



# Safety, tolerability, pharmacokinetics, and immunological activity of dual-combinations and triple-combinations of anti-HIV monoclonal antibodies PGT121, PGDM1400, 10-1074, and VRC07-523LS administered intravenously to HIV-uninfected adults: a phase 1 randomised trial

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## Summary

**Background** Preclinical and clinical studies suggest that combinations of broadly neutralising antibodies (bnAbs) targeting different HIV envelope epitopes might be required for sufficient prevention of infection. We aimed to evaluate the dual and triple anti-HIV bnAb combinations of PGDM1400 (V2 Apex), PGT121 (V3 glycan), 10-1074 (V3 glycan), and VRC07-523LS (CD4 binding site).

**Methods** In this phase 1 trial (HVTN 130/HPTN 089), adults without HIV were randomly assigned (1:1:1) to three dual-bnAb treatment groups simultaneously, or the triple-bnAb group, receiving 20 mg/kg of each antibody administered intravenously at four centres in the USA. Participants received a single dose of PGT121+VRC07-523LS (treatment one; n=6), PGDM1400+VRC07-523LS (treatment two; n=6), or 10-1074+VRC07-523LS (treatment three; n=6), and two doses of PGDM1400+PGT121+VRC07-523LS (treatment four; n=9). Primary outcomes were safety, pharmacokinetics, and neutralising activity. Safety was determined by monitoring for 60 min after infusions and throughout the study by collecting laboratory assessments (ie, blood count, chemistry, urinalysis, and HIV), and solicited and unsolicited adverse events (via case report forms and participant diaries). Serum concentrations of each bnAb were measured by binding antibody assays on days 0, 3, 6, 14, 28, 56, 112, 168, 224, 280, and 336, and by serum neutralisation titres against Env-pseudotyped viruses on days 0, 3, 28, 56, and 112. Pharmacokinetic parameters were estimated by use of two-compartment population pharmacokinetic models; combination bnAb neutralisation titres were directly measured and assessed with different interaction models. This trial is registered with ClinicalTrials.gov, NCT03928821, and has been completed.

**Findings** 27 participants were enrolled from July 31, to Dec 20, 2019. The median age was 26 years (range 19–50), 16 (58%) of 27 participants were assigned female sex at birth, and 24 (89%) participants were non-Hispanic White. Infusions were safe and well tolerated. There were no statistically significant differences in pharmacokinetic patterns between the dual and triple combinations of PGT121, PGDM1400, and VRC07-523LS. The median estimated elimination half-lives of PGT121, PGDM1400, 10-1074, and VRC07-523LS were 32·2, 25·4, 27·5, and 52·9 days, respectively. Neutralisation coverage against a panel of 12 viruses was greater in the triple-bnAb versus dual-bnAb groups: area under the magnitude-breadth curve at day 28 was 3·1, 2·9, 3·0, and 3·4 for treatments one to four, respectively. The Bliss-Hill multiplicative interaction model, which assumes complementary neutralisation with no antagonism or synergism among the bnAbs, best described combination bnAb titres in the dual-bnAb and triple-bnAb groups.

**Interpretation** No pharmacokinetic interactions among the bnAbs and no loss of complementary neutralisation were observed in the dual and triple combinations. This study lays the foundation for designing future combination bnAb HIV prevention efficacy trials.

**Funding** US National Institute of Allergy and Infectious Diseases, US National Institute on Drug Abuse, US National Institute of Mental Health, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

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## Introduction

HIV remains an important public health concern with an estimated global prevalence of 37·9 million infected

people worldwide and 1·5 million new HIV infections in 2020.<sup>1</sup> Despite extraordinary advances in HIV treatment, testing, and prevention, challenges in achieving a

*Lancet HIV* 2023; 10: e653–62

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## Research in context

### Evidence before this study

Passive immunisation with broadly neutralising antibodies (bnAbs) to prevent HIV acquisition is a rapidly expanding field. A single antibody targeting the CD4 binding site on the HIV envelope conferred protection against HIV strains susceptible to the antibody in the Antibody Mediated Prevention trials, even though prevention efficacy was limited due to circulating strains resistant to VRC01. To address this issue, several bnAbs are being tested alone or in combinations for safety, tolerability, and pharmacokinetic properties in phase 1 trials and in non-human primate models. Studies assessing neutralisation coverage and potential interactions of different bnAb combinations are important to guide the selection of optimal combinations and further inform the HIV prevention field. We did a systematic search in PubMed and ClinicalTrials.gov for the search terms PGT121, PGDM1400, 10-1074, and VRC07-523LS until October, 2022, and assessed publications for quality of evidence. The present study was designed to evaluate the safety and tolerability, neutralisation activity, and pharmacokinetics of dual and triple antibody

combinations that target three different envelope regions in people living without HIV.

### Added value of this study

This phase 1 study showed the feasibility of combining anti-HIV bnAbs targeting different sites on the HIV envelope in people living without HIV. It also showed that the dual-antibody and triple-antibody combinations were as effective as the individual antibodies at neutralisation, thus justifying a combination approach going forward for additional monoclonal antibody studies for HIV prevention. The fact that modelling predicted the combination neutralisation titre on the basis of the single antibody titres will be valuable for future trial design of different antibody combinations.

### Implications of all the available evidence

Our data show that a combination of three bnAbs can achieve the breadth and titre indicative of advanced clinical development. Addition of Fc mutations to increase the half-life of the V2 and V3 loop directed antibodies to match that of VRC07-523LS should enhance the feasibility of this type of intravenous regimen for global prevention of HIV.

meaningful and lasting effect on HIV incidence remain. Effective biomedical HIV prevention options that are safe, less dependent on individual adherence, and have sustained effectiveness are urgently needed. Passive immunisation with monoclonal antibodies (mAbs) is an important HIV prevention option that will potentially require dosing only a few times a year and eliminate the need for an individual's daily or event-driven adherence. If the effectiveness and durability of mAbs are similar to other forms of long-acting pre-exposure prophylaxis, they would offer additional choices in the prevention toolbox.

