Adjunctive passive immunotherapy in HIV-1-infected individuals treated with antiviral therapy during acute/early infection

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No similar paper has been or will be submitted elsewhere.

Informed consent was obtained from all patients and the study was approved by the Institutional Review Boards of the Rockefeller University. All clinical investigation was conducted according to the principles expressed in the Helsinki Declaration. Gabriela Stiegler, Brigitta Vcelar and Hermann Katinger are affiliated with Polymun Scientific, a private company involved in the development of the human monoclonal antibodies. All three monoclonal antibodies used in this trial- 2F5, 2G12 and 4E10 were provided by Polymun Scientific. T. Wrin, C.J. Petropoulos and J. Galovich are employees of Monogram Biosciences, Inc., the property company of the Phenosense Entry/Neutralization Assay used in this study.

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1 **ABBREVIATIONS**

2

3 ART  Antiretroviral therapy

4 GI  Gastrointestinal

5 mAbs  Monoclonal antibodies

6 PTT  Prothrombin Time

7 HIV  Human Immunodeficiency Virus

8 NtRTI  Nucleotide Reverse Transcriptase Inhibitor

9 NRTI  Nucleoside Reverse Transcriptase Inhibitor

10 SIV  Simian Immunodeficiency Virus

11 CHO  Chinese Hamster Ovary

12 AIEDRP  Acute Infection Early Disease Research Program

13 ELISA  Enzyme Linked Immunosorbent Assay

14 PCR  Polymerase Chain Reaction

15 DRVVT  Dilute Russel’s Viper Venom Time

16 MMC  Mucosal Mononuclear Cells

17 PBMC  Peripheral Blood Mononuclear Cells

18 HLA  Human Leucocyte Antigens

19 CCR5  Chemokine Receptor 5

20 MPER  Membrane Proximal Region

21

22

23
ABSTRACT

**Background:** Three neutralizing monoclonal antibodies (mAbs), 2G12, 2F5 and 4E10 with activity *in vitro* and *in vivo* were administered in an open label, non-randomized, proof-of-concept study to attempt to prevent viral rebound after interruption of antiretroviral therapy (ART). **Methods:** Ten HIV-1-infected individuals identified and treated during acute and early infection with ART were enrolled. The first 6 patients were administered 1.0 g of each of the three mAbs per infusion. The remaining 4 patients received 2G12 at 1.0 g/infusion and 2.0 g/infusion of 2F5 and 4E10. **Results:** mAbs were well tolerated. Grade I PTT prolongations were noted. Viral rebound was observed in 8/10 subjects (28 to 73 days post ART interruption) and 2/10 subjects remained aviremic over the course of the study. In 7/8 with viral rebound, clear resistance to 2G12 emerged whereas reductions in susceptibility of plasma-derived recombinant viruses to 2F5 and 4E10 were neither sustained nor consistently measured. Viral rebound was associated with a preferential depletion of CD4+ T cells within the GI tract. **Conclusions:** Though safe, the use of mAbs generally delayed but did not prevent virologic rebound. Consideration should be given to further pilot studies with alternative combinations of mAbs and perhaps additional novel treatment modalities.
BACKGROUND

Highly active antiretroviral therapy (HAART) has made a significant impact on the natural history of HIV-1 infection. A near-uniformly fatal infection has been transformed to one that is treatable and chronic when managed properly, particularly in resource abundant settings (42). Treatment regimens have evolved with less toxicity in the short- and long-term, as has compactness accompanying improved ease of administration. Contemporaneously new agents directed against both established and new targets, both viral and cellular, are on the therapeutic horizon.

Despite this apparent success over the past decade, we are still left with a treatment paradigm of life-long antiviral therapy for the majority of HIV-1 infected individuals. Concerns regarding current therapies include high cost (6), emergence of drug resistance in the face of less than perfect adherence (5), cardiovascular complications due to hyperlipidemia (15, 16), metabolic complications due to hyperglycemia and insulin resistance (10, 17, 31), the possibility of renal disease with chronic use of nucleotide reverse transcriptase inhibitors (NtRTI) (44), and perhaps persistent low level viral replication during therapy (20, 56). For all of these reasons, we and others have attempted to pilot alternative treatment paradigms- antiviral therapy for finite periods of time with therapeutic vaccination followed by treatment termination or structured treatment interruptions (32, 33, 40). Such efforts have not resulted in sustained control of viral replication in vivo in most of the participants in these studies, nevertheless, despite “failure”, lessons have been learned. One striking observation in our treatment interruption studies in patients treated during acute and early infection was that after viral rebound a spontaneous reduction in HIV-1 RNA levels in plasma was
observed, 1.7 log\textsubscript{10} on average (range 0.3 to 3.1) (33). This is comparable to what is seen with potent antiviral agents and we believe likely due to an anamnestic immune response in an already primed patient with an intact, inducible immune system.

Given these findings, we hypothesized that if we could complement the autologous cellular immune response induced by virologic rebound with a neutralizing serologic response obtained with infusions of potent neutralizing monoclonal antibodies (mAbs), perhaps sustained virological remission could be achieved. Three such mAbs were made available for clinical use- 2G12 which binds to a carbohydrate moiety on the silent face of gp120 (54), and 4E10, and 2F5, both of which bind to the membrane proximal ectodomain of gp41 (45, 52). At the time of the design of our study, these antibodies had been shown to prevent viral infection in the SIV/macaque model following oral (18, 24, 34), intravenous (12) or intravaginal (35) challenge. In addition, these mAbs were shown to reduce peak viremia post-infection and exhibit antiviral activity in established infection (3, 51). Subsequently, Trkola et al have reported on a passive immunization experiment using a combination of 2G12, 2F5 and 4E10 in acute and chronically HIV-1 infected humans and demonstrated that viral rebound was delayed in a subset of patients (53).

We selected a cohort of 10 individuals who were treated with combination antiretroviral therapy (ART) during acute and early infection and in whom undetectable viral loads were measured for at least 6 months prior to study entry. Patient selection was based on susceptibility of the baseline virus at the time of diagnosis, prior to ART-initiation, to each of the three antibodies. The aims of this proof-of-concept trial were:
1) To prevent or dramatically alter the dynamics of plasma HIV-1 RNA rebound post discontinuation of antiretroviral therapy with the use of a combination of monoclonal antibody infusions. 2) To establish the safety profile of the monoclonal antibody infusions and 3) To compare immunological and virological events in the peripheral blood prior to and post viral rebound with those in the tissue, specifically the lower gastrointestinal tract, a site identified in recent studies as critical to the pathogenesis of HIV-1 infection (7, 22, 36).
MATERIALS AND METHODS

Study subjects

Patients were recruited from the Aaron Diamond AIDS Research Center Primary Infection Program, a site of the Acute Infection and Early Disease Research Program (AIEDRP). Entry and infection duration criteria, all laboratory based, have been previously described (36). All patients were treated during acute or early infection with combination antiviral therapy including nucleoside reverse transcriptase inhibitors (NRTI) and either non-NRTI (NNRTI) or PI (Table 1) for at least 15 months and had HIV-1 RNA levels below detection in the 6 months preceding screening. Stored pretreatment patient plasma was tested for susceptibility to the three monoclonal antibodies using a recombinant assay as previously described (43). Susceptibility in this assay is scored as an inhibitory concentration at the 50% level below 50 µg/ml. Entry criteria allowed that patients be entered if their baseline virus was susceptible to 2G12 and at least to one of the remaining 2 antibodies, however all 10 subjects harbored viruses deemed susceptible to all 3 mAbs (Table 1). The treatment protocol and amendments were reviewed by the Rockefeller University Hospital Institutional Review Board and participants gave informed consent by signature.

