Small Molecule Mimetics of an HIV-1 gp41 Fusion Intermediate as Vaccine Leads

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We describe here a novel platform technology for the discovery of small molecule mimetics of conformational epitopes on protein antigens. As a model system, we selected mimetics of a conserved hydrophobic pocket within the N-heptad repeat region of the HIV-1 envelope protein, gp41. The human monoclonal antibody, D5, binds to this target and exhibits broadly neutralizing activity against HIV-1. We exploited the antigen-binding property of D5 to select complementary small molecules using a high throughput screen of a diverse chemical collection. The resulting small molecule leads were rendered immunogenic by linking them to a carrier protein and were shown to elicit N-heptad repeat-binding antibodies in a fraction of immunized mice. Plasma from HIV-1-infected subjects shown previously to contain broadly neutralizing antibodies was found to contain antibodies capable of binding to haptens represented in the benzylpiperidine leads identified as a result of the high throughput screen, further validating these molecules as vaccine leads. Our results suggest a new paradigm for vaccine discovery using a medicinal chemistry approach to identify lead molecules that, when optimized, could become vaccine candidates for infectious diseases that have been refractory to conventional vaccine development.

Immunologists have a long history of studying the diversity of antibodies and antibody-producing cells. First by empirical observation and subsequently through understanding at the molecular level, the basis for antibody diversity is now well understood (1–4). By contrast, the diversity of antigens recognized by individual antibodies has had limited study (5, 6). Polyreactive antibodies associated with autoimmune diseases have been identified, and, in certain cases, the molecular basis for autoreactivity has been linked to unusual antibody structures (e.g. the use of extended CDR3 regions (7, 8) or amino acid sequences in the VH region that result in highly charged cationic antibodies that can deposit on basement membranes and cause glomerulonephritis (9)). However, the number and diversity of antigens and epitopes recognized by a given antibody have received very little attention. Previous biomolecule mimotope approaches have often focused on discovery of short peptides as mimetics of polysaccharide, protein, or toxin structures (10, 11). Typically, screening approaches utilizing phage display libraries (12) have been employed for identification of peptide leads, with further optimization effected through synthetic manipulations of the sequence. Some instances of synthetic combinatorial libraries for di- and isopeptide mimetics have also been described (13). Although these approaches have generated some measure of success, peptide mimetics of HIV-1 neutralizing antibody targets have thus far not proven to be useful vaccine candidates (14–16), perhaps because peptide mimetics do not represent highly constrained molecular species. We sought to overcome the problems inherent in peptide-based approaches to developing an HIV-1 mimotope vaccine by searching for small molecule haptens that, when conjugated to a heterologous carrier protein, could potentially elicit antibodies similar in specificity and function to the corresponding monoclonal antibody used to screen for the small molecule itself. D5, an HIV-1-neutralizing human monoclonal antibody, is known to bind to a highly conserved hydrophobic pocket within the N-heptad repeat (NHR) region of gp41 (17, 18). Here we exploited the antigen-binding property of D5 to select complementary small molecules using a high throughput screen (HTS) of a diverse chemical collection. The resulting small molecule leads were rendered immunogenic by linking them to a carrier protein and are amenable to a medicinal chemistry approach to optimize their utility as a vaccine.

EXPERIMENTAL PROCEDURES

High Throughput Screen for Hapten Mimetics of HIV-1 NHR Pocket—An in vitro binding assay was developed using D5 IgG conjugated to europium chelate (Eu-D5) and a biotinylated gp41 mimetic molecule, 5-helix, that presents the hydrophobic pocket in a stabilized structural context (19). The assay readout is based on a time-resolved fluorescence reso-

