Discovery medicine – the HVTN's iterative approach to developing an HIV-1 broadly neutralizing vaccine

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Purpose of review
In the past two decades, there has been an explosion in the discovery of HIV-1 broadly neutralizing antibodies (bnAbs) and associated vaccine strategies to induce them. This abundance of approaches necessitates a system that accurately and expeditiously identifies the most promising regimens. We herein briefly review the background science of bnAbs, provide a description of the first round of phase 1 discovery medicine studies, and suggest an approach to integrate these into a comprehensive HIV-1-neutralizing vaccine.

Recent findings
With recent preclinical success including induction of early stage bnAbs in mouse knockin models and rhesus macaques, successful priming of VRC01-class bnAbs with eOD-GT8 in a recent study in humans, and proof-of-concept that intravenous infusion of VRC01 prevents sexual transmission of virus in humans, the stage is set for a broad and comprehensive bnAb vaccine program. Leveraging significant advances in protein nanoparticle science, mRNA technology, adjuvant development, and B-cell and antibody analyses, the HVTN has reconfigured its HIV-1 vaccine strategy by developing the Discovery Medicine Program to test promising vaccine candidates targeting six key epitopes.

Summary
The HVTN Discovery Medicine program is testing multiple HIV-1-neutralizing vaccine candidates.

Keywords
broadly neutralizing antibodies, discovery medicine, HIV-1 vaccines

INTRODUCTION

Despite over 20 years of intensive effort, developing an HIV-1 vaccine has proven to be one of the greatest challenges in vaccinology. Only one vaccine regimen has shown modest efficacy [1], and the efficacy has so far not been reproducible elsewhere [2]. Catalyzed by advancements in immunogen design, sequencing, and structural analyses, the field's attention has shifted to development of a multifocal broadly neutralizing antibody (bnAb) vaccine, and there is a growing pipeline of candidate products. The HIV Vaccine Trials Network (HVTN) has developed the Discovery Medicine Program to evaluate bnAb-inducing vaccine candidates efficiently and systematically, emphasizing streamlined processes for rapid vaccine design iteration.

Approximately 30% of persons living with chronic HIV-1 infection develop bnAbs, often with unique features such as long heavy chain complementary determining regions (HCDR3 s), high levels of somatic hypermutation (SHM), insertions and deletions, and rare and improbable mutations [3–18]. Six known epitope regions on the HIV-1 envelope (Env) are susceptible to bnAb development, some with very high breadth and potency: $CD4^{+}$-binding site (CD4bs), V2 apex, V3 glycan, gp120-gp41 interface, fusion peptide, and the membrane proximal external region (MPER) [1,3,19,20]. However, their naive B cell precursors can be extremely rare. Fortunately, the recent landmark eOD-GT8 study showed that, with the right immunogen, rare germline precursors [21,22] can be

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KEY POINTS

- Promising anti-HIV-1 bnAb vaccine candidates have emerged using germline approaches, mRNA and nanoparticle technologies.
- The HVTN is focusing on developing two to three candidate vaccines targeting at least three different HIV-1 epitopes.
- The sequential vaccine strategy is being employed through use of priming immunogens and multiple maturation inducing boosts, to develop mature potent and broad epitope specific bnAbs.
- Both multivalent nanoparticle and mRNA approaches are being pursued in creating a multiepitope anti-HIV-1 vaccine.

activated to induce first-step VRC01-class bnAbs in nearly all trial participants [23▪▪]. In addition, the Antibody Mediated Prevention (AMP) trials showed that passive infusion of a potent antibody like VRC01 is protective against HIV-1 strains that are neutralization-sensitive to VRC01 [24▪▪]. These results, together with the extraordinary success of the COVID-19 mRNA vaccines and improvements in immunogen design, have led to renewed enthusiasm for the development of HIV-1 neutralizing vaccines.