Antibodies

Monoclonal antibodies 2G12, 2F5 and 4E10 were produced by recombinant expression in Chinese hamster ovary (CHO) cells as immunoglobulin G1 (IgG1). The generation, production, and characterization of the mAbs were described previously (8, 27, 28).
Antibodies in concentrations of 8-15 mg/ml were provided in 1.0 and 2.0 gram doses in 10% maltose solution at pH 4.5 were provided by Polymun Scientific (Vienna, AT). They were shipped and stored at 4°C for subsequent use. 2G12, 4E10, and 2F5 were routinely infused intravenously over 20 minutes in order and were followed by a 50 ml normal saline infusion over 20 minutes. The initial 6 subjects received 1.0 gram of each antibody at each infusion. The remaining 4 patients received 2.0 grams of 2F5 and 4E10 and the same dose of 2G12 for reasons explained below.

**Study Procedures:**

Subjects were screened and if eligible entered the 28-week study which included 4 weeks of lead in and 24-weeks of infusions, treatment interruption, and post-infusion follow up. Infusions were given weekly for 3 weeks while ART was continued. At the 4th visit ART was discontinued and antibody infusions continued weekly for an additional 12 weeks. A total of 16 infusions were planned. Infusions were stopped if patient plasma HIV-1 RNA levels were above 10,000 copies/ml on 2 occasions at least 2 weeks apart. Patients were advised to resume antiviral therapy for HIV-1 RNA levels above 55,000 copies/ml or if CD4 cell counts fell below 350 cells/mm$^3$ or 50% of the count from the time of treatment interruption. Once infusions were complete, subjects were followed at 2 to 4 week intervals as indicated for 12 weeks (day 168). Subsequent visits occurred every 12 weeks as part of the ongoing AIEDRP-sponsored cohort study. Safety monitoring included weekly interval medical history and physical examination, hematology, chemistry, urinalysis, and serum complement levels. Longitudinal HIV-1 RNA determinations were performed with the Roche Ultrasensitive Amplicor COBAS assay with the lower limit of detection of 50 copies/ml plasma. Pre- and post-infusion plasma
was collected and stored at –80°C to measure antibody levels. Safety laboratory values during treatment and follow-up were examined according to the Division of AIDS (DAIDS) toxicity guidelines for adults (1).

**Antibody level determinations**

The plasma concentrations of mAbs 2G12, 4E10, and 2F5 were determined by specific double-sandwich enzyme-linked immunosorbent assays (ELISAs) by using gp160 (mAb 2G12) or peptide KKWNWFDITNWGGG (mAb 4E10) or GGGLELDKWASL (mAb 2F5) as the capture antigen. The measurement of 2G12 made use of anti-idiotypic antibody conjugated with biotin, whereas goat anti-human IgG conjugated with horseradish peroxidase was chosen to detect 4E10 and 2F5. Each sample was analyzed at eight different dilutions. The limit of detection was 3 ng/ml for each mAb. The methods were described in detail in previous publications (2, 3, 28).

**Determination of virus neutralization by mAbs 2F5, 4E10 and 2G12**

A previously described recombinant viral assay was used to measure virus-antibody neutralization (43). In brief, RNA derived from HIV-1-positive plasma was amplified by reverse transcription-PCR and incorporated into an expression vector (pCXAS) by conventional cloning methods. Recombinant HIV-1 stocks expressing patient virus envelope proteins were prepared by cotransfecting HEK293 cells with a replication-defective, luciferase expression cassette containing HIV-1 genomic viral vector and an appropriate envelope expression vector. Pseudotyped recombinant viruses were harvested 48 h post-transfection and incubated for 1 h at 37°C with serial fourfold dilutions of the three MAbs and plasma controls. U87 cells that express CD4, CCR5, and CXCR4 were inoculated with virus-antibody dilutions. Luciferase activity determined 72
hours post-inoculation was used as the indicator of infectivity. Neutralizing activity was displayed as the percent inhibition of luciferase production at each antibody concentration compared to that of an antibody-negative control. The 50% inhibitory concentration (IC<sub>50</sub>) is defined as the concentration of mAb required to inhibit virus infectivity by 50%. For the purposes of this study, viruses were classified as susceptible to neutralization if the IC<sub>50</sub> for that antibody was ≤50 µg/ml.

**Measurement of HIV-specific neutralizing antibody responses**

HIV-1-specific neutralizing antibody responses were measured by using a recombinant virus assay as described previously (43, 46), using recombinant viruses containing a firefly luciferase indicator gene. Virus infectivity was determined by measuring the amount of luciferase activity expressed in infected cells compared with an antibody-negative control after a single cycle of replication. Neutralizing antibody titers were calculated as the reciprocal of the plasma dilution conferring 50% inhibition (IC<sub>50</sub>). In addition to virus isolated from the study subjects, we also measured heterologous neutralization against the HIV-1 strains NL4–3 (GenBank accession no. AY669735 [GenBank] ) and JR-CSF (GenBank accession no. AY669726 [GenBank] ) as well as a virus pseudotyped with a non-HIV envelope (amphotropic murine leukemia virus) as a specificity control.

**Sequencing of the HIV-1 env gene**

HIV-1 genomic RNA was isolated from patient plasma by using oligo(dT) magnetic beads, and first-strand cDNA was synthesized in a standard reverse transcription reaction using oligo(dT) primers. The entire envelope (gp160) was PCR amplified by using forward and reverse primers located immediately upstream and
downstream of the env initiation and termination codons, respectively. The forward and
reverse primers contain unique recognition sites for PinAI and MluI. Env PCR products
were digested by using PinAI and MluI and ligated into the pCXAS expression vector,
which uses the cytomegalovirus immediate-early promoter enhancer to drive expression
of the env insert in transfected cells. Ligation products were introduced into competent
Escherichia coli cells (Invitrogen) by transformation, and DNA was purified from
bacterial culture. An aliquot of each transformation was spread onto agar plates, and
colony counts were used to estimate the number of env sequences represented in each
library (500–5,000 colonies). DNA was diluted, retransformed, and replated to allow easy
picking of individual colonies (usually 100–200 per plate). Sequencing analysis was
performed by using a thermocycling method with fluorescent dye-labeled
dideoxynucleotide chain terminator chemistry (Applied Biosystems). Sequencing reaction
products were resolved by using a 96 parallel capillary gel electrophoresis system
(Applied Biosystems 3700). Multiple sequence alignment was performed clustal W
algorithm, employing DNA star (Megalign) software.

