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Progress in HIV-1 Vaccine Development

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Abstract

The past two years have seen a number of basic and translational science advances in the quest for development of an effective HIV-1 vaccine. These advances include discovery of new envelope (Env) targets of potentially protective antibodies, demonstration that CD8+ T cells can control HIV-1 infection, development of immunogens to overcome HIV-1 T cell epitope diversity, identification of correlates of transmission risk in an HIV-1 efficacy trial, and mapping the co-evolution of HIV-1 founder Env mutants in infected individuals who develop bnAbs, thereby defining broad neutralizing antibody (bnAb) developmental pathways. Despite these advances, a promising HIV-1 vaccine efficacy trial published in 2013 failed to prevent infection, and the HIV-1 vaccine field is still years away from deployment of an effective vaccine. This review summarizes what some of the scientific advances have been, what roadblocks still remain, and what the most promising approaches are for progress in design of successful HIV-1 vaccine candidates.

Keywords

HIV-1; vaccine; T cells; B cells; broadly neutralizing antibodies

Introduction

Development of a safe and effective HIV-1 vaccine is a global priority [1]. The HIV-1 vaccine field is 30 years into the effort, yet there is no effective vaccine currently available. However, recent breakthroughs in the HIV-1 vaccine field have buoyed hopes that progress can now be made towards an effective vaccine. These advances include discovery of new envelope (Env) targets of potentially protective antibodies [2, 3], demonstration in proof of concept studies that CD8+ T cells can control HIV-1 infection [4, 5], development of immunogens to overcome HIV-1 T cell epitope diversity [6-9], identification of correlates of

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transmission risk in the first HIV-1 efficacy trial to show any protection [10-13], and mapping the evolution of the founder Env mutants in individuals who develop bnAbs, thereby defining broad neutralizing antibody (bnAb) development pathways [14].

Current roadblocks to HIV-1 vaccine development are the inability to induce antibody responses to desired conserved bnAb envelope regions [3] and difficulty in overcoming HIV-1 diversity [9]. Nonetheless, as outlined below, progress is being made in understanding the nature of the roadblocks and in devising strategies for overcoming these roadblocks.

New breakthroughs in HIV-1 vaccine research

This year the field had a major disappointment in the announcement of the lack of vaccine efficacy seen in a DNA prime, recombinant adenovirus type 5 (rAd5) boost HIV-1 vaccine trial developed by the NIH Vaccine Research Center [15]. This vaccine was designed primarily to test the hypothesis that high levels of CD8+ cytotoxic T cells (CTLs) could either protect against transmission or lead to control of plasma HIV-1 viral load. While a majority of vaccinees made T-cell responses as well as envelope-binding antibody levels, the trial showed no efficacy against HIV-1 acquisition [15]. Although additional efficacy trials with a new generation of vaccines are likely in future years, the two HIV-1 vaccine efficacy trial candidates that were primarily targeted to eliciting CD8+ T cells cytotoxic for HIV-infected CD4+ T cells that have been tested have both failed to demonstrate protective efficacy. The second failed trial, the Merck recombinant adenovirus type 5 trial, not only lacked vaccine efficacy, but also appeared to enhance infection in those vaccinees seropositive for Ad5 [16]. However, even HIV-1 efficacy trials that lack protective efficacy can provide information on the types of immune responses that are unlikely to be protective [17, 18].

A new set of studies by Hansen et al. [4, 5] have demonstrated in rhesus macaques that a replicating cytomegalovirus (CMV) vector expressing Simian Immunodeficiency Virus (SIV) antigens could eradicate early SIV infection in 50% of SIV-challenged rhesus macaques. Moreover, SIV-infected cell eradication was associated with an unusual form of CD8+ T cell killing in which CD8+ T cells recognized SIV peptides presented in the context of MHC class II molecules instead of the classical MHC class I [5]. Thus, the search is on to find safe CMV-like vectors that might recreate this activity in humans exposed to HIV-1, and intense research is ongoing to explain why the protective effect was only seen in 50% of rhesus macaques. Nonetheless, these data have demonstrated that indeed CD8+ T cells are associated with control and eradication of early retrovirus infections.