THE SCIENCE BEHIND BNAB VACCINES

Induction of bnAbs

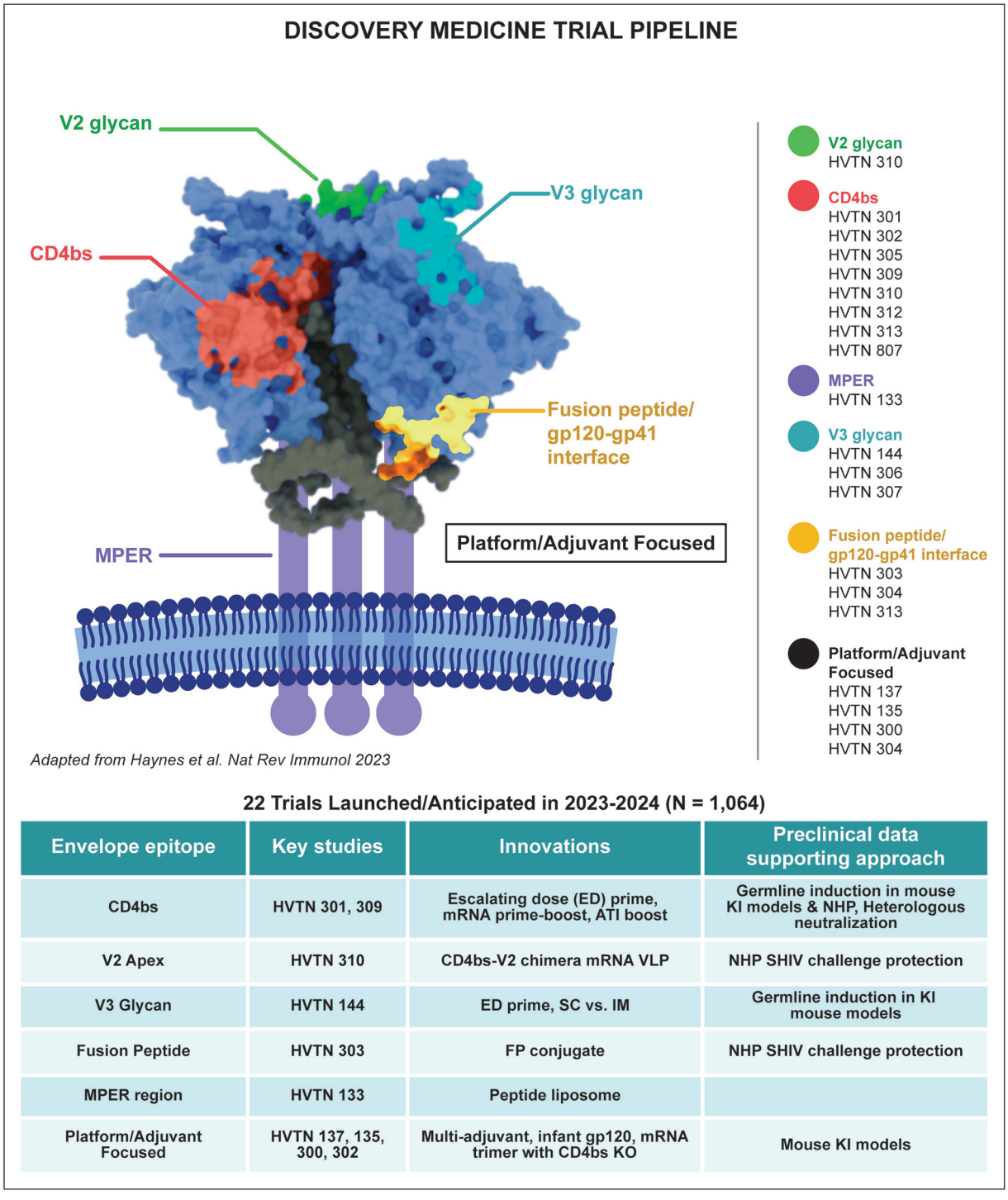
Upon antigen exposure, a diverse pool of naive B cells expressing unmutated B-cell receptors (BCRs) become activated, migrate to lymph nodes, and form productive germinal centers composed of groups of B cells with distinct V(D)J rearrangements [25]. Precursor frequency and affinity with the immunogen determine the competitive fitness of activated B cells in germinal centers [26] and ultimately drive quality and quantity of memory B cell (MBC) and plasma cell outputs [25,27,28]. The goal of a germline or lineage-targeting vaccine is to activate a pool of naive, precursor B cells which, with continued maturation, can ultimately yield bnAbs of high neutralization potency and breadth. This process is mimicked in sequential vaccine design processes known as 'priming', 'shepherding' (or 'shaping'), and 'polishing' [19,29]. These precursor B cells do not typically bind wild-type HIV-1 Env, nor do they neutralize the wild-type virus; therefore, the priming step must activate an unknown naive B cell whereafter boosting agents must bind unknown intermediate stage BCRs. In this way, subsequent heterologous boosting agents pull BCR development down a maturation pathway gradient, encouraging expression of rare key mutations that confer increasing affinity to the HIV trimer region that results in a fully developed bnAb. The B cell lineage for bnAbs, even those directed at the same region, are not uniform – for example, some people have multiple bnAbs directed at the same epitope such as the CD4bs. Whether there is an in vivo difference in the neutralizing efficiency of such bnAbs is unclear and will require further study, but the presence of multiple lineages provides optimism that the ability to prime and shepherd B cells into undergoing the uncommon mutations required of a bnAb can be achieved.