4 The abbreviations used are: NHR, N-heptad repeat; APC, allophycocyanin; SA, streptavidin; CDR, complementarity-determining region; CRM197, cross-reactive mutant protein of diphtheria toxin; FRET, fluorescence resonance energy transfer; HTS, high throughput screen; DCBA, D5 competitive binding assay; SPR, surface plasmon resonance; Aha, 6-amino-hexanoic acid; TMB, 3,3′,5,5′-tetramethylbenzidine; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.
nance energy transfer format. Biotin-5-helix binds to an allophycocyanin (APC)-conjugated streptavidin (SA) molecule to form a 5-helix-SA-APC complex. When Eu-D5 binds to the hydrophobic pocket of biotin-5-helix, it brings the europium into close proximity with the APC substrate, resulting in time-resolved fluorescence resonance energy transfer from europium to APC (340-nm excitation, 620-nm (europium) and 665-nm (APC) emission). Agents that interfere with the formation of the complex will cause a decrease in the ratio value. For the HTS screening campaign, the binding reaction was reduced to a 2.5-μl volume with final concentrations of 2.5 mM 5-helix, 1.2 mM Eu-D5, 3 mM SA-APC, 40 μM test compounds and 20-min binding time.

For the primary screen, an inhibition cut-off value of 31% was employed, along with the following filter criteria: 1) elimination of biotin-containing compounds, 2) elimination of compounds with undefined side chains (structures containing generic “R” or “X” groups), and 3) elimination of any compounds that scored in more than five unrelated screens. The number of positive compounds identified after application of the filters was 5,679. Two inhibition thresholds were used to score a compound as positive following F19 counter-screening: 1) >25% D5 inhibition and <20% F19 inhibition and 2) D5 inhibition > F19 inhibition + 20%. Using the more stringent filter (the first), 120 hits were identified, whereas 154 hits were found using filter 2.

Identification of Class II Compounds by Biacore A100
Screening—Surface plasmon resonance (SPR) experiments were carried out using a Biacore A100. Immobilization of all proteins (D5, 5-helix, 6-helix, and a nonspecific IgG1) was performed using amine coupling to a carboxymethylated dextran chip (CM5). Amine coupling on spots 1, 2, 4, and 5 (spot 1 used as reference) of each flow cell was accomplished by activating the chip surface with a 10-min injection of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and activating the chip surface with a 10-min injection of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and 1-ethyl-N,N,N'-tetramethyluronium tetrafluoroborate (Pierce). Briefly, CRM27 was dissolved at 1 mg/ml in 25 mM HEPES, pH 7.3, 0.15 M sodium chloride, 5 mM EDTA (HBS/EDTA) and mixed with a 10-fold molar excess of succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate (Pierce). Briefly, CRM27 was dissolved at 1 mg/ml in 25 mM HEPES, pH 7.3, 0.15 M sodium chloride, 5 mM EDTA (HBS/EDTA) and mixed with a 10-fold molar excess of succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate relative to free amine groups for 3 h at 22 °C. The maleimido-containing protein was purified from reaction components by desalting on a HiPrep 26/10 column (GE Biosciences) equilibrated in HBS/EDTA. Maleimide incorporation was quantified by measuring the free thiol consumption of N-acetylcysteine. The average derivatization was 139 nmol of maleimide/mg of CRM27. Thiocarboxylated hapten was dissolved in ethanol at a concentration of 10 mg/ml and subsequently mixed with maleimidated CRM27 (0.25 mg/ml) at a 3:1 molar ratio of thiol/maleimide in HBS/EDTA containing 10% ethanol. The conjugation reaction was allowed to proceed at 22 °C for 2 h, at which time any precipitated protein was removed by centrifugation. The clarified supernatant was dialyzed for 24 h at 22 °C against 25 mM HEPES, pH 7.3, 0.15 M sodium chloride and then concentrated ~4-fold using a 30,000 molecular weight cut-off membrane. Conjugation efficiency was determined by amino acid analysis for quantitation of 6-amino-hexanoic acid, and 5-dicarboxyethylcysteine, a unique residue generated by formation of a covalent bond between hapten and carrier (21).