The single trial of an HIV-1 vaccine that showed any efficacy was the RV144 canarypox prime, gp120 protein boost vaccine trial carried out in Thailand that reported an estimated vaccine efficacy of 31.2% [11]. This level of efficacy was not sufficient for deployment of the vaccine, but was encouraging to the field as it suggested that a preventive vaccine could be made [19]. An immune correlates study of the RV144 trial demonstrated that plasma antibodies to the second variable region of the gp120 envelope correlated with decreased HIV-1 transmission risk. In addition, plasma Env IgA responses correlated with decreased HIV-1 vaccine efficacy [10]. Follow-up correlates analyses demonstrated the robustness and breadth of the IgG correlate of risk across multiple subtypes of V1V2 antigens [13]. A genetic

analysis of RV144 breakthrough viruses in vaccinees and placebos demonstrated the site of immune pressure to be a single lysine residue (K169) in the second variable (V2) region of Env [20]. Isolation of V2 monoclonal antibodies demonstrated that antibodies that bound to K169 neither broadly bound transmitted/founder virions nor neutralized difficult-to-neutralize (tier 2) viruses, but did neutralize the vaccine strain virus, 92Th023 [21], mediated low level virion capture [21-23], and mediated antibody dependent cellular cytotoxicity (ADCC) [21, 24]. RV144 induced V1V2 IgG3 antibody responses correlated with decreased risk of HIV-1 infection [25] and correlated with ADCC in the RV144 trial [25, 26]. The Env IgG3 response declined quickly post vaccination [25] as did the overall vaccine efficacy [27], raising the question that the quantity of the antibody levels post vaccination may have contributed to a lowered vaccine efficacy.

Studies to understand correlates of HIV-1 risk in RV144 have also focused on understanding the mechanisms of specific Env IgA in decreasing HIV-1 vaccine efficacy. We found that HIV-1 Env IgA to a conformational C1 region in gp120 blocked IgG mediated ADCC, thus providing a rationale that vaccine-induced plasma IgA responses that bind to the same epitope on infected target cells as IgG could indeed block IgG NK mediated effector function [17]. Consequently, new vaccine candidates are now being designed to increase the breadth of induced FcR-mediated IgG anti-HIV activity, and to optimize the vaccine-induced antibody subclass (i.e. IgG3) and isotype profile [25]. Moreover, efforts are being made to increase antibody durability by incorporating a new adjuvant into the regimen, to determine if efficacy induced by an ALVAC prime, gp120 boost vaccine can be improved to the point of being clinically useful. However, the specific roles of ADCC-mediating antibodies and other FcR-mediated antibody functions in prevention of HIV-1 remains to be directly shown. Roederer et al. [28] have recently shown that current vaccines can induce antibodies that neutralize a subset of SIV viruses. These data suggested that partial efficacy in vaccine trials may be due to vaccine-induced neutralization of a small subset of sensitive viral quasispecies.

New progress has been made in overcoming HIV-1 diversity by induction of cross-reactive T cell responses to HIV-1 by vaccines designed *in silico* (called conserved and mosaic vaccines) [8, 29, 30]. These *in silico* designed immunogens are constructed to increase the coverage across both CD4+ and CD8+ T cell epitopes, and studies in non-human primates have demonstrated that indeed this is the case. Clinical trials with the conserved gene inserts are ongoing, and Phase I clinical trials with mosaic vaccines are planned to begin this year.

