Virological Control by the CD4-Binding Site Antibody N6 in Simian-Human Immunodeficiency Virus-Infected Rhesus Monkeys

Boris Julg,^{a,b} Amarendra Pegu,^c Peter Abbink,^b Jinyan Liu,^b Amanda Brinkman,^b Katherine Molloy,^b Shanell Mojta,^b Abishek Chandrashekar,^b Katherine Callow,^a Keyun Wang,^c Xuejun Chen,^c Stephen D. Schmidt,^c Jinghe Huang,^d Richard A. Koup,^c Michael S. Seaman,^b Brandon F. Keele,^e John R. Mascola,^c Mark Connors,^d Dan H. Barouch^{a,b}

Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard University, Cambridge, Massachusetts, USA^a; Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA^b; Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA^c; Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA^d; AIDS and Cancer Virus Program, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA^e

ABSTRACT Passive immunotherapy against HIV-1 will most likely require broadly neutralizing antibodies (bnAbs) with maximum breadth and potency to ensure therapeutic efficacy. Recently, the novel CD4 binding site antibody N6 demonstrated extraordinary neutralization breadth and potency against large panels of cross-clade pseudoviruses. We evaluated the *in vivo* antiviral activity of N6-LS, alone or in combination with the established V3-glycan antibody PGT121, in chronically simian-human immunodeficiency virus (SHIV)-SF162P3-infected macaques. A single dose of N6-LS suppressed plasma viral loads in 4 out of 5 animals at day 7, while the combination of both antibodies suppressed all animals. The combination of both antibodies had no additive antiviral effect compared to a single dose of PGT121, potentially reflecting the nearly 10-fold-higher potency of PGT121 against this SHIV. Viral rebound occurred in the majority of suppressed animals and was linked to declining plasma bnAb levels over time. In addition to the effect on plasma viremia, bnAb administration resulted in significantly reduced proviral DNA levels in PBMCs after 2 weeks and in lymph nodes after 10 weeks. Autologous neutralizing antibody (nAb) responses and CD8⁺ T-cell responses were not significantly enhanced in the bnAb-treated animals compared to control animals, arguing against their contribution to the viral effects observed. These results confirm the robust antiviral activity of N6-LS *in vivo*, supporting the further clinical development of this antibody.

IMPORTANCE Monoclonal antibodies (MAbs) are being considered for passive immunotherapy of HIV-1 infection. A critical requirement for such strategies is the identification of MAbs that recognize the diversity of variants within circulating but also reservoir viruses, and MAb combinations might be needed to achieve this goal. This study evaluates the novel bnAb N6-LS alone or in combination with the bnAb PGT121, in rhesus macaques that were chronically infected with SHIV. The results demonstrate that N6-LS potently suppressed plasma viral loads in the majority of animals but that the combination with PGT121 was not superior to PGT121 alone in delaying time to viral rebound or reducing peripheral blood mononuclear cell (PBMC) or lymph node proviral DNA levels. The occurrence of viral escape variants in an N6-LS-monotreated animal, however, argues for the need to maximize breadth and antiviral efficacy by combining bnAbs for therapeutic indications.

Received 5 April 2017 Accepted 15 May 2017
Accepted manuscript posted online 24 May 2017

Citation Julg B, Pegu A, Abbink P, Liu J, Brinkman A, Molloy K, Mojta S, Chandrashekar A, Callow K, Wang K, Chen X, Schmidt SD, Huang J, Koup RA, Seaman MS, Keele BF, Mascola JR, Connors M, Barouch DH. 2017. Virological control by the CD4-binding site antibody N6 in simian-human immunodeficiency virus-infected rhesus monkeys. *J Virol* 91:e00498-17. <https://doi.org/10.1128/JVI.00498-17>.

Editor Guido Silvestri, Emory University
Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Address correspondence to Dan H. Barouch, dbarouch@bidmc.harvard.edu.

KEYWORDS broadly neutralizing antibodies, antiviral activity *in vivo*, effect on tissue viral reservoir, autologous immune responses, bnAbs, cellular reservoir, immunotherapy

Over the past few years, potent broadly neutralizing monoclonal antibodies (bnAbs) against multiple sites of the human immunodeficiency virus type 1 (HIV-1) envelope trimer have been described. BnAbs targeting the CD4-binding site (CD4bs) (1–3), the V3-glycan supersite (4–6), and the V1V2 regions (7–9) have gained particular attention, and several antibodies are now in development for use in passive prophylaxis or immunotherapy (10–13). Of these, antibodies that bind to the CD4bs are among the broadest, reaching coverage of 70 to 90% against cross-clade viruses (14), while antibodies that target the V3-glycan or V1V2-apex region of the trimer are severalfold more potent, although generally less broad (4, 8, 9, 14). With the goal of utilizing bnAbs for therapeutic passive immunization strategies, a combination of maximized breadth and optimized potency seems to be critical.

Recently, the monoclonal CD4bs antibody N6, which combines both robust potency and remarkable breadth, was described. Specifically, N6 neutralized 96% of 181 cross-clade pseudoviruses with a median 50% inhibitory concentration (IC_{50}) of 0.038 μ g/ml, and 98% of 171 clade C pseudoviruses at a median IC_{50} of 0.066 μ g/ml, which is among the most potent thus far described (15). In addition, N6 neutralized many isolates that were highly resistant to other members of the VRC01-CD4bs antibody class due to its unique mode of Env recognition (15). N6 therefore possesses several characteristics that make the antibody an interesting candidate for HIV therapeutic and also prevention strategies. Its antiviral activity *in vivo*, however, has not been demonstrated.

Utilizing monoclonal antibodies (MAbs) to target the latent reservoir as part of eradication strategies that involve pharmacological reversal of latency followed by MAb-mediated clearance of infected cells is currently the focus of ongoing investigations. Several studies in animal models, including humanized mice and nonhuman primates (NHP), have reported potent bnAb-mediated suppression of plasma viremia; however, reduction of infected cells was more variable (16–21). A single dose of the V3-glycan antibody PGT121 suppressed plasma viremia in simian-human immunodeficiency virus (SHIV)-SF162P3-infected macaques and also reduced proviral DNA levels in peripheral blood mononuclear cells (PBMCs) and lymphatic tissue cells (16). While some animals maintained undetectable plasma viral loads, the antibody did not achieve complete eradication of the reservoir. These data demonstrate that MAb-based reservoir eradication strategies face several barriers, e.g., the existence or development of viral escape, or potentially insufficient MAb potency in tissues. Combining bnAbs with differing epitope specificities and potent IC_{80} s might overcome some of these limitations. Moreover, recent studies (22–24) have suggested that bnAb treatment could also boost autologous adaptive immunity, including increased autologous neutralizing Ab (nAb) breadth and cytotoxic T-cell responses, potentially due to enhanced antibody-bound antigen presentation by dendritic cells (DC) (22, 24). Boosted autologous immunity could by itself contribute to reservoir control and warrants careful evaluation during the assessment of MAb-based therapeutic approaches.

In this study, we examined the antiviral activity of N6-LS, alone or in combination with PGT121, in SHIV-infected rhesus macaques. Animals with chronic SHIV-SF162P3 infection and stable plasma viral RNA levels received a single infusion of either bnAb, a combination of both, or placebo, and the plasma viral load and tissue simian immunodeficiency virus (SIV) DNA were quantified. We also assessed changes of autologous nAb breadth and SHIV-specific CD8⁺ T-cell responses following bnAb treatment.

