## HIV THERAPY

# Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques

Ling Xu,[1]* Amarendra Pegu,[2]* Ercole Rao,[1] Nicole Doria-Rose,[2] Jochen Beninga,[1] Krisha McKee,[2] Dana M. Lord,[1] Ronnie R. Wei,[1] Gejing Deng,[1] Mark Louder,[2] Stephen D. Schmidt,[2] Zachary Mankoff,[2] Lan Wu,[1] Mangaiarkarasi Asokan,[2] Christian Beil,[1] Christian Lange,[1] Wulf Dirk Leuschner,[1] Jochen Kruip,[1] Rebecca Sendak,[1] Young Do Kwon,[2] Tongqing Zhou,[2] Xuejun Chen,[2] Robert T. Bailer,[2] Keyun Wang,[2] Misook Choe,[2] Lawrence J. Tartaglia,[3,4] Dan H. Barouch,[3,4] Sijy O'Dell,[2] John-Paul Todd,[2] Dennis R. Burton,[4,5] Mario Roederer,[2] Mark Connors,[6] Richard A. Koup,[2] Peter D. Kwong,[2] Zhi-yong Yang,[1] John R. Mascola,[2]† Gary J. Nabel[1]†

The development of an effective AIDS vaccine has been challenging because of viral genetic diversity and the difficulty of generating broadly neutralizing antibodies (bnAbs). We engineered trispecific antibodies (Abs) that allow a single molecule to interact with three independent HIV-1 envelope determinants: the CD4 binding site, the membrane-proximal external region (MPER), and the V1V2 glycan site. Trispecific Abs exhibited higher potency and breadth than any previously described single bnAb, showed pharmacokinetics similar to those of human bnAbs, and conferred complete immunity against a mixture of simian-human immunodeficiency viruses (SHIVs) in nonhuman primates, in contrast to single bnAbs. Trispecific Abs thus constitute a platform to engage multiple therapeutic targets through a single protein, and they may be applicable for treatment of diverse diseases, including infections, cancer, and autoimmunity.

A variety of broadly neutralizing antibodies (bnAbs) have been isolated from HIV-1–infected individuals (1–3), but their potential to treat or prevent infection in humans may be limited by the potency or breadth of viruses neutralized (4, 5). The targets of these antibodies have been defined according to an understanding of the HIV-1 envelope structure (6–9). Although bnAbs occur in selected HIV-1–infected individuals (usually after several years of infection), it remains a challenge to elicit them by vaccination because broad and potent HIV-1 neutralization often requires unusual antibody characteristics, such as long hypervariable loops, interaction with glycans, and a substantial level of somatic mutation. Strategies have thus shifted from active to passive immunization, both to protect against infection and to target latent virus (10–14).

We and others have begun to explore combinations of bnAbs that optimize potency and breadth of protection, thus reducing the likelihood of resistance and viral escape (15–17). Antibodies directed to the CD4 binding site (CD4bs), membrane-proximal external region (MPER), and variable-region glycans are among the combinations that so far provide optimal neutralization (18). In addition, alternative combinations have also been investigated for the immunotherapy of AIDS, specifically by directing T lymphocytes to activate latent viral gene expression and enhance lysis of virally infected cells (19, 20). Because multiple antibodies may help to reduce the viral replication that sustains chronic HIV-1 infection, we report here the generation of multispecific antibodies designed to increase the potential efficacy of HIV-1 antibodies for prevention or therapy.

### Design of bispecific antibodies and evaluation of neutralization breadth

Although individual anti–HIV-1 bnAbs can neutralize naturally occurring viral isolates with high potency, the percentage of strains inhibited by these monoclonal Abs (mAbs) varies (21, 22). In addition, resistant viruses can be found in the same patients from whom bnAbs were isolated, which suggests that immune pressure against a single epitope may not optimally treat HIV-1 infection or protect against it. We hypothesized that the breadth and potency of HIV-1 neutralization by a single antibody could be increased by combining the specificities against different epitopes into a single molecule. This strategy would be expected not only to improve efficacy but also to simplify treatment regimens, as well

as the regulatory issues required for clinical development.

To test this concept, we initially incorporated prototype bnAbs to the CD4bs and MPER sites into a modified bispecific Ab. When two variable regions are linked in tandem, the distal site typically retains its ability to bind antigen while the proximal binding is markedly diminished. We therefore used an alternative configuration, termed CODV-Ig, that introduced linkers and inverted the order of the antibody binding site in light and heavy chains to alter the orientation of the variable regions, allowing each region to interact with its target (23). Several known bnAbs, including VRC01, 10E8, PGT121, and PGT128 [reviewed in (1)], were evaluated for their ability to neutralize a select panel of viruses with known resistance or sensitivity to these antibodies (fig. S1). Initially, we determined whether the position of the variable regions from VRC01 and 10E8 in the proximal or distal positions (Fig. 1A) could affect neutralization breadth and potency. Inclusion of both variable regions in either orientation in the bispecific antibody reduced the number of resistant strains relative to the parental antibodies alone (Fig. 1B). Better potency was observed when VRC01 was proximal and 10E8 distal, although neither bispecific antibody was as potent as a mixture of the two antibodies alone.

To explore whether other bnAbs could perform better in the bispecific format, we evaluated two different combinations: VRC01 plus PGT121, or VRC01 plus PGT128. For PGT121, expression was observed only with VRC01 in the distal position. When this antibody was compared to the parental antibodies alone, it provided marginally better neutralization (Fig. 2A). In contrast, VRC01 could be expressed with PGT128 in both positions, with greater breadth observed when VRC01 was distal (Fig. 2B). Together, these data indicate that improvements in breadth could be achieved with a bispecific format; however, in this case, the potency was not consistently improved relative to each Ab alone. We therefore sought an alternative format to improve the potency and breadth of neutralization.

