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(57) **Abstract**: The present invention relates, in general, to an HIV-1 vaccine and, in particular, to a B cell lineage-based vaccination protocol.

VACCINE

This application claims priority from U.S. Provisional Application No. 61/542,469, filed October 3, 2011 and U.S. Provisional Application No. 61/708,503, filed October 1, 2012.

TECHNICAL FIELD

The present invention relates, in general, to an HIV-1 vaccine and, in particular, to a B cell lineage-based vaccination protocol.

BACKGROUND

The traditional strategies for vaccine development have been to make killed, attenuated or subunit preparations as homologous prime/boosts, and then to test them for safety and efficacy [1, 2]. Vaccines developed in this way are used world-wide for both bacterial and viral infectious diseases [1-4]. A number of viral targets have so far resisted this classical vaccine-development scheme -- HIV-1, dengue and hepatitis C among them [5-7]. Broadly protective influenza vaccines have also yet to be been achieved [8]. HIV-1 is thus a paradigm of those viral diseases for which inducing broadly neutralizing antibodies is especially difficult [9, 10].

For many of the viral vaccines in current use, induction of neutralizing antibodies is a principal correlate of protection [3, 4]. Efforts to find new vaccine-development strategies have therefore focused on design of immunogens bearing epitopes with high affinity for plasma antibodies produced by memory B cells. This strategy assumes that the antigens recognized by memory B cells in a vaccine boost are the same as those recognized by naïve B cells during the priming immunization. For both HIV-1 and influenza, however, this strategy has

not, as yet, led to induction in a majority of vaccinees of antibodies that neutralize a satisfactorily wide range of virus strains. The failure may stem in part from characteristics of the chosen immunogens (e.g., glycan masking of HIV-1 envelope protein epitopes [9]: Table 1) and in part from limited accessibility of conserved epitopes on the viral antigen [8] (e.g., the "stem" and sialic-acid binding epitopes on influenza HA). Mimicry of host antigens by some of these conserved epitopes may be another complication, leading to suppression of a potentially useful antibody response [11].

Table 1. Factors preventing induction of long-lasting broad neutralizing HIV-1 antibodies

• Neutralizing epitopes masked by carbohydrates
• Conformational flexibility of HIV-1 envelope
• Transient neutralizing epitope expression
• Molecular mimicry of Env carbohydrates and protein regions of host molecules
• Tolerance control of gp41 neutralizing epitope responses
• Half-life of all induced antibodies to Env are short; failure of Env to induce long-lived plasma cells
• Rapid viral escape from induced neutralizing antibodies
• Diversion of B cell responses from neutralizing determinants by immune dominant, non-neutralizing epitopes of Env
• Requirement for extensive somatic hypermutations, and requirement for complex maturation pathways

Making vaccines for infectious agents with transient, cryptic or host-mimicking epitopes may require detailed understanding of antibody affinity maturation -- in particular, of patterns of maturation that lead to rare, broadly protective antibodies [12-14]. It might then be possible to design immunogens that increase the likelihood of maturation along those pathways. Recent data from animal studies have demonstrated that the B cells that survive and persist in the germinal center reaction are those presenting B-cell receptors with the highest affinity for antigen [15-18]. Moreover, for some responses to viral antigens, the antigen that stimulates memory B cells during affinity maturation and the antigen that initially elicits naïve B cells may not be the same [12-14, 19-21]. Thus, to induce the processes that lead to such a protective response, it may be necessary to use one antigen for the vaccine prime (to trigger naïve B cells) and others in boosts that drive affinity maturation [12-14, 20-23].

Described herein is an approach to vaccine design based on insights from basic B cell biology, structural biology, and new methods for inferring unmutated ancestor antibodies as estimates of naïve B-cell receptors and their clonal lineage progeny. While the focus is on the biology of inducing broadly neutralizing antibodies to the HIV-1 Env, parallels are also drawn to issues for influenza vaccine development.

Biology of B cells and antibody responses.

Human B cells arise from committed progenitors that express the V(D)J recombinase, RAG1 and RAG2, to effect genomic rearrangements of the IGH gene loci [24-27]. In pre-B I cells, functional μH polypeptides formed by these rearrangements associate with surrogate light chains (SLC) [28-30] and Igα/Igβ heterodimers to form pre-B cell receptors (pre-BCR) [31] necessary for cell survival and proliferation [24, 32, 33]. These cells exit the cell cycle [25] as pre-B II cells, initiate rearrangements in the κ- or λ light (L)-chain loci [34, 35], and assemble a mature

BCR [36, 37] that binds antigen [24, 38] (Figure 1). The generation of a BCR by genomic rearrangement and the combinatorial association of IG V, D, and J gene segments ensures a diverse primary repertoire of BCR and antibodies but also produces self-reactive cells with significant frequency [39].

Most immature B cells are autoreactive; they are consequently eliminated or inactivated by immunological tolerance [40, 41]. The remaining B cells mature through the transitional 1 (T1) and T2 stages characterized by changes in membrane IgM (mIgM) density, mIgD expression, and the loss/diminution of CD10 and CD38 [42]. In the periphery, newly formed (T2) B cells are subject to a second round of immune tolerization before entering the mature B cell pools [40, 41]. Each of these stages in B-cell development is defined by a characteristic genomic and physiologic status (Figure 1); in concert, these events specify the potential of humoral immunity.

At least three mechanisms of immunological tolerance deplete the immature and maturing B-cell pools of self-reactivity: apoptotic deletion [43, 44], cellular inactivation by anergy [45, 46], and the replacement of autoreactive BCR by secondary V(D)J rearrangements [39, 47-49]. The great majority of lymphocytes that commit to the B-cell lineage do not reach the immature B cell stage because they express dysfunctional μH polypeptides and cannot form a pre-BCR [50, 51] or because they carry self-reactive BCR [40].

Autoreactive BCR frequencies decline with increasing developmental maturity [43, 47], even for cells drawn from peripheral sites [Figure 1] [52, 53]. The final stages of B-cell development and tolerization occur in secondary lymphoid tissues where newly formed (T2) B cells undergo selection into mature B-cell compartments [54, 55]. Tolerance mechanisms, especially apoptotic deletion [54-56], operate during the transitional stages of B-cell development, and the frequency of self-reactive cells decreases substantially after entry into the mature pools [40]. The effects of these tolerizing processes have been followed directly in humans by

recovering and expressing IgH and IgL gene rearrangements from individual immature, transitional, or mature B cells and determining the frequencies at which the reconstituted Abs react with human cell antigens [40, 47].

Despite the multiple tolerance pathways and checkpoints, not all autoreactive B cells are removed during development [41]. In mice, mature follicular B cells are substantially purged of autoreactivity, but the marginal zone (MZ) and B1 B cell compartments are enriched for self-reactive cells [57]. In humans, some 20% - 25% of mature, naïve B cells circulating in the blood continue to express autoreactive BCR [35, 40, 41].

Not all selection during B-cell development is negative. Careful accounting of V_H gene segment usage in immature and mature B-cell populations suggests that positive selection also occurs in the transitional stages of B-cell development [58, 59], but the mechanisms for such selection are obscure. The substantial selection imposed on the primary B-cell repertoire, negative and positive, by these physiologic events implies that the full potential of the primary, or germline, BCR repertoire is not available to vaccine immunogens. Only those subsets of naïve mature B cells that have been vetted by tolerance or remain following endogenous selection can respond. For microbial pathogens and vaccine antigens that mimic self-antigen determinants, the pool of mature B cells capable of responding can, therefore, be quite small or absent altogether.

This censoring of the primary BCR repertoire by tolerance sets up a road block in the development of effective HIV-1 vaccines as the success of naïve B cells in humoral responses is largely determined by BCR affinity [15-17]. If immunological tolerance reduces the BCR affinity and the numbers of naïve B cells that recognize HIV-1 neutralizing epitopes, humoral responses to those determinants will be suppressed. Indeed, HIV-1 infection and experimental HIV-1 vaccines are very inefficient in selecting B cells that secrete high affinity, broadly neutralizing, HIV-1 antibodies [5, 60-62].

The predicted effects of immune tolerance on HIV-1 BnAb production has been vividly illustrated in 2F5 VDJ "knock-in" (2F5 VDJ-KI) mice that contain the human VDJ gene rearrangement of the 2F5 BnAb [61, 62]. In 2F5 VDJ-KI mice, early B-cell development is normal, but the generation of immature B cells is severely impaired in a manner diagnostic of tolerization of auto-reactive BCR [43, 44]. Subsequent studies show that the 2F5 mAb avidly binds both mouse and human kynureninase, an enzyme of tryptophan metabolism, at an α-helical motif that matches exactly the 2F5 MPER epitope: ELDKWA [63] (SEQ ID NO: 1) (Yang, G., Haynes, B.F., Kelsoe, G. et al., unpublished)

Despite removal of most autoreactive B cells by the central and peripheral tolerance checkpoints [40, 41], antigen-driven, somatic hypermutation in mature, germinal center (GC) B cells generate de novo self-reactivity, and these B cell mutants can become memory B cells [64-66]. Thus, Ig hypermutation and selection in GC B cells not only drive affinity maturation [15, 18, 67-69], but also create newly autoreactive B cells that appear to be controlled only weakly [43, 70-72] by immunoregulation. At least two factors limit this de novo autoreactivity: the availability of T-cell help [18, 73] and the restricted capacity of GC B cells to accumulate serial mutations that do not compromise antigen binding and competition for cell activation and survival [18, 67, 74].

Eventually, V(D)J hypermutation approaches a ceiling, at which further mutation can only lower BCR affinity and decrease cell fitness [73-75]. The mean frequency of human Ig mutations in secondary immune responses is roughly 5% [20, 76, 77], and the significantly higher frequencies (10% - 15%) of mutations in Ig rearrangements that encode HIV-1 BnAbs [5, 11] therefore suggest atypical pathways of clonal evolution and/or selection. In contrast to clonal debilitation by high mutational burden [73-75], HIV-1 BnAbs appear to require extraordinary frequencies of V(D)J misincorporation [5, 11]. Perhaps the most plausible explanation for this unusual characteristic is serial induction of Ig hypermutation

and selection by distinct antigens. This explanation also suggests pathways for generating antibody responses that are normally proscribed by the effects of tolerance on the primary BCR repertoire.