RESULTS

N6-LS potently neutralizes SHIV-SF162P3. Among VRC01-class CD4bs MAbs, N6 has demonstrated unprecedented breadth and potency, e.g., N6 is almost 10-fold more

TABLE 1 Neutralization profile of SHIV-SF162P3 challenge stock^a

Epitope region	Antibody	IC ₅₀	IC ₈₀
CD4 binding site, VRC01 class	VRC01	2.43	6.75
	3BNC117	>50	>50
	VRC07-523	0.284	0.629
	N6	0.421	0.800
	N6-LS	0.480	1.03
V1/V2	CAP256-VRC26.25	>50	>50
	PGDM1400	>50	>50
Glycan V3	PGT121	0.085	0.177
	10-1074	0.073	0.155
Linear V3	447-52D	>50	>50

^aSensitivity of SHIV-SF162P3 challenge stock to a panel of broadly neutralizing monoclonal antibodies against different epitope regions is shown as IC₅₀s or IC₈₀s in $\mu\text{g/ml}$. The colored shading represents potency: 0.010 to 0.100, orange; 0.100 to 1.00, yellow; 1.00 to 10.0, green.

potent and has substantially more breadth than VRC01 (15), which is currently under evaluation in phase 2b clinical studies (NCT02840474 and NCT02664415). N6 is also more potent than VRC01 against SHIV-SF162P3, comparable to the CD4bs antibody VRC07-523, but less potent than certain V3-glycan MAbs, including PGT121 (Table 1). Given the unique neutralizing profile within the VRC01-like CD4bs antibody class, we selected N6 for *in vivo* testing, alone or in combination with the established V3-glycan MAb PGT121, which had previously demonstrated its activity against SHIV strains *in vitro* and *in vivo* in protection and therapeutic studies in rhesus macaques (16, 25, 26). N6-LS, used in this study, is a variant of N6 containing a methionine-to-leucine substitution and an asparagine-to-serine substitution at amino acid positions 428 and 434 that result in an increased affinity for FcRn, thereby increasing plasma half-life. The LS mutation has no effect on binding to HIV-1 Env, as reported previously for VRC01 (27), and we confirmed that N6-LS and N6 neutralized HIV-1 SF162P3 with similar potencies (IC₅₀s, 0.48 $\mu\text{g/ml}$ and 0.42 $\mu\text{g/ml}$ for N6-LS and N6, respectively) (Table 1). N6-LS was used here due to the short plasma half-life of the unmodified N6 in naive rhesus macaques, with a half-life for N6-LS of 8 days versus 3 days for N6 without the LS mutation (Fig. 1).

***In vivo* antiviral activity of N6-LS, PGT121, or a combination of both in SHIV-SF162P3-infected rhesus macaques.** To determine the ability of N6-LS, alone or in combination with PGT121, to reduce SHIV-SF162P3 levels in peripheral blood, as well as in cellular reservoirs and in tissues, we designed a therapeutic study using the rhesus

FIG 1 Serum PGT121, N6-LS, and N6 concentrations in naive, uninfected rhesus macaques. A single intravenous infusion of 10 mg/kg of either MAb was given on day 0. The half-life ($t_{1/2}$) for PGT121 was 13.4 days (standard error [SE], 3.8); for N6-LS, 8.2 days (SE, 1.7); and for N6, 2.8 days (SE, 0.4). The dotted line represents the assay limit of detection.

FIG 2 N6-LS and PGT121 therapeutic study schedule. PB, peripheral blood; LN, lymph node; bx, biopsy specimens.

macaque model (Fig. 2). A total of 18 animals that had been infected with SHIV-SF162P3 >6 months before monoclonal antibody administration and had shown stable plasma SHIV RNA levels were used. The animals were randomized to treatment or control groups, and baseline SHIV RNA levels were matched across the groups: two groups of 5 animals each received intravenously (i.v.) 10 mg/kg of body weight of N6-LS or PGT121 (Fig. 3A and B), and one group of 4 animals received intravenously both 10 mg/kg of N6-LS and 10 mg/kg of PGT121 (Fig. 3C). A control group of 4 animals received i.v. phosphate-buffered saline (PBS) instead of antibody and showed persistent infection (Fig. 3D). Overall viral loads on day 7 were significantly lower in the antibody-treated animals than in the placebo-treated control animals ($P = 0.03$; Mann–Whitney U test). Four out of 5 animals in the N6-LS group rapidly suppressed plasma SHIV RNA to undetectable levels within 7 days after MAb administration (mean, >1 log RNA copy ml^{-1} reduction) (Fig. 3A). The fifth animal (MGI), which had the highest baseline viral

FIG 3 Therapeutic efficacy of N6-LS, PGT121, or the cocktail of both. (A to D) Plasma viral RNA (log₁₀ copies per milliliter) in rhesus monkeys chronically infected with SHIV-SF162P3 after infusion on day 0 (vertical dotted lines) of N6-LS (A), PGT121 (B), N6-LS plus PGT121 (C), or PBS (placebo control) (D). (E) Median viral load declines in all groups were similar, ranging from 0.131 to 0.188 log₁₀ RNA copy/ml/day. The error bars indicate standard error of the mean (SEM). (F) Viral rebound was measurably delayed in the PGT121 and N6-LS/PGT121 groups compared to N6-LS alone. The assay sensitivity limit was 150 RNA copies per ml.

load ($5.1 \log_{10}$ RNA copies ml^{-1}) of all the animals, showed $1.45 \log_{10}$ RNA copies/ml reduction but did not achieve complete viral suppression. In the PGT121 group, 4 out of 5 animals achieved undetectable viral loads within 7 days ($>0.7 \log_{10}$ RNA copy/ml reduction), while the fifth animal became undetectable only on day 42 (DEKM) (Fig. 3B). In the N6-LS-PGT121 combination group, all the animals became readily undetectable within 7 days ($>0.92 \log_{10}$ RNA copy/ml reduction) (Fig. 3C). All except one animal (ML3 in the combination group) either rebounded from complete viral suppression or showed a viral load increase after an initial viral load drop (animal MGI). The kinetics of the initial decline of plasma viremia after infusion of N6-LS, PGT121, or the combination monoclonal antibody cocktails were similar, with a median of $0.169 \log_{10}$ RNA copy/ml/day (interquartile range [IQR], 0.152) for N6-LS, $0.188 \log_{10}$ RNA copy/ml/day (IQR, 0.224) for PGT121, and $0.131 \log_{10}$ RNA copy/ml/day (IQR, 0.182) for the combination N6-LS plus PGT121 (Fig. 3E). Interestingly, the animals in the N6-LS group rebounded after a median of 2.4 weeks, while the animals in the PGT121 and the combination groups rebounded only after a median of 7.4 and 7 weeks, respectively (Fig. 3F). No significant association between baseline plasma viral loads and time to viral rebound was found in this study (data not shown), but this association has been reported in previous NHP studies (16). These data demonstrate that both single antibodies potently suppressed viremia and that the combination resulted in potentially higher efficacy by suppressing all the animals. The data, however, also show that the bnAb combination did not result in more rapid viral RNA decline or in delayed viral rebound compared to PGT121 alone, suggesting that the addition of N6-LS did not amplify the antiviral activity of PGT121 against this highly PGT121-sensitive SHIV strain.