A minority of individuals living with HIV develop broadly neutralising antibodies (bnAbs) within 2 to 3 years after infection. Over the past decade, dozens of such antibodies have been identified and their target sites and mechanisms of action have been described,<sup>2-4</sup> paving the way to their evaluation as a passive immunisation HIV prevention strategy. These antibodies bind to highly conserved regions of the HIV envelope (Env), such as the CD4 binding site (eg, 3BNC117, VRC07-523LS, and N6), and other sites of vulnerability on the HIV-1 Env protein: V3 glycan (10-1074 and PGT121), the membrane-proximal external region (MPER) of gp41 (10E8), and the V2 glycan (PGDM1400 and CAP256-VRC26.25).<sup>3</sup>

Over time, bnAbs with improved breadth and potency have been identified, and modified antibodies have been engineered to extend antibody half-life or augment effector function.<sup>2,5,6</sup> Two seminal, proof-of-concept Antibody Mediated Prevention (AMP) trials showed that a single bnAb (VRC01) targeting the CD4 binding site could prevent infection with highly sensitive HIV strains.<sup>7</sup>

Overall, prevention efficacy was low given the predominance of resistant strains.<sup>7</sup> These data suggest that similar to combination antiretroviral therapy, either a combination of complementary antibodies binding different epitopes, or a single molecule engineered to bind two or three epitopes (bispecific or trispecific bnAbs),<sup>8</sup> will be needed to increase breadth and potency, and to limit viral resistance to ultimately enhance the prevention potential of these agents.<sup>9,10</sup>

Defining optimal combinations of bnAbs is an area of active investigation. Although many studies reporting the safety of single mAb therapy for HIV prevention in humans have been published,<sup>11-13</sup> data on combination bnAbs remain scarce. Early studies have suggested dual and triple combinations are safe and well tolerated.<sup>14-16</sup> The bnAbs VRC07-523LS, PGDM1400, PGT121, and 10-1074 were selected for this study on the basis of their neutralisation potency, breadth of HIV-1 strain coverage, and their complementarity. VRC07-523LS, which targets the CD4 binding site, was developed from a naturally occurring mAb and optimised to increase its breadth, potency, and half-life.<sup>17</sup> PGDM1400 protects against a large number of strains, targets the V2 glycan, and can prevent simian HIV transmission in non-human primates.<sup>18,19</sup> PGT121, which targets the V3 glycan, has high in-vitro potency and confers protection in non-human primate challenge models.<sup>19,20</sup> Similarly, 10-1074 (also targeting the V3 glycan) has high potency, blocks cell-free virus transmission, and protects against simian HIV in non-human primates.<sup>20-22</sup> PGDM1400 and PGT121 have complementary neutralisation profiles against global HIV-1 viruses.<sup>19,23</sup>

The HIV Vaccine Trials Network (HVTN) 130/HIV Prevention Trials Network (HPTN) 089 trial aimed to study the safety, tolerability, pharmacokinetics, and antiviral properties of these four human bnAbs targeting different sites of Env administered in dual and triple combination therapy.

## Methods

### Study design and participants

HVTN 130/HPTN 089 was a randomised, phase 1 study done at four centres in the USA (Fenway Health Clinical Research Site, Boston, MA; Harlem Prevention Center Clinical Research Site, New York, NY; Nashville Clinical Research Site; Columbia Physicians & Surgeons Clinical Research Site, New York, NY) between July 31, 2019, and March 25, 2021. The primary objective was to evaluate the safety, tolerability, pharmacokinetics, and serum neutralising activity of PGT121, PGDM1400, 10-1074, and VRC07-523LS antibodies when given sequentially in combinations of two (treatment 1, 2, and 3 [T1, T2, T3]) and in a combination of three (treatment 4 [T4]) bnAbs, dosed intravenously. Study products were administered sequentially (figure 1), with a 15–30 min time interval between individual antibodies. Volunteers were eligible for participation if aged 18–50 years, without HIV-1, in good general health, and not likely to be exposed to HIV during the study period based on behaviour reported within the 12 months before enrolment. Full eligibility criteria are in the appendix (p 3).

The study was approved by the Institutional Review Boards of each participating clinical research site listed above. All participants provided written informed consent in English. The trial was overseen by the HVTN Safety Monitoring Board and registered with ClinicalTrials.gov (NCT03928821). All products were manufactured under current Good Manufacturing Practice standards.

### Randomisation and masking

Participants were randomised through a internet-based randomisation system and the dual combination T1, T2, and T3 were randomised in blocks to ensure balance across groups. Enrolment of the triple combination T4 was contingent on review of safety data from T1–3 and thus T4 was not randomised with T1–3. Participants, clinical study staff, and laboratory programme staff were unmasked to participant treatment assignments.

### Procedures

Participants were screened for eligibility and randomly assigned to a treatment group within 56 days of screening. The first 18 participants were randomly assigned (1:1:1) to sequential infusion of PGT121 and VRC07-523LS (T1), PGDM1400 and VRC07-523LS (T2), or 10-1074 and VRC07-523LS (T3) on day 0. After review of safety data, an additional nine participants were assigned to T4 and received PGDM1400, PGT121, and VRC07-523LS on day 0 and again on day 112. All bnAbs

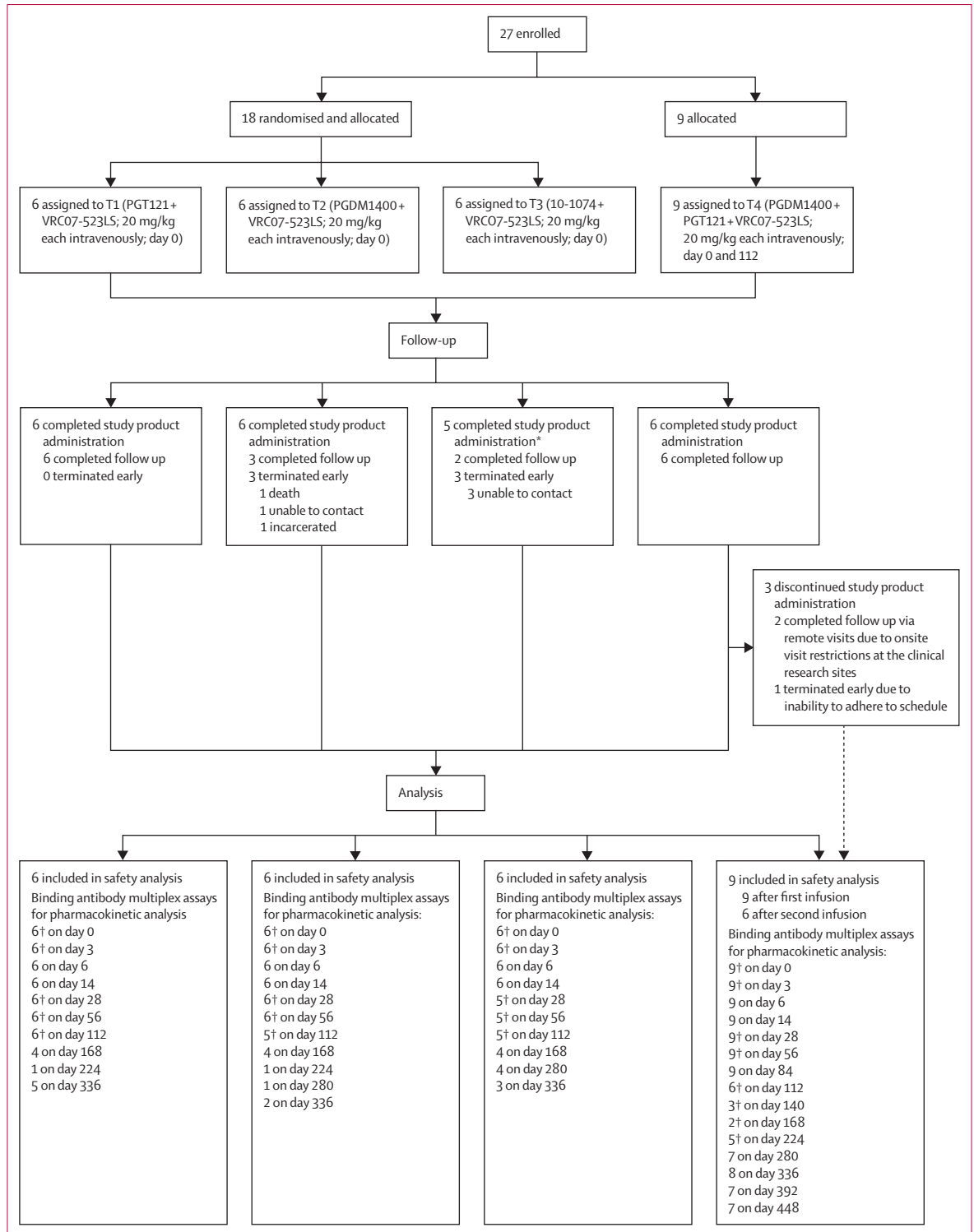
were administered intravenously at a dose of 20 mg/kg. The final infusion in HVTN 130/HPTN 089 was administered to the last enrolled participant on March 9, 2020. The antibodies were infused over 60 min at the 0-month visit. Infusions of PGT121 and PGDM1400 at the 4-month visit occurred over 30 min, and VRC07-523LS at 4 months was infused for 15–30 min. The time interval between individual study products was 15–30 min.