In GC, clonally related B cells rapidly divide; their clonal evolution is a Darwinian process comprising two component sub-processes: Ig hypermutation and affinity-dependent selection [18, 67, 78]. Selection is nonrandom of course, but even hypermutation is non-random, influenced substantially by local sequence context [79] due to the sequence specificity of activation-induced cytidine deaminase (AICDA) [80]. Furthermore, the codon bias exhibited by Ig genes increases the likelihood of mutations in the regions that encode the antigen-binding domains [81]. Even prior to selection, therefore, some evolutionary trajectories are favored over others. Continued survival and proliferation of GC B cells is strongly correlated with BCR affinity and appears to be determined by each B cell's capacity to collect and present antigen [18, 67] to local $CXCR5^{+}CD4^{+}$ T (T_{FH}) cells [82].

Unlike AICDA-driven hypermutation, where molecular biases remain constant, clonal selection in GC is relative to antibody fitness (affinity and specificity) and changes during the course affinity maturation. Individual GC, therefore, represent microcosms of Darwinian selection, and each is essentially an independent "experiment" in clonal evolution that is unique with regard to the founding B and T cell populations and the order and distribution of introduced mutations.

The poor efficiency with which either infection or immunization elicits BnAbs and the unusually high frequency of Ig mutations present in most BnAb gene rearrangements imply that BnAb B cells are products of disfavored and tortuous pathways of clonal evolution. Because BCR affinity is the critical determinant of GC B cell fitness, it should be possible to select a series of immunogens that direct GC B-cell evolution along normally disfavored pathways.

Any method for directed somatic evolution must take into account the complex and interrelated processes of Ig hypermutation, affinity-driven selection, and cognate interaction with T_{FH}. These hurdles are not insignificant, but neither are they necessarily insurmountable. Indeed, BnAb responses elicited by HIV-1 infection may represent an example of fortuitous sequential immunizations that, by chance, favor the development of BnAb B cells from unreactive, naïve populations.

Biology of antibody responses to HIV-1 as a paradigm of difficult-to-induce broadly neutralizing antibodies

The initial antibody response to HIV-1 following transmission is to non-neutralizing epitopes on gp41 [20, 83]. This initial Env antibody response has no anti-HIV-1 effect, as indicated by its failure to select for virus escape mutants [83]. The first antibody response that can neutralize the transmitted/founder virus in vitro is to gp120, is of extremely limited breadth, and appears only ~12-16 weeks after transmission [84, 85].

Antibodies to HIV-1 envelope that neutralize a broad range of HIV-1 isolates have yet to be induced by vaccination and appear in only a minority of subjects with chronic HIV-1 infection [5] (Figure 2). Indeed, only ~20% of chronically infected subjects eventually make high levels of broadly neutralizing antibodies, and then not until after ~4 or more years of infection [86]. Moreover, when made, broadly neutralizing antibodies are of no clinical benefit, probably because they have no effect on the well-established, latent pool of infected CD4 T cells [86].

Goals for an HIV-1 vaccine

Passive infusion of broadly neutralizing human monoclonal antibodies (mAbs) can protect against subsequent challenge with simian-human

immunodeficiency viruses (SHIVs) at antibody levels thought to be achievable by immunization [87-90]. Thus, despite the obstacles, a major goal of HIV-1 vaccine development is to find strategies for inducing antibodies with sufficient breadth to be practically useful at multiple global sites.

Recent advances in isolating human mAbs using single cell sorting of plasmablasts/plasma cells [20, 76] or of antigen-specific memory B cells decorated with fluorescently labeled antigen protein [91, 92], and clonal cultures of memory B cells that yield sufficient antibody for high throughput functional screening [22, 93, 94], have led to isolation of mAbs that recognize new targets for HIV-1 vaccine development (Figure 2). Those broadly neutralizing antibodies that are made in the setting of chronic HIV-1 infection have one or more of the following unusual traits: restricted heavy-chain variable region (V_H) usage, long HCDR3s, a high level of somatic mutations, and/or antibody polyreactivity for self or other non-HIV-1 antigens (rev. in [5, 11]). Some of these HIV BnAbs have been reverted to their unmutated ancestral state and found to bind poorly to native HIV-1 Env [12, 14]. This observation has suggested the notion of different or non-native immunogens for priming the Env response followed by other immunogens for boosting [12-14, 20-23]. Thus, the B cell lineage design strategy described herein is an effort to drive rare or complex B cell maturation pathways.

SUMMARY OF THE INVENTION

The present invention relates, in general, to an HIV-1 vaccine and, in particular, to a B cell lineage immunogen design.

In an aspect, the present invention relates to a method to identify prime and boost immunogens for use in a B cell lineage-based vaccination protocol comprising:

i) identifying pairs of VH and VL chain sequences expressed as B-cell receptors by a single cell of clonally related B cells from a subject producing broad neutralizing antibodies (bnAbs), including a pair of VH and VL chain sequences of a mature bnAb,

ii) inferring from the sequences of step (i), a pair of VH and VL chains of an unmutated ancestor antibody (UA) of the mature bnAb, and pairs of VH and VL chains of likely intermediate antibodies (IAs) of the mature bnAb,

iii) identifying a first immunogen with binding affinity for the UA, wherein the first immunogen is identified as a prime immunogen, and

iv) identifying one or more immunogens with enhanced binding affinity for one or more IAs relative to the first immunogen of step (iii), wherein the one or more immunogens is identified as one or more boost immunogens,

wherein the first immunogen identified as a prime immunogen and the one or more immunogens identified as one or more boost immunogens have different antigenic structures.

In an embodiment, the present invention relates to a method to identify prime and boost immunogens for use in a B cell lineage-based vaccination protocol comprising:

i) identifying pairs of variable heavy (VH) and variable light (VL) chain sequences expressed as B-cell receptors by a single cell of clonally related B cells from a subject producing broad neutralizing antibodies (bnAbs), including a pair of VH and VL chain sequences of a mature bnAb,

ii) inferring from the sequences of step (i), a pair of VH and VL chains of an unmutated ancestor antibody (UA) of the mature bnAb, and one or more pairs of VH and VL chains of likely intermediate antibodies (IAs) of the mature bnAb,

iii) expressing the pair of VH and VL chains of the UA inferred in step (ii) to produce the UA, and expressing the one or more pairs of VH and VL chains of the one or more likely IAs inferred in step (ii) to produce the one or more likely IAs,

iv) performing one or more UA binding assays, wherein the binding affinity of the expressed UA of step (iii) for one or more immunogens is

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determined,

v) identifying a first immunogen with binding affinity for the UA determined in step (iv), wherein the first immunogen is identified as a prime immunogen,

vi) performing one or more IA binding assays, wherein the binding affinity of the one or more expressed likely IAs of step (iii) for one or more immunogens is determined, wherein the one or more immunogens comprises the first immunogen identified as the prime immunogen in step (v), and

vii) identifying one or more second immunogens with enhanced binding affinity for one or more likely IAs relative to the first immunogen of step (v), wherein the one or more second immunogens is identified as one or more boost immunogens,

wherein the first immunogen identified as a prime immunogen and the one or more immunogens identified as one or more boost immunogens have different antigenic structures.

In a further aspect, the present invention relates to a combination comprising:

(a) the above-mentioned first immunogen, identified in step (iii) as a prime, and

(b) the above-mentioned one or more immunogens, identified in step (iv) as a boost,

for use in inducing an immune response in a mammal, wherein (a) and (b) are for administration to said mammal in an amount sufficient to effect said induction.

In an embodiment, the present invention relates to a combination for use in inducing an immune response in a mammal comprising:

(a) a first immunogen for administration as a prime comprising an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2; and

(b) one or more second immunogens for administration as a boost selected from: an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO:

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4, and an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5; and

(c) one or more additional immunogens for administration as a further boost selected from: an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3,

the first immunogen for administration as a prime, the one or more second immunogens for administration as a boost, and the one or more additional immunogens for administration as a further boost being for administration in an amount sufficient to effect said induction.

In a further aspect, the present invention relates to the envelopes listed "(i)" to "(iv)" below for use in inducing an immune response in a mammal, the envelopes being for administration to said mammal in an amount sufficient to effect induction in the sequence "(i)" followed by "(ii)" followed by "(iii)" followed by "(iv)" or in combination in an amount sufficient to effect said induction,

wherein envelope "(i)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 2,

envelope "(ii)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 3,

envelope "(iii)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 4, and

envelope "(iv)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 5.

In a further aspect, the present invention relates to a combination comprising:

(a) A244 gp120 as a prime,

(b) AE.RV144_42799 gp120 Δ11 as a boost,

(c) B.9021 envelope as a boost,

(d) A244 gp120 as a boost, and

(e) AE.RV144_42799 gp120 Δ11 and A244 gp120 as a final boost,

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for use in inducing an immune response in a mammal, wherein (a) to (e) are for administration to said mammal in an amount sufficient to effect said induction, and wherein B.9021 is B.9021 gp140C or B.9021 delta11 gp120.

In a further aspect, the present invention relates to one or more envelopes, wherein the one or more envelopes is/are A244 gp120, AE.RV144_42799 gp120 Δ11, a B.9021 envelope, or any combination thereof, alone or in combination, for use in inducing an immune response in a mammal, the one or more envelopes being for administration to said mammal in an amount sufficient to effect said induction, wherein B.9021 is B.9021 gp140C or B.9021 delta11 gp120, and A244 gp120 is for administration as a prime.

In a further aspect, the present invention relates to a use of a combination comprising:

(a) the above-mentioned first immunogen, identified in step (iii) as a prime, and

(b) the above-mentioned one or more immunogens, identified in step (iv) as a boost,

for inducing an immune response in a mammal, wherein (a) and (b) are for administration to said mammal in an amount sufficient to effect said induction.

In an embodiment, the present invention relates to a use of a combination for inducing an immune response in a mammal comprising:

(a) a first immunogen for administration as a prime comprising an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2; and

(b) one or more second immunogens for administration as a boost selected from: an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence in MRVKGIRKNCQQHLWRWGTMLLGILMICSA SEQ ID NO: 4, and an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5; and

(c) the one or more additional immunogens for administration as a further boost selected from: an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence in MRVKETQMNWPNLWKWGTLILGLVIICSA SEQ ID NO: 2 and an envelope polypeptide comprising all of the amino acids after the signal peptide sequence

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MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3,

the first immunogen for administration as a prime, the one or more second immunogens for administration as a boost, and the one or more additional immunogens for administration as a further boost being for administration in an amount sufficient to effect said induction

In a further aspect, the present invention relates to a use of the envelopes listed "(i)" to "(iv)" below for inducing an immune response in a mammal, the envelopes being for administration to said mammal in an amount sufficient to effect induction in the sequence "(i)" followed by "(ii)" followed by "(iii)" followed by "(iv)" or in combination in an amount sufficient to effect said induction,

wherein envelope "(i)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 2,

envelope "(ii)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 3,

envelope "(iii)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 4, and

envelope "(iv)" comprises all the consecutive amino acids after signal peptide sequence in SEQ ID NO: 5.