Pharmacokinetics of N6-LS and PGT121 *in vivo*. Serum samples were obtained throughout the study, and concentrations of N6-LS and PGT121 were determined by sandwich enzyme-linked immunosorbent assay (ELISA) using anti-idiotypic antibody for N6 or PGT121. The results show that the animals administered N6-LS or PGT121 alone had mean serum Ab concentrations of $48 \mu\text{g/ml}$ and $100 \mu\text{g/ml}$, respectively, at day 3 (Fig. 4A and B). The antibody half-lives for N6-LS and PGT121 were 6 and 10 days, respectively, in the single-MAb groups. In the combination group, the mean serum N6-LS concentration on day 3 was $58 \mu\text{g/ml}$, and the mean serum PGT121 concentration was $120 \mu\text{g/ml}$ (Fig. 4C). Both antibodies showed a slightly prolonged half-life when given in combination (10 days for N6-LS and 12 days for PGT121). The area under the concentration-time curve (AUC) for each MAb correlated with the time until viral rebound (Spearman r , 0.5; $P = 0.03$) (Fig. 4D), demonstrating the significance of persistent antibody levels for viral control. Interestingly, for both animals that did not completely suppress viremia (MGI) or required an extended period for full suppression (DEKM), MAb kinetics were indistinguishable from those of the other animals, suggesting that MAb levels do not explain the observed virological kinetics in these animals (Fig. 4E).

Antibody effects on proviral DNA in various tissue compartments. We next determined the impact of MAb infusion on proviral DNA in various tissue compartments and examined PBMCs and lymph node and colorectal tissue cells before and 2 and 10 weeks after the MAb administration. Overall, MAb administration significantly reduced proviral DNA levels in PBMCs at week 2 ($P = 0.002$); this effect was lost at week 10 (Fig. 5A). In contrast, MAb administration reduced proviral DNA levels in lymph node cells at week 2 (although not statistically significantly; $P = 0.057$), but the effect persisted with significantly lower SIV DNA levels in these lymphatic tissues at week 10 ($P = 0.002$) (Fig. 5C). However, no effect on proviral DNA levels was seen in colorectal tissue following MAb administration (data not shown). PBMC proviral DNA levels correlated with plasma virus levels at baseline and on day 70 (Spearman $r = 0.81$; $P < 0.001$), while lymph node DNA levels were associated with plasma viral loads only at baseline (Spearman $r = 0.57$; $P < 0.02$). In general, we observed large interanimal variability and did not detect any enhanced effects on the reservoir in the combination MAb group compared to the single-MAb groups (Fig. 5E). Interestingly, animal ML3,

FIG 4 (A to C) Serum N6-LS concentrations in animals that had received N6-LS alone (A), PGT121 levels in animals that had received PGT121 alone (B), and N6-LS and PGT121 levels in animals that had received the cocktail of both (C). The half-life for N6-LS alone was 6 days, for PGT121 alone was 10 days, for N6-LS in the combination was 12 days, and for PGT121 in the combination was 12 days. The dotted lines represent the assay limit of detection. (D) Correlation of the AUC for each MAb in each animal and the time to viral rebound for each animal. (E) Kinetics of viral RNA levels and serum antibody concentrations per animal throughout the study (top row, all animals that received N6-LS alone; middle row, all animals that received PGT121 alone; bottom row, animals that received the combination). The error bars indicate standard deviations.

which lacked viral rebound, demonstrated, among the animals in the MAb combination group, the strongest proviral DNA reduction in the lymph node cells on day 14, but DNA levels rebounded to baseline levels on day 70 in the absence of detectable plasma virus.

FIG 5 (A to D) Proviral DNA was significantly reduced after 2 weeks in PBMCs in monkeys that received monoclonal antibodies (N6-LS, PGT121, or both) (A) versus placebo controls (B), and proviral DNA levels were also significantly lower in lymph node mononuclear cells (LNMC) after 10 weeks in MAb-pretreated monkeys (C) versus placebo controls (D). The error bars indicate standard deviations. (E) Proviral DNA levels for each animal in PBMCs and lymph node mononuclear cells.

Sequence analysis of rebound viruses. Since 13 out of 14 animals that had received a monoclonal antibody (or a combination of two MAbs) showed rebound virus, we next determined if antibody-induced selection could be detected, acknowledging the limited time with sufficient antibody concentrations in this single infusion study to mediate sizable restriction. A total of 196 full-length envelope sequences (mean, 17 sequences per animal) pre-antibody administration and 220 sequences at the time of viral rebound (mean, 20 sequences per animal) were generated by single-genome amplification from all except 3 animals (ML3, MZA, and DEPV), for which RNA levels were too low to amplify. We then focused on gp120 regions relevant for N6-LS and PGT121 binding (for N6, loop D residues 276 to 283, CD4 BLP residues 362 to 374, and variable region 5 residues 458 to 469; for PGT121, variable region 3 residues 296 to 334, according to HXBc2 numbering). Overall, the majority of mutations were found within these regions, with evidence of strong selection in only a few animals (MGI, V3, D loop, and V5; MEW, V5; OOL, V3) (Fig. 6). We therefore focused on the 2 animals that had shown incomplete (MGI) or delayed (DEKM) viral suppression. Animal MGI, which failed to fully suppress viremia, initially showed a robust viral decline of 1.45 log₁₀ RNA copies/ml until 7 days after receiving N6-LS. While the pre-MAb virus did not harbor known viral escape mutations, a fixed mutation in position D/E279 in the D loop, which has been associated with N6 resistance (15), was present on day 14, when the animal developed viral rebound. In contrast, animal DEKM, which only fully suppressed viremia after 42 days following PGT121 administration, demonstrated 2 mutations in the V3

FIG 6 HIV-1 envelope sequence analysis before and after N6-LS, PGT121, or combination MAb infusion. HIV-1 envelopes were cloned from plasma samples. The logogram shows Env gp120 regions (amino acid positions: V3 region, residues 296 to 334; loop D, residues 276 to 283; CD4 binding site, residues 362 to 374; and V5 region, residues 458 to 469, according to HXBc2 numbering) indicating sequence changes from week -1 (Pre) to the time of viral rebound (Post). The frequency of each amino acid is indicated by its height. Red residues represent mutations that differ from the consensus sequence. Viruses from 2 animals in the PGT121 group did not amplify. A mutation in position 279 (D/E) was observed in the animal MGI, which showed virological failure following N6-LS infusion.

FIG 7 Anti-SHIV-specific adaptive immune responses before and after MAB/placebo administration. (A to C) Autologous serum neutralization titers against tier 1B viruses in all bnAb-treated animals (A), by bnAb or placebo group (B), and against the challenge stock SHIV-SF162P3 (C) as determined by TZM-bl assay. The numbers of antigen-specific T cells were measured in PBMCs by *in vitro* stimulation with HIV-1 Env, SIV Gag, and Pol peptide pools, followed by fluorescence-activated cell sorter (FACS) detection of intracellular IFN- γ , IL-2, and TNF- α . The numbers of responding cells were summed across the cytokines within CD8⁺ T cell subsets and are reported as percentages. (D and E) Animals that received bnAbs were compared to untreated animals at week -1 and week 4, and significant differences in total (D) and protein-specific (E) responses are indicated. (F) The proportions of cells that expressed Boolean combinations of IFN- γ , IL-2, and TNF- α are depicted as pie charts, averaged across bnAb versus placebo groups at the indicated weeks.

region, P/T309 and F/I316, at baseline while these mutations were not found in any of the other PGT121-monotreated animals. No classical 332-to-334 mutants were observed in any of the PGT121-treated animals.