### Generation and comparison of broad and potent trispecific antibodies

To achieve our goal, we used a previously undescribed trispecific Ab format. Three specificities were combined by using knob-in-hole heterodimerization (24) to pair a single arm derived from a normal immunoglobulin (IgG) with a double arm generated in the CODV-Ig. A panel of bnAbs was evaluated, including those directed against the CD4bs that included VRC01 and N6, as well as PGT121, PGDM1400, and 10E8 (fig. S1). A modified version of the latter, termed 10E8v4, was used because of its greater solubility (25). We first determined which bispecific arms showed the best potency, breadth, and yield. This screening analysis revealed that combinations containing PGDM1400, CD4bs, and 10E8v4 showed the highest level of production and greatest potency of neutralization (fig. S2).

[1]Sanofi, 640 Memorial Drive, Cambridge, MA 02139, USA. [2]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA. [3]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA. [4]Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139, USA. [5]Department of Immunology and Microbiology, International AIDS Vaccine Initiative (IAVI) Neutralizing Antibody Center, Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, The Scripps Research Institute, La Jolla, CA 92037, USA. [6]National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA.
*These authors contributed equally to this work.
†Corresponding author. Email: gary.nabel@sanofi.com (G.J.N.); jmascola@mail.nih.gov (J.R.M.)

We then evaluated different combinations of single-arm and double-arm specificities from PGDM1400, CD4bs, and 10E8v4 Abs for their expression levels and activity against a small panel of viruses (fig. S3), leading ultimately to the identification of trispecific antibodies VRC01/PGDM1400-10E8v4 and N6/PGDM1400-10E8v4 as lead candidates. When analyzed against a panel of 208 viruses (18) and compared to the parental antibodies alone, the highest neutralization potency and breadth were observed with N6/PGDM1400-10E8v4, with only 1 of the 208 viruses showing neutralization resistance and a median inhibitory concentration ($IC_{50}$) of less than 0.02 μg/ml (Fig. 3A). VRC01/PGDM1400-10E8v4 also displayed high potency and breadth, and only four resistant viruses were found. Some parental mAbs displayed either high breadth (e.g., 10E8, N6) or high potency (PGDM1400), but none displayed a combination of breadth and potency as optimal as the trispecific Abs (Fig. 3B). For example, the most potent and broad parental mAb, N6, was around 20% as potent as the N6/PGDM1400-10E8v4 trispecific Ab and targeted only a single epitope, which could increase the chance of viral escape mutations. As a single recombinant protein, the trispecific Abs demonstrated potency and breadth superior to any single antibody yet defined (Fig. 3 and fig. S4).

We also determined the binding affinity of each component of the trispecific Ab and compared each to its parental Fab. The equilibrium binding constant $K_d$ of each binding site in the trispecific Ab, determined by surface plasmon resonance, was comparable to the affinity of the parental Fab, with PGDM1400 showing a slight decrease in affinity (factor of ~3) and VRC01 and 10E8v4 exhibiting approximately a logarithmic increase (fig. S5). In addition, the trispecific Ab was able to bind sequentially to each of the three antigens (Fig. 3C), indicating that there is independent binding of each epitope.
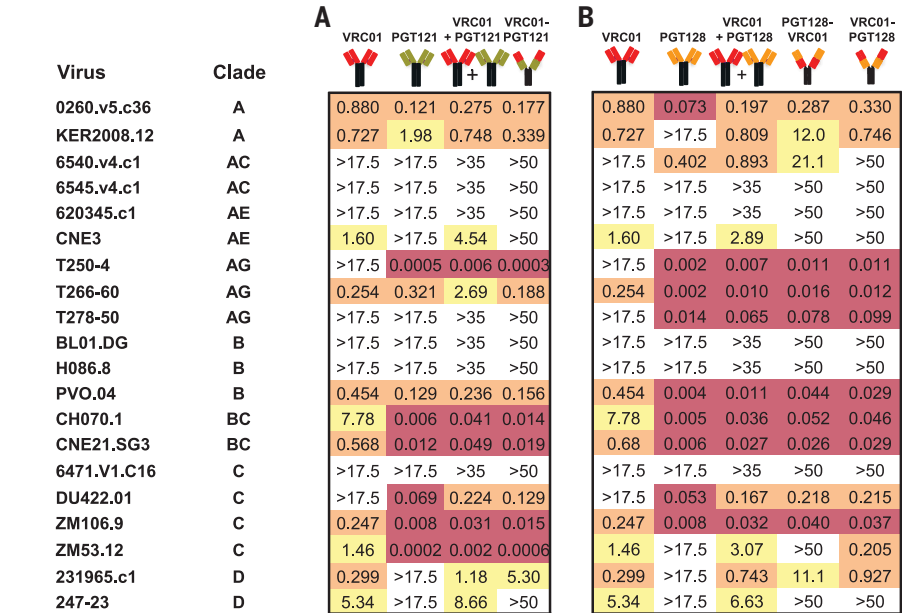
The N6 trispecific Ab also showed greater potency and breadth relative to three related bispecific Abs when tested against a panel of 20 viruses that were selected for resistance to bnAbs (table S1). This finding is consistent with previous studies comparing the efficacy of mixtures of two versus three bnAbs (18) and provides additional support for the multitargeting concept. In addition to their greater efficacy, the trispecific Abs also yielded higher protein levels and greater solubility than the bispecific model (compare fig. S2A and fig. S3), which would facilitate large-scale production and clinical translation.