In a further aspect, the present invention relates to a use of a combination comprising:

(a) A244 gp120 as a prime,

(b) AE.RV144_42799 gp120 Δ11 as a boost,

(c) B.9021 envelope as a boost,

(d) A244 gp120 as a boost, and

(e) AE.RV144_42799 gp120 Δ11 and A244 gp120 as a final boost,

for inducing an immune response in a mammal, wherein (a) to (e) are for administration to said mammal in an amount sufficient to effect said induction, and wherein B.9021 is B.9021 gp140C or B.9021 delta11 gp120.

In a further aspect, the present invention relates to a use of one or more of envelopes, wherein the one or more envelopes is/are A244 gp120, AE.RV144_42799 gp120 Δ11, a B.9021 envelope, or any combination thereof, alone or in combination, for inducing an immune response in a mammal, the one or more envelopes being for administration to said mammal in an amount sufficient to effect said induction, wherein B.9021 is B.9021 gp140C or B.9021 delta11 gp120, and A244 gp120 is for administration as a prime.

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In an embodiment, the present invention also relates to a combination for use in inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4.

In an embodiment, the present invention also relates to a combination for use in inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3.

In an embodiment, the present invention also relates to a combination for use in inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

In an embodiment, the present invention also relates to a use of a combination for inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4.

In an embodiment, the present invention also relates to a use of a combination for inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost

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immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3.

In an embodiment, the present invention also relates to a use of a combination for inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

In an embodiment, the present invention also relates to a composition comprising an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3.

Objects and advantages of the present invention will be clear from the description herein.

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BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1. Human B cells arise from committed progenitor cells that proliferate following expression of functional immunoglobulin heavy- (H-) chain polypeptides that associate with surrogate light chains (SLC). In pre-B I cells. H-chain and SLC pairs associate with Igα/Igβ heterodimers to form pre-B cell receptors (pre-BCR) and initiate cell proliferation. When these proliferating cells exit the cell cycle as pre-B II cells, increased RAG1/2 expression drives light-(L-) chain rearrangements and the assembly of mature BCR capable of binding antigen. Most newly generated immature B cells are autoreactive and consequently lost or inactivated at the first tolerance checkpoint; the remainder mature as transitional 1 (T1) and T2 B cells characterized by changes in membrane IgM (mIgM) density, increased mIgD expression, and the loss/diminution of CD10 and CD38. Newly formed T2 B cells are subject to a second round to immune tolerization before entering the mature B cell pools. Mature B cells activated by antigens and TFH characteristically down-regulate mIgD and increase CD38 expression as they enter the germline center (GC) reaction, GC are sites on intense B-cell proliferation, AICDA dependent Ig hypermutation and class-switch recombination, and affinity maturation.

Figure 2. Schematic diagram of trimeric HIV-1 Env with sites of epitopes for broadly neutralizing antibodies. The four general specificities for BnAbs so far detected are: the CD4 binding site; the V2,V3 variable loops; certain exposed

glycans; and the MPER. Red ovals: gp120 core; dark-red ovals, V1-V2 loops; magenta ovals: V3 loop; blue oval, gp41; bright red stripe: MPER of gp41; light brown, curved stripe: viral membrane bilayer. The PGT glycan antibodies depend on the N-linked glycan at position 332 in gp120; like the V2,V3 conformational antibodies, they also depend on the glycan at position 160.

Figure 3. Clonal lineage of V2,V3 conformational antibodies, CH01-CH04, their inferred intermediate antibodies (IAs, labeled 1, 2, and 3), and the inferred unmutated ancestor antibody (UA). Design of immunogens to drive such a pathway might involve producing the UA and IAs and using structure-based alterations in the antigen (i.e., changes in gp120 or gp140 predicted to enhance binding to UA or IA) or deriving altered antigens by a suitably designed selection strategy. Vaccine administration might prime with the antigen that binds UA most tightly, followed by sequential boosts with antigens optimized for binding to each IA. For this clonal lineage, an Env known to bind the UA (AE.A244 gp120: ref 21) could be a starting point for further immunogen design.

Figure 4. Monkey study 62.1.

Figure 5. Monkey study 34.1.

Figure 6. Levels of binding antibodies to A244 gp120D11 induced by A244gp120D11 alone (NHP #34.1) and sequential Env immunization (NHP #62.1).

Figure 7. HIV neutralization: comparisons of isolate means (in $\log_{10}$).

Figure 8. Sequences of A244 gp120 (SEQ ID NO: 2), AE. 427299Δ11gp120 (SEQ ID NO: 3), and B.9021 gp140C (SEQ ID NO: 4).

Figure 9. Sequence of 9021 Δ11 gp120 (SEQ ID NO: 5).

DETAILED DESCRIPTION OF THE INVENTION

Definitions of Terms

Autologous neutralizing antibodies: Antibodies that are produced first after transmission of HIV-1 and that selectively neutralize the transmitted/founder virus.

B-cell anergy: A type of B cell tolerance that renders potentially responding B cells unresponsive to antigen.

B-cell tolerance: The activity of the immune system to suppress B cells that are dangerously host reactive. These cells are either deleted from the B cell repertoire or rendered unresponsive or anergic. A third tolerance mechanism is swapping of either light chains (light chain editing) or heavy chains (heavy chain editing) to prevent self-reactivity of antibodies.

Broadly neutralizing antibodies (BnAbs): Antibodies produced by B cells that neutralize diverse strains of a particular infectious agent.

CD4-binding-site gp120 broadly neutralizing antibodies: The T-lymphocyte surface antigen, CD4, is the cellular receptor of HIV-1. It binds at a defined, conserved site on gp120. Although many antibodies recognize the region on the surface gp120 that includes the CD4 binding site, their footprint also covers adjacent parts of the surface, where mutation can lead to escape from neutralization by those antibodies. A few, broadly neutralizing antibodies (the VRC01-VRC03 clonal lineage, PG04, the CH30-CH34 clonal lineage) bind

gp120 in a way that closely resembles the contact made by CD4: the heavy-chain VH region of these antibodies (nearly all are V_H 1~2) mimics the N-terminal, Ig-like domain of CD4, with relatively few interactions outside the conserved, CD4-binding pocket.

Germinal center: Location in immune tissues at which dendritic and other cells present B cell contact antigen, helper T cells make contact with B cells, and immunoglobulin class switching and somatic hypermutation take place.

Heavy chain third complementary determining region (HCDR3): Three loops from each of the two immunoglobulin polypeptide chains contribute to its antigen-binding surface. The third of these "complementarity determining regions" (CDRs) on the heavy chain is particularly variable and often makes a particularly important contribution to antigen recognition.

Hemagglutinin broadly neutralizing determinants: The influenza virus hemagglutinin (HA), one of the two principal surface proteins on influenza A and B, has, like HIV-1 Env, both strain- specific and conserved determinants for neutralizing antibodies. Like HIV-1 Env neutralizing antibodies, most hemagglutinin neutralizing antibodies are strain specific and not broadly neutralizing. The conserved targets of broadly neutralizing influenza antibodies are the binding pocket for the receptor, sialic acid, and the "stalk" of the rod-like HA trimer.

Immunoglobulin class switching: The process in germinal centers -by which antigen drives switching of immunoglobulin made by a developing memory B cell from IgM to IgG, IgA or IgE. This process, which requires activation of the recombination activating genes I and II (RAGI, RAGII), is independent of somatic hypermutation. Not all memory B cells undergo class switching, however, and some memory B cells retain surface IgM.

Intermediate antibodies (IAs): Antibodies made by intermediates in the clonal lineage generated by affinity maturation of a naïve B cell in a germinal center.

Membrane-proximal-external-region (MPER) gp41 broadly neutralizing antibodies: The MPER is a site on HIV-1 Env gp41 near the viral membrane at which a number of neutralizing antibodies bind. Isolated natural antibodies that bind this region (2F5, 4E10, CAP206-CH12) are polyreactive; the tip of their HCDR3 associates with the viral lipid membrane while awaiting exposure of the gp41 intermediate neutralizing determinant.

Polyreactivity: the common characteristic of those virus-specific antibodies that also bind either host self antigens or other non-viral antigens.

V2,V3 conformational (quaternary) HIV-1 envelope gp120 broadly neutralizing antibodies: A group of HIV-1 broadly neutralizing antibodies recognizing an epitope on gp120 that is properly configured only (or primarily) when gp120 is part of the complete Env trimer. Mutational analysis of regions of gp120 that bind quaternary antibodies show that most of them recognize the second variable (V2) and third variable (V3) loops of HIV-1 Env. Examples include PG9, PG16 and the CH01-04 clonal lineage of human mAbs.

Somatic hypermutation: The process in germinal centers, mediated by the enzyme activation-induced cytidine deaminase (AID), that leads to affinity maturation of the antibody-antigen contact.

Third variable loop neutralizing antibodies: The third variable loop of HIV-1 envelope (V3) is part of the binding site for the CCR5 and CXCR4 Env co-receptors; it is a frequent target of neutralizing antibodies. Examples of V3 neutralizing antibodies isolated from chronically infected subject are 447, 19b and CH19. The V3 loops is masked on the envelopes of most transmitted/founder viruses, and thus V3 loop antibodies by themselves are likely to be of limited value as a vaccine response. V3 loop antibodies are easily elicited, however, and

they could be useful in combination with an antibody that induced V3 loop exposure (e.g., a CD4-binding-site antibody).

Unmutated ancestor antibodies (UAs): Antibodies that represent the B cell receptors (BCRs) on naïve B cells. UAs can be isolated from naïve or transitional B cell populations or inferred from memory B-cell mutated clonal lineages.

VH restriction: occurrence of the same V_H in the antibody responses of many different individuals to the same epitope.

B cell lineage vaccine design

Figure 3 shows a general outline for B-cell lineage vaccine design. There are several points that distinguish this approach from previous vaccination strategies. First, existing vaccines generally use the same immunogens for prime as for boosts. In the scheme outlined in Figure 3, different antigens can be used for multiple steps. Design of the priming antigen can utilize the B cell receptor from the inferred unmutated ancestor (UA, see below) or from an actual, isolated naïve B cell as a template, while design of boosting antigens can use the B-cell receptor from inferred (or isolated) maturation intermediates as templates (see immunogen design section below) [68]. Second, the B cell lineage notion targets, for the priming immunogen, the earliest stages of B cell clonal development, following the basic understanding of B cell antigen drive reviewed above (Figure 1). Third, for boosting immunogens, the scheme in Figure 3 anticipates choosing components that might have the highest affinity for early stages of B cell maturation.