Participants were monitored for at least 60 min after study product administration at study sites to assess for solicited local and systemic adverse events, including pain or tenderness at the infusion site, fever, malaise, myalgia, headache, chills, arthralgia, nausea, urticaria, non-exertional dyspnoea, non-exertional tachycardia, generalised pruritus, facial flushing, and unexplained diaphoresis. Participants subsequently completed daily diary entries to document adverse events for 3 days following infusion. Focused physical examinations and laboratory assays (complete blood count with differential, creatinine, alanine aminotransferase, urine dipstick, pregnancy testing, and HIV testing) were done for safety monitoring at prespecified intervals throughout the study. Unsolicited adverse events were documented throughout the duration of the study.

Although enrolment was completed during the COVID-19 pandemic in the USA in March 2020, follow-up was not completed, and three participants had not yet received their second (and final) infusion. As all attention turned to the COVID-19 response, the protocol was amended to minimise study staff and participant risk of COVID-19 exposure: visit windows were extended and remote clinic visits were permitted (eg, telephone calls, text messages, emails, or by other electronic means).

Serum PGT121, PGDM1400, and VRC07-523LS concentrations before and after administration were quantified by a validated binding antibody multiplex assay;<sup>24</sup> serum 10-1074 concentrations were quantified by a validated ELISA assay.<sup>14</sup> Serum bnAb concentrations were determined using standard curves for the corresponding mAb run on the same assay plate. Assays were done under the Good Clinical Laboratory Practice standards,<sup>25</sup> and performance of mAb standard curves and spiked quality control samples were tracked using Levey-Jennings charts.

Neutralising antibody activity against HIV-1 was measured as a function of reductions in Tat-regulated luciferase (Luc) reporter gene expression in TZM-bl cells as described.<sup>26,27</sup> The assay done in TZM-bl cells measured neutralisation titres against four Env-pseudotyped viruses that enables individual bnAb activities to be determined when multiple bnAbs were present (ie, bnAb-specific viruses; appendix p 3) in each infusion group. An additional panel of 12 Env-pseudotyped viruses, each one selected as being highly susceptible to all antibodies used in this study (appendix p 4), was used to generate a rich dataset for validation of the predictive modelling, and the assumption that combined effects of these



**Figure 1: Trial profile and specimen collection schedule**

From day 112 until the end of study, there were many missed visits due to local and national COVID-19 pandemic closure and isolation protocols. T1–4=treatments one to four. \*One participant in T3 received a partial infusion of 10-1074 and did not receive VRC07-523LS because of infiltration of the intravenous site.

†Neutralising antibody assay also.

three antibodies are mostly additive. The non-infused antibodies were assayed against this latter panel of viruses at concentrations and ratios that are consistent with their pharmacokinetics after intravenous infusion in healthy human volunteers as comparator values for activity in post-infusion serum samples. Serum neutralisation titre was defined as the serum dilution that reduced relative luminescence units by 50% and 80% (ID50 and ID80) relative to the relative luminescence units in virus control wells (cells+virus only) after subtraction of background relative luminescence units (cells only).

Antibodies against 10-1074, PGDM1400, PGT121, or VRC07-523LS were detected and quantified by use of a qualified bridging electrochemiluminescence assay as previously described.<sup>28</sup> Samples were tested in duplicate along with a panel of anti-idiotypic and negative controls and data accepted based on meeting pre-established criteria.

### Outcomes

The primary clinical objective was to evaluate safety and tolerability of the bnAbs administered in various combinations via sequential intravenous infusion. The primary endpoints included frequency of local and systemic solicited adverse events, laboratory measures of safety, unsolicited adverse events, and serious adverse events were recorded by clinicians at every visit as described above. The primary laboratory objectives included evaluating serum concentrations of each bnAb and serum neutralising activity against bnAb-specific and the global panel of viruses. A complete list of primary, secondary, and exploratory objectives and endpoints is described in the appendix (p 5).

### Statistical and pharmacokinetics analysis

All data were analysed according to participants' assigned treatment in the modified intention-to-treat cohort that included all enrolled participants receiving the first infusion. For each bnAb, serum concentrations after intravenous administration exhibited bi-exponential decay and were described by an open two-compartment disposition model with first-order elimination from the central compartment. The model was parameterised in terms of four parameters: clearance from the central compartment (litres per day), volume of the central compartment (litres), intercompartmental distribution clearance (litres per day), and volume of the peripheral compartment (litres). An exponential between-individual random effect was considered for clearance from the central compartment, volume of the central compartment, intercompartmental distribution clearance, and volume of the peripheral compartment on the basis of patterns observed in the data. Non-linear mixed effects modelling with the stochastic approximation of expectation-maximisation estimation method was employed. For each bnAb used in the triple combination (PGT121,

PGDM1400, and VRC07-523LS), pharmacokinetic data collected from both the dual-administration and triple-administration were pooled for the pharmacokinetic modelling; the effect of dual-administration versus triple-administration on each of the pharmacokinetic parameters was tested using the likelihood ratio test in the pharmacokinetic model. For 10-1074, only pharmacokinetic data from the dual combination was used for the modelling. Pharmacokinetic modelling was done using Monolix (version 2019R1, Lixoft SAS, Antony, France); all other analyses used SAS or R3.5.1. Combination bnAb neutralisation interactions were assessed using the maximum, additive, and Bliss-Hill models.<sup>29</sup> The concordance correlation coefficient was used to quantify the agreement between observed and estimated combination neutralisation titres under each of the interaction models.