Kinetics of autologous immune responses. It has been suggested that MAB administration can boost autologous adaptive immune responses (23, 24), presumably by improved antibody-bound antigen delivery to and presentation by DC (22). We therefore measured humoral responses to HIV-1 Env in animals' day 0 and week 22 sera, a time point where N6-LS and PGT121 had been long cleared, against a panel of tier 1 ($n = 3$) and tier 2 ($n = 4$) HIV-1 pseudoviruses and the SHIV-SF162P3 challenge stock. While nAbs to tier 2 viruses were rare, we observed significant increases in serum titers against tier 1 viruses in all the animals independently of MAB or placebo administration (median titer increases, 122.5 and 206 for the MAB groups and the placebo group, respectively; $P < 0.0001$ to 0.002) (Fig. 7A and B). In contrast, autologous neutralizing antibody responses against SHIV-SF162P3 did not change or were reduced (e.g., in PGT121-pretreated animals) (Fig. 7C). We also quantified antigen-specific CD8⁺ T cells from week -1 and day 28 following MAB administration by *in vitro* stimulation of PBMCs with HIV-1 Env and SIV Gag and Pol peptide pools, followed by intracellular cytokine staining for gamma interferon (IFN- γ), tumor necrosis factor alpha (TNF- α), and interleukin 2 (IL-2). Interestingly, overall frequencies of HIV-specific IFN- γ plus CD8⁺ T-cell responses (as percentages of total CD8⁺ T cells) significantly declined over time

in animals that received MAbs (mean, 0.43% to 0.26%; $P = 0.03$) (Fig. 7D), and the decline was primarily driven by Pol-specific responses (0.44% to 0.1%; $P = 0.004$) (Fig. 7E). No differences in frequencies of memory subsets (effector memory, central memory, naive, effector memory RA) or change in PD-1 expression was observed (data not shown). We also did not detect any substantial change in the ability of CD8⁺ T cells to secrete multiple cytokines simultaneously, a characteristic that has been previously associated with enhanced functionality and virus control (28) (Fig. 7F). In this study, the majority of T-cell-secreted IFN- γ and/or TNF- α was typical during chronic antigen exposure. Together, these data suggest that a single dose of MAb did not have any boosting effect on autologous immune responses in this animal model.

DISCUSSION

The results of this study validate the antiviral capacity of the N6-LS antibody in chronically SHIV-infected macaques. One dose of N6-LS resulted in rapid viral suppression in the majority of animals, with viral decay kinetics similar to those of PGT121 (16). The time to viral suppression was comparable to that for other CD4bs antibodies, e.g., 3BNC117 and VRC01, which are currently in clinical development (10–13, 19, 29). Interestingly, viral kinetics did not change when N6-LS was given in combination with PGT121, suggesting that there was no additive antiviral effect. This is consistent with previous studies that have tested bnAb combinations in SHIV-SF162P3-infected macaques (16, 19) and might reflect the fact that in the setting of a highly sensitive virus, a single MAb already executes maximal antiviral activity that is not amplified by additional MAbs, in particular if they are less potent.

While viral suppression occurred at comparable rates, viral rebound differed between groups, with relatively rapid rebound in the animals treated with N6-LS only and delayed rebound in the animals treated with PGT121 and the MAb combination. The time to viral rebound in general was quite variable between the animals, even within each bnAb group, and was not determined by baseline plasma viral loads; however, the sample size might have been too small in this study to detect a significant association. Instead, persistent bnAb levels over time (AUC) correlated with the time to reoccurrence of plasma virus. N6, which is highly somatic, hypermutated in both heavy (31%) and light (25%) chains (15), might be cleared more rapidly due to potentially increased antigenicity in the macaques, as has been previously observed with other CD4s bnAbs (30). Interestingly, when N6-LS was combined with PGT121, the half-lives of both substantially increased, suggesting that envelope protein may act as an antigenic sink. In this case, it is possible that a reduction of antigen by one antibody may spare the clearance of the second antibody through phagocytic removal of immune complexes.

All the animals in the bnAb combination group suppressed plasma viremia at day 7; however, 2 animals stood out in the mono-bnAb groups. Animal MGI dropped plasma viremia by 1.45 log₁₀ RNA copies/ml but then rebounded to pre-N6-LS levels within 14 days. The D/E279 mutation in the D loop found in the rebound virus was not present in any of the 18 pre-bnAb Env sequences but was present in 29/30 of the week 2 (first viral rebound time point) Env sequences, suggesting that the mutation developed rapidly during therapy. This residue has been shown to be critical for resistance to N6 and has been seen in the few viruses that were resistant within a panel of 181 tested pseudoviruses, as well as in the original patient's sequence in which N6 was identified (15). Sensitivity of these viruses to N6 neutralization was only recovered by reverting 279 to D. While we cannot exclude the possibility that this mutation had developed at low frequency during natural infection and was selected upon N6-LS treatment, our data highlight how rapidly virological escape can occur following single-bnAb treatment. The other animal that showed slow viral response to PGT121 did not show any typical mutations that would reduce susceptibility to PGT121, e.g., in the N-332 glycan site, but, interestingly, had variations in 2 positions in the V3-loop that have been described as being characteristic of SF162 viruses that had undergone a coreceptor tropism switch to CXCR4 (31, 32). As was recently reported, CXCR4-using viruses are substantially less susceptible to PGT121 neutralization (33), due to changes

specifically in the V3 base that are otherwise important for binding to CCR5 (34). Both examples underline how viral sequence diversity can impact single-bnAb activity.

BnAb effects on the cellular viral reservoir have been reported in NHP and humanized mice (16, 21), and bnAb-mediated clearance of infected cells is thought to potentially play a role in longer-term viral control and viral eradication (27). Here, we confirmed that bnAbs reduced cellular SIV DNA levels in PBMCs, but also in lymph nodes, suggesting that bnAb levels in lymphatic tissues were sufficient to achieve antiviral activity. Interestingly we did not observe an enhanced antireservoir effect of the combined compared to single bnAbs, suggesting that augmenting bnAb breadth and potency alone might not be sufficient but that other aspects, such as exposure of cell surface Env through latency reversal, might be critical for optimized reservoir cell recognition and eradication. In addition to a direct effect of Env binding, anti-reservoir activity might be mediated through enhancement of autologous immune responses. It has recently been reported that a single dose of the CD4bs antibody 3BNC117 resulted in a boost in autologous neutralizing antibody responses in HIV-infected individuals, at least compared to matched historical controls (35). In this study, we did detect increased autologous nAb titers, but this was independent of bnAb treatment and most likely a function of extended viral exposure in these chronically infected animals. In contrast, SHIV-specific CD8⁺ T-cell frequencies significantly declined in animals that had received bnAbs, most likely due to memory cell contraction in the setting of reduced antigen, as has been described for patients on antiretroviral therapy (ART) (36). It should be noted that the duration of antibody therapy in this model is brief, due to the potential for cross-species immune responses to the passively administered antibody. It remains to be seen whether quantitative or qualitative changes in the CD8⁺ T cell response are observed during more prolonged therapy in humans.

In summary, our results validate the antiviral activity of N6-LS *in vivo*. Our data also indicate that combining bnAbs does not inevitably result in superior plasma virus suppression or enhanced reduction of the cellular reservoir, at least in this SHIV model. As broad coverage of viral diversity is a major objective for combining bnAbs, future NHP studies should consider SHIV swarm infections to measure the full antiviral potential of bnAb combinations.