Immunogen design and sequential vaccination strategy

Three major approaches for bnAb germline initiation are being tested in the HVTN Discovery Medicine Program (Fig. 1). The first, 'structure-based immunogen design', uses a retrograde approach to immunogen development [8,11,30,31]. The priming antigen corresponding to selected bnAb germlines is computationally designed based on iterative affinity improvements with a pool of bnAb inferred germline (iGL) precursor antibodies. Successive Env immunogens are tested for binding to ultimately select a priming immunogen that can activate as many iGLs as possible [21,32–35,36▪▪].

The second approach, 'mutation guided immunogen design', uses an anterograde strategy for epitope-targeting prime and boost development [37,38]. HIV-1 envelopes thought to activate undifferentiated common ancestor (UCA) B cells are modified for adequate UCA activation, while simultaneously having higher affinity for the next intermediate stage B cell in the bnAb developmental pathway. The UCA/Env pairs are selected from individuals with chronic HIV-1 infection that develop potent and broad bnAbs. This affinity gradient-driven approach seeks to select rare but essential mutations necessary for maturation. Both programs use epitope-specific bnAbs with high potency and high breadth to model immunogen design. Preclinical testing includes knockin mouse models expressing human bnAb variable heavy and variable light chains [39▪], followed often by testing in nonhuman primates (rhesus macaques).

The third strategy, a 'germline/lineage agnostic immunofocusing approach', uses conserved Env peptides from the fusion peptide or MPER regions to induce broad, multigermline responses. Multiple distinct classes of fusion peptide bnAbs have been



22 Trials Launched/Anticipated in 2023-2024 (N = 1,064)

Envelope epitope	Key studies	Innovations	Preclinical data supporting approach
CD4bs	HVTN 301, 309	Escalating dose (ED) prime, mRNA prime-boost, ATI boost	Germline induction in mouse KI models & NHP, Heterologous neutralization
V2 Apex	HVTN 310	CD4bs-V2 chimera mRNA VLP	NHP SHIV challenge protection
V3 Glycan	HVTN 144	ED prime, SC vs. IM	Germline induction in KI mouse models
Fusion Peptide	HVTN 303	FP conjugate	NHP SHIV challenge protection
MPER region	HVTN 133	Peptide liposome	
Platform/Adjuvant Focused	HVTN 137, 135, 300, 302	Multi-adjuvant, infant gp120, mRNA trimer with CD4bs KO	Mouse KI models

FIGURE 1. HVTN Discovery Medicine Program. There are 22 trials in the HVTN pipeline targeting five epitopes using several platforms and adjuvants (top). Key innovations include the use of chimeric trimers, escalating dose priming, and comparisons between protein, protein nanoparticle, and mRNA immunogen platforms. Immunogen design is supported by rigorous preclinical data including use of KI mouse and NHP models (bottom). Adapted in part from [1].

induced by fusion peptide immunogens in animal models [40,41]. The MPER peptide has both proximal and distal epitope targets, and an immunogen targeting the proximal region has induced Tier 2 antiviral responses in HVTN 133 (NCT03934541) [42].

A final interesting approach in advanced development is to couple immunogens designed with one of the above strategies to the Fc portion of dendritic cell targeting antibodies to better amplify the germinal center reaction for specific antigens [43,44].

