mRNA platform for the development of an HIV vaccine: Curb your enthusiasm

Sheila Balinda
Background

Most candidate HIV-1 vaccines have been designed using a combination of protein subunit, viral vectors or DNA vaccine delivery platforms.

History of HIV vaccine efficacy

Vaccine Modality | No. of Trials
--- | ---
DNA/Pox Virus | 8
DNA/Ad Virus | 5
DNA/Pox Protein | 3
Viral vector - Pox | 3
Viral vector - Ad | 11
Protein | 8
Protein/Pox | 3

Adenovirus (Ad) vectors: Based on adenovirus, highly immunogenic. Prime-boost: Ad-specific immunity; major hurdle e.g., Ad5, Ad26, Ad35

Subunit proteins: Recombinant HIV proteins e.g., gd120, trimode gp140, gp111, Gag

Pox-virus vectors: Highly attenuated, replication-incompetent, extensive safety record; efficient expression vectors with large capacity for a broad DNA e.g., MVA, NYVAC, ALVAC

DNA Vaccines: Non-replicating plasmid DNA encoding HIV proteins. Employed as a prima in heterologous prime-boost vaccination modalities e.g., JD40205 encoding RV1-Gag, Pol, Env

Clinical presentation of HIV/AIDS

1981
1996
2003
2007
2009
2017
2020
2020, 2021
2023
2024

Ad5 gag/pol/nef HIV
Ad5VAX
ALVAC/HIV
ALVAC/HIV + Ad5
ALVAC/HIV + subfasc C
 gp120/MFG S 2700
VRC01 (IV)
Ad26M06 HIV gp140
Ad26M06 HIV gp140
DNA, CN54 gp140, ADSVAX B/E
MVA, MPLA
PV1PEVacc

Courtesy: Kundai, LDP workshop, 2023
Covid-19 mRNA vaccines

<table>
<thead>
<tr>
<th>COVID-19 mRNA vaccine</th>
<th>Target</th>
<th>mRNA dose (µg)</th>
<th>Ref.</th>
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<tr>
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<td>RBD</td>
<td>–</td>
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<tr>
<td>BNT162c2</td>
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<tr>
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<td>S-2P</td>
<td>15–135</td>
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<tr>
<td>EXG-5003</td>
<td>RBD</td>
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</tbody>
</table>

*in vitro* expression of mRNA molecules in mouse skeletal muscle cells for sensitization purpose was conducted in 1990

mRNA vaccines have been extensively studied in protection against viral infections caused by *influenza virus H7N9*, *Zika virus*, *Ebola virus*, *dengue virus*, *respiratory syncytial virus*, *cytomegalovirus*, *rabies virus*, *flaviviruses*
Current mRNA HIV-1 vaccines

- tHIVConsrvX
- mRNA-1644 (eOD-GT8 60mer mRNA)
- BG505 MD39.3 mRNA
- BG505 MD39.3 gp151 mRNA
- BG505 MD39.3 gp151 mRNA
- BG505 MD39.3 gp151 CD4KO mRNA

These are just a few examples but I think you can add more
RECAP: mRNA vaccine structure

Modified nucleosides such as pseudouridine (Ψ) and 5-methylcytosine are commonly used in non-amplifying mRNA to reduce innate immune response stimulation and proinflammatory responses.
RECAP: mRNA formulation methods

Advantages

HIV env trimer synthesis using protein subunit and viral vectors is relatively complex and time-consuming while pre-existing immunity against viral vectors and poor immunogenicity of DNA are key limitations of these platforms.

mRNA enables the rapid synthesis of safe vaccines using a cell/virus culture-free process.

mRNA vaccines can induce both B and T-cells; nucleoside-modified LNP mRNA can activate Tfh cells.

The greatest advantage is that the mRNA platform allows rapid iteration of candidate vaccines. The ease and speed of production as well as the robust immunogenicity provided by mRNA make it an ideal platform for the in vitro and in vivo evaluation of HIV env trimers.
Disadvantages

- allergy, renal failure, heart failure, and infarction remain a risk, the vaccine mRNA may also be degraded quickly after administration or cause cytokine storms.

- HIV-1 Diversity

- At IC80>0.05ug/ml, most of the T/F sequences were resistant.
- A1 T/Fs became more sensitive to the bnAbs as the concentration increased (1 to 50ug/ml)
- A1 T/F sequences from the GHWP are more sensitive to N6, VRC07_523_Ls, N49P7, VRC01, 3BNC117, and ePGDM1400_V9 than CAP256_VRC26_25, ePGT121_V1, BG18 (IC80>50ug/ml).

You could also talk about the cold chain requirement for mRNA vaccines which could limit their accessibility in resource-limited settings.

Most TF virus variants were more CCR5 tropic followed by dual tropism (X4R5), CXCR6 and CXCR4 (Figure 2A) respectively
All the TF virus variants identified as R5 tropic, X4 tropic and dual tropic were resistant to both Maraviroc and AMD3100
Overall, Inhibition is higher in historical (7 of 10 mAbs) than in contemporary strains.