MATERIALS AND METHODS

Animals and study design. (i) Therapeutic study. Eighteen Indian origin, outbred, young adult male and female rhesus monkeys (*Macaca mulatta*) that did not express the class I alleles Mamu-A*01, Mamu-B*08, and Mamu-B*17 associated with spontaneous virological control were housed at Alphagenesis Inc., Yermasse, SC. The animals were infected by the intrarectal route with our rhesus-derived SHIV-SF162P3 challenge stock 6 months before monoclonal antibody administration. The animals were randomly allocated to the different antibody dose and PBS control groups. Single monoclonal antibodies or the antibody cocktail were administered to the monkeys once by the intravenous route at a dose of 10 mg/kg for each monoclonal antibody. The control animals received a single i.v. administration of PBS. Serum samples for antibody detection and viral load determination were obtained at week -1 and days 0, 1, 3, 7, 14, 28, 42, 56, and 70. Lymph node and mucosal (sigmoid colon) biopsy specimens were taken at weeks -1, 2, and 10. The animal studies were approved by the appropriate Institutional Animal Care and Use Committees (IACUC).

(ii) Pharmacokinetics study. All animal experiments were reviewed and approved by the Animal Care and Use Committee of the Vaccine Research Center, NIAID, NIH, and all the animals were housed and cared for in accordance with local, state, federal, and institute policies in an American Association for Accreditation of Laboratory Animal Care-accredited facility at the NIH. Eight Indian origin rhesus macaques were administered low-endotoxin antibody preparations (<1 endotoxin unit [EU]/mg) intravenously at 10 mg of Ab/kg of body weight. Whole-blood samples were collected prior to injection and at multiple time points until week 4 postadministration.

Challenge virus. The challenge virus was SHIV-SF162P3 propagated in concanavalin A-activated rhesus macaque PBMCs. The virus was quantified by SIV p27 ELISA (Zeptometrix), and the 50% tissue culture infectious dose (TCID₅₀) was determined in TZM-bl cells (37).

Antibody production. N6 and PGT121 monoclonal antibodies were generated as previously described (4, 15) and purified by using a protein A affinity matrix (GE Healthcare). PBS was used as a control in this study. All the monoclonal antibody preparations were endotoxin free. The N6-LS antibody included the Fc region mutation (LS), as previously described (30), which increases the circulating half-life. The LS mutation does not affect the antibody-combining site, and thus, the neutralization potency is unaffected.

Neutralization assays. Neutralization of the SHIV-SF162P3 challenge stock and detection of autologous nAbs against the tier 1 pseudoviruses BaL.26, SS1196.1, and 6535.3 or the tier 2 pseudoviruses TRO.11, RHPA4259.7, SC422661.8, and PVO.4 was evaluated *in vitro* by using TZM-bl target cells and a luciferase reporter assay, as described previously (26, 38, 39). All the pseudoviruses were produced in 293T cells (38). Briefly, the SHIV stock was incubated with the antibody for 30 min at 37°C before the TZM-bl cells were added. Similarly, the tier 1 and 2 viruses or the challenge stock was incubated with macaque serum at week 0 and week 22 before the addition of TZM-bl cells. The protease inhibitor indinavir was added to a final concentration of 1 μ M to limit infection of target cells to a single round of viral replication. Luciferase expression was quantified 48 h after infection upon cell lysis and the addition of luciferin substrate (Promega).

ELISA. N6-LS and PGT121 serum concentrations were measured by a validated sandwich ELISA (40). Briefly, microtiter plates were coated with anti-idiotypic antibody specifically recognizing N6 or PGT121 and incubated overnight at 4°C. The plates were washed with PBS-0.05% Tween 20 and blocked with PBS-casein (Pierce). After blocking, serial dilutions of serum samples were added to the plate and incubated for 2 h at 37°C. Binding was detected with a horseradish peroxidase (HRP)-conjugated goat anti-human IgG secondary antibody (Fisher Scientific) and visualized with SureBlue tetramethylbenzidine (TMB) microwell peroxidase (KPL Research Products).

Tissue proviral DNA assay. Proviral DNA was quantified as previously reported (41). Lymph node and gastrointestinal mucosal biopsy specimens were processed as single-cell suspensions as previously described (42). Tissue-specific total cellular DNA was isolated from 5×10^6 cells using a QIAamp DNA blood minikit (Qiagen). The absolute quantification of viral DNA in each sample was determined by quantitative PCR (qPCR) using primers specific to a conserved region of SIVmac239. All samples were directly compared to a linear virus standard and the simultaneous amplification of a fragment of the human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene. The sensitivity of linear standards was compared to the 3D8 cell line as a reference standard as described previously (41). All PCR assays were performed with 100 and 200 ng of sample DNA.

Single-genome amplification and analysis. Single-genome amplification followed by direct sequencing of the Env gene was used to eliminate *Taq*-induced errors and *in vitro* recombination as described previously (43). Briefly, viral RNA was isolated from plasma using a QIAamp viral RNA kit (Qiagen). Reverse transcription (RT) of RNA to single-stranded cDNA was performed using SuperScript III reverse transcriptase according to the manufacturer's recommendations (Invitrogen), in brief, a cDNA reaction with 1 $\times$ RT buffer, 0.5 mM each deoxynucleoside triphosphate (dNTP), 5 mM dithiothreitol (DTT), RNaseOUT (RNase [recombinant RNase] inhibitor; 2 U/ml), SuperScript III reverse transcriptase (10 U/ml), and 0.25 mM antisense primer SIVEnvR1 (5'-TGT AAT AAA TCC CTT CCA GTC CCC CC-3'). RNA, primers and dNTPs were heated at 65°C for 5 min and then chilled on ice for 1 min; then, the entire reaction mixture was incubated at 50°C for 60 min, followed by 55°C for an additional 60 min. Finally, the reaction mixture was heat inactivated at 70°C for 15 min and then treated with 1 μ l RNase H at 37°C for 20 min. Then, cDNA templates were serially diluted until only a fraction (~25%) of amplicons were PCR positive under the following PCR conditions: PCR amplification was carried out using Platinum *Taq* (Invitrogen) with 1 $\times$ buffer, 2 mM MgCl₂, 0.2 mM each deoxynucleoside triphosphate, 0.2 μ M each primer, and 0.025 U/ μ l Platinum *Taq* polymerase. The primers for the first-round PCR were SIVEnvF1 (5'-CCT CCC CCT CCA GGA CTA GC-3') and antisense primer SIVEnvR1. The primers for the second-round PCR were SIVEnvF2 (5'-TAT AAT AGA CAT GGA GAC ACC CTT GAG GGA GC-3') and SIVEnvR2 (5'-ATG AGA CAT RTC TAT TGC CAA TTT GTA-3'). The cycle parameters were 94°C for 2 min, followed by 35 cycles of 94°C for 15 s, 55°C for 30 s, and 68°C for 4 min, followed by a final extension of 68°C for 10 min. The product of the first-round PCR (1 μ l) was subsequently used as a template in the second-round PCR under the same conditions but with a total of 45 cycles. All PCR-positive amplicons were directly sequenced using BigDye Terminator chemistry (Applied Biosystems). Any sequence with evidence of double peaks was excluded from further analysis.