The holy grail of HIV-1 vaccine development continues to be the induction of HIV-1 broadly neutralizing antibodies (bnAbs) [3, 31]. Although the HIV-1 envelope does have conserved regions to which neutralizing antibodies can bind [32], no current vaccine candidates have been able induce high levels of bnAbs [2, 31, 32]. The recent development of methods for generating recombinant antibody from single cells [33], the efficient isolation of individual plasmacytes and antigen-specific B cells by flow cytometry sorting [34-36], and high throughput clonal memory B cell cultures [37, 38] has permitted a host of new bnAbs to be recovered from HIV-1 infected individuals. HIV-1 bnAbs define four conserved Env targets for HIV neutralization [2, 3] (**Figure 1**). More than 30 bnAbs specific for conserved neutralizing Env epitopes have been isolated and characterized [3]. It has become clear that all

bnAbs share one or more unusual characteristics: extraordinary levels of somatic hypermutation **(Figure 2),** autoreactivty for host molecules, and long antibody heavy chain complementarity determining region 3s (HCDR3s) [31, 32, 39]. All of these traits are associated with direct or indirect control by host tolerance and immunoregulatory mechanisms, raising the hypothesis that a major regulator of HIV-1 bnAb generation is immune tolerance [31, 40, 41].

In 2005, Haynes and colleagues made the observation that two human recombinant bnAbs, called 2F5 and 4E10, that bind near the virion membrane to envelope gp41 were reactive in human autoantibody assays [40]. In a subsequent study, 2F5 was shown to avidly bind the human protein kynureninase (KYNU), and 4E10 was shown to react with the mammalian RNA splicing factor 3B3 [42]. For 2F5 reactivity with KYNU, the molecular mimicry is striking—the nominal gp41 epitope of the 2F5 bnAb is the linear peptide ELDKWAS and an identical six-residue sequence is present in KYNU (ELDKWA). This ELDKWA motif in KYNU is conserved in nearly all mammalian species and absent in all proteins other than the HIV Env [42]. Thus, the autoantigens for these two bnAbs, 2F5 and 4E10, have been identified, suggesting that expression of these bnAbs is limited by host tolerance mechanisms.

To determine directly whether expression of 2F5-like antibody is indeed controlled by immune tolerance, Verkoczy et al. constructed knockin mouse strains carrying the 2F5 bnAb genes [43-45]. BnAb knockin mice exhibited a severe block in B-cell development at the transition between pre-B and immature B cells. This developmental blockade represented the first tolerance checkpoint and was consistent with physiologically significant autoreactivity by both the mature and germline forms of the 2F5 antibody (**Figure 3**). The 2F5 knockin mouse strain also offered potentially good news for vaccine development. Although the vast majority (95%) of B cells expressing the 2F5 antibody were deleted at the first tolerance checkpoint, a small but significant fraction (5%) of 2F5+ B cells escaped this checkpoint but were functionally silenced (anergic) [43-45]. Remarkably, these anergic B cells could be activated by an immunogen that mimicked the membrane proximal region of gp41 to elicit plasma 2F5 bnAbs [45, 46]. Recently, it has been shown that the 4E10 HIV-1 bnAb is similarly controlled by tolerance deletion and anergy control mechanisms [46, 47]. A naturally occurring 2F5-like mAb in a HIV-1-infected individual has been isolated as well [48, 49], lending plausibility for gp41 neutralizing antibody induction by a vaccine.

BnAbs specific for the HIV-1 envelope gp120 V1V2 glycan bnAb Env region uniformly carry unusually long antibody HCDR3 sequences that appear to be necessary for neutralization [3]. It is likely that these rare HCDR3 motifs are necessary for the bnAb paratope to reach in and around glycans for avid binding at the variable loops of HIV-1 Env [50-57]. In humans, the population of B cells expressing antigen receptors with exceptionally long antibody HCDR3s are controlled by tolerance mechanisms, and this population is commonly reduced by deletion at the first tolerance checkpoint in bone marrow [58]. Therefore, the precursors of V1V2 glycan antibodies are similarly derived from a rare pool of B cells controlled by tolerance mechanisms.