**Examination of HIV-1 peptide specific cellular immune responses**

Pooled, overlapping 15-mer clade B peptides representing GAG, VPR and NEF
proteins were obtained from the NIH AIDS repository. Aliquots of 0.5 X 10^6 to 2 X 10^6
cryopreserved PBMCs were thawed and resuspended in culture medium. PBMCs were
incubated alone, in the presence of SEB, and HIV-1 peptides as previously described
(33). After a 6 hour stimulation, cells were harvested and stained with anti-CD3 Pacific
Blue (BD pharmingen, clone UCHT-1)-, anti-CD4 Alexa Flour 700 (BD Pharmingen,
clon RPA-T4) and anti-CD8 APC-Cy7 (BD Pharmingen, clone SK1) antibodies. After
surface staining, cells were permeabilized using cytofix-cytoperm solution (BD biosciences) as per manufacturer’s instructions. Intracellular staining was performed using anti-IL-2 FITC (BD Fastimmune, clone 5344.111) and anti-IFN-γ PE (BD Fastimmune, clone 25723.11). Cells were acquired using BD LSRII flow-cytometer (BD Biosciences, La Jolla, CA) and data was analyzed using BD FACS DIVA software.

**Gastrointestinal biopsy sample acquisition**

Peripheral blood and recto-sigmoid colonic mucosal tissue were collected from the study patients sequentially, prior to ART discontinuation, 2 to 6 weeks after ART discontinuation and 8-12 weeks post ART discontinuation. Informed consent was obtained from all patients and the study was approved by the Institutional Review Board of the Rockefeller University Hospital. All clinical investigation was conducted according to the principles expressed in the Helsinki Declaration.

Endoscopic biopsies were obtained from macroscopically normal colonic mucosa and were processed as described previously (36). Briefly, the biopsies were taken using large-cup endoscopic-biopsy forceps (Microvasive Radial Jaw, Boston Scientific, Boston, Massachusetts, United States) (outside diameter 3.3 mm) and placed immediately in tissue-culture medium (RPMI 1640, Mediatech, Herndon, Virginia, United States). Phlebotomy was undertaken immediately prior to endoscopy. Immediately after acquisition, mucosal mononuclear cells (MMCs) were enzymatically isolated from mucosal biopsies using a 30-min incubation in collagenase type II (Clostridiopeptidase A, Sigma-Aldrich, St. Louis, Missouri, United States) followed by mechanical separation through a blunt-ended 16-gauge needle. The digested cell suspension was strained through a 70-μm disposable plastic strainer. Immediately after isolation, cells were
washed with PBS and resuspended in PBS containing antibodies for flow cytometry. Peripheral blood mononuclear cells (PBMCs) were prepared by centrifugation on a Ficoll-Hypaque density gradient (Mediatech). PBMCs were stained for flow cytometry immediately after isolation.

**Flow cytometry**

Cell surface expression of lymphocyte antigens was identified by mAb staining of freshly isolated MMCs and PBMCs, followed by flow cytometry using a FACSCalibur® (Becton Dickinson Immunocytometry Systems (BDIS), Mountain View CA) with analysis using CellQuest® software (BDIS, Mountain View CA). MAbs used in this study included; anti-human CD3 fluorescein isothiocyanate (FITC) (clone UCHT1) (BDIS, Mountain View, CA), anti-human CD4- allophycocyanin (APC) (clone RPA T4) (Pharmingen, San Diego, CA), anti-human CD8 PE (clone RPA T8) (Pharmingen, San Diego, CA). During flow cytometry, lymphocytes, initially identified by their forward and side scatter characteristics, were subject to phenotypic analysis. Dead cells were excluded from analysis using 7-aminoactinomycin D (Calbiochem, La Jolla, CA). To determine the percentages of CD4+ and CD8+ cells in the T-cell population, gated lymphocytes were initially examined for the expression of CD3. The CD3+ lymphocytes were then analyzed for expression of CD4 and CD8 receptors.

**Statistical analysis**

Baseline characteristics as well as clinical and laboratory adverse experiences were recorded and analyzed descriptively. Additional statistical analyses included paired and unpaired Student T tests, Mann-Whitney test, and simple regressions and were performed with StatView (SAS Institute, Carey, NC).
RESULTS:

Study subjects

Ten subjects were deemed eligible for study. All were men who have sex with men, 9 Caucasian, 1 Asian, and were treated for anywhere from 15 to 60 months (Table 1) with PI-based (N=2) or NNRTI-based therapy (N=8). The estimated interval from infection to treatment initiation ranged from 23 to 120 days (mean: 54). Baseline virologic and immunologic data reflected duration of infection (Table 1). All pretreatment viruses were susceptible to all 3 mAbs, defined as above, with mean IC$_{50}$s lowest for 2F5 and highest for 2G12 (Table 1). Suppression of viral replication during ART was accompanied by CD4+ T cell increases of 350 cells/mm$^3$ on average (range: 37 to 563 cells/mm$^3$).

Safety and tolerability of antibody infusions:

A total of 149 infusions were performed. There were no serious adverse events recorded. Fifty-five adverse events occurred, all mild and transient, and were possibly related to the monoclonal antibody infusions. These included body aches (9), fatigue (1), flushed sensation (1), joint soreness (10), limb pain (1), low back ache (5), myalgia (2), redness at infusion site (1), tachycardia without change in blood pressure (14 episodes in 1 subject, likely not related to mAb infusion), tiredness (11), and “floaters in visual field”(1). There was no difference in the occurrence or frequency of adverse effects between patients who received 1gm of 2F5/4E10 and those who received 2gm of these two antibodies.

There were no laboratory adverse events reported other than PTT prolongations as described below. Complement levels remained constant pre- and post- infusion and...
within normal limits in all 10 subjects. Following the report by Haynes et al (23) describing in vitro anticardiolipin antibody activity of 2F5 and 4E10, 12 pre- and post-partial thromboplastin time (PTT) determinations were performed in 4 subjects. All patients had normal coagulation profiles pre-study entry. Of the 12 determinations pre-infusion, 9/12 were within normal limits. Of the 3 outside the normal range, all were Grade 1 (1.1 to 1.25 times the upper limit of normal) (1). Post-infusion PTT levels were drawn 20 minutes after completing antibody infusions at a site on the opposite arm from the infusion site and were prolonged (Grade 1) in 6/9 that were normal pre-infusion. Three that were prolonged pre-infusion were similarly prolonged post infusion and remained prolonged at the Grade 1 level. A mixing study was subsequently performed on one post infusion sample from one patient to ascertain whether the PTT prolongation was due to the presence of an inhibitor. The patient PTT pre-mix was 56.9 sec and with mixing was 53.0 sec. After 1-hour incubation the patient PTT was 53.1 sec and the 50/50 mix was 49.4 sec. These results, partial correction with mixing, are consistent with the presence of an inhibitor. The dilute Russel’s Viper Venom time (DRVVT) was only slightly prolonged 1.38 (normal less than 1.2) and consistent with the presence of a low level inhibitor. PTT prolongations were transient, that is, returned to normal range within a week of infusion on 6 of 9 occasions and returned to the normal range once infusions were completed. Of note, there were no thrombotic complications associated with mAb therapy.