Cellular immune assays. SHIV-specific cellular immune responses were assessed by multiparameter intracellular cytokine staining (ICS) assays as described previously (44). Nine-color ICS assays were performed with predetermined titers of monoclonal antibodies (Becton Dickinson) against CD3 (SP34; BV421), CD4 (L200; allophycocyanin [APC]-H7), CD8 (SK1; BV711), CD28 (L293; phycoerythrin [PE]-Cy7), CD95 (DX2; ECD; Beckman Coulter), IL-2 (MQ1-17H12; PE), IFN- γ (B27; APC), TNF- α (MAb11; Alexa 700), and PD-1 (EH21.1; BV605). IFN- γ backgrounds were consistently <0.01%.

Statistical analyses. Analyses of independent data were performed by two-tailed Mann-Whitney U tests and Wilcoxon rank sum tests. *P* values of less than 0.05 were considered significant. Statistical analyses were performed using GraphPad Prism. Pharmacokinetic parameters for MAbs kinetics in uninfected macaques were calculated in WinNonlin software using the noncompartment model. T-cell data were analyzed using Flowjo 10.1, as well as PESTLE and SPICE 5.3, which were both obtained from Mario Roederer, Vaccine Research Center, NIH.

ACKNOWLEDGMENTS

We thank W. Rinaldi (Alphagenesis, Inc.) for assistance in the execution of the animal studies and D. Ng'ang'a for assisting in the measurement of plasma viral loads.

This work was supported by NIH (K08 AI106408) (B.J.), NIH (UM1 AI126603 and UM1 AI124377) (D.H.B.), NIH (UM1 AI100663) (D.H.B.), and NIH (U19 AI096040) (M.S.S. and D.H.B.); amfAR (109219) (D.H.B.); the Ragon Institute of MGH, MIT, and Harvard; the intramural research program of the Vaccine Research Center, NIAID, NIH; and in part

with Federal funds from the National Cancer Institute, National Institutes of Health, under contract no. HHSN261200800001E.

The content of the publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Project planning was performed by B.J., J.R.M., R.A.K., M.C., and D.H.B.; antibodies were generated by J.H. and P.A.; viral neutralization assays were performed and analyzed by A.P. and S.D.S.; plasma viral loads and tissue cell SIV DNA levels were measured by P.A.; PK-ELISA was performed by A.P., X.C., and K.W.; SHIV Env sequences were generated and analyzed by B.F.K.; autologous nAb level analysis was performed by M.S.S.; T-cell analysis was performed by B.J., J.L., A.B., K.C., K.M., S.M., and A.C.; and the manuscript was written by B.J., J.R.M., M.C., and D.H.B.

We declare that we have no competing interests.

REFERENCES

- Scheid JF, Mouquet H, Ueberheide B, Diskin R, Klein F, Oliveira TY, Pietzsch J, Fenyo D, Abadir A, Velinzon K, Hurley A, Myung S, Boulad F, Poignard P, Burton DR, Pereyra F, Ho DD, Walker BD, Seaman MS, Bjorkman PJ, Chait BT, Nussenzweig MC. 2011. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* 333:1633–1637. <https://doi.org/10.1126/science.1207227>.
- Wu X, Yang ZY, Li Y, Hogerkorff CM, Schief WR, Seaman MS, Zhou T, Schmidt SD, Wu L, Xu L, Longo NS, McKee K, O'Dell S, Louder MK, Wycuff DL, Feng Y, Nason M, Doria-Rose N, Connors M, Kwong PD, Roederer M, Wyatt RT, Nabel GJ, Mascola JR. 2010. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* 329:856–861. <https://doi.org/10.1126/science.1187659>.
- Bonsignori M, Montefiori DC, Wu X, Chen X, Hwang KK, Tsao CY, Kozink DM, Parks RJ, Tomaras GD, Crump JA, Kapiga SH, Sam NE, Kwong PD, Kepler TB, Liao HX, Mascola JR, Haynes BF. 2012. Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: implications for vaccine design. *J Virol* 86: 4688–4692. <https://doi.org/10.1128/JVI.07163-11>.
- Walker LM, Huber M, Doores KJ, Falkowska E, Pejchal R, Julien JP, Wang SK, Ramos A, Chan-Hui PY, Moyle M, Mitcham JL, Hammond PW, Olsen OA, Phung P, Fling S, Wong CH, Phogat S, Wrin T, Simek MD, Protocol G Principal Investigators, Koff WC, Wilson IA, Burton DR, Poignard P. 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature* 477:466–470. <https://doi.org/10.1038/nature10373>.
- Mouquet H, Scharf L, Euler Z, Liu Y, Eden C, Scheid JF, Halper-Stromberg A, Gnanaprasagam PN, Spencer DI, Seaman MS, Schuitemaker H, Feizi T, Nussenzweig MC, Bjorkman PJ. 2012. Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc Natl Acad Sci U S A* 109:E3268–E3277. <https://doi.org/10.1073/pnas.1217207109>.
- Sok D, Doores KJ, Briney B, Le KM, Saye-Francisco KL, Ramos A, Kulp DW, Julien JP, Menis S, Wickramasinghe L, Seaman MS, Schief WR, Wilson IA, Poignard P, Burton DR. 2014. Promiscuous glycan site recognition by antibodies to the high-mannose patch of gp120 broadens neutralization of HIV. *Sci Transl Med* 6:236ra63. <https://doi.org/10.1126/scitranslmed.3008104>.
- Walker LM, Phogat SK, Chan-Hui PY, Wagner D, Phung P, Goss JL, Wrin T, Simek MD, Fling S, Mitcham JL, Lehrman JK, Priddy FH, Olsen OA, Frey SM, Hammond PW, Protocol G Principal Investigators, Kaminsky S, Zamb T, Moyle M, Koff WC, Poignard P, Burton DR. 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* 326:285–289. <https://doi.org/10.1126/science.1178746>.
- Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky BJ, Erndes MJ, Georgiev IS, Kim HJ, Pancera M, Staup RP, Altae-Tran HR, Bailer RT, Crooks ET, Cupo A, Druz A, Garrett NJ, Hoi KH, Kong R, Louder MK, Longo NS, McKee K, Nonyane M, O'Dell S, Roark RS, Rudicell RS, Schmidt SD, Sheward DJ, Soto C, Wibmer CK, Yang Y, Zhang Z, Program NCS, Mullikin JC, Binley JM, Sanders RW, Wilson IA, Moore JP, Ward AB, Georgiou G, Williamson C, Abdool Karim SS, Morris L, Kwong PD, Shapiro L, Mascola JR. 2014. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature* 509:55–62. <https://doi.org/10.1038/nature13036>.
- Sok D, van Gils MJ, Pauthner M, Julien JP, Saye-Francisco KL, Hsueh J, Briney B, Lee JH, Le KM, Lee PS, Hua Y, Seaman MS, Moore JP, Ward AB, Wilson IA, Sanders RW, Burton DR. 2014. Recombinant HIV envelope trimer selects for quaternary-dependent antibodies targeting the trimer apex. *Proc Natl Acad Sci U S A* 111:17624–17629. <https://doi.org/10.1073/pnas.1415789111>.
- Caskey M, Klein F, Lorenzi JC, Seaman MS, West AP, Jr, Buckley N, Kremer G, Nogueira L, Braunschweig M, Scheid JF, Horwitz JA, Shimeliovich I, Ben-Avraham S, Witmer-Pack M, Platten M, Lehmann C, Burke LA, Hawthorne T, Gorelick RJ, Walker BD, Keler T, Gulick RM, Fatkenheuer G, Schlesinger SJ, Nussenzweig MC. 2015. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature* 522:487–491. <https://doi.org/10.1038/nature14411>.
- Scheid JF, Horwitz JA, Bar-On Y, Kreider EF, Lu CL, Lorenzi JC, Feldmann A, Braunschweig M, Nogueira L, Oliveira T, Shimeliovich I, Patel R, Burke L, Cohen YZ, Hadrihan S, Settler A, Witmer-Pack M, West AP, Jr, Juel B, Keler T, Hawthorne T, Zingman B, Gulick RM, Pfeifer N, Learn GH, Seaman MS, Bjorkman PJ, Klein F, Schlesinger SJ, Walker BD, Hahn BH, Nussenzweig MC. 2016. HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 535:556–560. <https://doi.org/10.1038/nature18929>.
- Lynch RM, Boritz E, Coates EE, DeZure A, Madden P, Costner P, Enama ME, Plummer S, Holman L, Hendel CS, Gordon I, Casazza J, Conancibotti M, Migueles SA, Tressler R, Bailer RT, McDermott A, Narpala S, O'Dell S, Wolf G, Lifson JD, Freemire BA, Gorelick RJ, Pandey JP, Mohan S, Chomont N, Fromentin R, Chun TW, Fauci AS, Schwartz RM, Koup RA, Douek DC, Hu Z, Capparelli E, Graham BS, Mascola JR, Ledgerwood JE, VRC 601 Study Team. 2015. Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. *Sci Transl Med* 7:319ra206. <https://doi.org/10.1126/scitranslmed.aad5752>.
- Caskey M, Schoofs T, Gruell H, Settler A, Karagounis T, Kreider EF, Murrell B, Pfeifer N, Nogueira L, Oliveira TY, Learn GH, Cohen YZ, Lehmann C, Gillor D, Shimeliovich I, Unson-O'Brien C, Weiland D, Robles A, Kummerle T, Wyen C, Levin R, Witmer-Pack M, Eren K, Ignacio C, Kiss S, West AP, Jr, Mouquet H, Zingman BS, Gulick RM, Keler T, Bjorkman PJ, Seaman MS, Hahn BH, Fatkenheuer G, Schlesinger SJ, Nussenzweig MC, Klein F. 2017. Antibody 10-1074 suppresses viremia in HIV-1-infected individuals. *Nat Med* 23:185–191. <https://doi.org/10.1038/nm.4268>.
- Burton DR, Mascola JR. 2015. Antibody responses to envelope glycoproteins in HIV-1 infection. *Nat Immunol* 16:571–576. <https://doi.org/10.1038/ni.3158>.
- Huang J, Kang BH, Ishida E, Zhou T, Griesman T, Sheng Z, Wu F, Doria-Rose NA, Zhang B, McKee K, O'Dell S, Chuang GY, Druz A, Georgiev IS, Schramm CA, Zheng A, Joyce MG, Asokan M, Ransier A, Darko S, Migueles SA, Bailer RT, Louder MK, Alam SM, Parks R, Kelsoe G, Von Holle T, Haynes BF, Douek DC, Hirsch V, Seaman MS, Shapiro L, Mascola JR, Kwong PD, Connors M. 2016. Identification of a CD4-binding-site antibody to HIV that evolved near-pan neutralization breadth. *Immunity* 45:1108–1121. <https://doi.org/10.1016/j.immuni.2016.10.027>.
- Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J, Stephenson KE, Chang HW, Shekhar K, Gupta S, Nkolola JP, Seaman MS, Smith KM, Borduc-