Mouse immunogenicity study—4–5-week-old female Balb/c mice (Taconic, Hudson, NY) were maintained in the animal facilities of Merck Research Laboratories in accordance with institutional guidelines. All animal experiments were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. Hapten-CRM27 conjugates were formulated with 450 μg of Merck aluminum adjuvant and 0.5 mg of IMO-2055 (Idera Pharmaceuticals, Inc., Cambridge, MA) per ml in PBS. Mice (10 mice/group) were immunized intramuscularly with 100 μl of the vaccine containing 25 μg of total conjugate protein four times at 2-week intervals. Serum samples obtained from tail vein punctures were collected in Microtainer® serum separator tubes (BD Biosciences) preimmunization and at weeks 4, 6, and 8. Serum samples were stored at −20 °C until tested.

ELISA for Mouse Serum—Binding activity of mouse antisera were carried out by ELISA. 96-well streptavidin-coated Reacti-Bind plates (Pierce) were coated with 50 μl/well biotinylated test antigens, including individual hapten and 5-helix; alternatively, uncoated Maxisorp Immunoplate plates (Nunc)
were coated with 50 μl/well non-biotinylated test antigens, including (CCIZN17)_3. To quantify antibodies that recognized the Aha linker, a non-relevant biotinylated influenza peptide, HA022B (Ac-Aha-EGPAKLLKRGFFGAIGFLEECNH), was used as coating antigen. In addition, mouse antisera were tested against CRM197 alone. Each substrate was coated at a concentration of 2 μg/ml (or 4 μg/ml for HA022B) overnight at 4 °C. Plates were washed six times with PBS containing 0.05% Tween 20 (PBST) and blocked with 3% skim milk in PBST (milk-PBST). Mouse test antisera (100 μl/well) was prepared in milk-PBST starting at 1:5000 dilution in milk-PBST was added per well and incubated at room temperature for 1 h. Plates were washed six times, followed by the addition of 100 μl/well SuperBlu™ TMB solution (Virolabs, Chantilly, VA). After a 3–5-min incubation at room temperature, the reaction was stopped by adding 100 μl of stop solution for TMB (Virolabs) per well. Plates were read at 450 nm in a microplate reader. Titors were determined by the reciprocal of the dilution that was above background plus two S.D. values.

**ELISA for Human Plasma**—Plasma from a well characterized panel of HIV-1-infected subjects with documented broadly neutralizing antibody activity (22) and plasma from 10 control subjects were tested for binding to biotinylated hapten, 5-helix, and control antigens. For biotinylated antigens, Neutravidin-coated plates (Pierce) were used instead of Streptavidin-coated plates because the latter gave high background responses with the HIV-1 plasmas. Neutravidin plates were washed three times, and then 50 μl of test antisera at a concentration of 2 μg/ml (or 4 μg/ml for HA022B) was added and incubated overnight at 4 °C. Plates were washed six times with PBST and blocked with milk-PBST. Plasma samples were diluted 1:500 in milk-PBST and then added at 100 μl/well. The plates were incubated for 2 h at room temperature and then washed six times with PBST. HRP-conjugated goat anti-mouse IgG (H+L) secondary antibody (Invitrogen) at 1:5000 dilution in milk-PBST blocks the intramolecular folding of gp41 into a six-helix bundle structure that is essential for membrane fusion and viral entry. A protein mimetic of the NHR trimmer, termed 5-helix (19), was used as the ligand for D5, and the assay was based on inhibition of fluorescence resonance energy transfer (FRET) from europium-labeled D5 to APC-labeled 5-helix. The HTS conducted with a Merck screening library of >1.5 million small molecule compounds resulted in 5679 “hits” that disrupted the FRET signal. To eliminate compounds that caused nonspecific inhibition, a counterscreen was conducted using F19, a non-neutralizing monoclonal antibody that binds to 5-helix outside of the D5 epitope and is non-neutralizing.

