Octet, Your Friend in Vaccine Development: Assessing Antigenicity and Immunogenicity of HIV-1 Envelope Proteins by Bio-Layer Interferometry

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ABL Overview

- Located in Rockville, MD
- CMO/CRO that provide diverse services for biologics development, virus/protein GMP manufacturing, and clinical testing
- Approximately 200 employees in U.S. (Maryland) and Europe (Strasbourg and Lyon, France)
- Conducting contract R&D for 50+ years, GMP manufacturing for 25+ years
  - Co-discovery of HIV-I
  - Isolation and characterization of HTLV-I and HTLV-II
  - Developed the first HIV-1 serological diagnostic assay under contract with the NCI
Talk Outline

• Preclinical HIV vaccine study in NHPs
  • Antigenicity of HIV envelopes
  • Immunogenicity of HIV envelopes

• How we plan on expanding our ability to evaluate immunogenicity using Octet

• How we plan to translate what we have learned from the preclinical studies into manufacturing using Octet
HIV envelope “the primary target”

- HIV envelope is the only protein on the virus surface
- A major goal of HIV-1 vaccine research is to design vaccine regimens capable of inducing broadly neutralizing antibodies (bnAbs) that bind to the viral envelope glycoprotein (Env)

**Problem:** The env gene codes for the gp160 protein which forms a homotrimer, and is cleaved into gp120 and gp41 by the host cell protease, Furin. This is not very stable.

- One approach for generating recombinant stable trimeric immunogen is to abolish the gp120-gp41 cleavage sites of the precursor gp160 terminating the sequence at the end of the gp41 extracellular domain. This is referred to as uncleaved gp140 or gp145.
Immunogens

• The majority of new global HIV-1 infections are subtype C
• We selected 3 subtype C envelopes for this study
  • 93IN101 – Indian Clade C isolate from chronically infected patient
  • IN301 – Indian Clade C isolate from early transmitter
  • 1086 – African Clade C transmitted founder
Vaccine priming regimen

• “Jump start” the adaptive immune response by inducing T-cell memory

• Avirulent strains of newcastle disease virus (NDV) like the LaSota strain are suitable for use as viral vectors

• NDVs can be engineered to express vaccine immunogens

• NDV is ideal for use as a viral vector
  • Does not cause disease in humans
  • There is no preexisting immunity in humans
  • NDV infects via intranasal route and induces both systemic and mucosal immune responses
Study objective

The goal of this study is to generate proof-of-concept data addressing whether an NDV prime/protein boost protocol can elicit robust systemic and mucosal immune responses and how such responses are different than those induced following immunization with Env proteins alone.
Study design: rhesus macaque study

Mixture of IN101 + IN301 + 1086 gp145 in Adjuplex Adjuvant

Groups
1. NDV-NDV-Protein-Protein
2. Protein-Protein

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Expression and purification of immunogens

• Gp145 proteins were expressed in HEK-293 or CHO-S cells by PEI transfection and purified by lectin affinity chromatography

• The initial purification by lectin affinity results in a mixture of dimers and trimers

• The trimeric fractions then purified by gel filtration on a Superdex 200 SEC
Characterization of multimeric forms of HIV Env gp145s

SDS PAGE

Blue Native PAGE

MW (kDa)

250
150
100
75
50
37
25
20
15

Marker IN101gp145 IN301gp145 1086gp145

1,236
1,048
720
480
242
146
66
20

Trimer

Western Blot

Blue Native PAGE
Antigenicity of HIV Env gp145s

Antigenicity is the capacity of an antigen to bind specifically with a group of certain products that have adaptive immunity.

**Binding of CD4**
- CD4 Binding site

**Binding of bNAb**
- V1/V2/V3 region
- Glycan patch
- Membrane-proximal external region (MPER)
Antigenicity assay setup

- Binding of CD4 and bNAbs:
  - BLI was performed on Octet RED 96
  - CD4-HIS was immobilized to Anti-HIS biosensors (5 µg/ml)
  - Antibodies were immobilized to Protein G biosensors (5 µg/ml)
  - Ligands and analytes were diluted in 1 X PBS

- Binding of immobilized antibodies was evaluated against IN101 gp145 envelope
  - Two fold serial dilution of analyte starting at 800 nM
  - Association and Dissociation: 3 min
  - Binding kinetics were determined by performing 1:1 global curve fit analysis
HIV envelopes bind to soluble human CD4

\[ K_D = 4.7 \text{ nM} \]

\[ K_D = 2.5 \text{ nM} \]

\[ K_D = 0.9 \text{ nM} \]
Antigenicity of immunogens

CD4-BS

VRC01
B12
2G12
PGT121
PGT145
PG16
4E10

IN101

IN301

1086

IN101 gp145

IN301 gp145

1086 gp145

KD= 1.9 nM
KD= 7.9 nM
KD= 4.8 nM
KD= 1.9 nM
KD= 75.9 nM
KD= ND
KD= 6.3 nM
KD= 0.5 nM
KD= ND
KD= ND
KD= 2.2 nM
KD= 2.8 nM
KD= 6.7 nM
KD= 6.7 nM