CLINICAL TRIAL DESIGN

Discovery Medicine clinical trials are uniquely shaped by the overarching program goals. In contrast to more traditional clinical trials, they focus on gaining knowledge to facilitate iterative advancement in vaccine development. Trial sample size usually falls in the range of 10–20 participants in each study arm, with an emphasis on rapid assessment of safety and immunogenicity [45]. While placebos are sometimes included [46], they are often not necessary as the focus is on deep individual-level analysis to determine vaccine effects compared to baseline. An important feature of these trials is the ability to expand study scope based on early immune response data, collected typically after the second or third vaccination, to allow sufficient time for the emerging of bnAb precursors. This is accomplished by a Part B or Part C extension of the trial, with additional participants enrolled for the assessment of related regimens.

KEY DISCOVERY MEDICINE TRIALS

HVTN 302: mRNA BG505 MD39.3 trimers

BG505 MD39.3 is a modified Clade A BG505 SOSIP, which includes stabilizing and glycan hole masking mutations [34]. HVTN 302 (NCT05217641) tests three versions of BG505 MD39.3, including a soluble gp140 trimer, a membrane-bound gp151 trimer, and a membrane-bound gp151 with a CD4bs knock-out mutation that confers a 1000-fold reduced avidity for $CD4^+$ T cells [47]. This trial includes two doses, 100 and 250 μg of RNA, the higher of which is near the upper limits tested with current COVID-19 mRNA vaccines [48]. This study will help determine the relative advantages of mRNA-delivered soluble trimers vs. membrane-bound trimers. The first hypothesis is that membrane-bound bound trimers will present a more favorable orientation for an immune-focusing strategy, masking the immunogenic base, and therefore limiting the nonneutralizing, base-targeting B-cell response and improving B-cell responses targeting nonbase bnAb epitopes. Second, the CD4bs knockout immunogen reduces steric changes that occur during $CD4^+$ binding that expose nonproductive immune sites and maintain bnAb epitopes. Results of this trial will improve understanding of the magnitude and quality of immune responses to mRNA-encoded HIV-1 trimers and inform further iterative immunogen development.

HVTN 301: 426c.Mod.Core-C4b nanoparticle – a CD4bs VRC01-class immunogen

HVTN 301 (NCT05471076) uses a VRC01-class-germline targeting immunogen known as the 426c.Mod.Core-C4b, a self-assembling 7-mer nanoparticle, adjuvanted with 3M-052-AF and alum. VRC01-class of bnAbs are desirable because they have been isolated from many individuals across the globe and despite 30% amino acid divergence and use of different angles of approach, they retain high potency and breadth [16,49–51] attributed to their $CD4^+$ mimicking strategy. Several VRC01 targeting immunogens are in clinical trials, including the outer domain eOD-GT8 NP [23▪▪,32,52,53] and a trimer approach with BG505 GT1.1 [54]. The 426c.Mod.Core-C4b immunogen consists of the core inner and outer domains of the HIV-1 Clade C 426c transmitted founder viral envelope, including removal of the V1-V3 loops and several glycans, around the CD4bs, and importantly N276, required for germline binding [33,55–57].

The study goals are to assess whether naive B cells expressing VRC01-like B-cell receptors proliferate following immunization with a germline-targeting recombinant envelope; whether escalating dose priming [58–60], a strategy where the complete priming dose is divided up into multiple smaller escalating doses and delivered over two weeks, improves B cell activation over standard bolus dosing; and whether a high vs. low-dose boost improves VRC01-class B cell affinity maturation.

HVTN 144: N332-GT5 – testing a V3 glycan prime and generalizable HCDR3 bnAb-dependent design approach

HVTN 144 tests the N332-GT5 gp140 immunogen, a Clade A BG505 SOSIP trimer derivative, designed to induce V3 glycan BG19-class bnAb precursors, and is the first study targeting this epitope. This will also be the first human test of a generalizable strategy to prime HCDR3-dominant binding bnAbs through use of a computational engineering approach outlined by Steichen *et al.* [36▪▪]. While preclinical results were promising [34,35], if successful in humans it will validate the same approach for other HCDR3-dominant bnAb approaches targeting the V2 Apex, MPER, and FP regions. In addition, the trial will test a promising new adjuvant, saponin/monophosphoryl lipid A (MPLA) nanoparticle (SMNP), wherein saponin matrix technology is combined with the TLR4 agonist MPLA [61]. It will also test two dosing strategies, specifically whether subcutaneous dosing enhances drainage to axillary lymph nodes compared to intramuscular injection [62,63], and whether escalating dose priming reproduces improvements seen in macaques [59].