**RESULTS**

Selection of Small Molecule Mimetics of the HIV-1 NHR Pocket—The D5 competitive binding assay (DCBA) used as the basis for HTS is shown schematically in Fig. 1. Monoclonal antibody D5 specifically recognizes and binds to a well characterized hydrophobic pocket contained within the NHR domain of HIV-1 envelope glycoprotein gp41. This binding blocks the intramolecular folding of gp41 into a six-helix bun-

**Kinetic Binding Parameters for Small Molecule Mimetics**—Selection of molecules for limited SAR was based on evalu-
Molecular Modeling of Hapten Binding to D5—In order to render a small molecule hapten immunogenic, it must first be covalently coupled with a carrier protein to provide helper T cell epitopes required for generation of antibodies. The haptens must be derivatized in such a way as to preserve the ability to represent the native antigen and bind the antibody while providing a linker to the protein carrier. Computational chemistry studies were utilized to model binding of select haptens to D5 as a mimic of 5-helix. Using so-called sphere points, which were chosen to serve as mimotopes of the critical residues in gp41, conformations of the haptens were docked in and ranked using the FLOG algorithm (24). The published crystal structure of D5 bound to 5-helix (18) was used in all modeling studies. An N-Boc aminohexanoic acid motif (Aha-Boc) attached to the hapten ligand was used to represent the linked carrier for modeling and binding studies. The epitope for D5 antibody binding lies in the hydrophobic pocket region located near the carboxyl-terminal half of the NHR trimer. Amino acids Leu<sup>568</sup>, Trp<sup>571</sup>, and Lys<sup>574</sup> of gp41 (strain HXB2 numbering) are critical for antibody binding, whereas Val<sup>570</sup> contributes to a lesser extent. In the pose shown in Fig. 3C, Aha-Boc-derivatized compound 6 (aqua) is shown overlaid on the three 5-helix residues (gold) that make critical contacts in the CDR pocket of D5. Importantly, the region of the hapten incorporating the Aha-Boc spacer arm is predicted to point away from the contact residues of the antibody-combining site. Accordingly, parental compounds 4 – 8 were prepared as their Aha and Aha-Boc derivatives, and their binding to D5 was confirmed by SPR, with no loss of affinity observed (Fig. 3B and supplemental Fig. S2).

Immunogenicity of Hapten-Carrier Conjugates—CRM<sub>197</sub>, a mutant diphtheria toxin (20), was used as the carrier protein for conjugation of compounds 4 – 8. Covalent coupling between hapten and protein was confirmed by gel electrophoresis (Fig. 4) and identification of 5-dicarboxyethylcysteine by quantitative amino acid analysis (data not shown). Typical hapten loading on carrier protein was ∼15% (w/w). Groups of 10 BALB/c mice were immunized with individual conjugates four times at biweekly intervals with 25 μg of total protein co-formulated with Merck aluminum adjuvant and a TLR-9 agonist (IMO-2055), and an additional group received a mixture of all five conjugates dosed at 5 μg of each component. A peptide mimetic, (CCIZN17)<sub>3</sub>, which presents the HIV-1 NHR hydrophobic pocket in the context of a structured and highly thermostable trimer (25), was used as a positive control for the mouse immunogenicity studies. The results shown in Table 1 indicate that the hapten-carrier conjugates elicited very high titered antibodies to individual self-haptens and that the antiseras cross-reacted strongly to each heterologous hapten. We tested the sera for binding to 5-helix because the NHR pocket was the only common sequence element present in both (CCIZN17)<sub>3</sub> and 5-helix. We found that ∼90% of individual serum samples were negative for binding to 5-helix, even after 4 doses; however, 7 of 60 individual serum samples from the conjugate-immunized mice did show detectable binding to 5-helix (Fig. 5). All mice immunized with (CCIZN17)<sub>3</sub> produced detectable antibodies to 5-helix, although the geometric mean titer to 5-helix was >10-fold.
FIGURE 3. D5 binding parameters of HTS hits and structures for selected derivatized haptens. A, hits were tested for binding to D5 by SPR, and $k_{\text{on}}/k_{\text{off}}$ rates are plotted against $k_{\text{off}}$ rates. Compounds with the desired fast-on, slow-off kinetic profile are in the upper left quadrant. B, generalized chemical structures of derivatized haptens prepared for conjugation to carrier protein and to biotin are shown as compounds 1–3; specific structures of the benzylpiperidine haptens (compounds 4–8) selected for conjugation are displayed with their SPR binding parameters ($k_D$/$\log k_{\text{on}}$) for parent, Aha-derivatized, and Aha-Boc-derivatized compounds. C, FLOG model of Aha-Boc-derivatized 6 in the D5 antibody-combining site. Molecular modeling was performed utilizing the published crystal structure of D5 bound to 5-helix. In this pose, Aha-Boc 6 (aqua) is shown overlaid on the three 5-helix residues (gold) that make critical contacts in the CDR pocket of the antibody.
lower than that to the immunizing peptide (Table 1), similar to the observation with the hapten-carrier conjugates.