Clinical, virologic and immunologic profiles post treatment interruption

Eight of 10 subjects had rebound plasma viremia during the course of the 28 week study (Fig. 1, Table 2). All subjects experiencing rebound were asymptomatic and no
HIV-1 related conditions were documented after 24 months of follow-up. Three subjects terminated mAb infusions prematurely based on virologic criteria. The remaining 5 subjects had virologic rebound to low levels and did not meet stopping criteria. No patients reinitiated ART during the 24 week study. Virologic rebound occurred 72 days after treatment interruption, on average (range 28 to >168), that is to go from levels that were undetectable to levels that could be detected using the Roche AmpliCor Ultrasensitive assay. This was substantially prolonged from the 27-day interval previously published (33). Once rebound was detected, the subsequent peak viremia post rebound (mean: 4.3 log_{10} copies/ml) occurred at day 24 on average and spontaneously fell approximately 1.0 log_{10} copies/ml (range 0.4 to 2.2). Once rebounding, the initial plasma doubling times ranged from 1.5 to 3.9 days (2.3 on average, figure 2), a value not substantially different from that observed in our earlier treatment interruption studies (33).

After 24 months of follow up, 5 of 10 subjects have either restarted antiviral therapy or have met criteria for restarting therapy based on either CD4+ T cell counts below 350 cells/mm³ (N=4) or plasma viremia above 100,000 copies/ml (N=1). Four subjects remain off therapy with one subject remaining aviremic, and two with HIV-1 RNA levels below 1000 copies/ml. One individual remains asymptomatic with CD4+ T cell counts in the mid 400 cells/mm³ range and HIV-1 RNA levels of approximately 60,000 copies/ml. One subject is lost to follow up.

Pharmacokinetics:
Peak plasma concentrations of each of the three antibodies were measured a half hour post infusion and trough plasma concentrations were determined pre-infusion. The first 6 subjects (700-705) received 1.0 gm of each of the three antibodies. On day 0, peak plasma levels of the three mAbs were 701.3 µg/ml ± 45.7 for 2G12, 448.2 µg/ml ± 50.8 for 2F5 and 378.6 µg/ml ± 45.0 for 4E10 (p<0.01 for 2G12 levels vs 2F5 levels and p<0.01 for 2G12 levels vs. 4E10 levels). Similarly, the mean trough concentrations of 2F5 (47.8 µg/ml ± 26.9) and 4E10 (71.5 µg/ml ± 20.1) were significantly lower than the mean trough concentration of 2G12 (368.0 µg/ml ± 70.4) at baseline (day 0, p<0.01 and p<0.01 respectively).

Three of the initial 6 study participants received all 16 infusions (701, 703 and 705) whereas in 3 patients (700, 702 and 704), infusions were discontinued at week 10, 9 and 10 respectively. Inter-individual differences in the trough or peak plasma concentrations of each of the three antibodies did not correlate with clinical outcome. In these 6 subjects it was evident that 2G12 trough levels increased over time whereas 4E10 and 2F5 levels did not (figure 3A) Based on these data, we attempted to augment the accumulation of 2F5 and 4E10 by increasing the dose of both to 2.0 gm/infusion in the next 4 subjects.

In this group, the mean peak levels of 2G12 (739.6µg/ml ± 102.1), 2F5 (886.5µg/ml ± 108.4) and 4E10 (882.1µg/ml ± 178.2) were comparable on day 0 in the last 4 subjects. However, mean trough levels of 2G12 (336.6µg/ml ± 44.1) were significantly higher than levels of 2F5 (93.6µg/ml ± 62.4, p=0.001) and 4E10 (144.4µg/ml ± 71.9, p<0.01). Three out of four subjects completed the study and one (708) discontinued antibody infusions at week 11 due to virologic stopping criteria. Inter-
individual differences in the antibody levels did not account for clinical outcome as in the previous group. We did not observe a significant accumulation of either 2F5 or 4E10, as reflected by plasma trough levels, despite dose escalation to 2.0 gram per infusion (figure 3B).

Based on the above data, elimination half lives ($T_{1/2}$) of each of the three antibodies were determined as described (25) by fitting to a two-compartment first-order elimination model (Table 3). The mean $T_{1/2}$ of 2G12, 2F5 and 4E10 was $19.9 \pm 4.3$ days, $5.8 \pm 1.4$ days and $7.8 \pm 1.5$ days respectively, similar to previously published data (25).

**Susceptibility of the rebounding virus to mAbs**

A recombinant viral assay (43) was used to test the susceptibility of baseline and rebounding virus. Delay in viral rebound did not correlate with plasma neutralization titers of baseline virus (Spearman’s Rho -0.1, p=0.79). Assigning the 2 patients who did not rebound during the 24-week infusion/ART interruption/observation phase of the study, a value of 168 days, the time to rebound was negatively and significantly correlated with baseline susceptibility to 2G12 (Spearman’s rho –0.7, p=0.03). There was no relationship between the time to rebound and baseline susceptibility to 2F5 or 4E10.

In 7 of 8 subjects, a decrease in susceptibility to 2G12 accompanied virologic rebound, 2.8-fold on average, whereas reductions in susceptibility to 2F5 and 4E10 were neither sustained nor consistently measured (Fig 4). Full length envelope sequencing was performed to characterize the rebounding virus populations. In 6 of the 7 patients who developed 2G12 resistance, N-linked glycosylation site mutations (49) were found at baseline or post treatment (Fig 5). In the one patient with rebound viremia in whom 2G12
resistance was not detected, no such mutations were noted. There were no amino acid substitutions detected in the gp41 binding domains for 2F5 or 4E10, though a deletion of lysine in ELDWKA, the 2F5 binding domain, was detected in patient 704. This deletion, first appearing at day 56 and maintained to day 140, was not temporally associated with the emergence of measured changes in 2F5 susceptibility (11.2 µg/ml at baseline, 14.8 µg/ml at day 56, 17.6 µg/ml at day 140), though, this individual did exhibit a brief though non-sustained 2.5-fold reduction (27.5 µg/ml) in susceptibility to 2F5 at day 94 (24 days after infusions were discontinued).

**Neutralization of patient virus by patient plasma**

In order to examine the overall neutralization capacities of patient plasma, fold-dilutions that neutralized 50% of recombinant viruses constructed from patient plasma-derived envelopes at multiple time points were determined (Supplemental data). In general, we observed that patient plasma neutralized contemporaneous recombinant virus less well when compared to recombinant viruses constructed from circulating plasma virus from earlier time points. Furthermore, viruses became less neutralizable by plasma over time, consistent with rapid escape from either the effects of the combination of mAbs and/or from an autologous antibody response.