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Immuneogenicity testing in NHPs

• Characterization of the humoral response
  • Antibody titer
  • Neutralizing antibody titer
  • Serum antibody affinity

• Cell-mediated responses
  • TFh phenotyping
  • PBMC CD4 and CD8 ICS
  • BAL CD4 and CD8 ICS
  • Rectal CD4 and CD8 ICS
  • Vaginal CD4 and CD8 ICS
Antibody response in immunized macaques following protein boosts

Anti-IN101gp145 titers

Weeks post immunization

Group 1 (NDV + Protein) vs. Group 2 (Protein)
Neutralizing antibody titer in NHP sera

Neutralizing antibody responses were determined against a panel of viruses pseudotyped with Tier 1 clade C envelopes, Tier 2 clade C viruses, and heterologous clade A and B tier 2 HIV strains.
Neutralizing antibody titer in guinea pig sera

These data indicate these envelopes are capable of eliciting Tier 2 neutralizing antibodies and that there is room to grow in the NHPs.
Serum antibody affinity

Measuring antibody affinity for polyclonal sera posses some challenges:

• The affinity of monoclonal antibodies can be measured accurately because they are homogeneous and selective for a single epitope.

• Polyclonal antibodies are heterogeneous and will contain a mixture of antibodies of different affinities recognizing several epitopes — therefore only an average affinity can be determined.

• To determine antibody affinity you need to know the specific antibody concentration.

• Antigen specific antibody concentration was determined by ELISA using a purified standard.
Serum antibody affinity assay

- IN101 gp145 was biotinylated and immobilized on SAX sensors
  - Loaded slowly (10ug/ml) for 3 minutes. 0.2nM shift
- Sensors were blocked and baselined with 1XPBS + 4% normal NHP serum
- Samples were 2 fold serially diluted starting at 1:20 in 1XPBS + 4% normal NHP serum
- Association and dissociation: 3 min

Adapted from ForteBio
Kinetic analysis of post-HIV Env vaccinated NHP sera

Prebleeds

Group 1 (NDV + Protein)

Group 2 (Protein)

$K_D = 40.1$ pM

$K_D = 10.9$ pM

$K_D = 2.5$ pM

$K_D = ND$

$K_D = 6.1$ pM

$K_D = ND$

$K_D = 10.3$ pM

$K_D = 30.4$ pM

$K_D = 60.9$ pM

$K_D = 56.3$ pM

$K_D = 18.3$ pM

$K_D = ND$
Does NDV-priming increase vaccine induced antibody affinity?

Maybe

$K_D$ with $P = 0.09$

KD is approximating near significance. More boosting could drive affinity further.
Conclusions

• Stable trimeric HIV gp145 envelopes from Indian and African isolates can be expressed in HEK 293 and CHO-S cells and can be purified to homogeneity by lectin affinity and SEC

• These trimers display conformational integrity by their high affinity binding to soluble CD4 and HIV bNAbs. They displayed high affinity binding to CD4 binding site antibodies (i.e. B12 and VRC01) and glycan patch antibodies (2G12 and PGT121). Improvements in envelope design and or purification methods could enhance binding to quaternary epitope specific antibodies.

• NDV prime boost regimen elicits a potent antibody response in NHP. We observed neutralization against Tier 1 and Tier 2 isolates.

• Priming appears to have a modest enhancing effect on serum antibody affinity but more boosting maybe required.
Future assays in development for analyzing immunogenicity

• Binding of serum antibodies to HIV Env V1 and V2 loops
• Determining isotype of antigen specific antibody responses
• Examination of antibody function by measuring binding of antibodies to Fcy receptors
Antibodies bind to Fcy receptors to mediate effector functions

Binding to FcgRIIA = ADCP
Binding to FcgRIIIA = ADCC
ADCP assay group 1 and 2 post protein boost

Pre-immune

2 week post-boost

4 week post-boost
Future assays for Octet use on the manufacturing side

• Octet work performed in preclinical vaccine studies will serve as a foundation for using Octet in the manufacturing space

Upstream applications

• Using a standardized antigenic panel of bNabs we can:
  • Assess the antigenicity of HIV envelopes from different isolates, different stages of infection and different designs (gp120, gp145, SOSIP, and NFL)
  • Optimize cell line development to improve output of envelopes with certain characteristics
  • Evaluate feeding strategies to enhance posttranslational modifications i.e. glycosylation profile

Downstream applications

• The antigenic panel can be used to screen antibodies for use in immunoaffinity affinity chromatography (IAC) purification of HIV envelopes
  • Test IAC binding and elution conditions
  • Assess antigenicity of same HIV envelopes purified by different means
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