- chi EN, Cabral C, Smith JY, Blackmore S, Sanisetty S, Perry JR, Beck M, Lewis MG, Rinaldi W, Chakraborty AK, Poignard P, Nussenzweig MC, Burton DR. 2013. Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* 503:224–228. <https://doi.org/10.1038/nature12744>.
17. Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournazos S, Mouquet H, Spatz LA, Diskin R, Abadir A, Zang T, Dorner M, Billerbeck E, Labitt RN, Gaebler C, Marcovecchio PM, Incesu RB, Eisenreich TR, Bieniasz PD, Seaman MS, Bjorkman PJ, Ravetch JV, Ploss A, Nussenzweig MC. 2012. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature* 492:118–122. <https://doi.org/10.1038/nature11604>.
 18. Horwitz JA, Halper-Stromberg A, Mouquet H, Gitlin AD, Tretiakova A, Eisenreich TR, Malbec M, Gravemann S, Billerbeck E, Dorner M, Buning H, Schwartz O, Knops E, Kaiser R, Seaman MS, Wilson JM, Rice CM, Ploss A, Bjorkman PJ, Klein F, Nussenzweig MC. 2013. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. *Proc Natl Acad Sci U S A* 110:16538–16543. <https://doi.org/10.1073/pnas.1315295110>.
 19. Shingai M, Nishimura Y, Klein F, Mouquet H, Donau OK, Plishka R, Buckler-White A, Seaman M, Piatak M, Jr, Lifson JD, Dimitrov DS, Nussenzweig MC, Martin MA. 2013. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature* 503:277–280. <https://doi.org/10.1038/nature12746>.
 20. Halper-Stromberg A, Lu CL, Klein F, Horwitz JA, Bournazos S, Nogueira L, Eisenreich TR, Liu C, Gazumyan A, Schaefer U, Furze RC, Seaman MS, Prinjha R, Tarakhovsky A, Ravetch JV, Nussenzweig MC. 2014. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. *Cell* 158:969–999. <https://doi.org/10.1016/j.cell.2014.07.043>.
 21. Lu CL, Murakowski DK, Bournazos S, Schoofs T, Sarkar D, Halper-Stromberg A, Horwitz JA, Nogueira L, Golijanin J, Gazumyan A, Ravetch JV, Caskey M, Chakraborty AK, Nussenzweig MC. 2016. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science* 352:1001–1004. <https://doi.org/10.1126/science.aaf1279>.
 22. Platzter B, Stout M, Fiebigler E. 2014. Antigen cross-presentation of immune complexes. *Front Immunol* 5:140. <https://doi.org/10.3389/fimmu.2014.00140>.
 23. Schoofs T, Klein F, Braunschweig M, Kreider EF, Feldmann A, Nogueira L, Oliveira T, Lorenzi JC, Parrish EH, Learn GH, West AP, Jr, Bjorkman PJ, Schlesinger SJ, Seaman MS, Czartoski J, McElrath MJ, Pfeifer N, Hahn BH, Caskey M, Nussenzweig MC. 2016. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science* 352:997–1001. <https://doi.org/10.1126/science.aaf0972>.
 24. Nishimura Y, Gautam R, Chun TW, Sadjadpour R, Foulds KE, Shingai M, Klein F, Gazumyan A, Golijanin J, Donaldson M, Donau OK, Plishka RJ, Buckler-White A, Seaman MS, Lifson JD, Koup RA, Fauci AS, Nussenzweig MC, Martin MA. 2017. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature* 543:559–563. <https://doi.org/10.1038/nature21435>.
 25. Moldt B, Rakasz EG, Schultz N, Chan-Hui PY, Swiderek K, Weisgrau KL, Piaskowski SM, Bergman Z, Watkins DI, Poignard P, Burton DR. 2012. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *Proc Natl Acad Sci U S A* 109:18921–18925. <https://doi.org/10.1073/pnas.1214785109>.
 26. Pegu A, Yang ZY, Boyington JC, Wu L, Ko SY, Schmidt SD, McKee K, Kong WP, Shi W, Chen X, Todd JP, Letvin NL, Huang J, Nason MC, Hoxie JA, Kwong PD, Connors M, Rao SS, Mascola JR, Nabel GJ. 2014. Neutralizing antibodies to HIV-1 envelope protect more effectively in vivo than those to the CD4 receptor. *Sci Transl Med* 6:243ra88. <https://doi.org/10.1126/scitranslmed.3008992>.
 27. Julg B, Barouch DH. 2015. Novel immunological strategies for HIV-1 eradication. *J Virus Erad* 1:232–236.
 28. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA. 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8⁺ T cells. *Blood* 107:4781–4789. <https://doi.org/10.1182/blood-2005-12-4818>.
 29. Bar KJ, Sneller MC, Hraner LJ, Justement JS, Overton ET, Petrone ME, Salantes DB, Seamon CA, Scheinfeld B, Kwan RW, Learn GH, Proschan MA, Kreider EF, Blazkova J, Bardsley M, Refsland EW, Messer M, Claridge KE, Tustin NB, Madden PJ, Oden K, O'Dell SJ, Jarocki B, Shiakolas AR, Tressler RL, Doria-Rose NA, Bailer RT, Ledgerwood JE, Capparelli EV, Lynch RM, Graham BS, Moir S, Koup RA, Mascola JR, Hoxie JA, Fauci AS, Tebas P, Chun TW. 2016. Effect of HIV antibody VRC01 on viral rebound after treatment interruption. *N Engl J Med* 375:2037–2050. <https://doi.org/10.1056/NEJMoa1608243>.
 30. Ko SY, Pegu A, Rudicell RS, Yang ZY, Joyce MG, Chen X, Wang K, Bao S, Kraemer TD, Rath T, Zeng M, Schmidt SD, Todd JP, Pensak SR, Saunders KO, Nason MC, Haase AT, Rao SS, Blumberg RS, Mascola JR, Nabel GJ. 2014. Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* 514:642–645. <https://doi.org/10.1038/nature13612>.
