A Phase I/IIA Clinical Study with a Chimeric Mouse-Human Monoclonal Antibody to the V3 Loop of Human Immunodeficiency Virus Type 1 gp120

Huldrych F. Günthard,* Peter L. Gowland, Jörg Schüpbach, Michael S. C. Fung, Jürg Böni, Ruey-Shyan Liou, Nancy T. Chang, Peter Grob, Peter Graepel, Dietmar G. Braun,* and Ruedi Lüthy

Divisions of Infectious Diseases and of Clinical Immunology, Department of Medicine, University Hospital of Zurich; Swiss National Center for Retroviruses, Institute of Medical Virology, University of Zurich; and Ciba-Geigy Ltd., Pharmaceuticals Division, Basel, Switzerland; Tanox Biosystems, Inc., Houston, Texas

A phase I/IIA clinical trial with the chimeric mouse-human monoclonal antibody CGP 47 439 to the principal neutralization determinant in the V3 region of human immunodeficiency virus type 1 (HIV-1) strain IIIB envelope protein gp120 is reported. The trial was an uncontrolled single-center, open-label, multidose tolerability, immunogenicity, and pharmacokinetic study in homosexual men with advanced HIV disease. Patient groups were formed on the basis of the reactivity of the antibody with the gp120 of their HIV-1 isolates. Intravenous infusions of 1, 10, and 25 mg of antibody were followed by seven escalated doses of 50, 100, and 200 mg, every 3 weeks. The antibody was well tolerated; no toxicity was observed. Some patients showed a transient but insignificant antibody response to the antibody with no apparent adverse reactions or accelerated elimination of it. Substantial serum levels of the antibody were maintained with a mean $t_{1/2}\beta$ of 8–16 days. A virus burden reduction was observed in some patients.

There is evidence that neutralizing antibodies play a role in prevention of human immunodeficiency virus type 1 (HIV-1) infection and in delaying disease progression. In symptomatic primary infection, plasma and cellular viremia decrease while the levels of HIV-1 antibodies rise [1, 2]. Passive immunization is known to be effective for certain viral diseases [3–6]. In patients with AIDS or AIDS-related complex, the administration of plasma containing hyperimmune immunoglobulins can reduce viremia and delay disease progression [7–10]. Furthermore, studies with animal models support evidence that antibodies may prevent HIV-1 infection and related immunodeficiency viruses. Chimpanzees were protected from challenge with HIV-1 by a mouse-human chimeric antibody elicited against the principal neutralizing determinant (PND) in the V3 region of the homologous strain [11]. In cynomolgus monkeys challenged with simian immunodeficiency virus and HIV-2, infection was prevented by administration of the respective antisera [12]. In humans, the use of hyperimmune globulin from asymptomatic infected persons is beset with potential problems relating to production, availability, safety, and quality control. Therefore, monoclonal HIV-1 neutralizing antibodies are being considered as a possible alternative for passive immunization.

We conducted a phase I/IIA trial of a chimeric mouse-human monoclonal antibody (MAb; CGP 47 439) directed to the V3 loop of HIV-1 gp120. The antibody was intravenously infused into 12 homosexual men with advanced HIV disease. The variable regions of this chimeric antibody are derived from BAT123, a mouse MAb specific for the PND of HIV-1_{IIIB} and HIV-1_{MN} [13, 14]. Preclinical data showed that the mouse and chimeric antibodies had almost identical specificity and reactivity in antigen binding, in vitro neutralization of HIV-1 infectivity, and inhibition of the fusion between uninfected $CD4^+$ cells and HIV-1–infected cells to form syncytia [13, 15]. However, unlike BAT123, the antibody also mediated antibody-dependent cellular cytotoxicity in vitro.

Both the chimeric antibody and BAT123 protected mice with severe combined immunodeficiency transplanted with normal human peripheral blood leukocytes (hu-PBL-SCID mice [16, 17]) from infection with HIV-1 [18]. Preclinical toxicity testing of the antibody in rats and cynomolgus monkeys revealed no pyrogenicity, excellent tolerability, and absence of immunogenicity [19]. Studies of this chimeric antibody in cynomolgus monkeys also demonstrated favorable pharmacokinetic properties [20].

These encouraging preclinical data warranted a clinical trial to test the tolerability, immunogenicity, and pharmacokinetic properties of the chimeric antibody in humans. Surrogate markers (e.g., virus load, $CD4^+$ cell numbers, neopterin, and β_2-microglobulin) were also monitored. Participants

Received 7 March 1994; revised 18 July 1994.

Presented in part: VIII International Conference on AIDS/III STD World Congress, Amsterdam, 19–24 July 1992 (abstract PoB 3445).