Because our hapten mimetics were selected to promote a D5-like antibody response, we wished to determine whether D5-like antibodies are elicited during the course of natural HIV-1 infection. To do this, we tested a panel of plasma samples from HIV-1-infected individuals that were previously shown to contain broadly neutralizing antibody responses against clade B and C viruses (22) in the DCBA. As shown in Table 2, 18 of 19 plasmas had IC$_{50}$ values (reciprocal dilution with 50% inhibition) of ≥ 20, suggesting the presence of D5-like antibodies. The HIV-1 plasmas were also screened for the ability to bind directly to 5-helix and to biotinylated hapten 4–8 as well as to HA022B, a non-relevant influenza virus peptide conjugated to biotin using the same chemistry as was employed for preparation of the hapten-carrier conjugates, which served as a control for nonspecific recognition of the Aha linker portion of the conjugate. When tested at a dilution of 1:500, plasma from 16 of 19 HIV-1-infected subjects was found to contain antibodies that bound to at least one of the benzylpiperidine hapten with an $A_{450}$ nm value > 3-fold above the average background response of 12 non-infected control plasma samples. The results suggest that the benzylpiperidine hapten may be relevant mimotopes for the induction of D5-like antibodies.

**DISCUSSION**

Although potent broadly neutralizing antibodies against HIV-1 have been identified, construction of complementary immunogens has been problematic (14, 26–28). This is an inherent problem with peptide-based vaccines due to rotational flexibility of peptide bonds. Attempts have been made to constrain HIV-1 peptide mimetics into coiled coil trimers by adding non-HIV-1 sequences, such as the GCN4 sequence from yeast zinc finger proteins, but with limited success as a vaccine. A second problem is the immunodominance of non-neutralizing epitopes, which is an issue during natural infection as well as for vaccine constructs that may contain non-native features added to provide structure to the immunogen.

Because of their size, small molecule hapten displays a much more limited amount of molecular flexibility when compared with an extended polypeptide sequence (29–32). Furthermore, many small molecules comprising screening collections contain a variety of ring systems with a reduced number of rotatable bonds, further restricting the conformational space available to them. For small molecules acting as mimotopes of biomolecules, this restricted structural flexibility offers a potential advantage from an immunological viewpoint in terms of conformational display because a larger fraction of the response could be directed toward the biologically relevant epitope. Additionally, the small size of the mimotope may reduce misdirection of the immune response to non-neutralizing epitopes.

Although only a small fraction of immunized mice responded to benzylpiperidine hapten-carrier conjugates to produce antibodies that cross-reacted with 5-helix, the results from this investigation provide proof of concept that small molecule hapten may be relevant building blocks for a vaccine. It is possible that other hits identified in the current screen would make better mimotopes than those originally

**FIGURE 4.** Gel shift analysis of hapten-carrier conjugates. Thiolated hapten 4–8 were conjugated to CRM$_{197}$ and after the removal of free hapten, conjugates were electrophoresed on a 4–12% NuPAGE BisTris gel in MOPS running buffer (Invitrogen), and protein bands were visualized with a colloidal Coomassie Blue stain. Hapten-carrier conjugates (lanes 3–7) showed a clear shift in migration toward higher molecular mass relative to a CRM$_{197}$-only control (lane 2), indicative of covalent coupling of hapten to protein. Lane 1, molecular weight markers.