Despite continued infusions of mAbs we did not observe sustained changes in susceptibility of rebounding viruses to either 2F5 or 4E10 despite trough levels in excess of the neutralization IC$_{50}$. To attempt to understand the relative effects of 2F5 and 4E10, we analyzed the neutralization capacity of plasma after the emergence of resistance to 2G12 but prior to antibody discontinuation. This was compared with plasma neutralization capacity after antibody infusions were discontinued (Table 4). In 5/8
patients, there was no decline in plasma neutralization capacity after discontinuation of 4E10 and 2F5 infusions. Of the 3 patients in whom a decline in plasma neutralization capacity was observed, there was a modest (0.5 and 0.4 log_{10}) increase in plasma viral load in two of the three subjects after antibody infusions were discontinued (701 and 704 respectively), consistent with some in vivo neutralization effect of the 2 mAbs.

**Cellular immune responses during mAb infusions and viral rebound**

To determine the role of cellular immune responses in study subjects, we measured intracellular IFN-γ secretion in CD4+ and CD8+ T cells when pulsed with pooled, overlapping, 15-mer HIV-1 clade B peptides constituting GAG, NEF and VPR proteins. Five representative subjects were chosen for study. These included the 2 patients with persistent plasma HIV-1 RNA levels below detection (705 and 706), one patient with early viral rebound requiring premature antibody infusion discontinuation (700) and a genetically unrelated transmission pair with near identical viruses at baseline (708 and 709). Within the transmission pair, 708 experienced early virologic rebound, 28 days after ART discontinuation, whereas subject 709 demonstrated late rebound, 73 days after ART discontinuation. Thus, we had the rare opportunity to examine cellular immune responses in a genetically unrelated transmission pair with near identical viruses at baseline and somewhat disparate clinical outcomes. The time points chosen for these experiments were- 1. Acute and early HIV-1 infection prior to ART initiation, 2. At the time of mAb study enrollment, 3. After 3 infusions of mAb, prior to ART discontinuation, 4. After ART discontinuation, 5. At the time of initial viral rebound and, 6. At the end of the mAb study.
In general, the magnitude of the cellular immune responses was related to the rebounding plasma viral load, and the breadth of these responses remained relatively unchanged over time (Fig 6A). For example, in subject 700, a strong response directed against NEF, was observed during acute HIV-1 infection. This NEF-predominant response waned during the aviremic period and re-appeared after discontinuation of antiretroviral therapy, during viral rebound. Of note, the breadth of the CD8+ T cell responses in this patient or others did not change over time.

We observed a broad CD8+ T cell response in subject 708, who experienced early viral rebound whereas in subject 709, low level responses were observed during acute and early HIV-1 and after viral rebound.

In the 2 patients with persistent plasma HIV-1 RNA levels below detection (705 and 706), broad HIV-1 specific CD8+ T cell responses were observed in the peripheral blood of one, 705 but not 706. Subject 706 consented to gastrointestinal biopsy facilitating quantitation of IFN-γ-secreting CD4+ and CD8+ T cells pulsed with HIV-1 peptides as above. While responses in the peripheral blood were detected at low levels, we observed a strong, constitutive as well as antigen-specific CD4+ T cell-dominated response in the mucosal mononuclear cell population (Fig 6B).

We were concerned that perhaps delivery of mAbs to tissue compartments could affect treatment response, in that perhaps inadequate mAb levels at these sites could be correlated with viral outcome. We therefore attempted to determine mAb levels in tissue using immunohistochemistry, but were unable to do so because of non-specific tissue binding of detection antibodies.

**Examination of the gastrointestinal associated lymphoid tissue (GALT).**
Recent studies have demonstrated an important role played by mucosal sites such as the GI tract in establishing HIV-1 infection in a new host (22, 36). Along these lines we thought that in patients discontinuing ART after prolonged periods, the re-ignition of rapid rounds of viral replication could similarly emerge in the GI mucosa where higher levels of activated CD4+ T cells expressing CCR5 reside. We performed serial biopsies of the rectosigmoid colon to 1. Examine and compare CD4+ T cell subsets in tissue with peripheral blood, and 2. Determine the role of tissue compartments during virologic rebound.

Seven of 10 patients consented to sequential flexible sigmoidoscopy with multiple biopsies. Percent CD4+ T cells were measured using flow-cytometry in the peripheral blood and GI tract prior to, and after viral rebound (Table 5). In the two subjects who remained aviremic, GI-mucosal CD4+ T cell percentage did not decline after discontinuation of antiretroviral therapy. In contrast, in the 5 patients examined, where viral rebound followed ART discontinuation, there was a rapid, preferential and significant decline in the percentage of GI CD4+ T cells. In fact, in subject 700, CD4+ T cell percentage in the GI tract declined from 66% at baseline to 30% within 10 weeks of ART discontinuation. In contrast, peripheral blood CD4+ T cells declined from 61% to 52% over the same duration. A similar pattern was seen in every patient studied in whom viral rebound occurred.

In summary, it appears that akin to primary HIV-1 infection, ART interruption resulted in an early and preferential depletion of CD4+ T cells at mucosal sites in patients experiencing viral rebound. Interestingly, this was not seen in those who remained aviremic.
DISCUSSION

There is much interest in the antiviral activity of mAbs both as therapeutics, particularly in the post exposure prophylaxis setting, as well in the area of vaccine development- as the identification of broadly neutralizing antibodies may allow for the design of immunogens capable of stimulating this potential protective response. In the context of SIV infection, mAbs have been used for pre-and post-exposure prophylaxis with success (34, 35, 50). Akin to previous therapeutic vaccine studies, we performed this trial to provide humoral immunity passively whilst anticipating the emergence of a host-derived anamnestic cellular immune response to control viral replication in vivo following treatment interruption.

Given that this was a proof of concept study, we carefully selected patients who were treated during acute and early HIV-1 infection. Advantages in choosing this population includes the likelihood that prolonged antiretroviral therapy initiated early, could preserve autologous cellular immune responses (41) (47) and that the pretreatment viral population was relatively homogeneous (57), resulting in the low likelihood that susceptibility to the mAbs would be compromised by the presence of complex mixtures of viral quasispecies. The three neutralizing antibodies, 2G12, 2F5 and 4E10 were chosen solely on the basis of availability. Data from our laboratory (38) and additional studies by others (4, 9) demonstrated that 2F5 and 4E10 demonstrated broad and potent neutralizing activity against a panel of nearly 100 primary isolates. However, the neutralization capacity of 2G12 was variable and limiting, with only a third of the primary viral isolates tested showing in-vitro susceptibility (4, 38). Notably, the neutralization activity of all mAbs tested (including 2F5 and 4E10) was greater in
pseudovirus based assays compared to assays that were PBMC based {Binley, 2004 #26}. For the purposes of this study, we selected patients where the baseline virus was neutralized by all three antibodies in vitro, defined as an IC_{50} at or below 50 µg/ml. Finally, the trial design provided for mAb infusions overlapping with ART for three weeks to allow for accumulation of the three IgG subtype 1 antibodies in extravascular spaces prior to withdrawal of antiretroviral therapy.