## Fc modification to extend half-life and crystal structure

To identify the optimal candidate for further development, we determined the half-life of the trispecific Abs in nonhuman primates (NHPs). We previously showed that in the context of the VRC01 mAb, mutations that increased binding to the neonatal Fc receptor (FcRn), which recycles IgG in intestinal epithelial cells and increases levels in the serum, extended half-life, enhanced
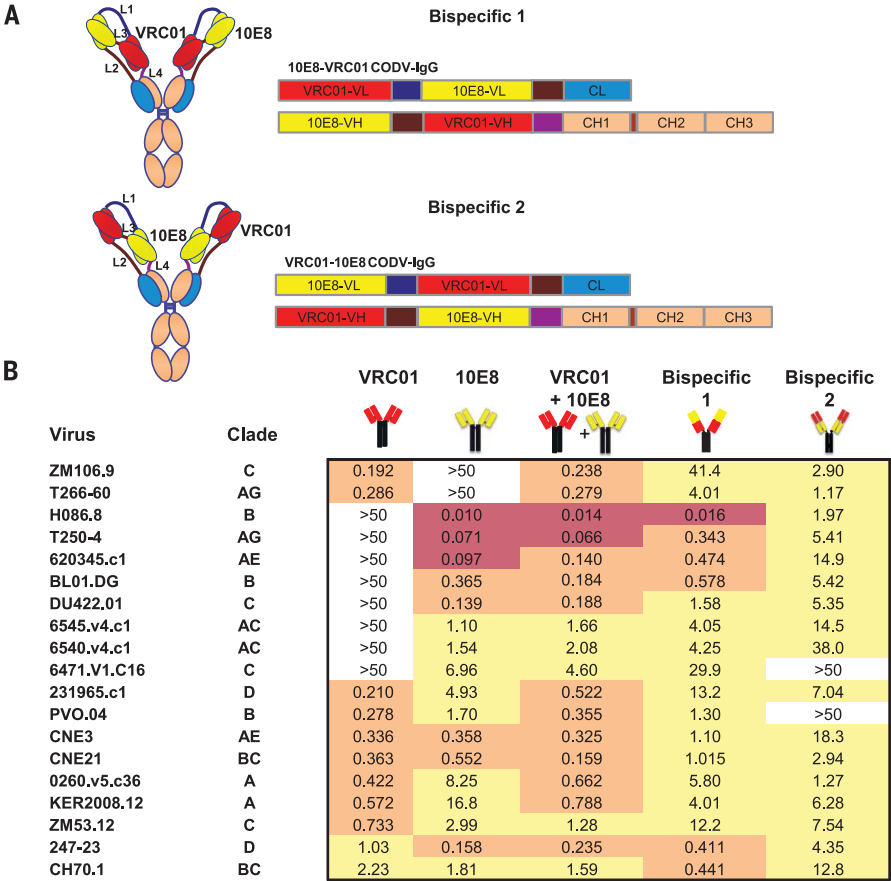


**Fig. 1. CODV-Ig bispecific antibody design and neutralization titers of the VRC01/10E8 bispecific antibodies.** (**A**) CODV-Ig bispecific antibody design with two different orientations of 10E8 and VRC01. (**B**) Neutralization titers ($IC_{50}$), in μg/ml, of VRC01/10E8 bispecific Abs and parental Abs against a select panel of 19 circulating HIV-1 strains; values highlighted in red, orange, and yellow indicate highest, medium, and lowest potency, respectively.

| Virus | Clade | VRC01 | 10E8 | VRC01 + 10E8 | Bispecific 1 | Bispecific 2 |
|---|---|---|---|---|---|---|
| ZM106.9 | C | 0.192 | >50 | 0.238 | 41.4 | 2.90 |
| T266-60 | AG | 0.286 | >50 | 0.279 | 4.01 | 1.17 |
| H086.8 | B | >50 | 0.010 | 0.014 | 0.016 | 1.97 |
| T250-4 | AG | >50 | 0.071 | 0.066 | 0.343 | 5.41 |
| 620345.c1 | AE | >50 | 0.097 | 0.140 | 0.474 | 14.9 |
| BL01.DG | B | >50 | 0.365 | 0.184 | 0.578 | 5.42 |
| DU422.01 | C | >50 | 0.139 | 0.188 | 1.58 | 5.35 |
| 6545.v4.c1 | AC | >50 | 1.10 | 1.66 | 4.05 | 14.5 |
| 6540.v4.c1 | AC | >50 | 1.54 | 2.08 | 4.25 | 38.0 |
| 6471.V1.C16 | C | >50 | 6.96 | 4.60 | 29.9 | >50 |
| 231965.c1 | D | 0.210 | 4.93 | 0.522 | 13.2 | 7.04 |
| PVO.04 | B | 0.278 | 1.70 | 0.355 | 1.30 | >50 |
| CNE3 | AE | 0.336 | 0.358 | 0.325 | 1.10 | 18.3 |
| CNE21 | BC | 0.363 | 0.552 | 0.159 | 1.015 | 2.94 |
| 0260.v5.c36 | A | 0.422 | 8.25 | 0.662 | 5.80 | 1.27 |
| KER2008.12 | A | 0.572 | 16.8 | 0.788 | 4.01 | 6.28 |
| ZM53.12 | C | 0.733 | 2.99 | 1.28 | 12.2 | 7.54 |
| 247-23 | D | 1.03 | 0.158 | 0.235 | 0.411 | 4.35 |
| CH70.1 | BC | 2.23 | 1.81 | 1.59 | 0.441 | 12.8 |