Written informed consent was obtained from all study participants for each part of the trial. The ethical review board of the Department of Medicine, University Hospital of Zurich agreed to the study.

Financial support: Ciba-Geigy, Basel.

Reprints or correspondence: Dr. Ruedi Lüthy, Division of Infectious Diseases, Dept. of Medicine, University Hospital of Zurich, CH-8091 Zurich, Switzerland.

* Current affiliations: Medzin, Stadtspital Triemli, Zurich (H.F.G.) and Immuno, Vienna (D.G.B.).

The Journal of Infectious Diseases 1994;170:1384–93

0022–1899/94/7006–0005$01.00

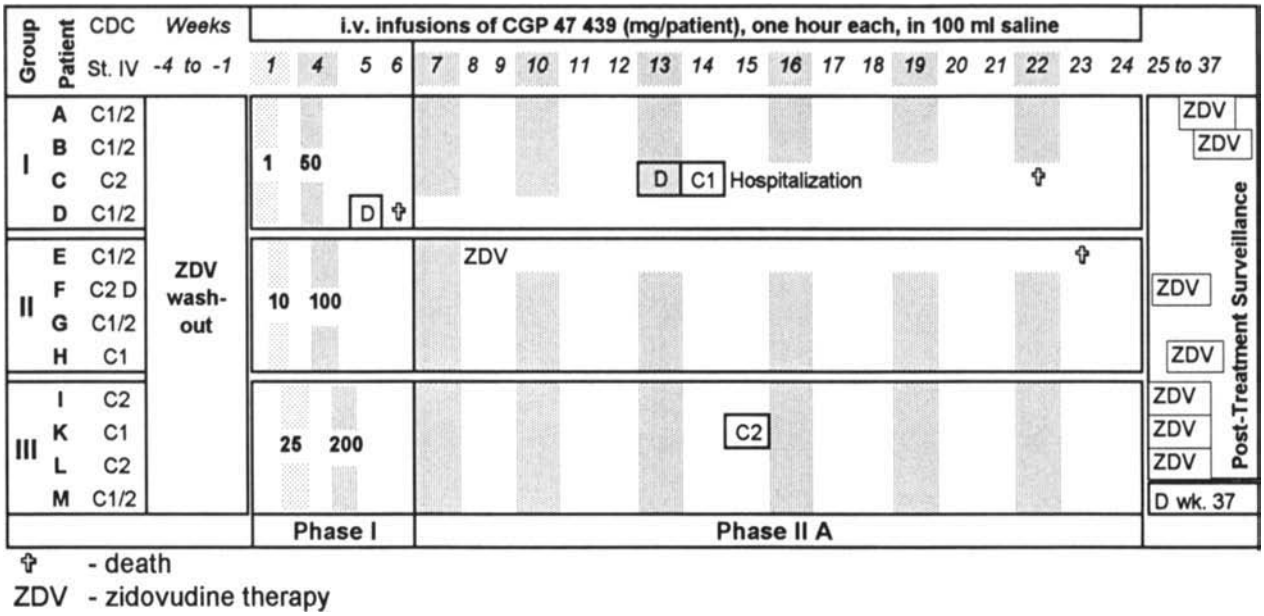

Figure 1. Study design. Column 1: patient treatment groups and pretrial stage (St) of opportunistic infections (IV-C1, IV-C2, or IV-D) according to Centers for Disease Control and Prevention (CDC) classification system; boxes with C1, C2, or D indicate changes in level of opportunistic infections during and after trial. Column 2: zidovudine (ZDV) withdrawal before trial. Columns 3 and 4: dosing (vertical bars) was done sequentially with 1 week between groups, fully escalated within 6 weeks. Patients then received 6 more infusions of their high dose. Column 5: ZDV boxes indicate approximate time (≥3 weeks after trial) zidovudine treatment resumed by patients. G and M never resumed zidovudine. Patients C–E died.

could not receive antiretroviral therapy for at least 4 weeks before, during, and 3 weeks after the trial.

Materials and Methods

Patient Enrollment and Study Design

Participants (men ≥18 years old) were selected from the Zurich AIDS patient cohort study [21]. They had stage IV (advanced) HIV disease according to the Centers for Disease Control and Prevention (CDC) classification system [22, 23] and a history of ≤3 C1-type opportunistic infections. For each patient, antibody to HIV-1 had to be confirmed, p24-antigenemia had to be detectable, and HIV-1 had to be isolated. Any antiretroviral therapy with nucleoside analogs or cytokines was to be discontinued 4 weeks before the first and until 3 weeks after the last antibody infusion. Four weeks before the first infusion, blood samples were obtained from 27 persons who had volunteered for the trial; HIV-1 was isolated from 17 of the volunteers, from whom 12 were selected to participate in the trial. Participants were selected on the basis of their reactivity with the antibody in a specially designed gp120 capture immunoassay (see below). They were allocated to 3 treatment groups, each containing 4 patients.