- 10 mAbs; 226 Viruses (80 Contemporary and 146 Historical, 8 HIV Clades) screened

- Overall, PGT151 and VRC01.23 are the most potent inhibitors across historical and contemporary

- Clade differences are evident across A1 and C

- Statistically meaningful Clade-based Comparisons only possible for clades A1 and C

- Critical relevance gaps (clades D, A/D and A) which circulate in this region are underrepresented and not statistically analysable
Establishment of an mRNA vaccine platform at MUL to support the preclinical evaluation of HIV-1 immunogens
HIV env trimer development under ADVANCE

Screening and selection of HIV env sequences
UKZN/MUL

HIV env trimer production and characterisation
ATRP

Immunogenicity Assessment
MUL and collaborating partner

mRNA synthesis and characterisation
MUL

- HIV env sequencing
- Pseudovirus production
- Assessment of of sensitivity to inferred germline, intermediate and mature bNAbs

- KI mice vaccination and sample collection
- HIV-1 env binding antibodies
- HIV-1 neutralising antibodies
- B-cell receptor sequencing
- B-cell phenotypic analysis

- Trimer design
- Timer expression
- Timer purification-chromatography
- Timer characterisation
  - Native PAGE and Western blot
  - Size distribution-DLS
  - Trimer stability-DSC
  - Ab binding-BLI
  - Conformation-NSEM
  - Timer design
  - Timer expression
  - Timer purification-chromatography
  - Trimer characterisation
    - Native PAGE and Western blot
    - Elisa
    - Flow cytometry
mRNA synthesis laboratories set up
mRNA vaccine development pathway

A. Synthesis of mRNA HIV vaccine

1. DNA sequence of HIV env antigen
2. Clone
3. Linearise
4. IVT reaction, purification
5. mRNA encoding HIV env
6. Add a m^G cap, purify

B. HIV-env trimer characterisation

1. Western blot
2. Elisa
3. NSEM
4. SPR
5. Purification
6. Cell culture
7. Transfect
8. m^G

C. Assessment of immune responses

1. Lipid nano particles
2. m^G
3. formulation
4. Immunisation
5. immune responses
BG505 DS-SOSIP mRNA & saRNA characterisation

BG505 DS-SOSIP mRNA (2.4Kb)

BG505 DS-SOSIP saRNA (9.7Kb)

HIV env trimer sequence design

1. 1 kb DNA loading ladder
2. BG505 DS-SOSIP_VRC VEEV
3. BG505 DS-SOSIP_VRC pCDNA3.1+

BLI octet showing binding of BG505 DS-SOSIP saRNA HIV env to PGT145 bNAb
<table>
<thead>
<tr>
<th>Sub-Activity</th>
<th>Description</th>
<th>Status</th>
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<tbody>
<tr>
<td>1</td>
<td>Training of a laboratory technologist in saRNA/mRNA production, development of SOPs and set up of equipment</td>
<td>Completed</td>
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<tr>
<td>2</td>
<td>Cloning BG505 DS SOSIP into mRNA and saRNA pDNA vector</td>
<td>Completed</td>
</tr>
<tr>
<td>3</td>
<td>Synthesis of mRNA &amp; saRNA encoding BG505 DS-SOSIP</td>
<td>Completed</td>
</tr>
<tr>
<td>4</td>
<td>Assessing in vitro antigen expression of saRNA and mRNA encoding BG505 DS-SOSIP HIV env immunogen</td>
<td>Ongoing</td>
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<tr>
<td>5</td>
<td>Synthesize mRNA encoding HIV env trimers selected from the HIV trimer workstream and characterize expressed proteins</td>
<td>Pending</td>
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</tbody>
</table>
Challenges, mitigation & next steps

- Limited capacity of characterisation of HIV-env trimers
  - HIV env trimer purification
  - Antibody binding assays such as SPR
  - Negative stain electron microscopy
- Lack of LNP formulation equipment
- Challenges with obtaining suitable animal models
  - Humanised mice required to test germline targeting vaccine approach
  - High background in local BALB/c mice
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And many other generous individuals and partners around the world

As of July 2023
Overall, Inhibition is higher in historical (7 of 10 mAbs) than in contemporary strains.

PGT 151, VRC01.23 and other VRC mAbs are the most potent.

*<0.05; ** , 0.001; *** < 0.0001; ns >0.05
MUL Summary: Virus Surveillance progress

• 10 mAbs; 226 Viruses (80 Contemporary and 146 Historical, 8 HIV Clades) screened

• 2 Historical Envs to be repeated

• Neutralising TCID50 and TCID80 for PGT151 and VRC01.23 against the Historical and contemporary panel is next

• Overall, PGT151 and VRC01.23 are the most potent inhibitors across historical and contemporary

• Clade differences are evident across A1 and C

• Statistically meaningful Clade-based Comparisons only possible for clades A1 and C

• Critical relevance gaps (clades D, A/D and A) which circulate in this region are underrepresented and not statistically analysable; thus, the sensitivity of the local region virus epidemic is not addressed