Three general steps are contemplated for any lineage-based approach to vaccine design. First, identify a set of clonally related memory B cells, using single cell technology to obtain the native variable heavy (V_H) and variable light (V_L) chain pairs. Second, infer with the computational methods described below,

the unmutated ancestral B-cell receptor (i.e., the presumptive receptor of the naïve B cell to be targeted), along with likely intermediate antibodies (IAs) at each clonal lineage branch point (Figure 2, circular nodes 1-3). Finally, design immunogens with enhanced affinity for UA and IAs, using the UA and IAs as structural templates (Figure 3). Thus, in contrast to the usual vaccine immunogens that prime and boost with the same immunogen, a B cell lineage-based vaccination protocol can prime with one immunogen and boost with another, and potentially boost with a sequence of several different immunogens [12-14, 20-23] (Figure 3). In recent work, a gp140 Env antigen that did not bind the UA of a BnAb was modified by native deglycosylation; unlike the untreated native Env antigen, the deglycosylated gp140 Env bound the BnAb UA with reasonable efficiency. Immunization of rhesus macaques showed that the Env that bound well to the UA was the superior immunogen [19].

It is important to note that variability of the antibody repertoire among individuals poses a potential problem for this strategy: a clonal lineage isolated from one subject may not be relevant for inducing a similar antibody in another subject. Recent observations of limited VH usage summarized above suggest that for some viral neutralizing epitopes the relevant immunoglobulin repertoire is restricted to a very small number of VH families and that the maturation pathways may be similar among individuals or require the same immunogens to drive similar pathways of affinity maturation. One example of convergent evolution of human antibodies in different individuals comes from work on B cell chronic lymphocytic leukemia (B cell CLL), in which similar B CLL VH HCDR3 sequences can be found in different people [95, 96]. A second comes analysis of influenza and HIV-1 VH1-69 antibodies, in which similar VH1-69 neutralizing antibodies can be isolated from different subjects [97-101]. A third example comes from structures of V2,V3 conformational (quaternary) antibodies in which the antibodies have very similar HCDR3 structures but arise from different VH

families [22, 101, 102]. Recently, use of 454 deep sequencing technology has shown convergent evolution of VH1-2 and VH1-46 CD4 in maturation of broadly neutralizing antibodies, but determining how distinct the affinity maturation pathways are for each specificity of HIV-1 broadly neutralizing antibodies requires experimental testing. Nonetheless, for major classes of such antibodies, the data summarized suggest commonalities among affinity maturation pathways in different individuals.

Inferring UAs and intermediates of BnAb clonal lineages

B cell lineage immunogen design requires that it be possible to infer from the sequences of the mature mutated antibodies in a lineage those of the intermediate and unmutated ancestors, as in the reconstructed clonal lineage in Figure 3. Antibody genes are assembled from a fixed set of gene segments; that there are relatively small numbers (i.e., non-astronomical) of possible genes ancestral to any given set of clonally-related antibody genes allows one to infer the ancestor antibodies [20-23].

The starting point for any likelihood-based phylogenetic analysis is a model for the introduction of changes along the branches. For the inference of unmutated ancestor antibodies of a clonal lineage (See UA, Figure 3), a model is needed for somatic mutation describing the probability that a given nucleotide (for example, the one at position 21 in the V region gene) that initially has state n_1 will, after the passage of t units of evolutionary time, have state n_2. This substitution model makes it possible to compute the probability of the observed data given any hypothesized ancestor. From there, the application of Bayes' rule provides the posterior probability for any hypothesized ancestor. The posterior probability at each position in the unmutated ancestor can now be computed from the posteriors over the gene segments and over other parameters of the rearrangement. The complete probability function provides a measure of the

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certainty of the inference at each position in addition to the most-likely nucleotide state itself. This additional information may be crucial to ensuring the relevance of subsequent assays performed on the synthesized unmutated ancestor. Some of the intermediate forms of the antibody genes through which a given member of the clone passed can be similarly inferred, though not all of them (antibodies at nodes 1-3, Figure 3). The more members of the antibody clone that it is possible to isolate, the higher the resolution with which the clonal intermediates can be reconstructed [20]. 454 deep sequencing has recently proved useful for expanding the breadth and depth of clonal lineages [20, 23].

Using UAs and IAs as templates for immunogen design

The goal of the immunogen-design strategy described herein is to derive proteins (or peptides) with enhanced affinity for the unmutated common ancestor of a lineage or for one or more of the inferred intermediate antibodies. The method of choice for finding such proteins will clearly depend on the extent of structural information available. In the most favorable circumstances, one might have crystal structures for the complex of the mature antibody (Fab) with antigen, structures of the UA and of one or more IAs, and perhaps a structure of an IA:antigen complex. It is likely that the native antigen will not bind tightly enough to the UA to enable structure determination for that complex. In the absence of any direct structural information, consideration can also be given to cases in which the antibody footprint has been mapped by one or more indirect methods (e.g., mass spectrometry).

Computational methods for ligand design are becoming more robust, and for certain immunogen-design applications, they are likely to be valuable[103]. It is anticipated that for the epitopes presented by HIV Env, however, the available structural information may be too restricted to allow one to rely primarily on a computational approach. The area of the interface between an antibody and a

tightly-bound antigen is generally between 750 and 1000 $Å^2$, and on the surface of gp120, for example, such an interface might include several loops from different segments of the polypeptide chain. Even if both the structure of the mature-antibody:Env complex and that of the UA were known, computational design of a modified Env with enhanced affinity for the UA would be challenging. Selection approaches should, in the near term at least, be more satisfactory and more reliable.

For continuous epitopes, phage display is a well-developed selection method for finding high-affinity peptides [104] . The best-studied continuous epitopes on HIV Env are those for the antibodies, 2F5 and 4E10, directed against the membrane proximal external region (MPER) of gp41. Efforts to obtain neutralizing antibodies by immunization with peptides bearing the sequence of these epitopes have been generally unsuccessful, presumably in part because the peptide, even if cyclized, adopts only rarely the conformation required for recognition in the context of gp41. In a computational effort to design suitable immunogens, the 2F5 epitope was grafted onto computationally selected protein scaffolds that present the peptide epitope in the conformation seen in its complex with the 2F5 antibody. These immunogens indeed elicited guinea-pig antibodies that recognize the epitope in its presented conformation [105]. The MPER epitopes are exposed only on the fusion intermediate conformation of gp41, however, not on the prefusion trimer [106] , and to have neutralizing activity, these antibodies must have a membrane-targeting segment at the tip of their heavy-chain CDR3 in addition to a high-affinity site for the peptide epitope [107]. Thus, more complex immunogens (e.g., coupled to some sort of membrane surface) may be necessary to elicit antibodies that have both properties.

Differences between antibody 2F5 and its probable unmutated ancestor have been mapped onto the 2F5 Fab:peptide-epitope complex. The side chains on the peptide that contact the antibody are all within a ten-residue stretch, and

several of these (a DKW sequence in particular) must clearly be an anchor segment even for a complex with the UA. Randomization of no more than 5 positions in the peptide would cover contacts with all the residues in the UA that are different from their counterparts in the mature antibody. Phage display libraries can accommodate this extent of sequence variation (i.e., about 3 x 10^6 members), so a direct lineage-based, experimental approach to finding potential immunogens is possible, by selecting from such libraries peptides that bind the UCA of a lineage or one of the inferred intermediates.

For discontinuous epitopes on gp120 that are antigenic on cell-surface expressed, trimeric Env, a selection scheme for variant Envs can be devised based on the same kind of single-cell sorting and subsequent sequencing used to derive the antibodies. Cells can be transfected with a library of Env-encoding vectors selectively randomized at a few positions, and the tag used for sorting can be, for example, be a fluorescently labeled version of the UA antibody. An appropriate procedure can be used to select only those cells expressing an Env variant with high affinity for the antibody. In cases for which a comparison has been made of the inferred UA sequence with the structure of an antigen-Fab complex, partial randomization of residue identities at 3-5 positions, as in the linear-epitope example, can be expected to generate the compensatory changes one is seeking.

Recognition of HIV-1 envelope by several classes of broadly neutralizing antibodies includes glycans presented by conformational protein epitopes. Such antibodies account for ~25% of the broadly neutralizing activity in the plasma of subjects selected for broad activity [108, 109]. By analogy with selection from phage-displayed libraries, synthetic libraries of glycans or peptide-glycan complexes can be screened to select potential immunogens with high affinity for UAs and IAs of clonal lineages [110]. Large-scale synthesis of chosen glycoconjugates can then yield the bulk material for immunization trials [111, 112].

The various approaches described herein are equally applicable to influenza-virus vaccine design. On the influenza-virus hemagglutinin (HA), two conserved epitopes have received recent attention -- one, a patch that covers the fusion peptide on the "stem" of the elongated HA trimer [97, 98, 113], the other, the pocket for binding sialic acid, the influenza-virus receptor[114]. Screens of three phage-displayed libraries of human antibodies, each from a quite different source, yielded similar antibodies directed against the stem epitope, and additional human mAbs of this kind have been identified subsequently by B-cell sorting. Conservation of the stem epitope may be partly a consequence of low exposure, due to tight packing of HA on the virion surface, and hence low immunogenicity on intact virus particles. An antibody from a vaccinated subject that binds the sialic-acid binding pocket and that mimics most of the sialic-acid contacts has been characterized [114]. It neutralizes a very broad range of H1 seasonal strains.

In summary, HIV-1 is a paradigm for a number of viruses that acquire resistance to immune detection by rapid mutation of exposed epitopes. These viruses do have conserved sites on their envelope proteins but a variety of mechanisms prevent efficient induction by vaccines of antibodies to these conserved epitopes. Some of these mechanisms, at least in the case of HIV-1, appear to be properties of tolerance control in the immune system. It is, therefore, clear that conventional immunization strategies will not succeed. Only rarely does the B-cell response follow the affinity maturation pathways that give rise to HIV-1 or influenza broadly neutralizing antibodies, and until recently there were no technologies available to define the maturation pathways of a particular antibody type or specificity. With recombinant antibody technology, clonal memory B-cell cultures, and 454 deep sequencing, clonal lineages of broadly neutralizing antibodies can now be detected and analyzed. Immunogens can be optimized for high affinity binding to antibodies (B-cell receptors of clonal lineage B-cells) at multiple stages of clonal lineage development, by combining

analysis of these lineages with structural analysis of the antibodies and their ligands. This combination provides a viable strategy for inducing B-cell maturation along pathways that would not be taken in response to conventional, single-immunogen vaccines.

Certain aspects of the present invention are described in greater detail in the non-limiting Example that follows.