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

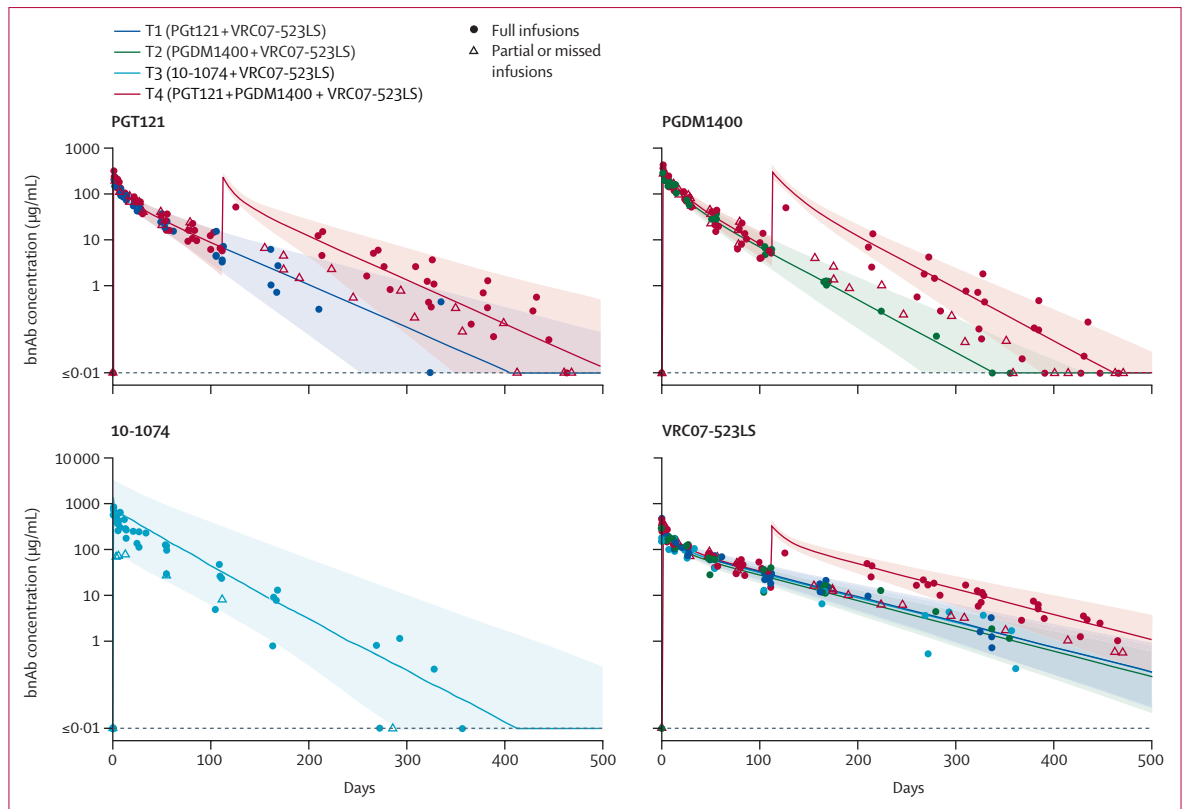
### Results

27 healthy participants without HIV were enrolled between July 31, 2019, and Dec 20, 2019, at four US-based clinical research sites affiliated with the HVTN and HPTN. Duration of follow-up was 12 months for participants

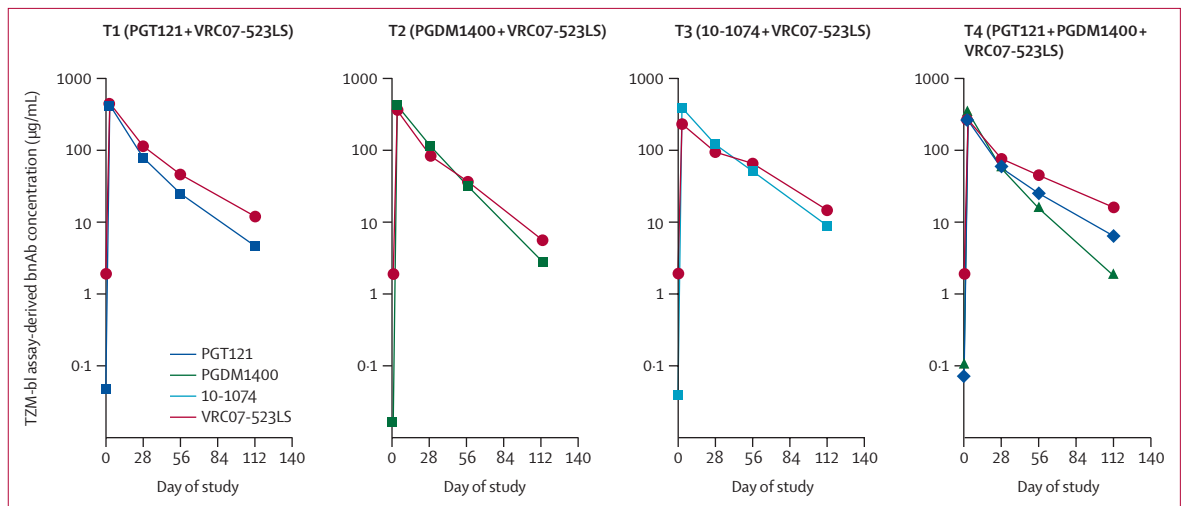
	T1 (n=6)	T2 (n=6)	T3 (n=6)	T4 (n=9)	Total (n=27)
Sex at birth					
Female	5	3	3	5	16 (59%)
Male	1	3	3	4	11 (41%)
Gender*					
Female	3	3	3	4	13 (48%)
Male	2	3	3	3	11 (41%)
Other†	1	0	1	2	4 (15%)
Age, years	25 (21–25)	29 (21–50)	28 (25–49)	30 (19–49)	26 (19–50)
Weight, kg	72.3 (55.7–84.4)	61.6 (55.8–74.4)	67.8 (51.3–77.6)	71.2 (53.5–86.4)	68.0 (51.3–86.4)
Ethnicity					
Hispanic or Latino	0	1	0	2	3 (11%)
Not Hispanic or Latino	6	5	6	7	24 (89%)
Race‡					
Black	0	0	2	0	2 (7%)
White	5	3	4	7	19 (70%)
Other§	1	3	0	2	6 (22%)

Data are n, n (%), or median (range). T1=PGT121 and VRC07-523LS (day 0). T2=PGDM1400 and VRC07-523LS (day 0). T3=10-1074 and VRC07-523LS (day 0). T4=PGDM1400, PGT121, and VRC07-523LS (day 0 and day 112). \*Participants could self-report more than one gender identity. †Other categories are transgender male, transgender female, gender queer, gender variant or gender non-conforming, self-identify, or prefer not to answer. ‡Participants were not required to answer these questions, so counts might not match the number of participants; missingness is not included explicitly as it is minimal. §Other categories are Asian, Native Hawaiian or other Pacific Islander, American Indian or Alaska Native, other, or multiracial.