Though the results fell far short of our goal of sustained control of viremia after ART discontinuation in at least 6 subjects, we believe that our findings could support further studies along this line.

First and foremost, mAb infusions were very well tolerated with essentially no toxicity. We had the opportunity to test coagulation parameters in 4 subjects as described above. In all 4 subjects transient, reversible, grade 1 PTT prolongations were noted post infusion. Mixing studies performed in one patient suggested the presence of a low level lupus anticoagulant-like inhibitor. Notably, no thrombotic events were noted. Detailed in-vitro analyses of 2F5 and 4E10 have been performed and suggest that 4E10 and not 2F5 exhibit anti-cardiolipin like activity (55).

Viral rebound, though not prevented, was significantly delayed in the majority (8/10) of our study subjects when compared to historic controls. Importantly, baseline susceptibility to 2G12 was inversely associated with the time to viral rebound and escape from 2G12 was clearly associated with viral rebound during antibody infusions. Examination of the rebounding virus population revealed that of the 8 patients, decreased susceptibility to 2G12 developed in 7. The only patient (709) where 2G12 resistance was not observed, low level viral rebound occurred on study day 73 and mAb infusions were
stopped on day 84 as per study protocol - at this point the patient was essentially lost to follow-up. It is conceivable that with time a more resistant viral population may have emerged. Taken together, our results confirm that the activity of 2G12 was dominant amongst the mAbs, and substantially delayed rebound, concordant with the findings of Trkola et al (53). We remain unsure, however, the degree to which the neutralizing activity of 2F5 and 4E10 contributed to our in vivo observations.

Though susceptibility to 2F5 and 4E10 was demonstrated in the recombinant assay, and the antibody levels at trough were in excess of the IC$_{50}$, antiviral activity was not clearly demonstrated for the following reasons: 1. changes in susceptibility (resistance) to 2F5 and 4E10 were not consistently measured despite ongoing viral replication in the presence of mAbs infusions, 2. no consistent increase in levels of plasma HIV-1 RNA were observed in patients when the antibody infusions were stopped, and 3. despite continued infusions of 2F5 and 4E10 in the face of 2G12 resistance, initial doubling times of plasma viremia were essentially identical to that seen in ART-treated subject interrupting therapy without adjunctive therapies (33). Furthermore, unlike 2G12, both 2F5 and 4E10 failed to accumulate with repeated infusions for reasons that remain unclear. Of note, in a Phase Ib study of 2F5 and 2G12 infusions in 7 chronically infected subjects, 2F5 clearly exerted antiviral activity in vivo in 3 of 5 subjects with 2F5 susceptible-2G12 resistant HIV-1, though the duration of antiviral activity was generally brief (51). In 2 patients in whom a response was followed by rebound, a shift in 2F5 susceptibility (IC 90) was demonstrated using a PBMC-based viral neutralization assay, however no genotype data were generated. We believe it is critical to better understand the relative contributions of both 2F5 and 4E10 in vivo as these mAbs have generated
considerable attention in the area of vaccine development and settings where passive immunization as prophylaxis may be desirable. Currently we are performing these studies to address this critical issue.

Although we cannot make generalizable conclusions from the prolonged control of viral replication in 2 of 10 study participants, prolonged viral suppression to undetectable levels in the absence of antiretroviral treatment is rare. In previous studies conducted by us and others, viral rebound following ART interruption has been more or less universal (13, 21, 33) with only anecdotal reports of control of viremia to levels below 500 copies in the absence of therapy (29, 40). That 3 of 10 individuals appear to be controllers over the longer term presents a glimmer of optimism.

Our results are consistent with the findings of Trkola et al {Trkola, 2005 #3} although there are some differences that merit attention. Specifically, Trkola et al, examined 8 patients treated during chronic HIV-1 infection and 6 patients treated during acute infection. One gram of mAbs 2G12 and 4E10 and 1.3 gram of 2F5 were infused to patients on a weekly basis. Two of 8 patients with chronic HIV-1 showed delay in viral rebound whereas all 6 patients treated during acute HIV showed viral rebound delay similar to what was noted in the present study. This observation underscores a potential limitation of passive immunization strategies and highlights the fact mAb neutralization potential may be significantly curtailed by viral quasispecies diversity generated during chronic HIV-1 infection. In both the present study and the one by Trkola et al, viral rebound correlated with susceptibility to 2G12, although two different neutralization assays were used. While Trkola et al used a PBMC based assay to determine IC90
neutralization titers, the present study employed a pseudovirus based assay and measured IC50 levels.

In order to understand the mechanisms behind persistent viral control in subjects 705 and 706, we performed detailed virological, genetic and immunological studies. In both, 705 and 706 the baseline virus was highly susceptible to 2G12 with IC50s of ~1µg/ml (Table 1). In addition, patient 705 had high plasma neutralization capacity at baseline (~1/1000). This appeared to decrease after mAb infusions were stopped suggesting that mAbs contributed to autologous plasma neutralization capacity (1/1039 on the day of last mAb infusion to 1/639 two weeks following mAb discontinuation).

Genetic studies performed on the study subjects were not relevant to the clinical outcome data (supplemental data). Both 705 and 706 did not have any known HLA class I alleles associated with good prognosis or slow clinical progression (11, 19) or a 32-base pair deletion in the open reading frame of the CCR5 gene (Δ32-CCR5) that induces a frame shift, and loss of a HIV-1 coreceptor activity (14, 30, 48). Actually, patient 706 was homozygous for the HLA class I allele, A*0301. Patient 702 with high level virologic rebound harbored both one copy of the Δ32-CCR5 allele but was also homozygous at the HLA class I loci B*3801 and C*1203.

To characterize cellular immune responses in both peripheral blood and the GALT, we measured the percent of CD4+ and CD8+ T cells secreting IFN-γ in response to HIV-1 overlapping 15-mer peptides representing Clade B GAG, VPR, and NEF. Though it is now clear that these responses may not correlate with levels of plasma viremia, this assay was considered the standard in the field at the time these studies were performed. In subject 705, broad CD8+ T cell responses were observed (Fig 6). In subject
706, we detected lower level, narrower CD8+ T cell responses in the peripheral blood.

However, on detailed examination of the mucosal mononuclear cells isolated from the GI tract, we observed a significant constitutive and antigen-specific, CD4-dominated response. This finding is unusual and in our experience has been observed to date in but one of 5 acutely- and none of 10 chronically- HIV-1 infected subjects similarly studied. Clearly the significance of this finding requires further study.