Study Conduct and Posttreatment Surveillance

The chimeric antibody was administered by intravenous infusion at 21-day intervals within groups (figure 1). Dose was escalated stepwise between groups at 7-day intervals. Group 1 (patients A-D), group 2 (E-H), and group 3 (I, K-M) received 1-, 10-, and 25-mg doses, respectively. Patients tolerated the first dose. The second dose was escalated within groups (i.e., 50, 100, and 200 mg, respectively). After completion of this phase (1), an interim analysis was done, and the trial was continued for another six doses.

Each antibody dose was administered in 100 mL of saline (Laboratories Hausmann, St. Gallen, Switzerland) by time-controlled infusion (60 min) into a forearm vein. For each patient, a medical history was recorded and a physical examination was done weekly by the same physician of the study team. Venous blood was collected between 8 and 10 A.M. before each infusion and in weekly intervals between the infusions. Patients were followed for 3 months after the eighth infusion (weekly for the first 3 weeks, then once every 4 weeks). Antiretroviral medication could be resumed 3 weeks after the last infusion. All patients, including those who participated in the prestudy selection phase, received their medical care in our outpatient clinic and were seen regularly after the study end.

Construction, Production, and Purification of the Antibody

The construction of the chimeric heavy- and light-chain genes and the method of generating transfectomas secreting the chimeric antibody are described in detail [15]. The manufacture and purification of the clinical grade antibody is also described [19].

Laboratory Tests

Routine tests. Hematology and clinical chemistry tests, urinanalysis, and testing of β_2-microglobulin, neopterin, and the major immunoglobulin classes (IgG, IgM, IgA) were done in laboratories at the University Hospital of Zurich.

Pharmacokinetics and anti-CGP 47 439 antibody response. A sandwich ELISA using a specific antiidiotypic MAb (AB19-4) to the chimeric antibody was used to determine serum concentrations of the antibody. The chimeric antibody was used in a double-antibody capture ELISA to detect antiidiotypic antibodies to the chimeric antibody as a measure of immunogenicity. These two methods, the pharmacokinetic calculations, and results of this study were described earlier [20, 24].

gp120 quantification and the chimeric antibody capture ELISAs. Peripheral blood mononuclear cells (PBMC, 2–5 × 10^6) from each patient were cocultivated with 10^7 phytohemagglutinin (PHA)–stimulated PBMC from healthy blood donors. The cultures were maintained in RPMI 1640 containing 15% fetal calf serum (FCS) and 40 IU/mL recombinant interleukin-2 (IL-2; Boehringer, Mannheim, Germany) for ≥3 weeks; 5 × 10^6 PBMC were added once a week. Culture supernatant was assayed for HIV-1 p24 antigen by ELISA (Du Pont NEN, Wilmington, DE). Cultures yielding ≥20 ng/mL p24 antigen were harvested by centrifugation, and the cell pellet was lysed with a buffer containing nonionic detergent (1% Nonidet P-40; Sigma, St. Louis). gp120 in the cell lysate was measured by ELISA: Solubilized gp120 molecules were captured onto the solid phase via adsorbed sheep antibody D7324 to the C-terminal 15 amino acids of HIV-1 gp120 (Aalto BioReagents, Dublin). Bound gp120 was detected with the murine MAb G3-508, which binds to the relatively conserved C4 region of gp120 of diverse HIV-1 isolates [25]. Bound murine antibody was detected with peroxidase–conjugated goat anti-mouse immunoglobulin (Jackson ImmunoResearch, West Grove, PA).

The amount of gp120 in the lysates was determined from a standard curve with purified HIV-1_{IIIB} gp120. Lysates containing >15 ng of gp120/mL were tested in an antibody capture ELISA to determine the specific binding of the antibody to the gp120 of the patient's virus isolate. The lysates were incubated in wells coated with antibody or with normal human IgG1κ (Sigma) as a negative control. Specific binding was detected with biotinylated MAb G3-519, another anti-C4 region of HIV-1 gp120 antibody that binds to the gp120 of diverse HIV-1 variants [25]. Peroxidase-conjugated streptavidin (Jackson) was used to detect bound antibody. A ratio of the optical density (antibody vs. normal human IgG1κ) was determined. According to this ratio, three capture strengths were arbitrarily defined: no capture (ratio 1), weak capture (ratio between 1 and 2.5), and strong capture (ratio >2.5).