EXAMPLE 1

Fig. 4 shows the set of immunizations in NHP study 62.1 wherein immunogens were chosen based on how well they bound to the antibody members of the CH01-CH04 broad neutralizing clonal lineage. A244 gp120 delta 11 was used as the prime and the boost was the placebo breakthrough infection in the RV144 trial, 427299.AE gp120 env delta 11, then a further boost with the 9021.B gp140Cenv (but could have been delta 11 gp120—either one), another boost with A244 gp120 Env delta 11 and then another boost with a combination of A244 gp120 delta 11 + 427299 Env. As shown in Fig. 6, when the NHP study 34.1, in which A244 gp120 delta 11 alone was used (see Fig. 5)), was compared to NHP study 62.1, in terms of binding of antibodies to A244 gp120 delta 11, similar binding titers are observed. However, the comparison shown in Fig. 7 yields a completely different result. The blue neutralizing antibody levels are with A244 gp120 D11 Env (study 34.1) and are what was seen in the plasma of the RV144 trial (Montefiori et al, J. Inf. Dis. 206:431-41 (2012)) high titers to the tier 1 AE isolate that was in the vaccine AE92TH023 , some other low level tier 1 neutralizing antibody levels, and the rest of the levels were negative (neutralizing antibody assay levels in this assay start at a plasma dilution of 1:20 such that levels on the graph of "10" are below the level of detection and are read as negative). In contrast, the titers to the tier 1s in the red bars from study 62.1 show

1-2 logs higher abs to the tier 1s but most importantly now significant neutralizing antibody levels to the two tier 2 transmitted founder breakthrough viruses from the RV144 trial (all assays in TZMBL assay, except for the two arrows indicate HIV isolates which were assayed in the A3R5 cell neutralizing antibody assay). Thus, by immunizing with sequential Envs chosen for their ability to optimally bind at UCA, IA and mature antibody member points of a broad neutralizing antibody lineage, the breadth of neutralizing antibody coverage has been increased by inducing new neutralizing antibodies to Tier 2 (difficult to neutralize) HIV strains AE.427299 and AE.703357, demonstrating proof of concept that the strategy of B cell lineage immunogen design can indeed induce improved neutralizing antibody breadth. Moreover, these data demonstrate a new discovery as a strategy for inducing greater breadth of neutralization in using the ALVAC/AIDSVAX type of vaccine (Haynes et al, NEJM 366: 1275-1286 (2012)) for future vaccine trials, and that is adding gp120 Envs to the primer and or the boost regimen made up of Env gp120s chosen from the breakthrough infections that did not match the original vaccine in RV144 to increase the potency of vaccine efficacy of a vaccine in Thailand. Rolland has shown that if the RV144 trial breakthrough viruses are compared from vaccinees and placebo recipients, those viruses that had similarity at the V2 region were controlled by 45% vaccine efficacy (Rolland M et al, Nature Sept. 10, Epub ahead of print, doi: 10.1038/nature 11519, 2012). Thus, screening the sequences of RV144 breakthrough viruses for the most common HIV strains with Env V2 regions that did not match the vaccine should demonstrate the Env V2 motifs that should be included in additional prime or boosting Envs in the next vaccine to increase the vaccine efficacy. In addition, the adjuvant used will be important. In the trials above in NHP study 34.1 and 62.1 the adjuvant used was a squalene based adjuvant with TLR7 + TLR9 agonists added to the squalene (see PCT/US2011/062055). Currently available adjuvants that are available and can

be considered for use in humans is MF-59 (Dell'Era et al, Vaccine 30: 936-40 (2012)) or AS01B (Leroux-Roels et al, Vaccine 28: 7016-24 (2010)). Thus, a vaccine can be designed based on a polyvalent immunogen comprising a mixture of Envs administered in sequence as shown, for example, in Fig. 4 or alternatively the sequentially chosen Envs can be administered all together for each immunization as describe (Haynes et al, AIDS Res. Human Retrovirol. 11:211-21 (1995)) to overcome any type of primer-induce suppression of Env responses. Thus, the present invention relates, at least in part, to an approach to improving the RV144 vaccine by adding gp120s or gp140Cs (with or without the delta 11 (D11) deletion) (e.g., 427299 Env gp120 sequences) to the A244 gp120 immunogen to expand the coverage of the the RV144 original vaccine. (See, for example Figs. 4 and 5.) It can be seen that this strategy of probing the breakthrough viruses of any partially successful vaccine trial can utilize this strategy to improve that vaccines coverage of infectious agent strains and in doing so, improve the vaccine efficacy of that vaccine.

The present invention also relates in part to demonstrating proof of concept of the general strategy of vaccine design known as "B Cell Lineage Immunogen Design" wherein the prime and boost immunogens are chosen based on the strength of binding of each vaccine component to an antibody template in the antibody clonal lineage that is desired to induce.

* * *

Also referred to herein are U.S. Provisional Application No. 61/542,469, filed October 3, 2011 and International Application No. PCT/US2011/000352, filed February 25, 2011.

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WHAT IS CLAIMED IS:

1. A method to identify prime and boost immunogens for use in a B cell lineage-based vaccination protocol comprising:

i) identifying pairs of variable heavy (VH) and variable light (VL) chain sequences expressed as B-cell receptors by a single cell of clonally related B cells from a subject producing broad neutralizing antibodies (bnAbs), including a pair of VH and VL chain sequences of a mature bnAb,

ii) inferring from the sequences of step (i), a pair of VH and VL chains of an unmutated ancestor antibody (UA) of the mature bnAb, and one or more pairs of VH and VL chains of likely intermediate antibodies (IAs) of the mature bnAb,

iii) expressing the pair of VH and VL chains of the UA inferred in step (ii) to produce the UA, and expressing the one or more pairs of VH and VL chains of the one or more likely IAs inferred in step (ii) to produce the one or more likely IAs,

iv) performing one or more UA binding assays, wherein the binding affinity of the expressed UA of step (iii) for one or more immunogens is determined,

v) identifying a first immunogen with binding affinity for the UA determined in step (iv), wherein the first immunogen is identified as a prime immunogen,

vi) performing one or more IA binding assays, wherein the binding affinity of the one or more expressed likely IAs of step (iii) for one or more immunogens is determined, wherein the one or more immunogens comprises the first immunogen identified as the prime immunogen in step (v), and

vii) identifying one or more second immunogens with enhanced binding affinity for one or more likely IAs relative to the first immunogen of step (v), wherein the one or more second immunogens is identified as one or more boost immunogens,

wherein the first immunogen identified as a prime immunogen and the one or more immunogens identified as one or more boost immunogens have different antigenic structures.

2. The method of claim 1, further comprising:

viii) performing one or more additional binding assays, wherein the binding

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affinity of the mature bnAb for one or more immunogens is determined, wherein the one or more immunogens comprises the one or more second immunogens identified as the one or more boost immunogens in step (vii), and

ix) identifying one or more additional immunogens with enhanced binding affinity for the mature bnAb relative to the one or more second immunogens of step (vii), wherein the one or more additional immunogens with enhanced binding affinity for the mature bnAb is identified as one or more boost immunogens.

3. The method of claim 1 or 2, wherein the broad neutralizing antibodies of step i) are broad neutralizing antibodies to HIV-1 and wherein the one or more immunogens of step iv) is an HIV-1 envelope polypeptide immunogen.

4. The method of claim 1 or 2, wherein the broad neutralizing antibodies of step i) are broad neutralizing antibodies to influenza and wherein the one or more immunogens of step iv) is an hemagglutinin polypeptide immunogen.

5. The method of any one of claims 1 to 4, using computational methods to infer the sequence of the VH and VL chains of the UA of step (ii).

6. The method of any one of claims 1 to 5, wherein the IAs are inferred at each branch point of the clonal lineage of the clonally related B cells.

7. The method of any one of claims 1 to 6, wherein the first immunogen identified in step
(v) and the one or more second immunogens identified in step (vii) are different proteins.

8. The method of any one of claims 2 to 6, wherein the first immunogen identified in step (v) and each of the one or more additional immunogens identified in step (ix) are different proteins.

9. The method of any one of claims 2 to 6, wherein each of the one or more second

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immunogens identified in step (vii) and each of the one or more additional immunogens identified in step (ix) are different proteins.

10. The method of claim 9, wherein the first immunogen identified in step (v) and each of the one or more second immunogens identified in step (vii) are different proteins, wherein the first immunogen identified in step (v) is a different protein than each of the one or more additional immunogens identified in step (ix), and wherein each of the one or more second immunogens identified in step (vii) is a different protein than each of the one or more additional immunogens identified in step (ix).

11. A combination for use in inducing an immune response in a mammal comprising:

(a) a first immunogen for administration as a prime comprising an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2; and

(b) one or more second immunogens for administration as a boost selected from: an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4, and an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5; and

(c) one or more additional immunogens for administration as a further boost selected from: an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3,

the first immunogen for administration as a prime, the one or more second immunogens for administration as a boost, and the one or more additional

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immunogens for administration as a further boost being for administration in an amount sufficient to effect said induction.

12. The combination for use according to claim 11 further comprising an adjuvant for administration to said mammal.

13. The combination for use according to claim 12 wherein said adjuvant is a squalene-based adjuvant.

14. Use of a combination for inducing an immune response in a mammal comprising:

(a) a first immunogen for administration as a prime comprising an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2; and

(b) one or more second immunogens for administration as a boost selected from: an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence in MRVKGIRKNCQQHLWRWGTMLLGILMICSA SEQ ID NO: 4, and an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5; and

(c) the one or more additional immunogens for administration as a further boost selected from: an envelope polypeptide comprising all of the consecutive amino acids after the signal peptide sequence in MRVKETQMNWPNLWKWGTLILGLVIICSA SEQ ID NO: 2 and an envelope polypeptide comprising all of the amino acids after the signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3,

the first immunogen for administration as a prime, the one or more second immunogens for administration as a boost, and the one or more additional immunogens for administration as a further boost being for administration in an

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amount sufficient to effect said induction.

15. The use according to claim 14, wherein the combination further comprises an adjuvant for administration to said mammal.

16. The use according to claim 15, wherein said adjuvant is a squalene-based adjuvant.

17. A combination for use in inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4.

18. The combination for use of claim 17, further comprising at least one further boost immunogen for administration to the mammal, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2, a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

19. The combination for use of claim 18, further comprising an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3 for administration to the mammal as an additional further boost.

20. A combination for use in inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal,

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wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3.