As mentioned, all HIV-1 bnAbs have been shown to carry significantly higher frequencies of V(D)J mutations than non-HIV-1 antibodies [3] (Figure 3). Among the known bnAbs, CD4 binding site (CD4bs) antibodies of the VRC01-type (a type of bnAb shaped like the CD4 molecule itself) have the highest levels of somatic hypermutation (SHM), often reaching [.approxequal]30%. Many of these antibodies are also autoreactive; interestingly, the most common self-antigen recognized by several CD4bs bnAbs are ubiquitin ligases [14, 59] (Kelsoe, G, unpublished observations). The extraordinary frequency of point as well as insertion/deletion mutations in HIV-1 bnAbs is both puzzling and, perhaps, a significant clue towards determining why bnAbs are so difficult to induce. Whereas V(D)J mutations and selection in germinal centers are necessary to increase Ab affinity and specificity, B cells that become heavily mutated (>5%-8%) often exhibit reduced fitness by either lowered affinity or the acquisition of autoreactivity. In both instances, these mutant B cells are selected against and become a minor component of the humoral response or disappear altogether [31]. The strong association of bnAbs with properties that are typically rare and in other types of antibodies, disfavored, is consistent with the absence or rarity of unmutated, naïve B cells capable of founding clonal lineages leading to bnAb production [31]. This tolerance hypothesis [31, 41] explains not only why bnAb production is uncommon, but also why bnAb B cell antigen receptors are so characteristically atypical.

B-cell-lineage immunogen design is a strategy that has been proposed to overcome the disfavored status of HIV-1 bnAb clonal lineages (**Figure 3**) [31]. B-cell-lineage immunogen design is based on the survival advantage exhibited by germinal center B cells expressing antigen receptors (BCR) with the highest affinity for antigen [60-62]. By defining optimal immunogens to guide clonal evolution in germinal centers, B-cell lineages that would normally be disfavored can be promoted. Briefly, the process of B-cell-lineage design for HIV-1 bnAb production is: 1) bnAb clonal lineages from a patient or vaccine are isolated or inferred; 2) the recovered bnAbs are expressed as recombinant antibodies for Env-binding assays; 3) panels of recombinant Envs are expressed to screen for their binding affinities to each crucial branch in the bnAb lineage, and 4) those Envs or Env fragments that bind the highest affinity to the BCR at critical lineage branches are selected as immunogens. The serial administration of the selected Env immunogens would favor those V(D)J mutations and evolutionary trajectories that are necessary to generate bnAbs. The Env immunogens that optimally bind to the germline or unmutated common ancestor (UCA) become the vaccine prime, and those that bind to intermediate antibodies the first boosts, and those that bind to the mature antibodies become the final boosts [31].

Screening with heterologous Envs for B-cell-lineage immunogen design can be effective, but heterologous Envs frequently do not react with the UCAs of bnAb lineages [63, 64]. This is likely because bnAbs lineages arise from the initial autologous Env antibody responses that are exquisitely specific for the infecting, founder virus Env [14, 65]. Consequently, the ideal immunogens for initiating bnAb responses will likely be autologous founder virus Envs from those individuals that make bnAbs during the course of infection [14], or Env immunogens specifically engineered to bind to specific UCA BCRs [66].

In the Center for HIV/AIDS Vaccine Immunology-Immunogen Discovery (CHAVI-ID) program at Duke, 17 individuals followed from the time of transmission of HIV-1 to the

development of bnAbs are being studied for the co-evolution of virus and immunity. The first of these individuals, CH505, has been extensively studied and the co-evolution of founder virus and bnAb clonal lineage maturation meticulously mapped (**Figure 4)**. In doing so, the evolution of HIV-1 Env in response to antibody-mediated selection has been elucidated in unprecedented detail [14]. Indeed, in CH505 a complete history of the sequential Env mutants that elicited bnAbs production was demonstrated, and now Envs and their sequence of administration can be recreated as serial immunogens to attempt to induce bnAb production by vaccination.