**Table: Demographic characteristics of participants in the HVTN 130/HPTN 089 trial**



**Figure 2: Observed antibody serum concentrations with 90% prediction interval from the population pharmacokinetic model**  
 Observed (data points) and predicted (line) concentrations of each bnAb. Serum concentration was measured by binding antibody multiplex assay and 10-1074 by ELISA. Shaded areas denote 90% prediction interval. One participant from T3 received partial infusion of 10-1074 and no VRC07-523LS. bnAb=broadly neutralising antibodies. T1-4=treatment one to four.



**Figure 3: Geometric mean ID80 neutralisation-effective serum concentrations of each bnAb in dual and triple bnAb combinations**  
 Neutralisation-effective serum concentrations of each bnAb were calculated by multiplying the ID80 neutralisation titre of the serum sample against a bnAb-specific isolate by the IC80 of the clinical lot of the bnAb against the corresponding isolate. bnAb=broadly neutralising antibodies.

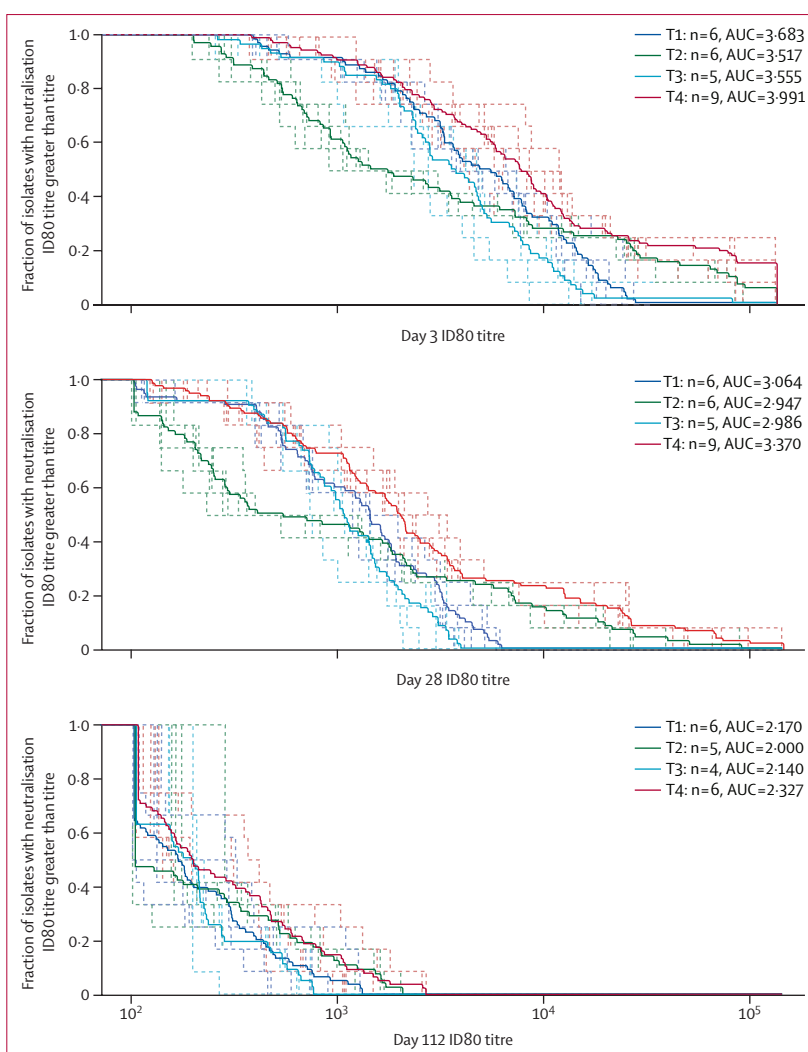
receiving dual combination bnAb therapy and 16 months for participants receiving triple combination bnAb therapy, and all visits were completed by March 25, 2021 (table). Median age was 26 years; and 16 were assigned female at

birth (table). 19 identified as White, two as Black, and three as Hispanic or Latino. Data unavailability was mostly caused by missed visits or remote visits due to the COVID-19 pandemic (appendix p 6).

All six participants in T1 received a single intravenous dose of PGT121 followed by VRC07-523LS, all six in T2 received a single intravenous dose of PGDM1400 followed by VRC07-523LS, and five of six in T3 received a single intravenous dose of 10-1074 followed by VRC07-523LS. All nine participants in T4 received a first intravenous dose of VRC07-523LS administered sequentially with PGDM1400 and PGT121, and six received the second intravenous dose 4 months later; three participants missed the second dose due to clinical research site operational disruptions from the COVID-19 pandemic, with one participant missing a visit and two participants undergoing remote visits, which precluded study product administration and collection of samples (figure 1). 26 participants received at least one dose of VRC07-523LS at 20 mg/kg.

Overall, intravenous administration of study products was generally well tolerated with no related serious adverse events, unexpected reactions, or safety events warranting study pause. 19 participants reported 52 unsolicited adverse events, the majority of which were grade 1 (31 in 16 participants) or grade 2 (20 in 13 participants) in severity. The most commonly reported adverse events were upper respiratory tract infection in four participants, decreased platelet count in three (one grade 2 at day 112, the rest grade 1 or mild), grade 1 pyrexia in two (during study follow-up, unrelated to study product), COVID-19 in two (one grade 1 and one grade 2), urinary tract infection in two, and increased alanine aminotransferase in two (occurring at days 14 and 112). In 18 participants, 51 unsolicited events were of mild or moderate severity. There was one serious adverse event of death due to respiratory failure in a participant in T2 with an undisclosed pre-existing history of asthma who had received his last study product administration more than 7 months earlier. This death was assessed by the principal investigator as not related to study products. Two of 27 participants developed two adverse events that were assessed as related to the study products; these resolved without residual effects. One participant in T3 developed mild and episodic bilateral hand paraesthesia the evening of study product administration, which resolved the following day. One participant in T2 developed a mild infusion-related reaction (chills, mild upper back muscle pain, mild joint pain in wrists, and a mild headache), which resolved within 1 h. Solicited local and systemic reactions in dual and triple combination treatment groups were mild to moderate (appendix p 15).

