Messenger RNA in Lipid Nanoparticles
For Induction of HIV Protective Antibodies

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HYPOTHESIS

Induction of high-titered, durable, polyclonal serum and mucosal bnAb responses to multiple Env epitopes will be protect against HIV transmission.
Goal for the Duke CHAVD 2022-2026

• Proof of concept that a bnAb vaccine can be made.

Criteria:
1) Induce at least one type of bnAb to achieve ≥ 50% serum breadth against R5 tier 2 transmitted/founder viruses with antibodies that bind to a bnAb epitope in monkeys or humans.
2) Induce durable serum and mucosal bnAb levels for a minimum ≥ 1 year in monkeys or humans.
Challenges of eliciting HIV broadly neutralizing antibodies (bnAbs)

• **Glycan (candy coated) shield:**
  HIV Envelope (Env) glycoprotein is the target for neutralizing antibodies. Env glycoprotein is covered in sugars, shielding the Env glycoprotein from immune system.

• **Immune tolerance:**
  Broadly neutralizing antibodies (bnAbs) can be disfavored by immune system thinking bnAbs should not be made.

• **Improbable mutations:**
  BnAbs have high levels of mutations (or changes), and many of these critical changes that turns antibodies into bnAbs rarely occur.

• **Easily made Env antibodies are not bnAbs and divert the immune system:**
  Non-neutralizing spots on the HIV envelope can induce antibodies that out-compete rare bnAb B cell precursors
HIV Env trimer with broadly neutralizing targets showing the need for a polyclonal multi-B lineage response to the CD4 binding site bnAb epitope and to at least two other epitopes such as the V1V2 glycan, the V3 glycan, fusion domain or the membrane proximal external region sites.
Strategy for induction of broadly neutralizing antibodies.

<table>
<thead>
<tr>
<th>HIV Envelope epitope</th>
<th>Antibody classes</th>
</tr>
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<tbody>
<tr>
<td>CD4 binding site</td>
<td>VRC01, ANC131/CH235</td>
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<tr>
<td>V3-glycan</td>
<td>PGT121, DH270</td>
</tr>
<tr>
<td>V1/V2-glycan</td>
<td>PG9, CH01</td>
</tr>
<tr>
<td>gp41 MPER</td>
<td>2F5, 10E8/DH511</td>
</tr>
<tr>
<td>Fusion domain</td>
<td>VRC34</td>
</tr>
<tr>
<td>FDG</td>
<td>DH851, DH1003, 2G12</td>
</tr>
</tbody>
</table>

Haynes, Burton, Mascola, Sci Transl Med 2019;11:eaa2686
Haynes, Kelsoe, Harrison, Kepler Nature Biotech. 30: 423-33, 2012
Nucleoside-modified mRNA in ionizable lipid nanoparticles (mRNA-LNP)

Two COVID-19 mRNA vaccines approved by the FDA

• **Safe:**
  No viral components other than the immunogen itself encoded as mRNA;
  No risk of genome integration;
  Neutrally charged under neutral pH.

• **High immunogenicity:**
  Low doses of mRNAs have been showed to induce protective immune responses to Zika, Influenza and SARS-CoV-2 viruses;

• **Ease of production:**
  Rapid and cost-effective large-scale manufacturing
The ability of the mRNA to be a good vaccine depends on the lipid nanoparticle to release the mRNA in the inside (cytoplasm) of the cell.

mRNA released from lipid nanoparticles is translated in the cytoplasm, meaning the protein that the mRNA encodes is produced and is either secreted or put into the membrane of the cell and then is recognized by the immune system as a vaccine.
Advantages of the mRNA/LNP platform

• Durable Ab responses (Pardi N. et al. Nature. 543: 248, 2017; Saunders K et al. NPJ Vaccines)

• Induce high levels of helper T cells for antibody production (Pardi N et al. J.Exp Med 215: 1571, 2018)

• Practical for multiple component vaccines; Cost-effective

• Short production time compared to proteins and live and inactivated viruses: therefore we can test many vaccine candidates faster.

• But the key issue for HIV vaccine development remains is immunogen design.
The Titers to Prevent HIV infection are High

The serum ID50 titers needed to achieve 95% protection for

- CD4 binding site were 529 (95% CI: 274, 1020),
- first and second variable (V1V2) region 547 (95% CI: 108, 2765), and for
- gp41 membrane proximal external region 15 (95% CI: 5, 42).

The Titers to Prevent HIV infection are High: the Antibody Mediated Protection Protection Trial

Studied infusion of the CD4 binding site broadly neutralizing antibody, VRC01, 10 IV administrations over 72 months at 10 mg/kg and 30 mg/kg.

Overall: no protection, but for susceptible isolates, it was protective.

The serum ID50 titers needed to achieve 80% protection from transmission of susceptible isolates was durable titer of $\sim215$.

Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination

Norbert Pardi1*, Michael J. Hogan1*, Rebecca S. Pelc2, Hiromi Muramatsu1, Hanne Andersen3, Christina R. DeMaso2, Kimberly A. Dowd3, Laura L. Sutherland3, Richard M. Searce3, Robert Parks4, Wendeline Wagner3, Alex Granados3, Jack Greenhouse3, Michelle Walker3, Elinor Willis3, Jae-Sung Yu3, Charles E. McGee4, Gregory D. Sempowski4, Barbara L. Mui6, Ying K. Tam8, Yan-Jang Huang4, Dana Vanlandingham4, Veronica M. Holmes1, Harikrishnan Balachandran8, Sujata Sahu8, Michelle Lifton8, Stephen Higgs2, Scott E. Hensley2, Thomas D. Madden6, Michael J. Hope6, Katalin Kariko6, Sampa Santra8, Barney S. Graham10, Mark G. Lewis3, Theodore C. Pierson3, Barton F. Haynes4 & Drew Weissman9

SIGNIFICANCES OF THIS MANUSCRIPT

• This was the first manuscript published that used the nucleoside-modified mRNA-lipid nanoparticle platform.