The studies in CH505 revealed that bnAbs emerged only after the extensive diversification of the founder virus Env in successive waves of virus escape from the serial production of autologous neutralizing antibodies (the HIV-1arms race) (**Figure 4**). Thus, CH505 sequential Envs are being produced for trials in rhesus macaques and in humans to determine if similar bnAb lineages can be driven in a vaccination setting. Since it takes ~2 years for bnAbs to develop in chronically HIV-1 infected individuals [35, 48, 67, 68]. We expect that multiple immunizations in macaques and humans will be necessary to drive bnAb development.

Various HIV-1 envelope immunogen types are now being developed that express epitopes for bnAbs and their precursors. There are three new structures proposed for the HIV-1 Env trimer [69-72] and the hope is that having the structure of a native Env trimer will be more antigenic and more immunogenic than previously used immunogens. In general, three categories of Env immunogens are in development—minimal immunogens that are fragments or scaffolded portions of HIV-1 Env neutralizing epitopes [66, 73, 74], intermediate Env immunogens such as monomeric Env gp120 [11], and various forms of Env trimers [75]. To date, however, not even those single immunogens with near native structures have been capable of inducing the immune system to generate bnAbs following vaccination.

It has been the view of the field that only ~10%-20% of HIV-1 chronically infected individuals are capable of making bnAbs [35, 48, 67, 68]. More recently, however, screens of large numbers of infected individuals for their breadth of plasma neutralization has demonstrated that there are not polar extremes of neutralizing capacity, but rather a gradation of neutralization breadth in chronically infected populations [76]. What is consistent is that those individuals who do make bnAbs, require years to do so. Early in HIV-1 infection, all infected individuals make autologous neutralizing Abs that are not different from patients that never generate bnAb. Thus, for vaccine trials, the concept is emerging that a successful vaccination for HIV-1, induction of bnAbs will require repetitive immunizations over a longer period of time than for currently licensed vaccines. This type of immunization poses the difficult question of how to design practical immunizations in order to replicate the evolution of the transmitted founder virus envelope over time of bnAb development.

The Way Forward

The way forward for HIV-1 vaccine development is centered on development of new immunogens to overcome diversity for T cell responses (mosaic and conserved immunogens), the induction of greater breadth and durability of immune responses induced

in RV144 to improve on the minimal efficacy seen in RV144, and the development of vaccine strategies to recreate the Env swarms that generate bnAbs when they do occur in the setting of infection (Table 1). In essence, the job of the HIV-1 vaccine development field is to convert subdominant immune responses to become dominant responses -- a task never before required of, or successfully accomplished by, an infectious disease vaccine. Thus, HIV-1 vaccine work is breaking new ground in vaccinology, and success in development of an HIV-1 vaccine will herald success for other difficult to make broadly reactive vaccines such as for influenza and hepatitis C.

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Abbreviations used

bnAb	broad neutralizing antibody
Env	envelope
HIV-1	human immunodeficiency virus 1
rAd5	recombinant adenovirus type 5
CTLs	cytotoxic T cells
CMV	cytomegalovirus
ADCC	antibody dependent cellular cytotoxicity
SIV	simian Immunodeficiency virus
HCDR3s	immunoglobulin third heavy chain complementarity determining region
KYNU	kynureninase
BCR	B cell antigen receptor
SHM	somatic hypermutation
HC	heavy chain
IA	intermediate antibody
UCA	unmutated common ancestor antibody