**TABLE 1**

Antibody response to hapten-CRM$_{197}$ conjugates in mice

| Immunogen | 4-Biotin | 5-Biotin | 6-Biotin | 7-Biotin | 8-Biotin | 5-Helix-biotin (CCIZN17)$_3$ |
|-----------|---------|---------|---------|---------|---------|-----------------------------|
| 4-CRM$_{197}$ | 569,832 | 572,349 | 390,902 | 577,991 | 660,798 | 85 |
| 5-CRM$_{197}$ | 351,550 | 721,366 | 1,141,213 | 939,158 | 1,003,875 | 79 |
| 6-CRM$_{197}$ | 240,282 | 237,640 | 670,769 | 323,664 | 989,409 | 54 |
| 7-CRM$_{197}$ | 259,769 | 314,699 | 305,457 | 254,665 | 191,631 | 64 |
| 8-CRM$_{197}$ | 147,705 | 226,279 | 270,264 | 255,218 | 328,073 | 65 |
| Mixture of 4–8-CRM$_{197}$ | 243,350 | 232,811 | 360,402 | 276,403 | 425,011 | 73 |
| CRM$_{197}$ | 234 | 150 | 140 | 160 | 183 | 50 |
| CCIZN17$_3$ | 50 | 74 | 50 | 50 | 54 | 53,459 |

**Post-dose 4 GMT to plates coated with**

| 1/dilution |
|-----------|
| 85 | 61 |
| 79 | 50 |
| 54 | 60 |
| 64 | 50 |
| 66 | 50 |
| 65 | 50 |
| 73 | 50 |
| 73 | 50 |
| 50 | 50 |

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selected or that the first set could be further optimized to more completely occupy the antibody combining site or to increase the affinity for D5. Alternatively, the low response rate may be due to the nature of the D5 binding “pocket,” which is hydrophobic and relatively flat. Epitopes that are more protruding, such as the CD4 binding site epitope on HIV-1 gp120 recognized by the neutralizing mAb 1b12 (33), might serve as better candidates for selection of small molecule mimotopes. In addition, other HIV-1-neutralizing human mAbs, such as 2F5, 2G12, 4E10, or the newly described PG9 and PG16 broadly neutralizing antibodies (34), might make better or complementary targets for additional HTS experiments.

This investigation represents the first attempt to discover potential small molecule vaccine mimetics based on high throughput small molecule library screening. The results show that the combining site of a single monoclonal antibody can accommodate an array of small molecule ligands with different chemical structures and affinities. We further show the utility of using SPR as a tool to select and optimize vaccine leads (e.g. synthesis of molecules with fast on-rates to mimic short lived fusion intermediates). The resulting small molecules can be rendered immunogenic by linking them to a carrier protein as demonstrated here. An optimized set of benzylicpiperidine molecules resulting from the screen were conjugated to a carrier protein and shown to elicit antisera

TABLE 2
Antibody binding to biotinylated haptens in plasma from HIV-1-infected subjects and controls as measured by ELISA