It is interesting that patients 708 and 709 were infected with the same virus, with the same susceptibilities to the 3 mAbs, yet patient 708 rebounded earlier than all subjects whereas patient 709 did not rebound until day 73. We expected to find that cellular immune responses would explain this difference in these genetically unrelated hosts. Patient 708 had low level, broad responses against all three antigens (GAG, VPR and NEF) tested (Fig 6) whereas patient 709 had low level cellular responses. We can only conclude that the differences between outcomes in these individuals could not be detected with the use of this assay. Furthermore, we did not observe a correlation between GAG-predominant, narrow cellular immune responses and a favorable viral outcome as has been recently demonstrated at the population level in a heterogeneous cohort of chronically HIV-1 infected individuals (26). Also, of interest was the fact that measured responses to HIV-1 antigens pre-ART and post-treatment interruption remained fixed. That is, a patient who had a NEF-dominated response at baseline, showed a similar response (with variable magnitude) post-ART discontinuation. These findings are consistent with the concept that the autologous cellular immune responses may indeed be fixed due to genetic determinants, and though the early use of ART may preserve these responses, it is unlikely that such responses will be substantially altered qualitatively.
Though we cannot pinpoint the exact mechanisms of persistent aviremia in subjects 705 and 706, our findings suggest a convergence of multiple factors, as opposed to one unifying observation—(high susceptibility to 2G12, potent autologous plasma neutralization activity and broad cellular immune response in the peripheral blood or in the GI tract) resulted in a favorable outcome. We urge caution in the over-interpretation of these data. However, we believe that the success of these two patients, as well as a third patient who exhibited modest rebound viremia that has been controlled over the long term, does raise the possibility that in the right clinical setting, with the use of a combination of potent mAbs to which the patient virus is highly susceptible (i.e., IC50 below 1.0 µg/ml), prolonged viral suppression in the absence of antiretroviral therapy may be feasible.

We sampled the gastrointestinal tract, the largest reservoir of immune cells in the body (39) and a site that has been demonstrated to play a critical role in the pathogenesis of acute (22, 36, 37) and chronic HIV-1 (7). In the two patients with persistent control of viremia, serial biopsies did not demonstrate a decline in GI CD4+ T cell percentage. In contrast, in 5 subjects with viral rebound who consented to sequential examination of GI tract, an early and preferential decline of CD4+ T cells was observed in the GI tract, prior to such changes in the peripheral blood. Though, at this point we cannot state whether rebounding virus emerges from a tissue reservoir or the tissue is seeded from elsewhere, these findings strengthen the argument that activated CD4+ T cells resident in the GI tract are critical targets as rapid rounds of viral replication become reignited after ART is withdrawn.
In summary this study provides a number of important conclusions: Firstly, we have confirmed the efficacy of 2G12 as a neutralizing mAb which has potential in future trials of passive immunization, though its utility may be limited by lack of broad efficacy against patient-derived viruses. Secondly, this study raises the possibility of discordance between the strong and broad in-vitro neutralization profiles of mAbs to the membrane proximal ectodomain of gp41, like 2F5 and 4E10, and their apparent in-vivo efficacy. Given the interest in these MAbS as models for vaccine-induced humoral responses, we believe it critical that we better understand the relative contributions of these MAbS to the virologic effects we have observed, and indeed these studies are in progress. Thirdly, though falling far short of our goal of long term control of viremia in at least 6 of 10 subjects, long term suppression of viremia was observed in 3/10 participants raising the possibility that with more careful selection of mAbS and trial subjects, the results of such pilot trials could be improved and pave the way for larger Phase II and III trials. Finally, the present study demonstrates that viral rebound in the absence of therapy is associated with a rapid decline in mucosal CD4+ T cells establishing the importance of the activated tissue effector memory CD4+ T cell population in establishing and perhaps fueling virologic rebound. We suggest that protection of these cells using innovative approaches such as CCR5 antagonists in addition to immune based adjuncts may alter the balance between innate, cellular and humoral responses on the one side and the reigniting of rapid rounds of viral replication on the other, such that our goal of long term control of HIV-1 replication in the absence of therapy may be realized.
# TABLES

Table 1. Baseline patient characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Symptoms to treatment (days)</th>
<th>Treatment duration (months)</th>
<th>Baseline HIV-1 RNA (log_{10} copies)</th>
<th>CD4+ T cell count (cells/mm$^3$) Baseline</th>
<th>IC$_{50}$ to mAbs (µg/ml)</th>
<th>2G12</th>
<th>2F5</th>
<th>4E10</th>
</tr>
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<tr>
<td>700</td>
<td>20</td>
<td>60</td>
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<td>120 (b)</td>
<td>15</td>
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<td>498</td>
<td>1061</td>
<td>1.5</td>
<td>2.0</td>
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</tr>
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<td>704</td>
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<td>801</td>
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<td>449</td>
<td>678</td>
<td>0.7</td>
<td>18.6</td>
<td>20.1</td>
</tr>
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<td>27</td>
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<td>7.2</td>
<td>1.8</td>
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</tr>
<tr>
<td>709</td>
<td>30</td>
<td>27</td>
<td>5.1</td>
<td>575</td>
<td>1106</td>
<td>6.1</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (c)</td>
<td>33 ± 23</td>
<td>29 ± 15</td>
<td>5.7 ± 1.0</td>
<td>440 ± 170</td>
<td>793 ± 223</td>
<td>13.7 ± 22.6</td>
<td>6.3 ± 6.9</td>
<td>7.1 ± 7.1</td>
</tr>
</tbody>
</table>

a) mAbs = monoclonal antibodies

b) Asymptomatic acute seroconversion, duration of infection based on a non-reactive detuned ELISA at presentation.

c) S.D. = standard deviation
Table 2: Characteristics of virologic rebound

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time to Rebound (days)</th>
<th>Peak viremia (log copies)</th>
<th>Change in viral load post-peak viremia (log copies)</th>
<th>Initial plasma viremia doubling time (days)</th>
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<td>49</td>
<td>5.1</td>
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<td>701</td>
<td>42</td>
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<td>5.4</td>
<td>1.1</td>
<td>1.7</td>
</tr>
<tr>
<td>703</td>
<td>49</td>
<td>3.9</td>
<td>2.2</td>
<td>1.9</td>
</tr>
<tr>
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<td>1.5</td>
</tr>
<tr>
<td>705</td>
<td>168 (b)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>706</td>
<td>168 (b)</td>
<td>-</td>
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<td>-</td>
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<td>709</td>
<td>73</td>
<td>3.7</td>
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<td>2.7</td>
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</table>

Mean 72 ± 53 4.3 ± 0.7 1.0 ± 0.4 2.3 ± 0.8

a) Reflects the time when plasma viremia was first detected
b) Patients did not rebound during the course of observation (24 weeks).
Table 3: Plasma half lives (days) of the three mAbs