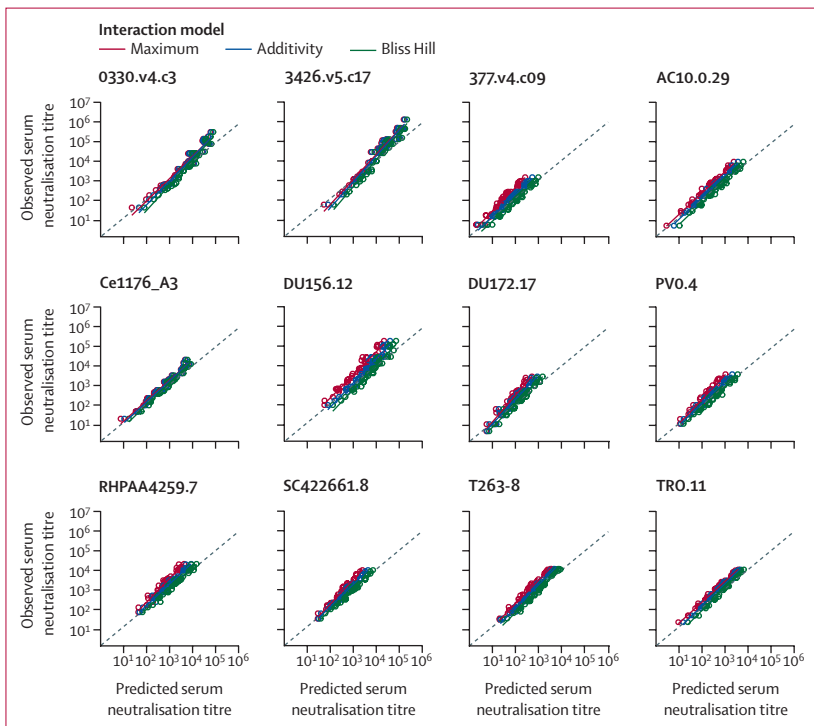
Observed antibody concentrations across treatment groups and at every timepoint after infusion are shown in figure 2 and summarised in the appendix (pp 7–8). The median elimination half-life estimates were 32.2 days (mean 33.0, 95% CI 28.9–36.5) for PGT121, 25.4 days (mean 25.7, 95% CI 23.9–27.7) for PGDM1400, 27.5 days (mean 30.1 days, 95% CI 27.7–33.0) for 10-1074, and 52.9 days (mean 53.3,



**Figure 4:** Individual participant and group average ID80 neutralisation magnitude and breadth against a 12 multiclade virus panel at 3 (A), 28 (B) and 112 days (C) after product administration

Participants received all scheduled product administrations. Dashed curves are for individual participant and solid curves are for group average. AUC are calculated for the group average magnitude-breadth curve. Env-pseudotyped virus panel includes: 0330.v4.c3; 3426.v5.c17; 377.v4.c09; AC10.0.29; Ce1176\_A3; DU156.12; DU172.17; PVO.4; RHPA4259.7; SC422661.8; T263-8; and TRO.11. T1–4=treatment one to four. AUC=area under the curve.

95% CI 49.5–57.8) for VRC07-523LS (appendix p 9). More details of other pharmacokinetic parameters are provided in the appendix (pp 11–14). There were no statistically significant differences in any pharmacokinetic parameters for PGT121, PGDM1400, and VRC07-523LS between dual-combination T1 and T2 compared with triple-combination T4. Coadministration in dual or triple combination did not affect individual antibody pharmacokinetics (appendix p 9). The median elimination half-life of VRC07-523LS in the triple combination (T4) of 54.9 days (SD 6.7) was not significantly different than in the dual combinations (T1, T2, or T3): T1, 53.2 days (SD 6.5); T2, 50.6 days (SD 4.7); and T3, 51.8 days (SD 8.3; appendix p 16).



**Figure 5: Concordance between observed and estimated combination bnAb neutralisation ID80 titres against a 12 multiclade virus panel of participants in the treatment four group**  
 The y axis shows the observed neutralisation ID80 titre at 3, 28, and 112 days after product administration in serum samples of participants who received the triple bnAb combination (PGT121 + PGDM1400 + VRC07-523LS). The x axis shows estimated combination neutralisation ID50 titre under the maximum (red), additive (blue) and Bliss-Hill (green) neutralisation interaction model. Circles denote individual datapoints. Solid lines denote the best fit linear regression line. The dashed lines denote the identity  $Y=X$  line (indicating perfect agreement).

An important objective of the study was to assess how much of the neutralisation activity was retained after passive administration of the bnAbs via estimating neutralisation-effective antibody serum concentration as the product of the observed ID80 (or ID50) neutralisation titre and the *in vitro* neutralisation potency IC80 (or IC50) of the clinical lot of the bnAbs against the same virus. The neutralisation-effective concentration of VRC07-523LS was 324.2 µg/mL in T1, 257.8 µg/mL in T2, 169.7 µg/mL in T3, and 193.4 µg/mL in T4 at the peak timepoint (day 3 after infusion; figure 3). These values were similar to those measured by binding antibody multiplex assay, suggesting that neutralisation function for each bnAb in the combinations was largely maintained in serum as predicted by binding antibody assay-based pharmacokinetic concentrations. Similar neutralisation-effective serum concentrations were observed for all dual and triple bnAb combinations. ID50 neutralising titres showed the same pattern (appendix p 17).

*In vitro* models suggest that administering bnAbs in combination produces more potent and broader neutralisation than administering a single bnAb.<sup>10,23,30</sup> Additionally, the triple combination of PGT121 + PGDM1400 + VRC07-523LS has been predicted to produce neutralisation superior to that of any of the dual

combinations.<sup>31</sup> Among participants who received all scheduled product administrations, we measured serum neutralisation against a panel of 12 Env pseudoviruses in which each virus was highly susceptible to all four bnAbs. As expected, participants in the triple combination (T4) had greater magnitude of neutralising activity against the panel than did those from the dual-bnAb combination groups (T1, T2, T3; figure 4). This pattern was seen at each timepoint through day 112, and ID50 titre showed a similar trend (appendix p 18).

Combination antibody neutralisation interactions were assessed in serum samples with three different mathematical modelling approaches (additive, maximum, and Bliss-Hill) that, *in vitro*, enabled prediction of bnAb combination neutralisation magnitude and breadth from single bnAb data.<sup>23,30</sup> We observed good agreement with a high concordance correlation coefficient (concordance correlation coefficient >0.9 mostly) between the observed and predicted combination serum neutralisation titres against a 12-virus panel for participants in T4 (figure 5; appendix pp 10, 19). bnAb combination serum neutralisation titres were best predicted by the Bliss-Hill model on the basis of individual bnAb neutralisation titres. Similar performances of the Bliss-Hill model were observed for the dual combination groups.

Serum samples for participants were evaluated for anti-drug antibodies before treatment and then at several follow-up visits. No treatment-induced or boosted antibodies against any of the four drug products were observed (appendix p 20).