• The manuscript convinced BioNTech and many academic labs to adopt this platform for clinical development.

• The first 2 COVID-19 vaccines approved used this platform. They have also been identified by the FDA as the first line vaccines for use.
How durable will mRNA induced HIV Env antibodies be?

Lipid nanoparticle encapsulated nucleoside-modified mRNA vaccines elicit polyfunctional HIV-1 antibodies comparable to proteins in nonhuman primates

Kevin D. Saunders1,2,3,4,5, Norbert Pardi6, Robert Parks1,2, Sampa Santra2,7, Zekun Mu8, Laura Sutherland9,10, Richard Sievers11, 
Maggie Bun12, Amanda Eaton12, Giovanna Hernandez13, Derrick Goodman14, Michael J. Hogan15, Ihsun Tomaszczuk, David N. Gordon16, 
R. Wes Reumertree17, Yunfei Wang18, Mark G. Lewis19, Theodore C. Pierson9, Chris Barbosa20, Ying Tam21, Gary R. Marquis21, 
Mahanta Rao22, Zoltan Beck23,24, Xiaoying Shen25, Guido Ferrante26, Georgia D. Tomaras27, David C. Montefiori28, 
Drew Weissman29 and Barton F. Haynes30,31,32

www.nature.com/npjvacines
Issues for encoding a complex trimer immunogen with mRNA/LNPs

• BnAb immunogens must be well folded to express bnAb epitopes and not express NNAb epitopes.

• Must define stabilization strategies of well-folded Envs that result in the majority of mRNA-encoded Env molecules being well folded.
mRNA-encoded HIV-1 Env trimer ferritin nanoparticles induce monoclonal antibodies that neutralize heterologous HIV-1 isolates in mice

Graphical abstract

- mRNA-LNP encoding multiple complex forms of HIV vaccines can be made by mRNAs
  - Trimers
  - Envelope-nanoparticles
  - Cell surface Env
MPER
Degree of Proximal MPER BnAb Lineage Maturation in HVTN 133

- Unmutated Ancestor
  - No lipid binding
  - Binds DKW epitope
  - Non-neutralizing

- Early Intermediate
  - No lipid binding
  - Binds DKW epitope
  - No Neut in TZM-bl assay
  - Neutralization Tier 2 viruses in TZM-bl assay

- Late Intermediate
  - Some lipid binding
  - Binds DKW epitope
  - Neutralization Tier 1 viruses in TZM-bl assay
  - Neutralization Tier 2 viruses in TZM-bl-Fcγ1 assay

- BnAb
  - Lipid binding
  - Binds DKW epitope
  - Neutralization Tier 2 viruses in TZM-bl assay
  - Neutralization Tier 2 viruses in TZM-bl-Fcγ1 assay
Status of MPER Immunogens

1. MPER peptide-liposome immunogen induced proximal gp41 MPER bnAb precursors in humans in HVTN 133.

2. Prime and boost MPER bnAbs are in preclinical development as mRNAs.
CD4 Binding Site
Vaccine-Induced BnAb Precursors in Rhesus Macaques: a key step to proof of concept that bnAb vaccine can be made

Victoria Stalls, Katarzyna Janowska, Priyamvada Acharya
Status of CD4 Binding Site Immunogens

1. Nanoparticle protein GMP mfr. CHAVD Qtr. 4, 2022.

2. Boost already produced GMP; Already being used as a prime in the HVTN 300 trials—has induced CD4 bs autologous NAbs in humans.
Status of CD4 Binding Site VH1-46 as mRNAs

Prime/boosts mRNA gp160s.
To be compared with proteins.
Boosts being developed in the CHAVD by Rory Henderson, Kevin Saunders, James Counts, Kevin Wiehe
Timelines for mRNA-LNP Pipeline

• Preclinical down-selection: 10 weeks

• GMP manufacturing: 5 months

• Dedicated team of regulatory, process development, analytics and manufacture for each product.

• Two teams: total of 12 FTEs for CHAVD products.

• Overlapping product development, such that 2-3 mRNAs per year tested in HVTN experimental medicine Phase 1 trials.
Proof of Concept that a BnAb Vaccine Can be Made-2022

- Germline targeting when precursors common (CD4 BS, MPER)
- Germline targeting when precursors are rare (V2 or V3-glycan)
- Design of boosts that can select for rare mutations
- Design of boosts that can “finish” or “polish” a bnAb lineage
- Durable bnAb responses
- Prime multiple bnAb types at the same time
- Combined “T and B” cell vaccine

No success
Success in bnAb KI mice or NHPs
Success in Humans
Suites designed with flexible single-use systems and ability to operate independently
Will people care about a HIV vaccine? Are their countermeasures to this issue or not?

Answers:

1. Yes, people will care about a vaccine if the preclinical and clinical data on efficacy are compelling and the safety data are convincing.

2. People will care about the vaccine if it is affordable, available, the immunization regimen is practical and the vaccine’s protective effect is durable for at least a year.
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