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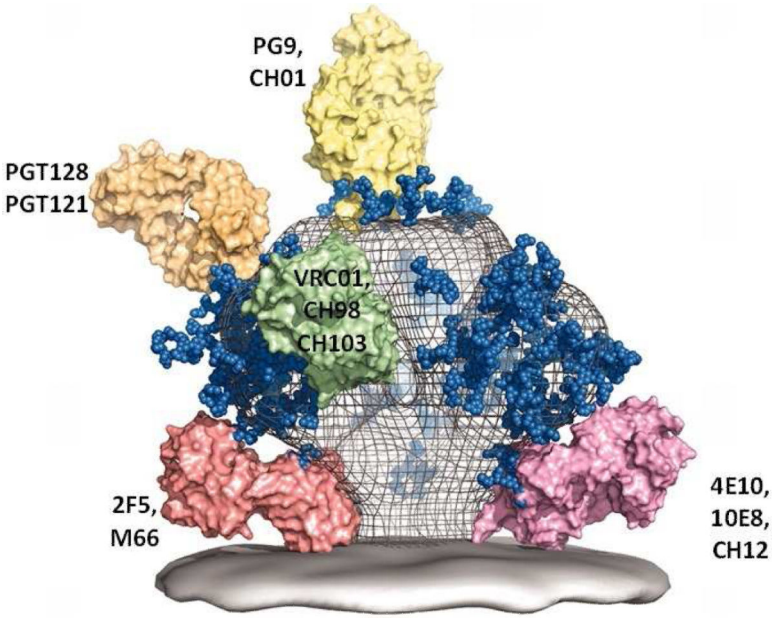

Fig. 1.

A model of the HIV-1-1 Env spike with select bnAb Fab molecules bound to bnAb sites [2].

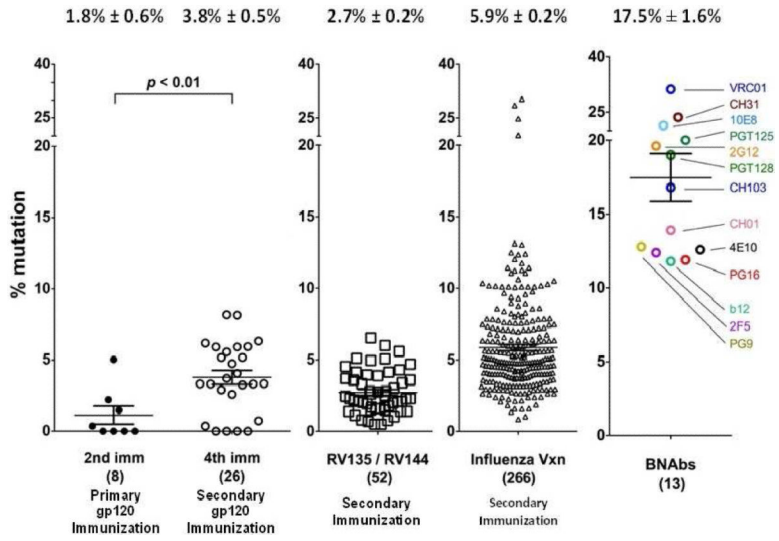

Fig. 2. Comparison of Heavy Chain Mutation Frequency in HIV-1 Immunization, Influenza Immunization, and HIV-1 Broad Neutralizing Abs

Heavy chain (HC) mutation frequencies were determined for three different vaccine studies and compared to that of well-characterized bnAbs. The left two columns show HC mutation frequencies induced by two or four immunizations of a gp120 immunogen [77], there was a rise in mutation observed with repeated immunization. The third column shows an intermediate degree of mutation frequencies observed among antibodies isolated from the canarypox-prime Env-boost RV144 regimen in Phase II and III trials [38]. The fourth column is the mutation frequency observed for influenza vaccine recipients [78]; mutation frequencies after repeated exposure to influenza are higher than those for HIV-1 vaccines. The last column shows 13 well characterized bnAbs all of which show an exceptional degree of mutation.

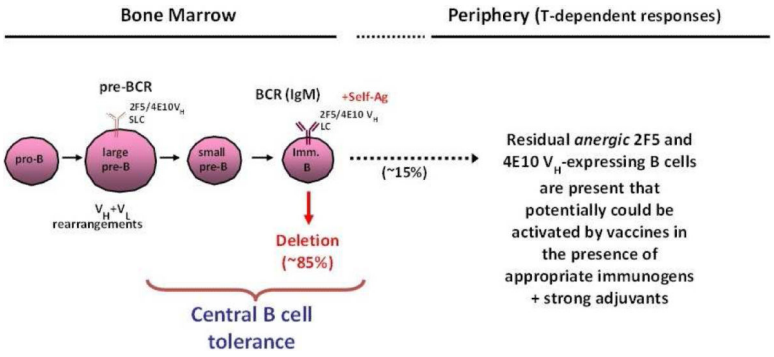

Fig. 3. Central deletion of B-cells expressing gp41 broadly neutralizing antibodies