The primary study outcomes include the proportion of participants that develop V3 glycan epitope-specific and BG18 class-specific MBCs, and to determine the BCR immunogenetics (variable heavy

allele, variable light allele, and HCDR3 length) and sequences in responders. Leukapheresis will be used for PBMC collection and fine needle aspiration (FNA) will be used to interrogate lymph node B and T follicular helper cells. If successful, this priming regimen will be used in combination with intermediate stage shaping and late-stage wild-type polishing immunogens to further induce promising BG18-like bnAb lineages.

HVTN 309/312 & 307/3XX: testing protein vs. mRNA for two lineage-targeting strategies, CD4bs CH235, and V3 glycan DH270

The HVTN will be testing two lineage-targeting strategies – the CD4bs CH235 bnAb program and the V3 glycan DH270 bnAb program – in both protein nanoparticle and mRNA vaccine platforms. The HVTN 309 study is the first in a series of studies to induce the VH1-46-dependent CD4bs CH235 lineage, with the series testing versions of the CH505 M5.G458K SOSIP prime and CH505 TF chSOSIP boost. The immunogens are based on the UCA for CH235 and CH303, both isolated from the same African individual [64]. HVTN 309 will test a ferritin nanoparticle (FeNP) 24-mer adjuvanted with 3M-052-AF + alum or with the empty lipid nanoparticle (LNP) ACU-026-001-1. HVTN 312 will test mRNA versions of the same immunogens in membrane-bound gp160 versions. Preclinical testing has successfully induced intermediate-step bnAbs in knockin mice and macaques [65▪▪,66] and the mRNA versions may offer some developmental advantages [67].

The second series targets the potent V3 glycan DH270 lineage [68], testing protein NP (HVTN 307) vs. mRNA versions (under development). The prime CH848 10.17DT, a modified transmitted founder virus, features a shortened V1 loop and removal of two V1 glycans (N133 and N138). Using the mutation-guided methodology, boosts are designed to favor induction of key activation-induced cytidine deaminase (AID) cold spot mutations. Preclinical testing induced Tier 2 heterologous neutralization and improbable mutations at HC G75R and LC S27Y in knockin mice [65▪▪], with mRNA providing further LC mutational advantages [69], and boosting contributing further intermediate stage key improbable mutations [38]. The human mRNA study will also include use of a novel chimeric prime with implantation of a V3 sequence from a second V3 bnAb lineage, testing whether two different lineages targeting the V3 glycan can be induced through one hybrid immunogen. A second chimeric priming approach will be tested in HVTN 310 described below.

HVTN 310: an mRNA virus-like particle comprehensive approach for 'priming-shaping-polishing'

HVTN 310, building on promising results in macaques, will test a series of mRNA-expressed virus-like particle (VLP) immunogens encompassing a 'priming, shaping, and polishing' approach within a single study to induce mature VRC01-class and CHO1-class bnAb responses, potentially within the same individual. This approach is based on work by Paolo Lusso *et al.* [70▪] that showed sequential immunization with mRNA-encoding envelopes derived from three HIV-1 clades (A/B/C), each co-formulated with SIVmac239 Gag mRNA, induced 79% per-exposure risk reduction against multiple intravaginal challenges. A follow-up study in mice was performed with adaptations to improve neutralizing responses [71]. HVTN 310 primes with a VLP expressed Clade C 426c Env missing glycans at positions 276 (loop D), 460, and 463 (V5 loop) around the CD4bs. An alternative prime replaces the 426c V1-V2-V3 loops with these sequences from the Clade A Q23.17 TF virus, designed to engage V2 apex CH01-class germline Abs [72]. Subsequent boosts at months 2, 4, 6, and 8 will use mRNA VLPS with increasingly intact glycans, followed by autologous then heterologous wild-type trimers. The primary outcomes include antigen specific B cells with both VRC01-class and CHO1-class bnAb features, and autologous and heterologous neutralizing responses. This 'prime to polish' study will be an important keystone test of the strategy and several important design features important to the program.