**Fig. 2. Neutralization titers of VRC01/PGT121- and VRC01/PGT128-based bispecific antibodies.** (**A** and **B**) Neutralization titers ($IC_{50}$), in μg/ml, of the VRC01/PGT121 (A) and VRC01/PGT128 (B) bispecific Abs against a select panel of 20 circulating HIV-1 strains, with colors as in Fig. 1.

| | | A | | | | B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Virus | Clade | VRC01 | PGT121 | VRC01 + PGT121 | VRC01-PGT121 | VRC01 | PGT128 | VRC01 + PGT128 | PGT128-VRC01 | VRC01-PGT128 |
| 0260.v5.c36 | A | 0.880 | 0.121 | 0.275 | 0.177 | 0.880 | 0.073 | 0.197 | 0.287 | 0.330 |
| KER2008.12 | A | 0.727 | 1.98 | 0.748 | 0.339 | 0.727 | >17.5 | 0.809 | 12.0 | 0.746 |
| 6540.v4.c1 | AC | >17.5 | >17.5 | >35 | >50 | >17.5 | 0.402 | 0.893 | 21.1 | >50 |
| 6545.v4.c1 | AC | >17.5 | >17.5 | >35 | >50 | >17.5 | >17.5 | >35 | >50 | >50 |
| 620345.c1 | AE | >17.5 | >17.5 | >35 | >50 | >17.5 | >17.5 | >35 | >50 | >50 |
| CNE3 | AE | 1.60 | >17.5 | 4.54 | >50 | 1.60 | >17.5 | 2.89 | >50 | >50 |
| T250-4 | AG | >17.5 | 0.0005 | 0.006 | 0.0003 | >17.5 | 0.002 | 0.007 | 0.011 | 0.011 |
| T266-60 | AG | 0.254 | 0.321 | 2.69 | 0.188 | 0.254 | 0.002 | 0.010 | 0.016 | 0.012 |
| T278-50 | AG | >17.5 | >17.5 | >35 | >50 | >17.5 | 0.014 | 0.065 | 0.078 | 0.099 |
| BL01.DG | B | >17.5 | >17.5 | >35 | >50 | >17.5 | >17.5 | >35 | >50 | >50 |
| H086.8 | B | >17.5 | >17.5 | >35 | >50 | >17.5 | >17.5 | >35 | >50 | >50 |
| PVO.04 | B | 0.454 | 0.129 | 0.236 | 0.156 | 0.454 | 0.004 | 0.011 | 0.044 | 0.029 |
| CH070.1 | BC | 7.78 | 0.006 | 0.041 | 0.014 | 7.78 | 0.005 | 0.036 | 0.052 | 0.046 |
| CNE21.SG3 | BC | 0.568 | 0.012 | 0.049 | 0.019 | 0.68 | 0.006 | 0.027 | 0.026 | 0.029 |
| 6471.V1.C16 | C | >17.5 | >17.5 | >35 | >50 | >17.5 | >17.5 | >35 | >50 | >50 |
| DU422.01 | C | >17.5 | 0.069 | 0.224 | 0.129 | >17.5 | 0.053 | 0.167 | 0.218 | 0.215 |
| ZM106.9 | C | 0.247 | 0.008 | 0.031 | 0.015 | 0.247 | 0.008 | 0.032 | 0.040 | 0.037 |
| ZM53.12 | C | 1.46 | 0.0002 | 0.002 | 0.0006 | 1.46 | >17.5 | 3.07 | >50 | 0.205 |
| 231965.c1 | D | 0.299 | >17.5 | 1.18 | 5.30 | 0.299 | >17.5 | 0.743 | 11.1 | 0.927 |
| 247-23 | D | 5.34 | >17.5 | 8.66 | >50 | 5.34 | >17.5 | 6.63 | >50 | >50 |

mucosal localization, and conferred more efficient protection against lentivirus infection relative to the wild-type antibody (26). One such mutation was incorporated into the trispecific Abs as well as the parental VRC01 and N6 Abs. Abs were then infused into rhesus macaques, and serum levels were analyzed over a 14-day time frame. Ab VRC01 displayed a longer half-life than the

more broad and potent N6, which was also directed to the CD4bs (Fig. 4, VRC01 versus N6). Similarly, the trispecific Ab containing VRC01 showed greater persistence and a longer half-life (7.43 days, based on day 1–day 14 serum concentrations) than the N6 trispecific (4.79 days) in vivo (Fig. 4, VRC01/PGDM1400-10E8v4 and N6/PGDM1400-10E8v4). For this reason, and because

the N6 trispecific Ab yielded less product with decreased solubility, we studied the VRC01/PGDM1400-10E8v4 trispecific Ab further.

Further characterization was performed by solving the crystal structure of the bispecific arm of the trispecific Ab, PGDM1400-10E8v4 CODV Fab, at 3.55 Å resolution (Fig. 5, A and B). The light chain was well resolved in the electron density (with the exception of the two most C-terminal residues), whereas the heavy chain showed some regions of dynamic disorder. The most notable region consisted of part of PGDM1400 complementary-determining region heavy chain 3 (CDRH3) and the linker between PGDM1400 Fv and the heavy chain constant domain (residues 280 to 305). Similar to the anti–interleukin-4 (IL-4)/IL-13 CODV Fab crystal structures (23), the PGDM1400 and 10E8v4 Fvs opposed one another, with the CDRs well exposed to the solvent. The distance between the CDRH3s of PGDM1400 and 10E8v4 exceeded 100 Å. The PGDM1400 and 10E8v4 Fvs superposed very well with their respective parental Fv structures [RMSD (Cα) ≈ 1 Å] (fig. S6) (25, 27), confirming that their antigen-binding properties have been well preserved in the CODV format. Most important, the orientations of the CDRs in two Fvs were 180° from each other, which suggests that each antibody-combining site can independently engage its antigen without obstructing the other Fv structure. A model for the trispecific Ab was constructed by combining the PGDM1400-10E8v4 CODV Fab with VRC01 (6) and the intact b12 (28) IgG crystal structures (Fig. 5C). Similar to a natural IgG, the distance between the monovalent fragment of antigen binding (Fab) and CODV Fab was about 150 Å. Two of three antigens (gp120 core and gp41 MPER) were also included in the model, although we do not have direct evidence that all three HIV epitopes can be engaged simultaneously by a single trispecific Ab.