Serum p24 antigen. Free serum p24 was determined undenatured with a commercial antigen test (p24-UD; Abbott, Abbott Park, IL). To measure total (i.e., free and immunocomplexed) antigen, serum samples were diluted with a twofold volume of 0.5% Triton X-100, heat-denatured at 100°C for 5 min (p24-HD), and tested by HIV-1 p24 core profile ELISA (Du Pont NEN), as described [26]. An ELISA amplification system (ELAST; Du Pont NEN) was used for signal amplification, resulting in a detection limit of ~0.5 pg/mL compared with 12.5 pg/mL in the routinely used Abbott kit.

Lymphocyte subsets. $CD3^+$, $CD4^+$, and $CD8^+$ lymphocytes were counted in peripheral blood using routine procedures with commercially available MAb reagents (Becton Dickinson, Basel; Coulter, Instrumenten Gesellschaft, Zurich) and flow cytometry (Coulter, Instrumenten Gesellschaft).

Plasma TCID. Plasma TCID was determined as described [27]. Decreasing volumes (500, 100, 10, and 1 μL) of cell-free plasma were incubated in duplicate with 2 × 10^6 PHA-stimulated PBMC in 1.5 mL of RPMI 1640 containing 15% FCS and 40 IU/mL recombinant IL-2 in 24-well plates. After 24 h, the cells were washed three times and maintained for 28 days, with replenishment of medium every 3–4 days. PHA-stimulated PBMC were added weekly, and p24 was monitored in the culture supernatants until day 28 using the Du Pont p24 core profile ELISA. Cultures with >200 pg of p24/mL on 2 occasions were considered positive. The smallest plasma volume required to produce a positive culture was taken as the end point (2, 10, 100, or 1000 TCID/mL of plasma).

Quantitative reverse polymerase chain reaction (qrPCR). qrPCR was done on virus particles pelleted from EDTA-plasma. Virus was resuspended in buffer and the RNA was extracted according to standard procedures [28, 29] using proteinase digestion, phenol-chloroform extraction, and addition of carrier tRNA for precipitation. Ten percent of the extracted viral RNA was reverse-transcribed in a total volume of 25 μL at 37°C for 5 h with MuLV reverse transcriptase and primer SK150 [30], using the reaction conditions recommended by the manufacturer (Boehringer Mannheim). For amplification by PCR, the complete reaction was added to 75 μL of buffer containing primer SK145 [28] and Taq DNA polymerase. After amplification for 30 cycles, DNA was analyzed by Southern blot with ^{32}P-end labeled oligonucleotide SK102 [28]. Radioactive bands were counted in a beta counter.

Branched DNA (bDNA) signal amplification for HIV-1 RNA quantitation. bDNA (Chiron; Emeryville, CA) was amplified according to the manufacturer's instructions. Virus was pelleted at 23,000 *g* for 1 h from 1 mL of plasma. A viral lysate was prepared and added to microwells. The RNA target was captured onto the microwell surface and detected via bDNA amplifier molecules and alkaline-phosphatase–labeled oligonucleotides, and signal was generated using a chemoluminescent substrate. Light emission is proportional to the number of HIV-1 RNA molecules (HIV-1 RNA equivalents) present in the specimen [31, 32].

Statistical analysis. Analysis of covariance was done for all surrogate markers and virus load parameters. Linear regression analysis and Spearman's rank order correlation coefficients were calculated to compare the different methods to measure virus load. Survival analysis was done by Kaplan-Meier estimates [33]. p24-UD, bDNA, and qrPCR results were more nearly log-normally distributed than normally distributed; therefore, log transformations were taken of these variables for correlation analysis.

Results

Patient selection. HIV-1 was isolated during the preselection period from 17 of 27 patients entering the zidovudine

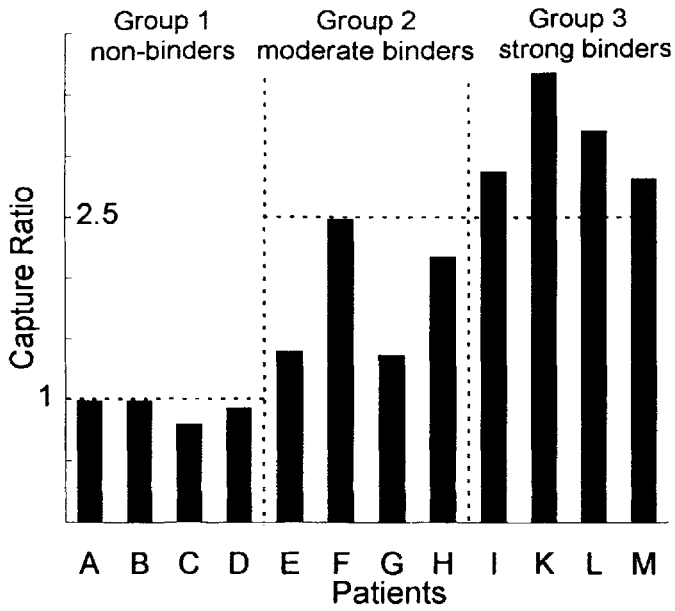

Figure 2. Results of gp120 capture assay. Capture ratio = ratio of reactivities (optical density) between CGP 47 439–coated wells and wells coated with normal human IgG. Patients allocated to groups on basis of ratios.