## Discussion

This study provides important data about safety and tolerability of dual and triple combination of bnAbs when administered sequentially via intravenous infusion and is among the first to describe the use of triple bnAb combinations. All combinations and infusions were generally well tolerated with no serious adverse events attributed to the study products. This finding is similar to that of previous reports evaluating dual or triple bnAb combinations for HIV prevention or for treatment in people living with HIV, and our study adds to the body of crucial data as we move towards future bnAb efficacy trials. Additionally, the data reflect the challenges of an interventional clinical trial for HIV prevention in the setting of the COVID-19 pandemic.

We noted that pharmacokinetic patterns were consistent for each bnAb between the dual or triple combinations, and these were similar to those in previous reports,<sup>32</sup> even accounting for different study population, timepoints, and dose. For example, another phase 1 study evaluating single-dose pharmacokinetics of VRC07-523LS administered via different routes and doses reported an estimated median elimination half-life of 54.8 days,<sup>32</sup> which is similar to what we report in our study (52.9 days). The finding that coadministration in dual or triple combination did not affect the pharmacokinetic



features of the individual bnAbs suggests that there was no clear evidence of pharmacokinetic interactions between any of the antibodies.

The neutralisation function was maintained as predicted by the pharmacokinetic data and the complementary neutralisation magnitude and breadth of bnAbs, with no antagonism or synergism among the bnAbs, were maintained in this study. We also confirmed that the neutralisation coverage was the greatest in the triple bnAb combination compared with any of the dual combinations. The day 112 concentration decays were not at very high titres, but we believe this issue can be resolved with the improved half-life of lysine/serine mutation versions of the V2 and V3 bnAbs.<sup>16,33</sup> At days 3 and 28, we observed ID80 titres of about 200, which was recently shown to be sufficient for prevention of HIV acquisition.<sup>33</sup> The average serum concentrations at 16 weeks after the second intravenous infusion at a dose level of 20 mg/kg were predicted to be 1.6 mcg/mL for PGDM1400, 1.8 mcg/mL for PGT121, and 3.4 mcg/mL for VRC07-523LS. These trough concentrations were believed to confer sufficient neutralisation against diverse panels of viruses. On the basis of the protective neutralisation threshold defined in the AMP study,<sup>33</sup> these concentrations collectively confer protection against a virus with *in vitro* neutralisation sensitivity of IC80 around 0.01 to 0.04 mcg/mL.

We found that combination bnAb neutralisation titres can be predicted by use of individual bnAb neutralisation titres, which validates the *in vitro* models. The ability to accurately predict the breadth and potency of antibody combinations without experimental validation is important to future rapid and iterative identification of optimal combinations, especially in resource-limited settings.

Our study has some limitations. Due to operational restrictions imposed during the COVID-19 pandemic, several participants had remote visits and therefore samples were missing from timepoints, including three of nine participants in group 4 (T4) having missed their second infusion. This reduced sample size influences the precision (not necessarily the accuracy) of our study results; however, we do not believe the interpretation of the presented results is meaningfully affected. Specifically, for the evaluation of the safety endpoints, data after the first infusion were available from all 27 participants, and available from six of nine participants in the triple-bnAb group (T4) after the second infusion. Two of three participants who missed the second infusion in T4 did attend at least one additional clinical or blood collection visit. Therefore, for laboratory endpoints including bnAb concentrations and neutralisation titres, we were able to account for the actual number of infusions that each participant received, and the actual visit and specimen collection dates based on all available data. Another drawback is that, as noted above, a third of the participants in T4 did not receive the second triple bnAb infusion due to disruption caused by the COVID-19

pandemic. It is important to note that the panel of viruses used in our neutralisation assays were chosen to be sensitivity to the four antibodies, and might not represent the diversity of circulating strains. Lastly, even though the point estimates of the pharmacokinetic parameters for the dual and triple combinations did not seem to differ in a clinically meaningful way, it is important to highlight the small sample size of the study. Given the sample size, we only had power to detect large differences in pharmacokinetic or neutralisation between the dual and triple combinations, as well as overall safety. These results will need to be further investigated in on-going (NCT04212091 and NCT05184452) and future bnAb trials evaluating lysine/serine formulations of all four antibodies in dual and triple combinations.

These data, building on AMP correlates results, provide an important basis for the prediction of prevention efficacy of the triple-mAb combination, and inform the field as we seek to identify optimal antibody combinations for HIV prevention. It is expected that future studies will evaluate mAb combinations incorporating lysine/serine-modified versions of all four antibodies, which are expected to evolve to improve function and administration, in preparation for a planned efficacy trial.

#### Contributors

MES, SM, CAP, TG, GDT, YH, DM conceptualised the study and provided supervision. KES, JH, AE, CY, LZ, and JAW did the lab assays or curated the data, or both. MES, CY, LZ, MDM, and YH contributed to data visualisation. CY, LZ, and YH formally analysed the data and were responsible for the methodology and software used. YH, DHB, GDT, and TG were responsible for funding acquisition. DHB, BJ, KS, MN, MC, and MEA contributed resources. MES, SM, DAT, WC, MY, KM, and SK contributed to the investigation. MES, YH, CAP, MDM, GDT, and DM contributed to the original draft and SM, CY, BH, TG, DAT, WC, MY, JH, KES, LZ, AE, KM, SK, KS, BJ, MC, MN, LG, DHB, JAW, and MEA did the review and editing. All authors had full access to all the data in the study. MES, YH, and CAP verified all the data in the study and had final responsibility for the decision to submit for publication.

#### Declaration of interests

MES received funding from the US National Institutes of Health paid to the institution for bnAb prevention studies. BJ is a part-time employee and equity holder of Leyden Laboratories, a company developing pandemic prevention therapeutics. All other authors declare no competing interests.

#### Data sharing

All data will be publicly available upon publication at <https://atlas.scharp.org/cpas/project/HVTN%20Public%20Data/begin.view>.

#### Acknowledgments

We thank the study participants. We also thank Kelvin Chiong, Lu Zhang, Mark Sampson, Martina Wesley, Caroline Brackett, and Nicole Yates for help with the laboratory assays. Overall support for the HIV Prevention Trials Network (HPTN) is provided by the US National Institute of Allergy and Infectious Diseases, US National Institutes of Health (NIH), Office of the Director, US National Institute on Drug Abuse, US National Institute of Mental Health, and the Eunice Kennedy Shriver National Institute of Child Health and Human Development under award numbers UM1AI068619 (HPTN Leadership and Operations Center), UM1AI068617 (HPTN Statistical and Data Management Center), and UM1AI068613 (HPTN Laboratory Center). Additional funding includes NIH grants UM1 AI068614 (HIV Vaccine Trials Network [HVTN] Leadership and Operations Center), UM1 AI068635 (HVTN Statistical and Data Management Center), UM1 AI068618 (HVTN Laboratory Center), UM1 AI069412, UM1 AI069439, and UM1 AI069470.