## Enhanced cross-protection and decreased viral escape in vivo

To evaluate the VRC01/PGDM1400-10E8v4 trispecific Ab for its ability to protect against infection, we used a mixture of two SHIVs that each differed in neutralization sensitivity to the parental bnAbs. In vitro assessment of the replication-competent SHIV challenge stocks showed that SHIV BaLP4 was sensitive to VRC01 and the trispecific antibody but was resistant to PGDM1400 (Fig. 6A). In contrast, SHIV 325C virus was sensitive to PGDM1400 and the trispecific Ab but was resistant to VRC01 (Fig. 6A). In a neutralization assay with an equal mixture of SHIV BaLP4 and SHIV 325c, we observed that the trispecific Ab could achieve complete neutralization of the viral mixture, whereas VRC01 or PGDM1400 could not (fig. S7). When naïve rhesus macaques were infused with the half-life–extended VRC01, PGDM1400, or VRC01/PGDM1400-10E8v4 (5 mg/kg), respectively, serum concentrations were maintained at levels of ≥1 μg/ml for more than 14 days for all Abs (Fig. 6B). A decrease in serum levels at later time points for
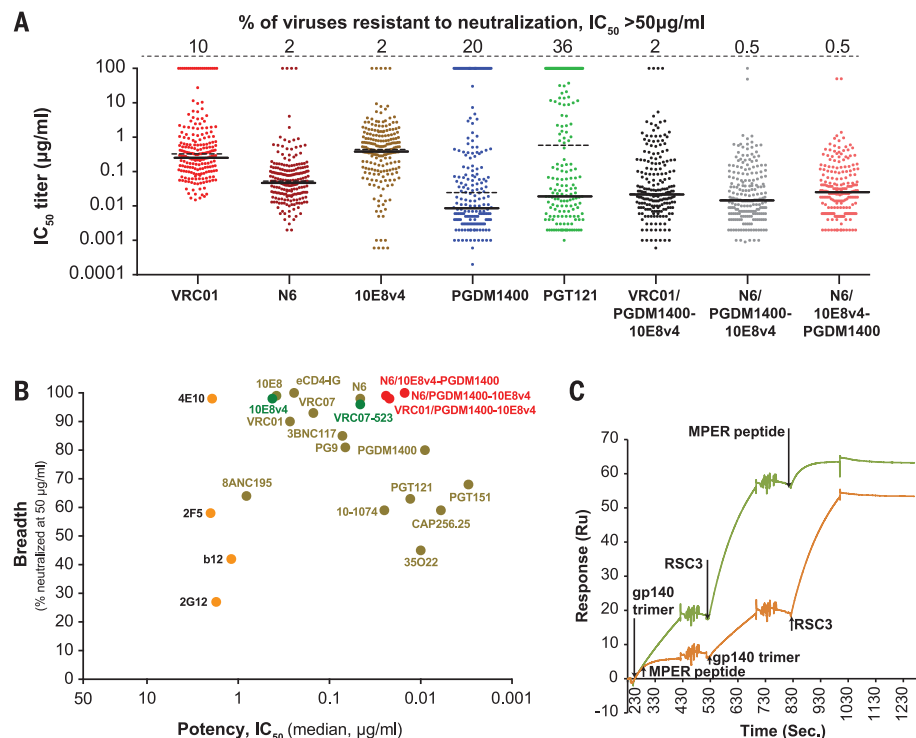
**Fig. 3. Neutralization titers of trispecific antibodies and broadly neutralizing antibodies, and sequential binding of alternative Env epitopes.** (**A**) Neutralization titers (IC$_{50}$) of different bnAbs and trispecific Abs against a genetically diverse panel of 208 circulating HIV-1 strains. The solid line denotes the median IC$_{50}$ neutralization titer of sensitive viruses; the dashed line indicates median titers of all 208 viral strains. Percentages of resistant viruses are shown in the top line. (**B**) Breadth and potency of the trispecific Abs relative to other bnAbs. Colors associated with each Ab designate first-generation Abs of lower breadth (orange); second-generation bnAbs or an engineered Ab-like molecule, eCD4-IgG (brown); structurally enhanced bnAbs (green); or trispecifics (red). (**C**) Sequential binding of three antigens to the trispecific Ab, VRC01/PGDM1400-10E8v4, in the indicated order. The RSC3 (45) antigen represents monomeric gp120 optimized for the CD4 binding site Ab VRC01. MPER peptide interacts with 10E8 (7); gp140 trimer for PGDM1400 was derived from the gp140ΔN6 (BG505) protein.