<table>
<thead>
<tr>
<th>Subject</th>
<th>2G12</th>
<th>4E10</th>
<th>2F5</th>
<th>2G12</th>
<th>4E10</th>
<th>2F5</th>
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<td>700</td>
<td>0.55</td>
<td>0.66</td>
<td>0.52</td>
<td>14.71</td>
<td>5.89</td>
<td>3.64</td>
</tr>
<tr>
<td>701</td>
<td>0.59</td>
<td>0.67</td>
<td>0.62</td>
<td>26.86</td>
<td>11.41</td>
<td>8.52</td>
</tr>
<tr>
<td>702</td>
<td>0.73</td>
<td>0.68</td>
<td>0.65</td>
<td>19.02</td>
<td>7.13</td>
<td>6.68</td>
</tr>
<tr>
<td>703</td>
<td>0.71</td>
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<td>0.65</td>
<td>20.64</td>
<td>7.04</td>
<td>7.45</td>
</tr>
<tr>
<td>704</td>
<td>0.73</td>
<td>0.71</td>
<td>0.67</td>
<td>19.11</td>
<td>7.77</td>
<td>4.80</td>
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<tr>
<td>705</td>
<td>0.61</td>
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<td>0.56</td>
<td>26.13</td>
<td>6.68</td>
<td>5.12</td>
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<td>706</td>
<td>0.65</td>
<td>0.45</td>
<td>0.47</td>
<td>17.97</td>
<td>8.32</td>
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</tr>
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<td>0.71</td>
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<td>5.24</td>
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<td>0.61</td>
<td>19.89</td>
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<td>5.79</td>
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<td>SD</td>
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<td>0.08</td>
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<td>1.50</td>
<td>1.40</td>
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Table 4: Plasma neutralization of autologous viruses

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma neutralization capacity</th>
<th>Δ plasma HIV-1 RNA post mAb discontinuation (log_{10} copies/ml)</th>
<th>Ratio of plasma trough level: IC_{50} (day of mAb discontinuation)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial viral rebound</td>
<td>First 2G12 resistance</td>
<td>2-weeks post mAb discontinuation</td>
</tr>
<tr>
<td>700</td>
<td>1:295</td>
<td>1:627</td>
<td>1:401</td>
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<tr>
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<td>1:167</td>
<td>1:167</td>
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<td>1:200</td>
<td>1:222</td>
</tr>
<tr>
<td>709</td>
<td>1:166</td>
<td>NA</td>
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</table>

a. DC= discontinuation

b. 1 week post mAb DC, patient lost to follow up
Table 5: T cell subsets in the peripheral blood and the GI tract

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>%CD4+ T cells</th>
<th>%CD8+ T cells</th>
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<td></td>
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<td>MMC</td>
</tr>
<tr>
<td></td>
<td>Biopsy #1</td>
<td>Biopsy #2</td>
</tr>
<tr>
<td></td>
<td>-3 to 0</td>
<td>5 to 7</td>
</tr>
<tr>
<td>700</td>
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<tr>
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</tr>
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<td>707</td>
<td>63.2</td>
<td>64.4</td>
</tr>
</tbody>
</table>

a) PBMC = peripheral blood mononuclear cells

b) MMC = mucosal mononuclear cells
FIGURE LEGENDS

Figure 1: Virologic and immunologic profiles of the 10 study subjects. Viral load (purple circles) and CD4+ T cell counts (blue rectangles), both represented on the Y-axis are plotted over time (X-axis) in each study subject. Yellow shaded areas represent the time period during which patients received mAb infusions and the orange line depicts the day of ART discontinuation. Three subjects with early viral rebound requiring mAb discontinuation are shown in the top row, 5 subjects with intermediate viral rebound are shown in the middle row and the three subjects with prolonged control of plasma viremia in the absence of antiretroviral therapy are shown in the bottom row.

Figure 2. Initial doubling times of rebounding plasma virus. Plasma viral load (Y-axis) before and after virologic rebound (X-axis) in eight study subjects. Linear regression analysis assuming first order kinetics was used to calculate the initial doubling times of virus rebound in plasma as previously published (33).

Figure 3: Plasma concentration of each of the three mAbs. Concentration of each of the three mAbs- 2G12 (green), 2F5 (purple), and 4E10 (orange), were measured half an hour post infusion (peak levels) and 7 days later, prior to the next infusion (trough levels). The first six subjects received 1gm/infusion of each of the three mAbs (figure 3A). In the last 4 subjects (figure 3B), the doses of 2F5 and 4E10 were increased to 2gm/infusion as described in the text.
mAb levels (µg/ml) are represented on the Y-axis and are plotted over time in days (shown on the X-axis)

**Figure 4: Neutralization profiles of patient-derived virus at baseline and after rebound.**
Using a recombinant viral assay (43), susceptibility of baseline and rebounding virus to the three mAbs (measured in µg/ml, shown on the Y-axis) was followed over time in days (X-axis) in 8 study subjects with rebound viremia. The range of susceptibility is shown by the arrows; values above 50 µg/ml are considered resistant and for sake of illustration plotted as 60 µg/ml.

**Figure 5: HIV-1 env amino acid sequences in rebounding plasma HIV-1.** Full length envelope sequencing was performed on plasma-derived HIV-1 prior to and after rebound. Sequences from each patient were aligned with the reference strain HXB2 and changes at N-linked glycosylation sites known to result in reduced susceptibility to 2G12 (49) are highlighted in red.

**Figure 6: Cellular immune responses at acute and early HIV-1 infection and during the course of study.**
**Panel A:** Five representative subjects were chosen and CD4+ and CD8+ T cell responses to pooled peptides representing GAG, VPR and NEF from HIV-1 clade B. Percent IFN-γ producing cells (Y-axis) are plotted at sequential time points (X-axis). **Panel B:** In subject 706, CD4+ and CD8+ T cell responses were compared between the peripheral blood and gastrointestinal tract (X-axis) and percent cytokine producing cells (depicted on the Y-axis) are examined.
REFERENCE:

1. 2004. Division of AIDS table for grading the severity of adult and pediatric adverse events.


T-cell responses to different HIV proteins have discordant associations with viral load.


recognizes a cluster of alpha1-->2 mannose residues on the outer face of gp120. J Virol
76:7306-21.


Figure 1

Plasma HIV-1 RNA (copies/ml) vs. Study day

CD4+ T cell count
Figure 2

Plasma HIV-1 RNA (copies/ml)

Slope: 0.41  
T2: 1.69 days

Slope: 0.26  
T2: 2.67 days

Slope: 0.42  
T2: 1.45 days

Slope: 0.37  
T2: 1.87 days

Slope: 0.45  
T2: 1.54 days

Slope: 0.32  
T2: 2.17 days

Slope: 0.18  
T2: 3.85 days

Slope: 0.26  
T2: 2.67 days
Figure 3B

Plasma antibody concentration (µg/ml)

Study day

ACCEPTED on August 30, 2017 by guest http://jvi.asm.org/ Downloaded from

706
707
708
709
Figure 4

Antibody IC50 (µg/ml)

Baseline virus

Days on study

Range of susceptibility
Figure 6A

%IFN-γ positive T cells

CD8+ T cells

CD4+ T cells
Figure 6B
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Study Day: 35, 56, 70, 77, 84, 91, 112
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