 31. Dejuq N, Simmons G, Clapham PR. 2000. T-cell line adaptation of human immunodeficiency virus type 1 strain SF162: effects on envelope, vpu and macrophage-tropism. *J Gen Virol* 81:2899–2904. <https://doi.org/10.1099/0022-1317-81-12-2899>.
 32. Kiselyeva Y, Nedellec R, Ramos A, Pastore C, Margolis LB, Mosier DE. 2007. Evolution of CXCR4-using human immunodeficiency virus type 1 SF162 is associated with two unique envelope mutations. *J Virol* 81:3657–3661. <https://doi.org/10.1128/JVI.02310-06>.
 33. Sok D, Pauthner M, Briney B, Lee JH, Saye-Francisco KL, Hsueh J, Ramos A, Le KM, Jones M, Jardine JG, Bastidas R, Sarkar A, Liang CH, Shivatare SS, Wu CY, Schief WR, Wong CH, Wilson IA, Ward AB, Zhu J, Poignard P, Burton DR. 2016. A prominent site of antibody vulnerability on HIV envelope incorporates a motif associated with CCR5 binding and its camouflaging glycans. *Immunity* 45:31–45. <https://doi.org/10.1016/j.immuni.2016.06.026>.
 34. Cormier EG, Dragic T. 2002. The crown and stem of the V3 loop play distinct roles in human immunodeficiency virus type 1 envelope glycoprotein interactions with the CCR5 coreceptor. *J Virol* 76:8953–8957. <https://doi.org/10.1128/JVI.76.17.8953-8957.2002>.
 35. Juhasz AB. 2016. Encouraging positive interactions in dementia care. *Br J Nurs* 25:1162. <https://doi.org/10.12968/bjon.2016.25.21.1162>.
 36. Conrad JA, Ramalingam RK, Duncan CB, Smith RM, Wei J, Barnett L, Simons BC, Lorey SL, Kalams SA. 2012. Antiretroviral therapy reduces the magnitude and T cell receptor repertoire diversity of HIV-specific T cell responses without changing T cell clonotype dominance. *J Virol* 86:4213–4221. <https://doi.org/10.1128/JVI.06000-11>.
 37. Chang HW, Tartaglia LJ, Whitney JB, Lim SY, Sanisetty S, Lavine CL, Seaman MS, Rademeyer C, Williamson C, Ellingson-Strauss K, Stamatatos L, Kublin J, Barouch DH. 2015. Generation and evaluation of clade C simian-human immunodeficiency virus challenge stocks. *J Virol* 89:1965–1974. <https://doi.org/10.1128/JVI.03279-14>.
 38. Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, Koutsoukos M, Voss G, Goepfert P, Gilbert P, Greene KM, Bilska M, Kothe DL, Salazar-Gonzalez JF, Wei X, Decker JM, Hahn BH, Montefiori DC. 2005. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 79:10108–10125. <https://doi.org/10.1128/JVI.79.16.10108-10125.2005>.
 39. Montefiori DC. 2009. Measuring HIV neutralization in a luciferase reporter gene assay. *Methods Mol Biol* 485:395–405. https://doi.org/10.1007/978-1-59745-170-3_26.
 40. Ledgerwood JE, Coates EE, Yamshchikov G, Saunders JG, Holman L, Enama ME, DeZure A, Lynch RM, Gordon I, Plummer S, Hendel CS, Pegu A, Conan-Cibotti M, Sitar S, Bailer RT, Narpala S, McDermott A, Louder M, O'Dell S, Mohan S, Pandey JP, Schwartz RM, Hu Z, Koup RA, Capparelli E, Mascola JR, Graham BS, VRC 601 Study Team. 2015. Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. *Clin Exp Immunol* 182:289–301. <https://doi.org/10.1111/cei.12692>.
 41. Whitney JB, Luedemann C, Hraner P, Rao SS, Mascola JR, Nabel GJ, Letvin NL. 2009. T-cell vaccination reduces simian immunodeficiency virus levels in semen. *J Virol* 83:10840–10843. <https://doi.org/10.1128/JVI.01202-09>.
 42. Li H, Liu J, Carville A, Mansfield KG, Lynch D, Barouch DH. 2011. Durable mucosal simian immunodeficiency virus-specific effector memory T lymphocyte responses elicited by recombinant adenovirus vectors in rhesus monkeys. *J Virol* 85:11007–11015. <https://doi.org/10.1128/JVI.05346-11>.
 43. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, Sun C, Grayson T, Wang S, Li H, Wei X, Jiang C, Kirchherr JL, Gao F, Anderson JA, Ping LH, Swanstrom R, Tomaras GD, Blattner WA, Goepfert PA, Kilby JM, Saag MS, Delwart EL, Busch MP, Cohen MS, Montefiori DC,

- Haynes BF, Gaschen B, Athreya GS, Lee HY, Wood N, Seoighe C, Perelson AS, Bhattacharya T, Korber BT, Hahn BH, Shaw GM. 2008. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc Natl Acad Sci U S A* 105:7552–7557. <https://doi.org/10.1073/pnas.0802203105>.
44. Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, Lynch DM, Lampietro MJ, SanMiguel A, Seaman MS, Ferrari G, Forthal DN, Ourmanov I, Hirsch VM, Carville A, Mansfield KG, Stablein D, Pau MG, Schuitemaker H, Sadoff JC, Billings EA, Rao M, Robb ML, Kim JH, Marovich MA, Goudsmit J, Michael NL. 2012. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature* 482:89–93. <https://doi.org/10.1038/nature10766>.