21. The combination for use of claim 20, further comprising at least one further boost immunogen for administration to the mammal, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRYKETQMNWPNLWKWGTLILGLVIICSA in SEQ 2, a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRYKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 4, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

22. The combination for use of claim 21, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRYKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 4, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

23. The combination for use of claim 22, further comprising an additional boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 for administration to the mammal.

24. The combination for use of claim 21 or 23, further comprising an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3 for administration to the mammal as an additional further boost.

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25. A combination for use in inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

26. The combination for use of claim 25, further comprising at least one further boost immunogen for administration to the mammal, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2, a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4.

27. The combination for use of claim 26, further comprising an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3 for administration to the mammal as an additional further boost.

28. The combination for use of any one of claims 17-27, further comprising an adjuvant for administration to the mammal.

29. The combination for use of claim 28, wherein the adjuvant is a squalene-based adjuvant.

30. The combination for use of claim 28, wherein the adjuvant further comprises TLR7 and TLR9 agonists.

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31. Use of a combination for inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4.

32. The use of claim 31, wherein the combination further comprises at least one further boost immunogen for administration to the mammal, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2, a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

33. The use of claim 32, wherein the combination further comprises an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3 for administration to the mammal as an additional further boost.

34. Use of a combination for inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3.

35. The use of claim 34, wherein the combination further comprises at least one further

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boost immunogen for administration to the mammal, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRYKETQMNWPNLWKWGTLILGLVIICSA in SEQ 2, a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRYKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 4, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

36. The use of claim 35, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRYKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 4, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCOOHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

37. The use of claim 36, wherein the combination further comprises an additional boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 for administration to the mammal.

38. The use of claim 35 or 37, wherein the combination further comprises an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3 for administration to the mammal as an additional further boost.

39. Use of a combination for inducing an immune response in a mammal comprising a prime immunogen and a boost immunogen for sequential administration to the mammal, wherein the prime immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and wherein the boost immunogen comprises all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 5.

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40. The use of claim 39, wherein the combination further comprises at least one further boost immunogen for administration to the mammal, wherein the at least one further boost immunogen is selected from the group consisting of a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2, a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3, and a boost immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKGIRKNCQQHLWRWGTMLLGILMICSA in SEQ ID NO: 4.

41. The use of claim 40, wherein the combination further comprises an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3 for administration to the mammal as an additional further boost.

42. The use of any one of claims 31 to 41, wherein the combination further comprises an adjuvant for administration to the mammal.

43. The use of claim 42, wherein the adjuvant is a squalene-based adjuvant.

44. The use of claim 42, wherein the adjuvant further comprises TLR7 and TLR9 agonists.

45. A composition comprising an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQMNWPNLWKWGTLILGLVIICSA in SEQ ID NO: 2 and an immunogen comprising all the consecutive amino acids after signal peptide sequence MRVKETQRSWPNLWKWGTLILGLVIMCNA in SEQ ID NO: 3.

46. The composition of claim 45, further comprising, an adjuvant.

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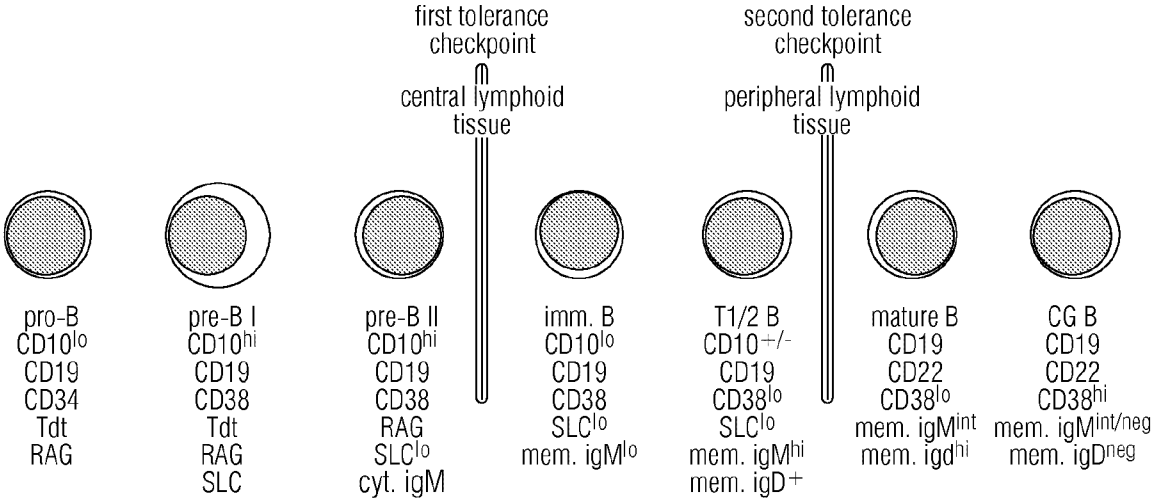
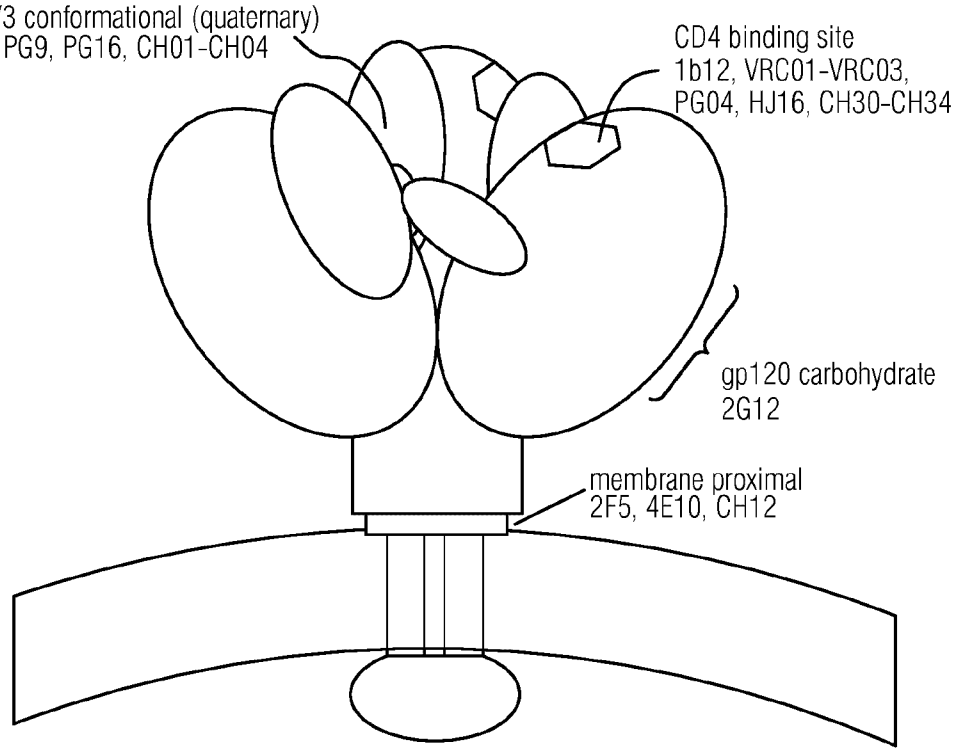


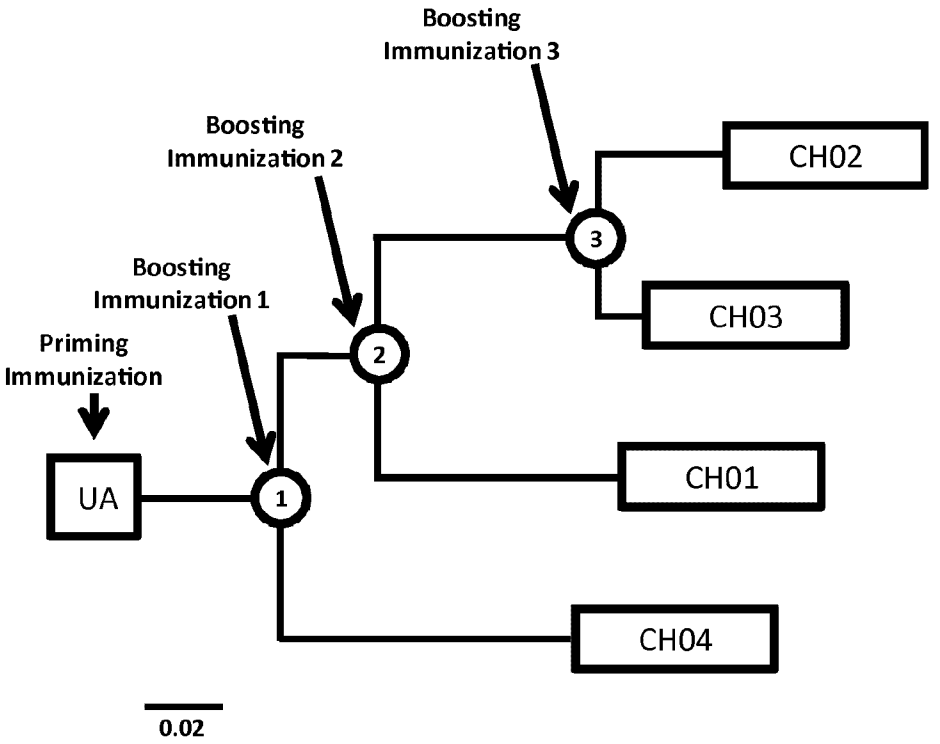
Human B cells arise from committed progenitor cells that proliferate following expression of functional immunoglobulin heavy- (H-) chain polypeptides that associate with surrogate light chains (SLC). In pre-B I cells. H-chain and SLC pairs associate with Igα/Igβ heterodimers to form pre-B cell receptors (pre-BCR) and initiate cell proliferation. When these proliferating cells exit the cell cycle as pre-B II cells, increased RAG1/2 expression drives light- (L-) chain rearrangements and the assembly of mature BCR capable of binding antigen. Most newly generated immature B cells are autoreactive and consequently lost or inactivated at the first tolerance checkpoint; the remainder mature as transitional 1 (T1) and T2 B cells characterized by changes in membrane IgM (mIgM) density, increased mIgD expression, and the loss/diminution of CD10 and CD38. Newly formed T2 B cells are subject to a second round to immune tolerization before entering the mature B cell pools. Mature B cells activated by antigens and TFH characteristically down-regulate mIgD and increase CD38 expression as they enter the germline center (GC) reaction, GC are sites on intense B-cell proliferation, AICDA dependent Ig hypermutation and class-switch recombination, and affinity maturation.

