeze-thaw, and Short term (at -20±5°C) and Long term stability (at -70±10°C)	15	One/two analysts
Method comparison between singlets and duplicates	2	One/two analysts
Edge effect	2	One/two analysts

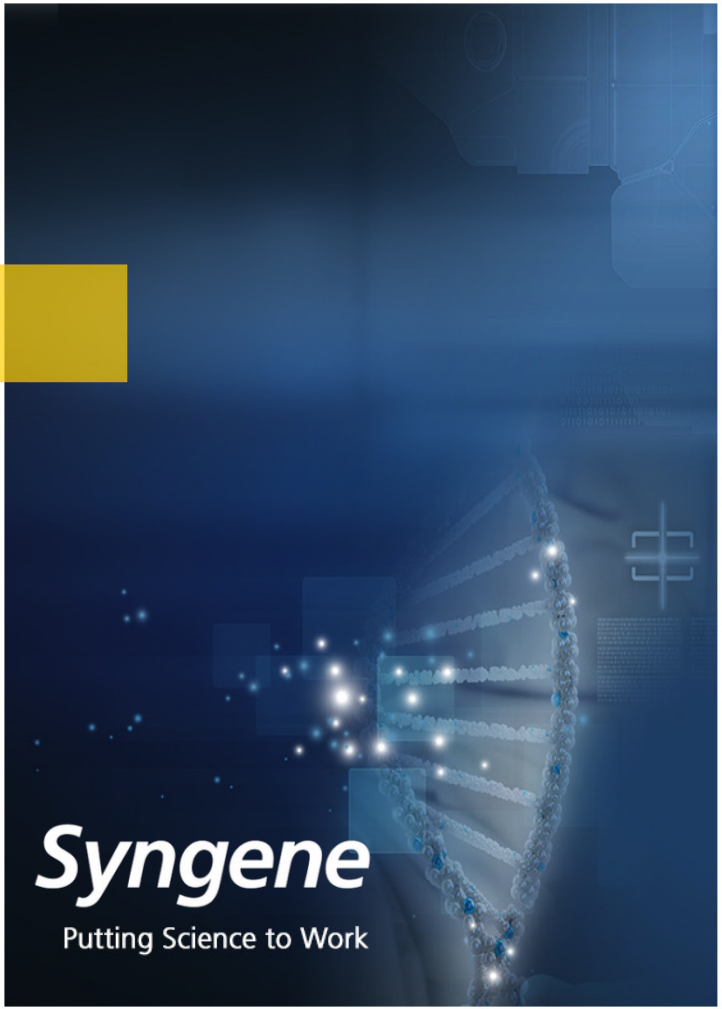
Conclusion

1. Wanted immunogenicity against Vaccines can be evaluated using re-purposed Ligand Binding Assays
2. In our experience, MSD and ELISA are optimum platforms to study anti-vax antibodies (non-functional nature)
3. Laboratories that are already running Ligand Binding Assays for Biologics can easily begin running Vaccine assays- with the same infrastructure, trained scientists, regression software
4. Additional training will be required for understanding ‘quantification of immunogenicity response in concentrations using reference standards and regression software’
5. SOPs will need to be revised to add method validation aspects as per ICH Q2 R1
6. It may indeed be a PK assay in an ADA assay!



Reference documents:

- Annex 9 Guidelines on clinical evaluation of vaccines: Regulatory Expectations Replacement of Annex 1 of WHO Technical Report Series, No. 924
- Validation of analytical procedures: Text and Methodology, Q2(R1) – ICH Harmonised Tripartite Guideline



Thank you for your patience

Questions and discussions