washout phase. All isolates had positive gp120 binding results (>15 ng gp120/mL of lysate); 8 of the 17 isolates were gp120 capture positive. Of these, 4 showed moderate but clear and 4 showed somewhat stronger gp120 capture ratios (figure 2). On the basis of these results, the patients were allocated to the 3 treatment groups. It was hoped that patients with the strongest virus reactivity were also those who were infected with a virus that could be neutralized by the antibody. Therefore, group 1 (capture negative) was given a low dose, 5 and 50 mg, of the chimeric antibody, group 2 (moderately capture positive) was given a medium dose, 10 and 100 mg, and group 3 (strongly capture positive) was given a high dose, 25 and 200 mg.

Clinical results and survival. Nine of the 12 patients completed the study (figure 1). Patient D died 3 weeks after the second infusion, 1 week after a retropharyngeal lymphoma was detected. Patient C was hospitalized 1 week after his fifth infusion, with signs of cerebral toxoplasmosis, of which he died 8 weeks later. Patient E quit the study to resume zidovudine treatment after having had three antibody infusions. At clinical follow-up, his cytomegalovirus (CMV) disease was exacerbated and, in addition to his preexisting CMV retinitis, a CMV stomach ulcer was diagnosed. Patient E then presented with a probably preexisting CMV-HIV encephalitis, which progressed rapidly; he died 22 weeks after the initial infusion. Apart from patients C, D, and K (who developed a candida stomatitis for the first time in week 15), no other patients progressed within the CDC HIV stage during the study.

A survival analysis comparing the 12 study patients with 15 who resumed their antiretroviral medication because they were not selected for the study (due to their negative capture ratio in the gp120 capture assays) showed no statistically significant difference between the groups ($P = .95$, log rank test) (table 1). The median survival time for the 12 antibody-treated patients was 562 days (95% confidence interval [CI], 149–857 days) versus 644 days (95% CI, 255–857 days) for the 15 patients receiving antiretroviral therapy. Patient B from group 1, patient F from group 2, and patients I, K, and L from group 3 were still alive 2.5 years after the study began. Disease progressed (i.e., new opportunistic infections or Kaposi's sarcoma) in all of these patients after study end.

Pharmacokinetics and immunogenicity. These results were published in detail elsewhere [24]. The mean elimination half-life ($t_{1/2}\beta$) of the chimeric antibody was 8–16 days. Patients B, K, and L had marginal and transient antibodies against the chimeric antibody. Except for patient K (see below), none of these patients complained about itching or skin rash. No correlation was observed between the presence of the transient antibodies and the $t_{1/2}\beta$ of the chimeric antibody. Substantial serum levels of the circulating antibody were present in all patients during the entire study (range, 0.3–30.7 μg/mL, depending on dose).

Safety. Patient K developed a skin rash over his entire body 4 days after the first infusion and 9 days after beginning treatment with amoxicillin-clavulanic acid for external otitis. At that time, levels of anti-CGP 47 439 antibodies were not elevated. Aminopenicillins frequently cause skin rashes in HIV-infected patients, in particular as the disease progresses [34, 35]. Antibody treatment was continued with a 200-mg dose at day 21, and six subsequent 200-mg doses were given according to schedule with good tolerability. Patient E complained about increasing tiredness after each infusion. He elected to quit the study and resumed zidovudine therapy

Table 1. Basic characteristics of HIV-infected patients in the pretrial selection of participants for the clinical study.

Characteristic	Group A	Group B
No. of patients	12	15
Age, years (range)	39.1 (28–56)	38.8 (24–53)
Time from AIDS stage IV-C2 diagnosis to study selection (months)	11.8 (3–26)	15.3 (1–36)
Duration of zidovudine treatment before study selection (months)	12.9 (4–27)	13.8 (0–36)
$CD4^+$ lymphocytes/mm^3 before study selection	80 (0–230)	111 (6–350)

NOTE. Group A patients, treated with CGP 47 439; group B patients, not selected. Data are mean (range).

Table 2. $CD4^+$ lymphocyte counts in HIV-infected patients receiving infusions of the chimeric mouse-human monoclonal antibody (CGP 47 439) directed to the V3 loop of HIV-1 gp120.