FIGURE 1



Schematic diagram of trimeric HIV-1 Env with sites of epitopes for broadly neutralizing antibodies. The four general specificities for BnAbs so far detected are: the CD4 binding site; the V2,V3 variable loops; certain exposed glycans; and the MPER. Red ovals: gp120 core; dark-red ovals, V1-V2 loops; magenta ovals: V3 loop; blue oval, gp41; bright red stripe: MPER of gp41; light brown, curved stripe: viral membrane bilayer. The PGT glycan antibodies depend on the N-linked glycan at position 332 in gp120; like the V2,V3 conformational antibodies, they also depend on the glycan at position 160.

FIGURE 2



Clonal lineage of V2,V3 conformational antibodies, CH01-CH04, their inferred intermediate antibodies (IAs, labeled 1, 2, and 3), and the inferred unmutated ancestor antibody (UA). Design of immunogens to drive such a pathway might involve producing the UA and IAs and using structure-based alterations in the antigen (i.e., changes in gp120 or gp140 predicted to enhance binding to UA or IA) or deriving altered antigens by a suitably designed selection strategy. Vaccine administration might prime with the antigen that binds UA most tightly, followed by sequential boosts with antigens optimized for binding to each IA. For this clonal lineage, an Env known to bind the UA (AE.A244 gp120: ref 21) could be a starting point for further immunogen design.

FIGURE 3

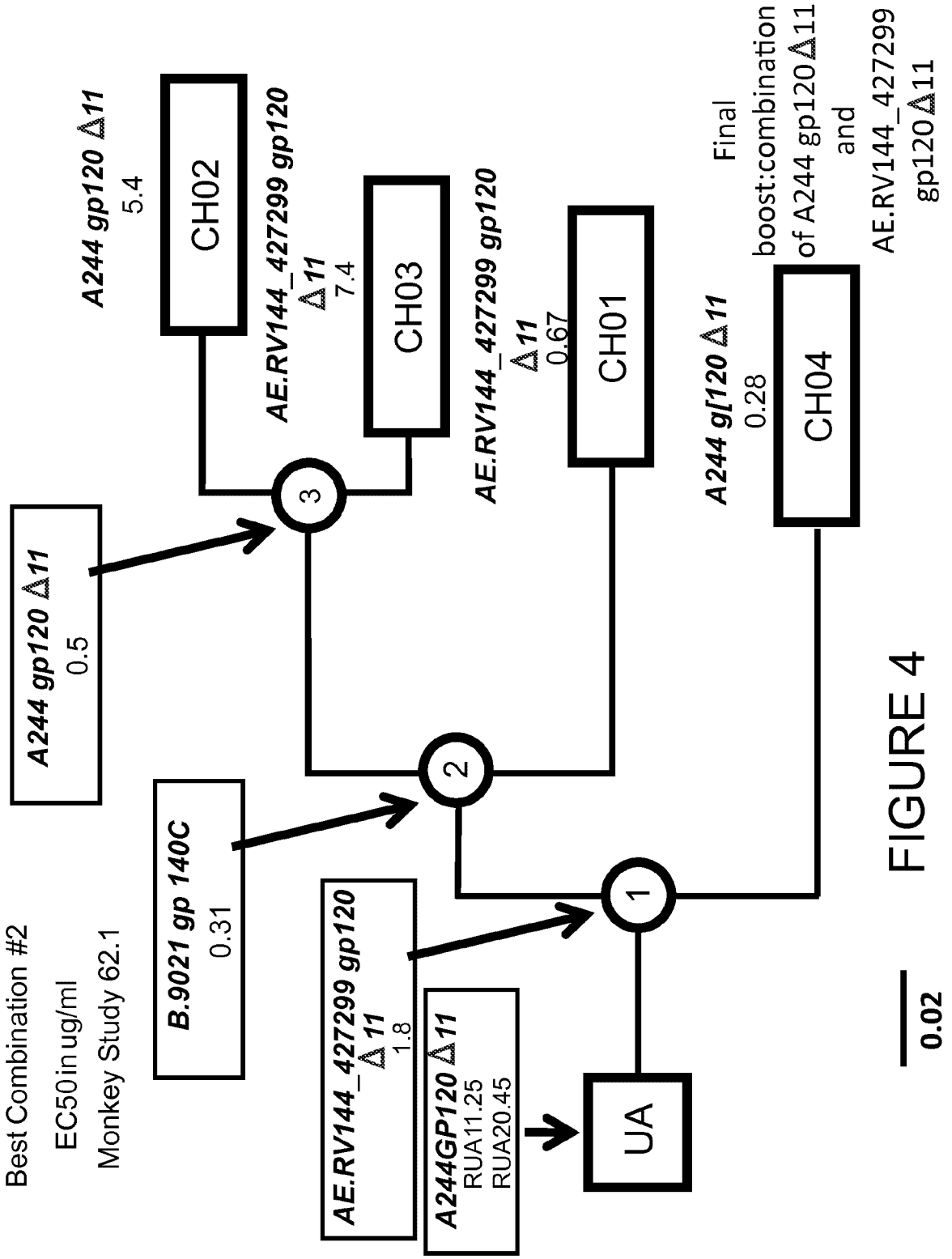

Best Combination #2
EC50 in ug/ml
Monkey Study 62.1
A244 gp120 Δ11
0.5
B.9021 gp 140C
0.31
AE.RV144_427299 gp120
Δ11
1.8
A244GP120 Δ11
RUA11.25
RUA20.45
UA
1
2
3
A244 gp120 Δ11
5.4
CH02
AE.RV144_427299 gp120
Δ11
7.4
CH03
AE.RV144_427299 gp120
Δ11
0.67
CH01
A244 g[120 Δ11
0.28
CH04
Final
boost:combination
of A244 gp120Δ11
and
AE.RV144_427299
gp120Δ11
0.02

FIGURE 4

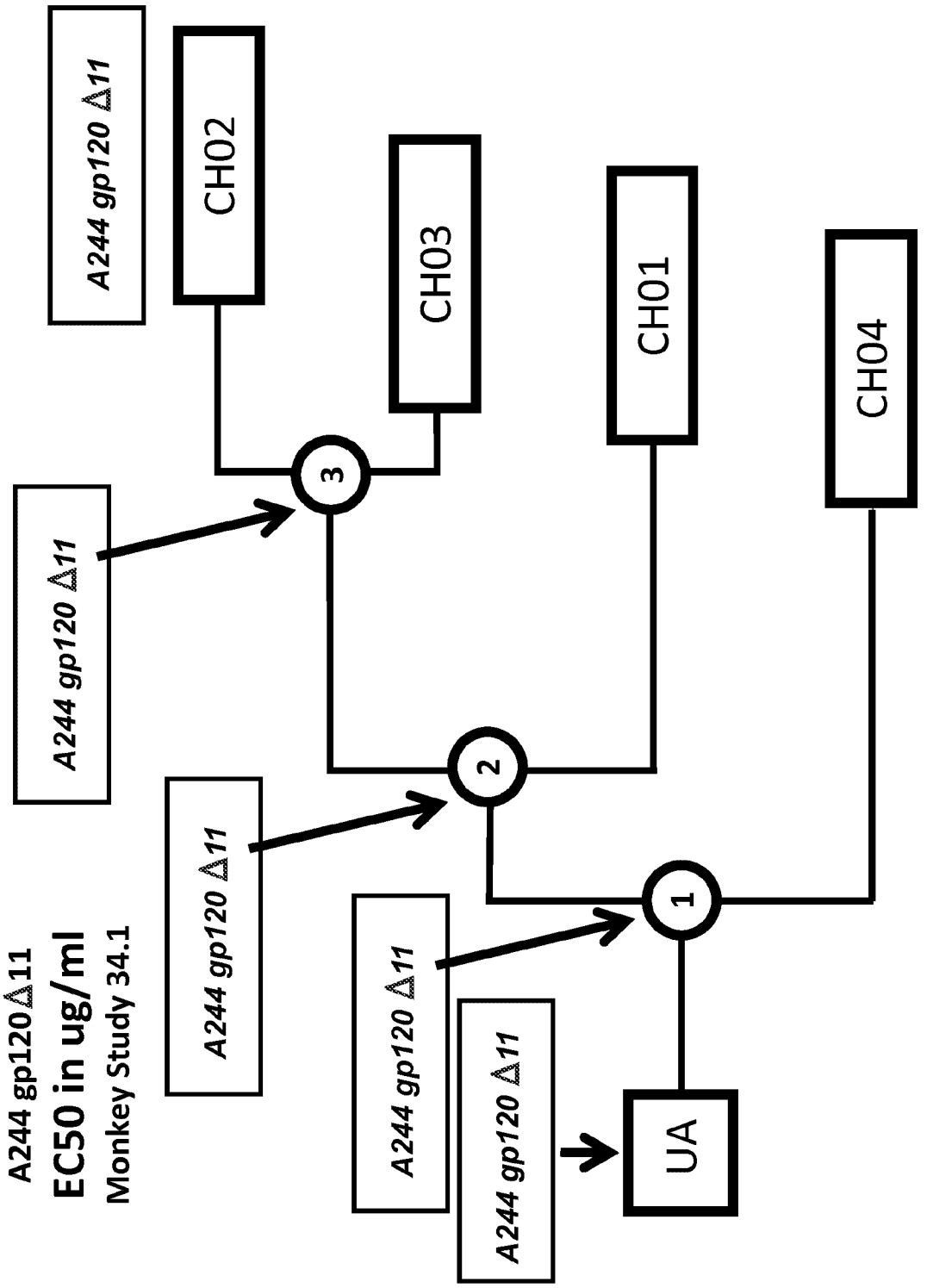

A244 gp120Δ11
EC50 in ug/ml
Monkey Study 34.1
A244 gp120 Δ11
A244 gp120 Δ11
A244 gp120 Δ11
A244 gp120 Δ11
UA
1
2
3
CH02
CH03
CH01
CH04
0.02

FIGURE 5

Similar Levels of Binding Antibodies to A244 gp120D11 Induced by A244 gp120D11 Alone (NHP #34.1) and Sequential Env Immunization (NHP #62.1)