**Fig. 4. Serum antibody levels in rhesus macaques infused with parental and trispecific antibodies.** Concentrations of VRC01 (green), N6 (black), and the two indicated trispecific Abs (brown, blue) containing a Fc mutation to extend half-life were measured in serum over the course of 14 days after intravenous administration of a single 10 mg/kg dose of each antibody. Each data point represents the mean ± SEM of the values from two to six animals per group (VRC01, n = 6; N6, n = 4; each trispecific Ab, n = 2) and determined in replicates from two independent experiments.
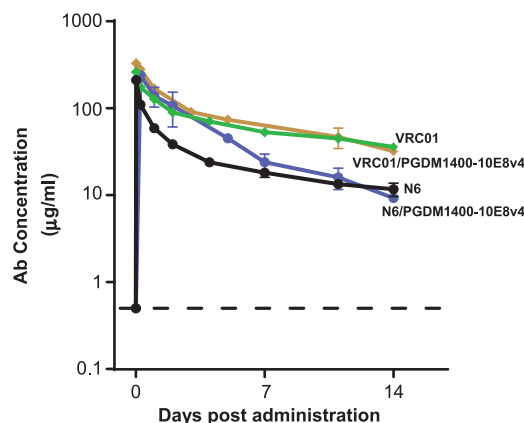
the trispecific Ab correlated with the development of monkey anti-human Abs but arose almost 2 weeks after the SHIV challenge.

To ensure an adequate challenge dose, we first challenged naïve animals with each virus independently. For SHIV 325c, four naïve rhesus macaques were inoculated one time intrarectally with 1 ml of undiluted viral stock. All four animals were infected and showed persistent viremia for up to 90 days (fig. S8). For SHIV BaLP4, the same stock and dose of virus were used as described in several of our prior publications (26, 29, 30). In total, 30 control animals were previously challenged with a single 1-ml intrarectal inoculation of SHIV BaLP4, and all became infected.

To assess in vivo protection, we challenged NHPs mucosally with a mixture of these differentially sensitive SHIVs, 5 days after Ab infusion in two separate experiments, with four animals in each group. In total, six of eight macaques (75%) infused with VRC01 alone, and five of eight animals (62%) treated solely with PGDM1400, became infected. In contrast, none of the eight animals in the trispecific-treated group were infected (Fig. 6C; $P$ = 0.0058, two-tailed Fisher exact test). These data confirm that the improved breadth and potency of the trispecific Ab conferred protection against viruses that otherwise show resistance to single bnAbs alone.

## Discussion
### Next-generation HIV-1 bnAbs

A hallmark of HIV-1 infection is the remarkable genetic diversity of the virus. Since 2010, substantial progress has been made in the identification of bnAbs that show exceptional breadth and potency [reviewed in (1)]. Several of these antibodies have progressed into clinical trials for prevention or treatment, and there is renewed interest in exploring their potential in the clinical management of HIV-1 infection (5, 12, 14). Here, we explored the potential of different bnAbs to combine into a single protein that confers protection against diverse HIV-1 strains. Among the classes of bnAbs, we found that trispecific Abs derived from bnAbs with CD4bs, MPER, and V1V2 glycan specificities had broad specificity, were potent, and could be produced in sufficient quantities to allow evaluation in NHPs and eventually in humans. When tested in NHPs with viruses resistant to individual parental bnAbs, the trispecific Ab demonstrated complete protection against both viruses, whereas infection was established in most animals treated with individual parental antibodies VRC01 and PDGM1400. In addition, the ability of this trispecific Ab to target three independent epitopes may improve treatment efficacy in humans.

In HIV-1–infected patients, reductions in viral load have been observed after one infusion of a single bnAb, thus demonstrating the biological activity of HIV-1 bnAbs (31–34). A modest extension of viral rebound was also observed when individual bnAbs were infused after antiretroviral drugs were discontinued in previously suppressed

HIV-1–infected subjects (32, 33). NHP and human passive transfer studies have also suggested that such bnAbs can enhance antiviral immunity that may contribute to improved viral control (35, 36). In addition, NHP studies demonstrate the importance of mAb potency and prolonged antibody half-life in mediating protection against infection (26, 29). The generation of trispecific Abs with improved potency and breadth may further enhance the efficacy of either passive immunity or passive-active immunization strategies.

Although bnAbs show exceptional breadth and potency, resistant viral strains have been detected in patients who make these Abs (6, 37) and among natural viral isolates (38–40), raising the concern that resistance and escape mutations may arise. Such escape mutations are produced frequently with antiviral drug therapy (41), and countermeasures to reduce the likelihood of escape would increase the likelihood of developing a globally relevant therapy. Such breadth of coverage might alternatively be generated by administering multiple bnAbs, and protective efficacy in a NHP model has recently been demonstrated against a mixture of SHIV viruses using an antibody cocktail (42), providing further support for the multitargeting concept. Combination mAb therapy increases the complexity, development pathway, cost, and regulatory burdens of their use for treatment or prevention, in contrast to a single biologic therapy. The potency of the

trispecific Abs described here also exceeds that of a broad and potent recombinant form of CD4 (43), termed eCD4-Ig (fig. S4), and this latter molecule is also directed to a single, albeit highly conserved, HIV-1 Env epitope. The availability of a single protein that targets multiple independent epitopes on virus also reduces the potential generation of escape mutations. This advantage could be related, in part, to the presence of three independent binding specificities at all times, in contrast to mixtures of antibodies where selective pressure by individual mAbs with shorter half-lives may wane.

### Clinical translation

The trispecific Abs have not yet been evaluated for safety and efficacy in humans. Initial characterization of their half-life in NHPs suggests that they behave similarly to conventional antibodies, but it remains unknown whether they could be immunogenic in vivo. The administration of a bispecific antibody to the human cytokines IL-4 and IL-13, which uses a related format and linkers (44), may provide guidance in this regard. This bispecific antibody has been evaluated in humans, where single subcutaneous doses of SAR156597, ranging from 10 to 300 mg/kg, were well tolerated in healthy subjects, with low titers of anti-drug antibody (ADA) in only 4 of 36 subjects (44). This trial showed a mean half-life of about 2 weeks (44), similar to natural mAbs.