	$CD4^+$ lymphocytes/mm^3			
Patients	At zidovudine withdrawal	Day 0	Immediately before resumption of zidovudine	Last visit of the trial*
Group 1				
A	60	30	40	40
B	70	60	20	50
C	70	60	NA	NA
D	0	60	NA	NA
Group 2				
E	10	10	40	30
F	170	100	80	70
G	0	0	(10)†	0
H	20	20	20	0
Group 3				
I	170	140	30	80
K	230	200	80	30
L	140	70	30	60
M	20	10	(0)†	10

NOTE. NA, not available (patients died). Patient E died 22 weeks after the first infusion.

* ~3 months after last infusion.

† Patients never resumed zidovudine therapy.

after the third infusion. We therefore considered that the antibody was safe for the patients.

Clinical chemistry and urinalysis. Clinical chemistry parameters included alanine transaminase, aspartate transaminase, alkaline phosphatase, bilirubin, prothrombin time, blood urea nitrogen, creatinine, glucose, and potassium. These parameters were monitored weekly during the first 3 weeks of the trial, every 2 weeks until week 6, and every 3 weeks thereafter until the trial end. Patients showed either changes within the normal range or no change compared with pretrial levels (data not shown). Specifically, liver and kidney functions yielded no evidence for immune complex disease.

Hematology. During zidovudine treatment, all patients had mild, moderate, or severe macrocytic anemia. The mean corpuscular volume normalized in all 12 patients, whereas the red blood cell counts and hemoglobin level remained stable or improved during treatment with the antibody. After resumption of antiretroviral therapy, the previously observed macrocytic anemia reappeared. Platelets, granulocytes, monocytes, and lymphocytes did not show significant changes over time (data not shown). However, platelet counts dropped 17% and 15% in patients A and L, respectively, during the zidovudine washout phase.

Surrogate markers. During the trial there was considerable inter- and intrapatient variability of the $CD4^+$ lymphocyte counts (table 2). After the trial, 5 patients (B, F, I, K, and L) had considerably lower counts compared with those at baseline, and 4 (A, G, H, and M) had counts that remained fairly stable. Of the 3 patients who died (C–E), only 1 (E) showed a slight improvement of his $CD4^+$ lymphocyte count. In group 3, the initial $CD4^+$ lymphocyte counts were markedly higher than those for groups 1 and 2.

Levels of serum β_2-microglobulin and neopterin were elevated in all patients during the entire study. An effect of treatment was not detectable (data not shown).

Major immunoglobulin classes. Serum levels of IgG, IgM, and IgA showed no relevant changes during the study (data not shown).

Virologic markers. Five methods were used to measure the patients' virus loads: concentration of undenatured viral antigen p24 in serum (p24-UD), p24 concentration after heat denaturation (p24-HD) for quantitative detection of both free and immunocomplexed antigen, virion RNA in plasma assessed by bDNA amplification or qrPCR, and the determination of infectious virus in plasma (TCID). Regression analysis of this data showed good correlations of p24-UD and p24-HD ($r = .78$, $P < .001$) and of p24-HD and bDNA ($r = .59$, $P < .001$). In contrast, correlations of p24-UD and bDNA ($r = .41$, $P < .001$), bDNA and qrPCR ($r = .30$, $P = .02$), and qrPCR and p24-HD ($r = -.06$, $P = .54$) were poor.

Plasma viremia as determined for patient K by bDNA and qrPCR analyses indicate loads of $1–6 \times 10^5$ and $3–6 \times 10^5$ virus particles/mL, respectively (figure 3). The mean TCID corresponded to a mere 0.002% of these particles (range, 0.0008%–0.05%), indicating that only a minor fraction of the particles were capable of replicating under the chosen in vitro culture conditions. There was a mean of 3.2×10^3 virus particles/μg of p24 (range, $1.4–6.1 \times 10^3$, estimated by p24-HD and bDNA). The mean was only 790 for undenatured antigen. This is considerably below the reported 10^4 particles/μg of p24 [36], thus indicating that there must be forms of antigen not associated with virus particles. Alternatively, there may be particles that contain less than the regular 2 copies of RNA.

Table 3 summarizes treatment-associated changes of the virus load as measured by bDNA and p24-HD, the two best correlating independent parameters. For statistical evaluation, a linear regression analysis of virus load over time was done. If available, day zero measurements were used as the baseline; otherwise, the last measurement before day zero was used. The analysis was otherwise restricted to the actual time during which the patients were under antibody treatment. Detailed data, expressed as percentage of the baseline value, of selected patients in whom virus load reductions were observed are shown in figure 4.