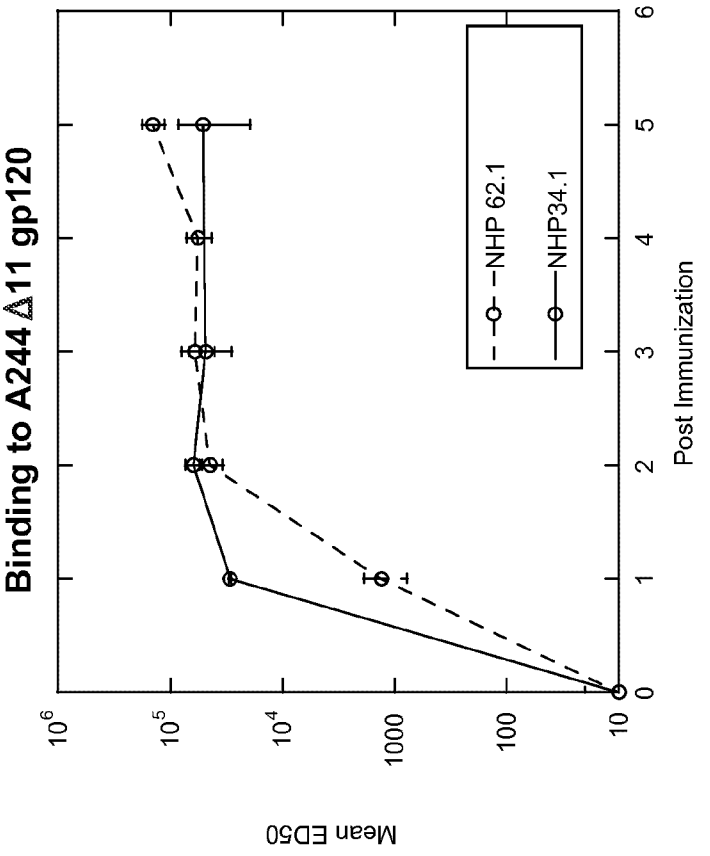


FIGURE 6

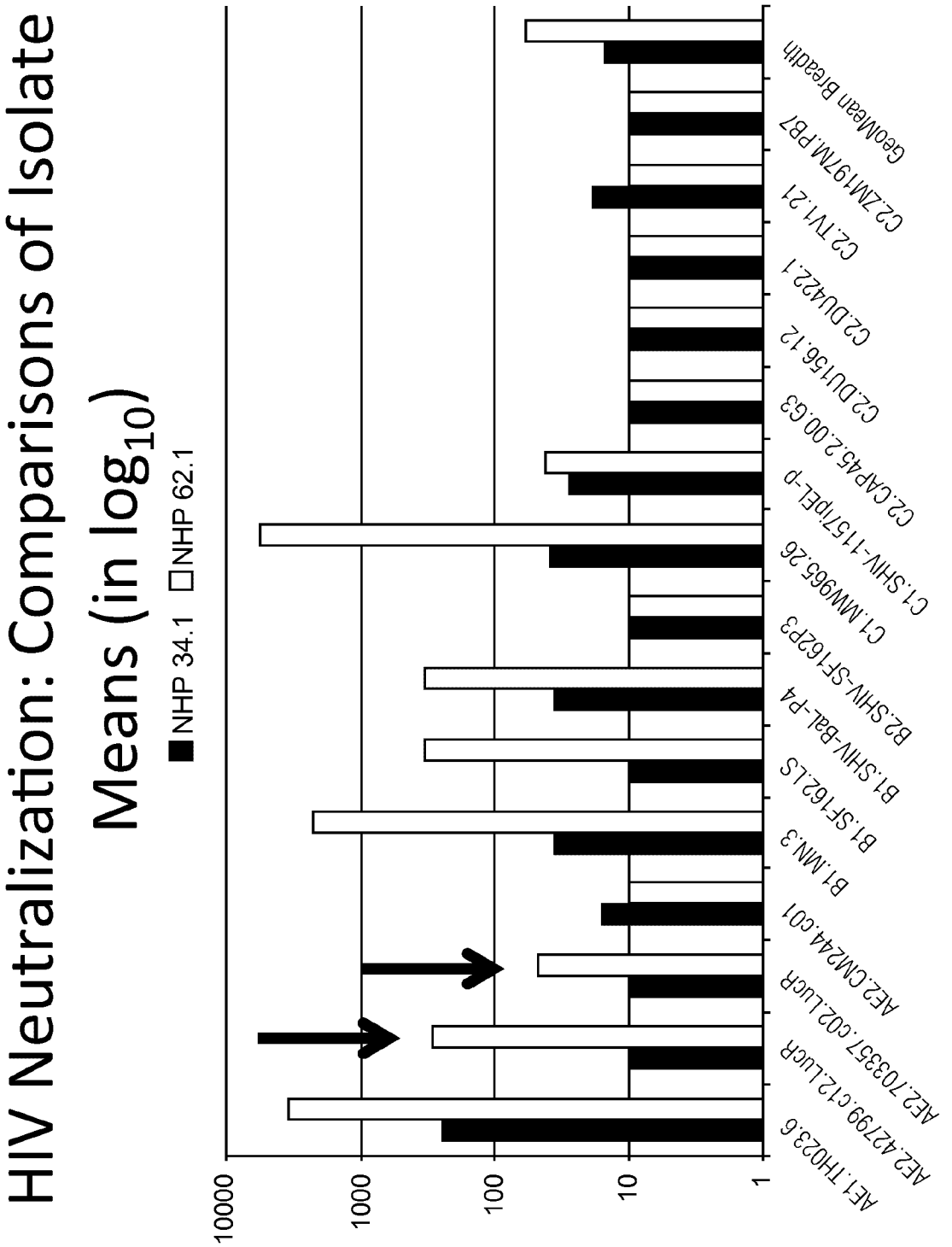

HIV Neutralization: Comparisons of Isolate Means (in log10)
NHP 34.1
NHP 62.1
10000
1000
100
10
1
A.E1.TH023.6
A.E2.4279.c12.LucR
A.E2.703357.c02.LucR
A.E2.CM244.c01
B1.MN.3
B1.SF162.LS
B1.SHIV-BaL-P4
B2.SHIV-SF162P3
C1.MM965.26
C1.SHIV-1157IPEL-P
C2.CAP45.2.00.G3
C2.DU156.12
C2.DU422.1
C2.TV1.21
C2.ZM197M.PB7
GeoMean Breadth

FIGURE 7

>A244 gp120
MRVKETQMNWPNLWKWGTLILGLVIICSASDNLWVTVYYGVPVWKEADTTLFCASDAKAHETEVHNVWATHAC
VPTDPNPQEIDLENVTENFNMWKNNMVEQMQEDVISLWDQSLKPCVKLTPPCVTLHCTNANLTKANLTNVNNR
TNVSNIIGNITDEVRNCSFNMTTELRDKKQKVHALFYKLDIVPIEDNNDSSEYRLINCNTSVIKQPCPKISFD
PIPIHYCTPAGYAILKCNDKNFNGTGPCKNVSSVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLTNNAKTI
IVHLNKSVVINCTRPSNNTRTSITIGPGQVFYRTGDIIGDIRKAYCEINGTEWNKALKQVTEKLKEHFNNKPI
IFQPPSGGDLEITMHHFNCRGEFFYCNTTRLFNNTCIANGTIEGCNGNITLPCKIKQIINMWQGAGQAMYAPP
ISGTINCVSNITGILLTRDGGATNNTNNETFRPGGGNIKDNWRNELYKYKVVQIEPLGVAPTRAKRRVVEREK
R
>AE.427299Δ11gp120
MRVKETQRSWPNLWKWGTLILGLVIMCNAVPVWRDADTTLFCASDAQAHVTEVHNIWATHACVPTDPNPQEIH
LENVTENFNMWKNNMAEQMQEDVISLWDQSLKPCVKLTPLCVTLKCTANITITNATTRTENTTKENLIGNITD
ELRNCSFNVTTELRDRQRKAYALFYKLDIVPINNEANSSEYRLINCNTSVIKQACPKVSFDPIPIHYCTPAGY
AILKCNDKNFNGTGPCKNVSSVQCTHGIKPVVSTQLLLNGSLAEDEIIIRSENLTDNSKNIIVHLNESVVINC
TRPSNNTVKSIRIGPGQTFYRTGDIIGDIRQAYCNVNGTKWYEVLRNVTKKLKEHFNNKTIVFQQPPPGGDLE
ITTHHFNCRGEFFYCNTTELFNNTCVNETINNGTEGWCKGDIILPCRIKQIINLWQEVGQAMYAPPVSGQIRC
ISNITGIILTRDGGNGKNGTLNNETFRPGGGNMKDNWRSELYKYKVVEIEPLGIAPSRAKERVVEMKREKE
>B.9021 gp140C
MRVKGIRKNCQQHLWRWGTMLLGILMICSAAENLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHA
CVPTDPNPQEMVLENVTEYFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLTCTDYEWNCTGIRNSIC
KYNNMTNNSSSGNYTGWERGEIKNCSFNSTISGIRDKVRKEYALLYKIDLVSIDGSNTSYRMISCNTSVITQS
CPKISFEPIPLHYCTPAGFALLKCNDKKFNGTGLCHNVSTVQCTHGIKPVVSTQLLLNGSLAEEEVVIRSKNF
TDNAKIIIVQLNETVEINCTRPGNNTRKSIHIAPGRTFYATGEIIGDIRRAHCNISREKWNTTLHRIATKLRE
QYNKTIVFNQSSGGDPEIVMHSVNCGGEFFYCNTSKLFNSTWNSTGGSISEDSENITLPCRIKQIVNMWQEVG
KAMYAPPIRGQIRCSSNITGLLLTRDGGINQSISETFRPGGGDMRDNWRSELYKYKVVKIEPLGIAPTKARER
VVQREKEAVGIGAVFLGFLGAAGSTMGAASLTLTVQARLLLSGIVQQQNNLLRAIEAQQHMLQLTVWGIKQLQ
ARVLALERYLRDQQLMGIWGCSGKLICTTAVPWNASWSNKSLNDIWNNMTWMQWEREIDNYTGLIYSLLEESQ
NQQEKNEQDLLALDKWANLWTWFDISNWLWYIK

FIGURE 8

>9021 Δ11 gp120
MRVKGIRKNCQQHLWRWGTMLLGILMICSAVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQE
MVLENVTEYFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLTCTDYEWNCTGIRNSICKYNNMTNNS
SSGNYTGWERGEIKNCSFNSTISGIRDKVRKEYALLYKIDLVSIDGSNTSYRMISCNTSVITQSCPKISFEP
IPLHYCTPAGFALLKCNDKKFNGTGLCHNVSTVQCTHGIKPVVSTQLLLNGSLAEEEVVIRSKNFTDNAKII
IVQLNETVEINCTRPGNNTRKSIHIAPGRTFYATGEIIGDIRRAHCNISREKWNTTLHRIATKLREQYNKTI
VFNQSSGGDPEIVMHSVNCGGEFFYCNTSKLFNSTWNSTGGSISEDSENITLPCRIKQIVNMWQEVGKAMYA
PPIRGQIRCSSNITGLLLTRDGGINQSISETFRPGGGDMRDNWRSELYKYKVVKIEPLGIAPTKARERVVQR
EKE

FIGURE 9