**Fig. 5. Crystal structure of the CODV Fab and a structure model of the trispecific antibody.** (**A**) Configuration of the trispecific antibody, color-coded by parental antibody. Dark shades (purple or green) denote heavy chain peptides; light shades denote light chain peptides. (**B**) Crystal structure of the PGDM1400-10E8v4 CODV Fab in side and top views. CDRH3s from the two Fvs are labeled to highlight the antigen-binding region gp41 MPER, which was modeled in by superposing PDB 5IQ9 onto the 10E8v4 Fv. (**C**) VRC01/gp120 structure (PDB 4LST) and the CODV Fab were modeled onto the b12 structure (PDB 1HZH) by overlaying the CH1-CL domains. Color codes are matched in (A), (B), and (C).
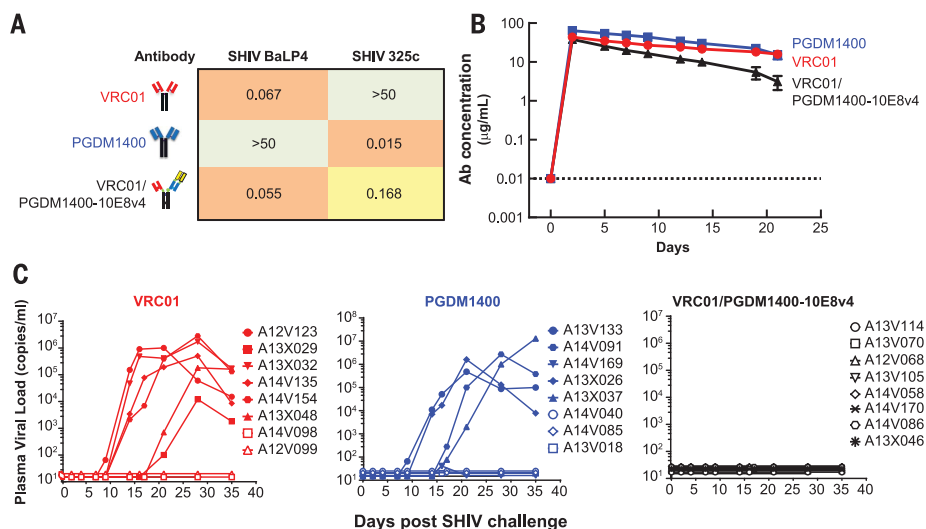
**Fig. 6. Trispecific and broad neutralizing antibody sensitivity of SHIVs, plasma antibody levels, and viremia in rhesus macaques.** (**A**) IC$_{50}$ neutralizing titers, in μg/ml, of VRC01, PGDM1400, and VRC01/PGDM1400-10E8v4 against replication-competent SHIV BaLP4 or SHIV 325c, with colors as in Fig. 1. (**B**) Plasma levels of VRC01, PGDM1400, and VRC01/PGDM1400-10E8v4 in rhesus macaques (*n* = 8 on each arm, done in two separate experiments with four animals each). All animals were administered 5 mg/kg of the indicated antibody intravenously. Each data point represents the mean ± SEM of the values from all eight animals per group. (**C**) Plasma viral loads in rhesus macaques (*n* = 8 per group) challenged with a mixture of SHIV BaLP4 and SHIV 325c, 5 days after intravenous administration of VRC01, PGDM1400, or VRC01/PGDM1400-10E8v4.

Although further human trials are needed to assess the full potential of the trispecific Ab platform, the data from the NHP challenge study described here, as well as the previous experience in humans with bispecific Abs (*44*), suggest that the approach merits further clinical investigation. Studies in HIV-infected subjects, alone or in combination with other immune interventions, will address the potential of trispecific Abs to provide durable protective immunity against infection or sustained viral control in HIV-infected subjects during drug holidays or in the absence of antiretroviral therapy. The recognition of independent target sites with multispecific antibodies can also be applied to other infectious diseases, cancer, and autoimmunity. These antibodies can promote recognition and binding to critical antigenic determinants on target cells while simultaneously allowing engagement of immune cells that can stimulate relevant effector function without the complications and expense of delivering multiple recombinant proteins.

