A Mass Spectrometry based “ELISA” Assay for Adenovirus Vaccine Potency Testing

Virus capsid session

Jonathan Knibbe | CASSS MS | 22 September 2017
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Our HIV Vaccine candidate:

Johnson & Johnson Announces Encouraging First-in-Human Clinical Data for Investigational HIV Preventive Vaccine

Jul 24, 2017

In Phase 1/2a APPROACH study, HIV-1 antibody response observed in all healthy volunteers

Mosaic-based vaccine regimen is designed to elicit an immune response against a wide variety of HIV subtypes prevalent worldwide

Positive clinical and preclinical results inform selection of lead mosaic HIV vaccine regimen for further evaluation in Phase 2b proof-of-concept study

Paris, France, 24 July 2017 — Johnson & Johnson today announced encouraging first-in-human clinical data for an investigational HIV-1 vaccine regimen in development at its Janssen Pharmaceutical Companies. In an oral presentation of the early stage Phase 1/2a APPROACH study at the 9th IAS Conference on HIV Science (IAS 2017), the “mosaic”-based vaccine regimen from Janssen Vaccines & Prevention B.V. (Janssen) appeared to be well-tolerated and elicited HIV-1 antibody responses in 100% of healthy volunteers (n=393).

“Finding a preventive vaccine has proven to be one of the biggest scientific challenges in the 35-year quest to end the HIV pandemic. A successful preventive vaccine for HIV will need to provide broad protection against a wide range of viral strains,” said Professor Dan Barouch, Harvard Medical School, Director of the Center for Virology and Vaccine Research at Beth Israel Deaconess Medical Center and a key collaborator for APPROACH. “These promising, early-stage results suggest that these vaccines utilizing mosaic immunogens should be evaluated further for their potential ability to achieve this historic goal.”

Our HIV Vaccine candidate: principle

Mode of action = transgene protein production
Our HIV Vaccine candidate:

Tetravalent adenovirus 26 (AdVac®) vector product

90% identical sequence
81% identical sequence

Our HIV Vaccine candidate: potency testing

Lot release requires potency test

Potency ... “the specific ability or capacity of the product, as indicated by appropriate laboratory tests ... to effect a given result” (21 CFR 600.3(s))

‘given result’ = transgene protein production

Transgene expression-ELISA

+ Suitable for Quality Control (QC) laboratory
+ Sensitive
+ Selective

Test sample → Standard sample → multiple dilutions → cell model → specific antibody

Detection

Dose response curves

dilution vs. transgene

High sequence homology = no specific antibody

USP<1032> Design and Development of Biological Assays.
Can we use Mass Spectrometry to quantify the transgene proteins?

Can we combine a bioassay with a MS detector?
TGE-MS: concept and challenges

Challenge#1: find proteotypic peptides

Challenge#2: QC-proof Sample prep

Challenge#3: QC-proof MS detection

Challenge#4: Test design

1. 'ELISA' infection
2. Tryptic digestion
3. Selected Reaction Monitoring (SRM)
4. Potency determination

Test sample
Reference sample
Cell model

Infectious Diseases and Vaccines
Challenge #1 find proteotypic peptides

In-silico digest

Proteome-Wide Screen

Peptide selection study

# of candidates

HIV1  36
HIV2  34
HIV3  71
HIV4  71

# of candidates

HIV1  7
HIV2  10
HIV3  4
HIV4  5

# of candidates

HIV1  1
HIV2  1
HIV3  1
HIV4  1
Challenge #2: QC-proof sample prep

1. Disruption and solubilization
2. Protein content determination
3. Reduction & alkylation
4. Enzymatic proteolysis
5. MS-compatible peptide recovery

Lot release test = Quality Control laboratory
→ Simple, robust

Dose response curve with replicates
→ High-throughput
Challenge #2: QC-proof sample prep

1. Disruption and solubilization
2. Protein content determination
3. Reduction & alkylation
4. Enzymatic proteolysis
5. MS-compatible peptide recovery

In-solution digestion in 96-well plate format

Optimized seeding, infection & lysis

96 samples/run
Simple, robust
Challenge#3: QC-proof MS detection

Requirements

→ **Selective and sensitive peptide quantification in complex matrix**

Lot release test = Quality Control laboratory

→ **Simple, robust, compatible software**

Dose response curve with replicates

→ **High-throughput**

Triple quadrupole MS ‘must-have’ instrument
Challenge#3: QC-proof MS detection

A LC-MS/MS method was developed:
<15min/run
Validated

HIV1  HIV2  HIV3  HIV4

Linearity (r²)
≥ 0.9991
Precision (total CV)
1.6 - 6.6%
Accuracy (overall bias)
0.9 - 6.2%

Chromatograms of HIV peptides spiked at LLOQ level
TGE-MS: concept and challenges

1. 'ELISA' infection
2. Tryptic digestion
3. Selected Reaction Monitoring (SRM)
4. Potency determination

96 well plate tryptic digestion

Challenge#4: test design

Single proteotypic peptides selected

Validated LC-MS/MS method

Test sample

Reference sample

Cell model

log(dose)
Challenge #4: Test design

When using a linear model estimation:
‘Use at least three and preferably four adjacent concentrations; require that the slope of the linear segment is sufficiently steep; and require that the lines fit to Standard and Test samples are straight and that the lines are parallel’ (USP <1032>)

ELISA
four-parameter logistic curve

MS
Simple linear model (y=α+βx)
Challenge #4: Test design

-When using a linear model estimation:
- ≥4 concentrations (dose)
- Steep straight lines
- Parallel lines

ELISA
four-parameter logistic curve

MS
Simple linear model (y=α+βx)
Challenge #4: Test design

-6 dilution levels
-6 points / dilution
-3 runs

-Parallel lines?
-Do we find 80% potency?
Challenge #4: Test design results

Parallelism test
- F-test: pass (F>0.05)
- ChiSquare: pass (>0.95)

All transgene proteins, all runs individually & combined

Potency target=80%

Found:
- HIV1: 76.4% ±7.1%
- HIV2: 78.6% ±1.0%
- HIV3: 81.2 ±3.4%
- HIV4: 81.4% ±5.3%
Can we use Mass Spectrometry to quantify the transgene proteins?

...Yes we can

Can we combine a bioassay with a MS detector?

...Yes we can
Should we combine a bioassay with a MS detector?

**Mass Spectrometry**
- Cheap reagents (SILS)
- Easy to multiplex
  (measure multiple targets/sample)
- Sensitivity

**TGE-ELISA**
- Sensitive
- Selective

• Need for antibodies
The People

- Janssen Vaccines Leiden
  - Arjen Scholten
  - Jonathan Knibbe
  - Annemiek Verwilligen
  - Hans Kanbier

- Janssen Biologics Leiden
  - Crina Balog
  - Lars Meijer

- Janssen Pharmaceuticals Beerse
  - Tom Verhaeghe
  - Lieve Dillen
  - Mark Verhemeldonck
Bioassay – Mass Spectrometry future?

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