Highlighted is the pre-B to immature B-cell transition, the stage of B-cell development in the bone marrow at which most B cells expressing 4E10 or 2F5 bnAbs (as BCRs) have been demonstrated in knockin mice to be profoundly impaired [44, 47, 79]. This stage also coincides with the first general checkpoint at which B-cell tolerance mechanisms, including apoptotic deletion, begin to occur [31, 39].

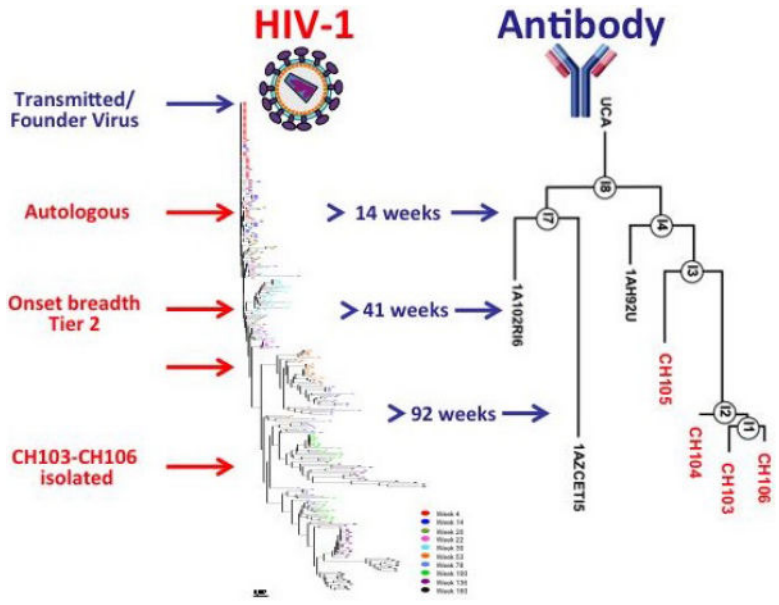

Fig. 4. Co-evolution of virus and a single antibody lineage in an HIV-1 seroconverter

Mature CD4+-binding site antibodies CH103-106 were isolated from circulating memory B cells at week 136 after infection. Longitudinal sampling allowed inference and reconstruction of the evolution of the infecting viral sequence and of the specific neutralizing antibody lineage. B-cell gene sequencing and bioinformatics analyses were used to infer early intermediates (IA) and the unmutated common ancestor (UCA) antibody. The left part of the figure displays a phylogenetic tree of Env sequences derived from week 4 through week 160. The UCA and IA heavy chain sequences of the CH103 antibody lineages are shown alongside viral evolution. This antibody lineage evolved to gain high-affinity Env binding, and virus neutralization evolved from strain-specific autologous virus activity to cross-reactive neutralization of heterologous viruses [14].

Table 1

Major New Directions in HIV-1 Vaccine Research

Induction of Protective T Cell Responses	Induction of Protective B Cell Responses
Defining strategies for overcoming T cell Immunity (mosaic, conserved immunogens)	Defining pathways of broadly neutralizing antibodies in HIV-infected individuals
Defining new conserved T cell epitopes for incorporation into T cell immunogens	Selecting immunogens that can bind to the naïve B cell receptors of broadly neutralized antibodies
Defining replicating vectors for T cell immunogens (attenuated cytomegalovirus, replicating adenovirus, poxviruses)	Selecting sequential and “swam” immunogens that can recapitate bnAb induction with vaccination
Design immunogens to induce T follicular helper cells to drive protective antibody responses	Design immunogens to improve on efficacy seen in RV144 canarypox prime clade B/E gp120 boost Thai trial