Antigen p24-HD was significantly reduced over time in 5 patients (B, F, G, I, and M). A parallel decrease of bDNA values occurred in patients B, F, and M; this decrease was statistically significant in M, although only five measure-

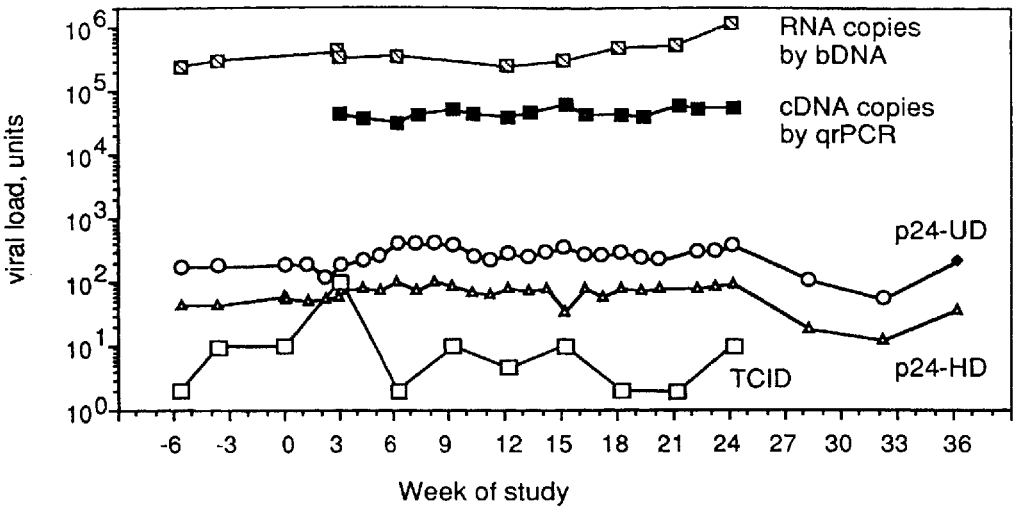

Figure 3. Typical virus load parameters shown by data for patient K. bDNA (branched DNA) signal amplification and qrPCR (quantitative reverse polymerase chain reaction) are expressed in RNA and cDNA copies/mL of plasma, respectively; p24-UD (undenatured p24) and p24-HD (heat-denatured p24) are expressed in pg/mL of serum; and TCID is expressed per milliliter of plasma.

ments were possible. A significant initial decrease in bDNA values up to day 86 was found in patient B; this decrease was, however, followed by an increase resulting in an insignificant overall decrease. Of particular interest is patient I who, despite a significant reduction in antigen, showed a significant rise in bDNA. A similar situation was found in patient G, although the increase in bDNA was not significant. The course was also remarkable in patient E, who had a highly significant drop in bDNA during the few weeks after receiving the antibody; p24 antigen during this time showed a transient peak. Patients A, D, H, and K exhibited an initially stable virus load, or even a decrease, followed by an increase. No clear trend, as demonstrated by divergent results of bDNA and p24-HD values, was observed in patients C and L; patient C had an overall increase in p24 antigen.

In conclusion, 10 patients exhibited a significant overall change in virus load; of these, 7 indicated a reduction (bDNA of E and M; p24-HD of B, F, G, I, and M) and only 3 (bDNA of I; p24-HD of C and H) indicated an increase. In addition, patients A and B initially had transient but significant reductions that lasted for 11–12 weeks.

In general, the load reductions were <1 log but still repre-

Table 3. Treatment-associated changes of the virus load parameters, measured by branched DNA (bDNA) signal amplification and heat-denatured p24 (p24-HD).

Treatment group, patient	p24-HD			bDNA			Remarks
	Coefficient	P	r_s	Coefficient	P	r_s	
Group 1							
A	0.065	.323*	.205	−0.072	.678	−.200	Stable
B	**−0.586**	**.000**	−.691	−0.144	.285†	−.370	Decrease, significant for bDNA‡
C	**1.100**	**.015**	.451	−0.475	.641	−.429	Divergent course
D	−0.028	.983	.029	−1.597	.147	−.400	No major trend (limited data)
Group 2							
E	−0.489	.205	−.292	**−0.902**	**.003**	−1.000	Decrease, significant for bDNA
F	**−0.285**	**.006**	−.582	−0.215	.371	−.400	Decrease, significant for p24-HD
G	**−0.216**	**.031**	−.411	0.461	.142	.345	Divergent course
H	**2.391**	**.000**	.865	0.422	.054	.619	Increase, significant for p24-HD
Group 3							
I	**−0.078**	**.023**	−.451	**0.683**	**.035**	.683	Divergent course
K	0.161	.097	.473	0.843	.076	.550	Increase§
L	0.174	.093	.298	−0.092	.271	−.367	Stable
M	**−0.344**	**.000**	−.745	**−0.378**	**.041**	−.900	Decrease, significant overall

NOTE. Bold type, significant virus load courses ($P < .5$). Group 1, received 1- and 50-mg doses of antibody; group 2, 10- and 100-mg doses; group 3, 25- and 200-mg doses.