## REFERENCES AND NOTES

1. D. R. Burton, L. Hangartner, *Annu. Rev. Immunol.* **34**, 635–659 (2016).
2. J. R. Mascola, B. F. Haynes, *Immunol. Rev.* **254**, 225–244 (2013).
3. P. D. Kwong, J. R. Mascola, *Immunity* **37**, 412–425 (2012).
4. L. E. McCoy, D. R. Burton, *Immunol. Rev.* **275**, 11–20 (2017).
5. D. M. Margolis, R. A. Koup, G. Ferrari, *Immunol. Rev.* **275**, 313–323 (2017).
6. T. Zhou et al., *Immunity* **39**, 245–258 (2013).
7. J. Huang et al., *Nature* **491**, 406–412 (2012).
8. J. S. McLellan et al., *Nature* **480**, 336–343 (2011).
9. A. B. Ward, I. A. Wilson, *Immunol. Rev.* **275**, 21–32 (2017).
10. B. F. Haynes, J. R. Mascola, *Immunol. Rev.* **275**, 5–10 (2017).
11. A. S. Fauci, *J. Am. Med. Assoc.* **316**, 143–144 (2016).
12. A. Pegu, A. J. Hessell, J. R. Mascola, N. L. Haigwood, *Immunol. Rev.* **275**, 296–312 (2017).
13. J. M. Brady, D. Baltimore, A. B. Balazs, *Immunol. Rev.* **275**, 324–333 (2017).
14. M. Caskey, F. Klein, M. C. Nussenzweig, *N. Engl. J. Med.* **375**, 2019–2021 (2016).
15. M. Asokan et al., *J. Virol.* **89**, 12501–12512 (2015).
16. S. Bournazos, A. Gazumyan, M. S. Seaman, M. C. Nussenzweig, J. V. Ravetch, *Cell* **165**, 1609–1620 (2016).
17. Y. Huang et al., *Cell* **165**, 1621–1631 (2016).
18. R. Kong et al., *J. Virol.* **89**, 2659–2671 (2015).
19. A. Pegu et al., *Nat. Commun.* **6**, 8447 (2015).
20. D. D. Sloan et al., *PLOS Pathog.* **11**, e1005233 (2015).
21. F. Gao et al., *Cell* **158**, 481–491 (2014).
22. N. A. Doria-Rose et al., *Nature* **509**, 55–62 (2014).
23. A. Steinmetz et al., *MAbs* **8**, 867–878 (2016).
24. A. M. Merchant et al., *Nat. Biotechnol.* **16**, 677–681 (1998).
25. Y. D. Kwon et al., *J. Virol.* **90**, 5899–5914 (2016).
26. S. Y. Ko et al., *Nature* **514**, 642–645 (2014).
27. D. Sok et al., *Proc. Natl. Acad. Sci. U.S.A.* **111**, 17624–17629 (2014).
28. E. O. Saphire et al., *Science* **293**, 1155–1159 (2001).
29. A. Pegu et al., *Sci. Transl. Med.* **6**, 243ra88 (2014).
30. K. O. Saunders et al., *J. Virol.* **89**, 5895–5903 (2015).
31. R. M. Lynch et al., *Sci. Transl. Med.* **7**, 319ra206 (2015).
32. K. J. Bar et al., *N. Engl. J. Med.* **375**, 2037–2050 (2016).
33. J. F. Scheid et al., *Nature* **535**, 556–560 (2016).
34. M. Caskey et al., *Nat. Med.* **23**, 185–191 (2017).
35. T. Schoofs et al., *Science* **352**, 997–1001 (2016).
36. Y. Nishimura et al., *Nature* **543**, 559–563 (2017).
37. X. Wu et al., *Cell* **161**, 470–485 (2015).
38. H.-X. Liao et al., *Nature* **496**, 469–476 (2013).
39. P. L. Moore et al., *J. Virol.* **87**, 4882–4894 (2013).
40. J. N. Bhiman et al., *Nat. Med.* **21**, 1332–1336 (2015).
41. World Health Organization, *WHO HIV Drug Resistance Report 2012* (2012); www.who.int/hiv/pub/drugresistance/report2012/en/.
42. B. Julg et al., *Sci. Transl. Med.* **9**, eaao4235 (2017).
43. M. R. Gardner et al., *Nature* **519**, 87–91 (2015).
44. C. Soubrane et al., paper presented at the 18th International Colloquium on Lung and Airway Fibrosis, Mont Tremblant, Quebec; www.iclaf.com/Final_ICLAF_Program.pdf, 127 (2014).
45. X. Wu et al., *Science* **329**, 856–861 (2010).

# Science

## Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques

Ling Xu, Amarendra Pegu, Ercole Rao, Nicole Doria-Rose, Jochen Beninga, Krisha McKee, Dana M. Lord, Ronnie R. Wei, Gejing Deng, Mark Louder, Stephen D. Schmidt, Zachary Mankoff, Lan Wu, Mangaiarkarasi Asokan, Christian Beil, Christian Lange, Wulf Dirk Leuschner, Jochen Kruip, Rebecca Sendak, Young Do Kwon, Tongqing Zhou, Xuejun Chen, Robert T. Bailer, Keyun Wang, Misook Choe, Lawrence J. Tartaglia, Dan H. Barouch, Sijy O'Dell, John-Paul Todd, Dennis R. Burton, Mario Roederer, Mark Connors, Richard A. Koup, Peter D. Kwong, Zhi-yong Yang, John R. Mascola and Gary J. Nabel

### A triple threat for HIV
The HIV virus continually evolves tricks to evade elimination by the host. Prevention and a cure will likely rely on broadly neutralizing antibodies that can recognize and conquer multiple viral strains or subtypes. Xu *et al.* engineered a single antibody molecule to recognize three highly conserved proteins needed for HIV infection (see the Perspective by Cohen and Corey). This "trispecific" antibody uses two sites (V1V2 and MPER) to bind HIV-infected cells, while the third site (CD4bs) recruits killer T lymphocytes that can eliminate the virus. When tested against >200 different HIV strains, trispecific antibodies were highly potent and broadly neutralized ~99% of HIV viruses. This approach could potentially simplify HIV treatment regimens and improve therapy response.
*Science*, this issue p. 85; see also p. 46

| | |
|---|---|
| **ARTICLE TOOLS** | http://science.sciencemag.org/content/358/6359/85 |
| **SUPPLEMENTARY MATERIALS** | http://science.sciencemag.org/content/suppl/2017/09/19/science.aan8630.DC1 |
| **RELATED CONTENT** | http://stm.sciencemag.org/content/scitransmed/9/408/eaao4235.full<br>http://science.sciencemag.org/content/sci/358/6359/46.full<br>http://stm.sciencemag.org/content/scitransmed/9/406/eaal1321.full<br>http://stm.sciencemag.org/content/scitransmed/9/402/eaaf1483.full<br>http://stm.sciencemag.org/content/scitransmed/9/405/eaam5441.full |
| **REFERENCES** | This article cites 46 articles, 14 of which you can access for free<br>http://science.sciencemag.org/content/358/6359/85#BIBL |
| **PERMISSIONS** | http://www.sciencemag.org/help/reprints-and-permissions |

Use of this article is subject to the Terms of Service