## References

- 1 UNAIDS. Global HIV & AIDS statistics—fact sheet. <https://www.unaids.org/en/resources/fact-sheet> (accessed Aug 18, 2021).
- 2 Karuna ST, Corey L. Broadly neutralizing antibodies for HIV prevention. *Annu Rev Med* 2020; **71**: 329–46.
- 3 Mahomed S, Garrett N, Baxter C, Abdool Karim Q, Abdool Karim SS. Clinical trials of broadly neutralizing monoclonal antibodies for human immunodeficiency virus prevention: a review. *J Infect Dis* 2021; **223**: 370–80.
- 4 Walsh SR, Seaman MS. Broadly neutralizing antibodies for HIV-1 prevention. *Front Immunol* 2021; **12**: 712122.
- 5 Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: a phase 1 open-label clinical trial in healthy adults. *PLoS Med* 2018; **15**: e1002493.
- 6 Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol* 2007; **7**: 715–25.
- 7 Corey L, Gilbert PB, Juraska M, et al. Two randomized trials of neutralizing antibodies to prevent HIV-1 acquisition. *N Engl J Med* 2021; **384**: 1003–14.
- 8 Padte NN, Yu J, Huang Y, Ho DD. Engineering multi-specific antibodies against HIV-1. *Retrovirology* 2018; **15**: 60.
- 9 Julg B, Liu PT, Wagh K, et al. Protection against a mixed SHIV challenge by a broadly neutralizing antibody cocktail. *Sci Transl Med* 2017; **9**: eaao4235.
- 10 Kong R, Louder MK, Wagh K, et al. Improving neutralization potency and breadth by combining broadly reactive HIV-1 antibodies targeting major neutralization epitopes. *J Virol* 2015; **89**: 2659–71.
- 11 Gaudinski MR, Houser KV, Doria-Rose NA, et al. Safety and pharmacokinetics of broadly neutralising human monoclonal antibody VRC07-523LS in healthy adults: a phase 1 dose-escalation clinical trial. *Lancet HIV* 2019; **6**: e667–79.
- 12 Mayer KH, Seaton KE, Huang Y, et al. Safety, pharmacokinetics, and immunological activities of multiple intravenous or subcutaneous doses of an anti-HIV monoclonal antibody, VRC01, administered to HIV-uninfected adults: results of a phase 1 randomized trial. *PLoS Med* 2017; **14**: e1002435.
- 13 Takuva S, Karuna ST, Juraska M, et al. Infusion reactions after receiving the broadly neutralizing antibody VRC01 or placebo to reduce HIV-1 acquisition: results from the phase 2b antibody-mediated prevention randomized trials. *J Acquir Immune Defic Syndr* 2022; **89**: 405–13.
- 14 Cohen YZ, Butler AL, Millard K, et al. Safety, pharmacokinetics, and immunogenicity of the combination of the broadly neutralizing anti-HIV-1 antibodies 3BNC117 and 10-1074 in healthy adults: a randomized, phase 1 study. *PLoS One* 2019; **14**: e0219142.
- 15 Mahomed S, Garrett N, Capparelli EV, et al. Safety and pharmacokinetics of monoclonal antibodies VRC07-523LS and PGT121 administered subcutaneously for HIV prevention. *J Infect Dis* 2022; **226**: 510–20.
- 16 Julg B, Stephenson KE, Wagh K, et al. Safety and antiviral activity of triple combination broadly neutralizing monoclonal antibody therapy against HIV-1: a phase 1 clinical trial. *Nat Med* 2022; **28**: 1288–96.
- 17 Rudicell RS, Kwon YD, Ko SY, et al. Enhanced potency of a broadly neutralizing HIV-1 antibody in vitro improves protection against lentiviral infection in vivo. *J Virol* 2014; **88**: 12669–82.
- 18 Julg B, Tartaglia LJ, Keele BF, et al. Broadly neutralizing antibodies targeting the HIV-1 envelope V2 apex confer protection against a clade C SHIV challenge. *Sci Transl Med* 2017; **9**: eaal1321.
- 19 Sok D, van Gils MJ, Pauthner M, et al. Recombinant HIV envelope trimer selects for quaternary-dependent antibodies targeting the trimer apex. *Proc Natl Acad Sci USA* 2014; **111**: 17624–29.
- 20 Scheid JF, Mouquet H, Ueberheide B, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science* 2011; **333**: 1633–37.
- 21 Gautam R, Nishimura Y, Pegu A, et al. A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. *Nature* 2016; **533**: 105–09.
- 22 Malbec M, Porrot F, Rua R, et al. Broadly neutralizing antibodies that inhibit HIV-1 cell to cell transmission. *J Exp Med* 2013; **210**: 2813–21.
- 23 Wagh K, Bhattacharya T, Williamson C, et al. Optimal combinations of broadly neutralizing antibodies for prevention and treatment of HIV-1 clade C infection. *PLoS Pathog* 2016; **12**: e1005520.
- 24 Wesley MS, Chiong KT, Seaton KE, et al. Validation of a triplex pharmacokinetic assay for simultaneous quantitation of HIV-1 broadly neutralizing antibodies PGT121, PGDM1400, and VRC07-523-LS. *Front Immunol* 2021; **12**: 709994.
- 25 Ezzelle J, Rodriguez-Chavez IR, Darden JM, et al. Guidelines on good clinical laboratory practice: bridging operations between research and clinical research laboratories. *J Pharm Biomed Anal* 2008; **46**: 18–29.
- 26 Montefiori DC. Measuring HIV neutralization in a luciferase reporter gene assay. *Methods Mol Biol* 2009; **485**: 395–405.
- 27 Sarzotti-Kelsoe M, Bailer RT, Turk E, et al. Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. *J Immunol Methods* 2014; **409**: 131–46.
- 28 Bharadwaj P, Riekofski C, Lin S, et al. Implementation of a three-tiered approach to identify and characterize anti-drug antibodies raised against HIV-specific broadly neutralizing antibodies. *J Immunol Methods* 2020; **479**: 112764.
- 29 Mayer BT, deCamp AC, Huang Y, et al. Optimizing clinical dosing of combination broadly neutralizing antibodies for HIV prevention. *PLoS Comput Biol* 2022; **18**: e1010003.
- 30 Wagh K, Seaman MS, Zingg M, et al. Potential of conventional & bispecific broadly neutralizing antibodies for prevention of HIV-1 subtype A, C & D infections. *PLoS Pathog* 2018; **14**: e1006860.
- 31 Stephenson KE, Wagh K, Korber B, Barouch DH. Vaccines and broadly neutralizing antibodies for HIV-1 prevention. *Annu Rev Immunol* 2020; **38**: 673–703.
- 32 Walsh SR, Gay C, Karuna ST, et al. Safety and single-dose pharmacokinetics of VRC07-523LS administered via different routes and doses. HIVR4P 2024, the 5th HIV Research for Prevention Conference; Jan 27, 2021 (abstr OA03.01).
- 33 Gilbert PB, Huang Y, deCamp AC, et al. Neutralization titer biomarker for antibody-mediated prevention of HIV-1 acquisition. *Nat Med* 2022; **28**: 1924–32.