ANALYSIS GOALS AND WORKFLOW

B cell assays are the most important immunological interrogation step for the Discovery Medicine program, with induction of paired variable heavy and variable light alleles suggesting epitope specific bnAbs and the development of characteristic mutations providing evidence of bnAb lineage maturation (Fig. 2). Serological studies provide supportive evidence of plasma cell responses, but negative results do not rule out B cell initiation. In addition, interim analyses of adequate, but incomplete data sets are used to make iterative changes.

B cell analyses

The first important vaccine responses are immunogen-binding and epitope-specific IgG+ B cells. Leukapheresis and lymph node fine needle aspiration are used to collect cells from the periphery and germinal center, respectively, and they are sorted

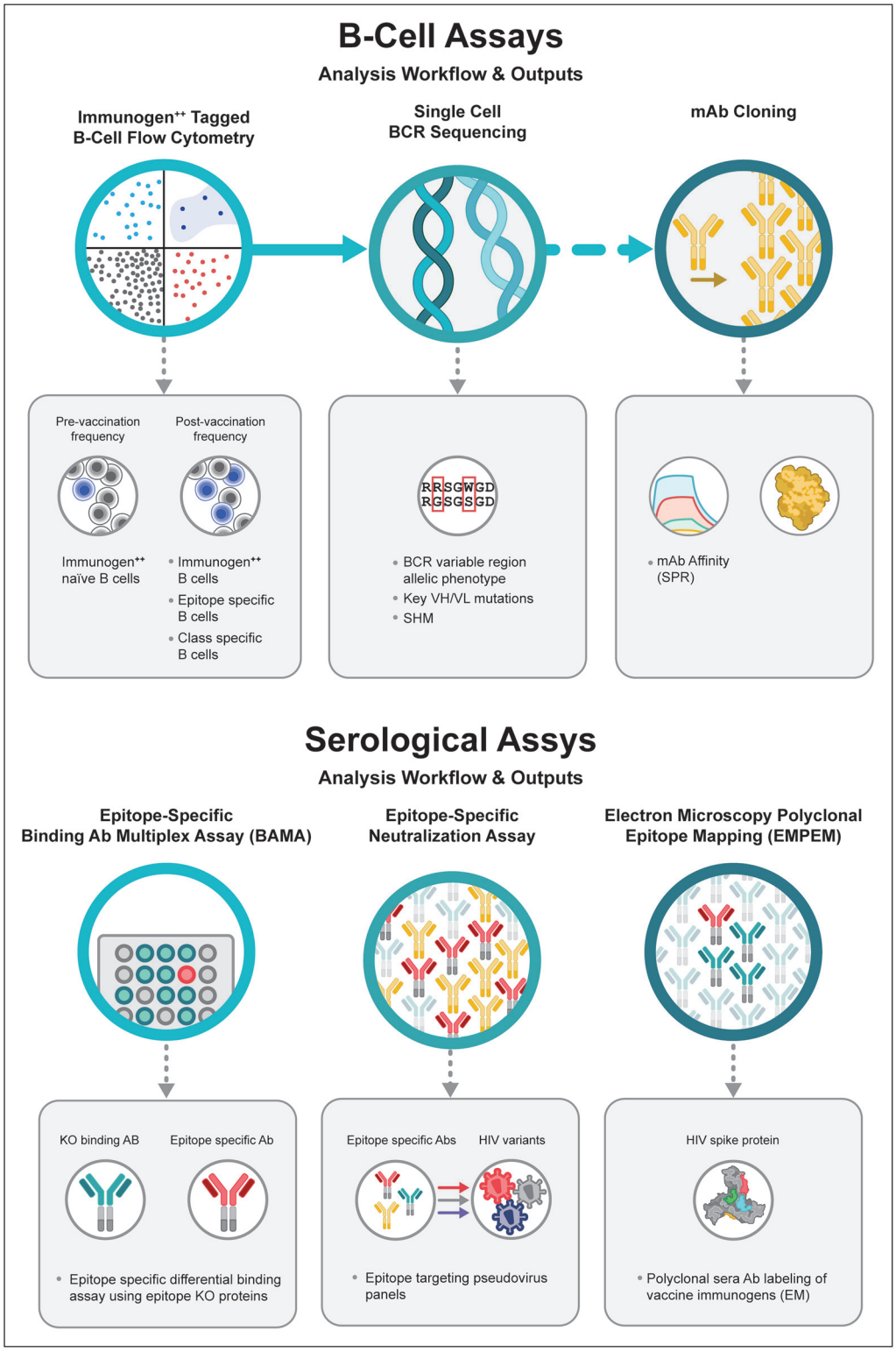


FIGURE 2. Laboratory analyses. The B cell analysis workflow outlined here includes FACS single cell sorting, BCR sequencing, and mAb cloning. These allow assessment of VH and VL allele usage, development of key mutations, and evaluation of binding affinity (top). Serological assays, which can be run in parallel, provide complementary and more rapid assessments of antibody development, including epitope-specific BAMA, epitope-specific neutralizing assay, and EMPEM (bottom). $^{++}$ = cell sorting using two different flourochrome labeled antigens.