| Human plasma | HIV-neutralizing | DCBA IC₅₀ | 4-Biotin | 5-Biotin | 6-Biotin | 7-Biotin | 8-Biotin | 5-Helix-biotin | HA022B-biotin (control) |
|--------------|-----------------|----------|---------|---------|---------|---------|---------|---------------|------------------------|
|              | activity        |          |         |         |         |         |         |               |                        |
| Z 1648       | +               | 26       | 0.71    | 0.81    | 0.41    | 1.28    | 0.10    | 2.58          | 0.10                   |
| Z 1652       | +               | 410      | 0.61    | 0.83    | 0.64    | 1.69    | 0.15    | 2.62          | 0.17                   |
| Z 1686       | +               | 207      | 0.93    | 0.67    | 0.52    | 1.49    | 0.20    | 2.54          | 0.17                   |
| Z 1702       | +               | 20       | 0.13    | 0.12    | 0.13    | 0.18    | 0.10    | 1.75          | 0.10                   |
| BB8          | +               | <20      | 0.84    | 0.43    | 0.34    | 1.42    | 0.28    | 2.35          | 0.31                   |
| BB12         | +               | 30       | 0.69    | 0.25    | 0.20    | 0.68    | 0.12    | 2.54          | 0.12                   |
| BB14         | +               | 66       | 0.79    | 0.49    | 0.28    | 1.00    | 0.25    | 2.33          | 0.24                   |
| BB21         | +               | 498      | 0.83    | 0.76    | 0.45    | 1.72    | 0.16    | 2.37          | 0.15                   |
| BB24         | +               | 75       | 0.45    | 0.32    | 0.18    | 0.93    | 0.15    | 2.51          | 0.10                   |
| BB28         | +               | 60       | 0.51    | 0.50    | 0.34    | 1.61    | 0.24    | 2.56          | 0.18                   |
| BB34         | +               | 57       | 0.59    | 0.63    | 0.49    | 1.33    | 0.34    | 2.13          | 0.29                   |
| BB47         | +               | 41       | 0.66    | 0.50    | 0.32    | 1.49    | 0.17    | 2.30          | 0.16                   |
| B55          | +               | 2732     | 0.83    | 0.58    | 0.54    | 1.17    | 0.30    | 2.62          | 0.21                   |
| BB68         | +               | 72       | 0.41    | 0.42    | 0.21    | 0.66    | 0.16    | 2.51          | 0.18                   |
| BB75         | +               | 685      | 0.79    | 0.65    | 0.74    | 1.19    | 0.28    | 2.47          | 0.17                   |
| BB80         | +               | 417      | 0.31    | 0.40    | 0.35    | 0.92    | 0.19    | 2.38          | 0.18                   |
| BB81         | +               | 592      | 0.35    | 0.26    | 0.31    | 1.22    | 0.20    | 2.34          | 0.15                   |
| BB105        | +               | 92       | 0.41    | 0.63    | 0.67    | 1.77    | 0.49    | 2.30          | 0.29                   |
| BB107        | +               | 46       | 0.24    | 0.37    | 0.15    | 0.97    | 0.11    | 2.22          | 0.11                   |
| Z 0210       | -               | <20      | 0.56    | 0.54    | 0.44    | 0.99    | 0.36    | 0.14          | 0.32                   |
| Z 0211       | -               | <20      | 0.41    | 0.40    | 0.43    | 0.48    | 0.36    | 0.12          | 0.33                   |
| S2407        | NT              | NT       | 0.15    | 0.48    | 0.17    | 0.20    | 0.13    | 0.10          | 0.14                   |
| S1749        | NT              | NT       | 0.15    | 0.17    | 0.18    | 0.14    | 0.12    | 0.10          | 0.11                   |
| S2975        | NT              | NT       | 0.12    | 0.20    | 0.14    | 0.19    | 0.12    | 0.11          | 0.10                   |
| S2898        | NT              | NT       | 0.27    | 0.49    | 0.45    | 0.24    | 0.15    | 0.14          | 0.29                   |
| M6397        | NT              | NT       | 0.19    | 0.12    | 0.09    | 0.10    | 0.07    | 0.07          | 0.24                   |
| M6429        | NT              | NT       | 0.53    | 0.28    | 0.26    | 0.35    | 0.23    | 0.21          | 0.22                   |
| S3534        | NT              | NT       | 0.21    | 0.22    | 0.23    | 0.25    | 0.21    | 0.21          | 0.25                   |
| S2407        | NT              | NT       | 0.15    | 0.52    | 0.17    | 0.22    | 0.13    | 0.10          | 0.14                   |
| M5360        | NT              | NT       | 0.11    | 0.16    | 0.11    | 0.43    | 0.10    | 0.10          | 0.12                   |
| S2908        | NT              | NT       | 0.46    | 0.44    | 0.57    | 0.42    | 0.41    | 0.43          | 0.54                   |
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capable of binding to the NHR of gp41. Of particular interest, infected human plasma IgG containing broadly neutralizing activity against HIV-1 was found to contain D5-like antibodies capable of binding to epitopes generated as a result of the HTS, thus reinforcing their candidacy as vaccine leads.

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