by flow cytometry. Antigen-specific MBCs are then applied to the 10X Genomics single-cell platform and next-generation sequencing (NGS) is used for BCR evaluation [73,74]. Paired variable heavy and variable light sequences are matched for allelic comparison to known antibody classes and CDR3 s sequences are evaluated for germline assignment [23▪▪,75] and key non-activation-induced cytidine deaminase (non-AID) derived mutations important for lineage development [76–78]. Sequencing is the most sensitive measure of vaccine response and is critical for iteration [38,77,78].

Monoclonal antibody (mAb) cloning is then used to measure affinity and determine 3D structural binding characteristics (angle of approach, epitope-paratope interactions). The affinity of mAbs as determined by surface plasmon resonance (SPR) or biolayer interferometry (BLI) [23▪▪]. mAb epitope mapping is then performed using a series of complementary techniques: binding antibody multiplex assay (BAMA), neutralization, and cryo-electron microscopy (for amino acid level mapping) [79,80]. This step links the mAb genetic signature to functional binding, allowing comparisons of sequence, affinity, and angle of approach of model bnAbs such as VRC01, CH235, or BG18. It also allows discovery of new bnAb-like mAbs and is particularly helpful for the epitope-agnostic designs where the outcomes of a polyclonal response are less predictable.

Serum analyses

The serum assays allow a rapid and cost effective assessment of antibody output, including individual response rate and magnitude of polyclonal, epitope-focused, and bnAb class-specific antibodies. The BAMA assesses polyclonal responses and when combined with knockout antigens allows determination of epitope-targeting responses [81]. Pseudo-virus neutralization assays, with and without knockout antigens, are critical for determining functional capacity against the autologous virus [82,83] and assessing development of cross neutralizing breadth

	Priming →	Shaping →	Polishing			
Visit	Month 0	Month 2	Month 4	Month 6	Month 12	Month 24
One Possible Combination Vaccine	CD4bs prime	CD4bs prime	CD4bs boost 1	CD4bs boost 2	CD4bs boost 3	CD4bs durability boost
	V3 glycan prime	V3 glycan prime	V3 glycan boost 1	V3 glycan boost 2	V3 glycan boost 3	V3 glycan durability boost
	MPER prime	MPER prime	MPER boost 1	MPER boost 2	MPER boost 3	MPER durability boost

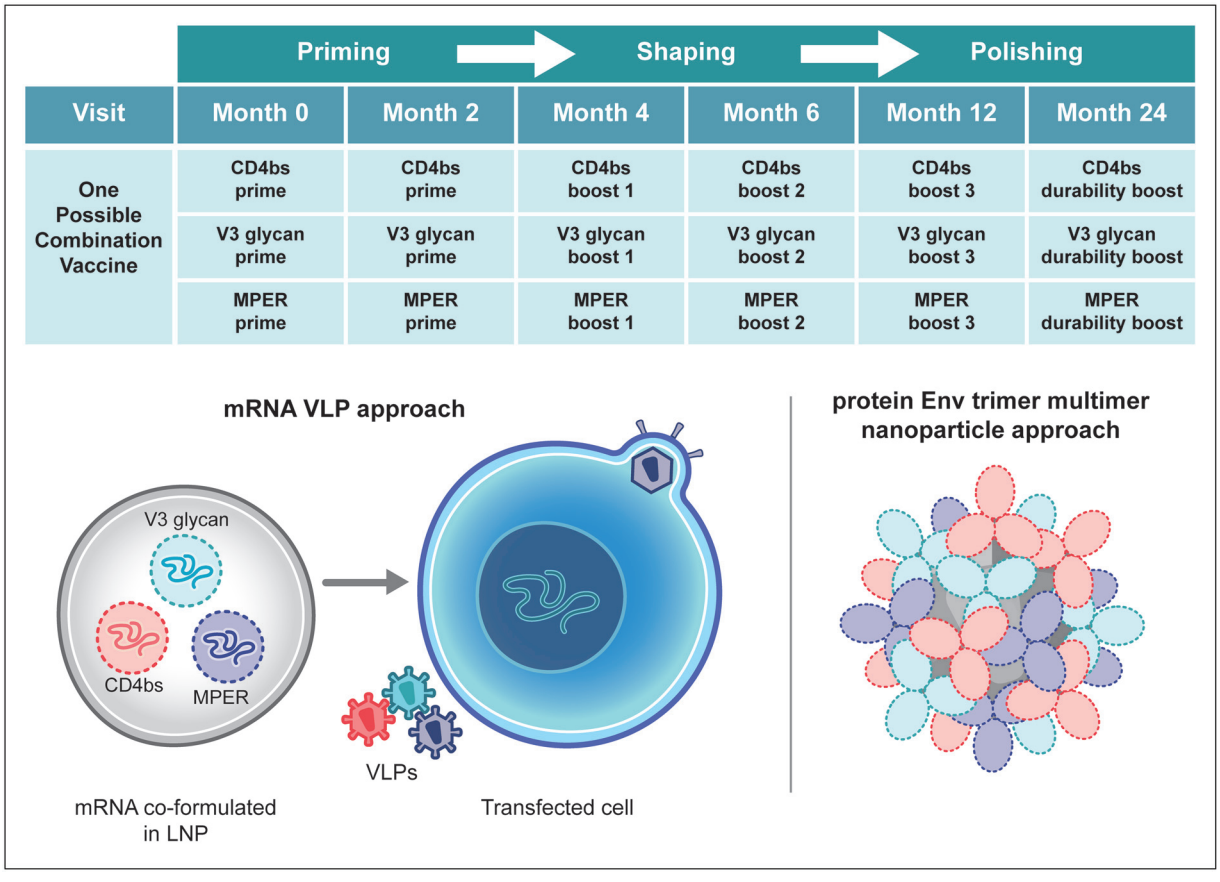


FIGURE 3. Hypothetical combination HIV-1 three-epitope vaccine. The table (top) illustrates a three-epitope combination featuring a two-dose prime targeting the CD4bs, V3 glycan, and MPER regions, then two different shaping boosts, followed by a polishing boost, all distributed over 12 months. A final durability boost is hypothesized at 24 months and periodically thereafter to maintain adequate bnAb levels. Two-model platform delivery options include a combination mRNA encoded CD4bs (red), V3 glycan (blue), and MPER (purple) single VLP (bottom left) or multimeric triple epitope nanoparticle (bottom right).

[84]. Electron microscope polyclonal epitope mapping (EMPEM) [85], a new technique, adds a wealth of structural information, including epitope specificity and off target responses, angles of approach, and visualization of the dynamics of Ab responses at individual level over time [86▪]. This last feature is useful for prioritizing expensive analyses like sequencing and mAb cloning for best responding individuals.

CONCLUSION

Success for this first round of Discovery Medicine trials is defined by how well they activate bnAb class responses, including whether they induce suggestive variable heavy and variable light alleles and key paratope defining mutations. The next round of studies will test a series of boosting agents to measure how far down the maturation pathway promising lineages can be pushed. To support this, more potent adjuvants are also required to improve the germinal center responses and durability [87,88] and the program has several studies evaluating 3M-052-AF [67,89–91], empty LNP [66,92,93], and SMNP [59,61]. A unified vaccine will likely require combining approaches into a multiepitope inducing prime-boost strategy (Fig. 3). VLPs are one promising option [94], as they provide durable immunogenicity [88], proven efficacy with multiple licenced vacines [95–100], and mRNA versions are attractive due to ease of manufacture, in-vivo expression, and technological advances like dose-sparing self-amplifying RNA [101,102]. Multimeric nanoparticles are another important alternative under investigation for COVID-19 and influenza [103–106] and could be adapted for HIV. Finally, adding a T cell component may be critical for ultimate success [107▪], and trials are already under way testing a promising $CD8^+$ approach [108–110].

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Conflicts of interest

T.M.M., S.R., and Y.H. have no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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