* Significant decrease of p24-HD to day 79 ($P = .013$), followed by increase in coincidence with erythema.

† Significant decrease of bDNA to day 86 ($P = .019$), followed by increase.

‡ p24-HD undetectable within 10 weeks.

§ bDNA stable to 12–15 weeks, followed by increase.

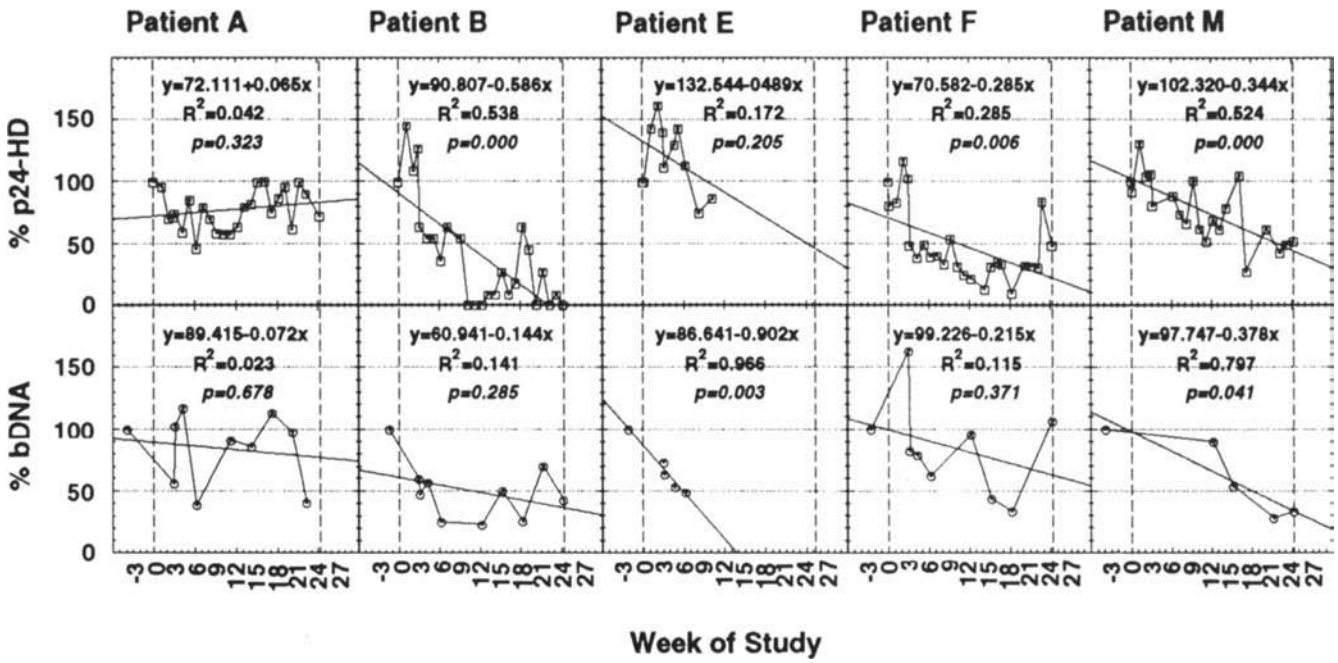

Figure 4. Changes in p24-HD (heat-denatured p24) and bDNA (branched DNA) signal amplification values for selected patients. Results expressed as % of value on day 0 (if available) or nearest pretrial measurement. Linear regression analysis restricted to period when patients were treated with CGP 47 439. Dashed lines = beginning and end of trial.

sented a major fraction of the virus load (mean, −56%; SD, 23%; range, −14% to −90%). In patient B, who started the trial with a p24-HD antigen level of only 1.5 pg/mL serum, the antigen became undetectable within 10 weeks. Reductions were not dose related; effects were evenly distributed among the 3 treatment groups. The fraction of increases in virus load were higher (mean, 163%; SD, 101%; range, 40%–379%); they also were unrelated to dose.

Discussion

To our knowledge, this phase I/IIA trial with the chimeric mouse-human monoclonal anti-gp120 antibody is the first of its kind reported. It was conducted as an uncontrolled single-center, open-label, multidose tolerability, immunogenicity, and pharmacokinetic study in 12 patients with advanced HIV disease. Despite the obvious limitations of such a small study, the extension from phase I to phase IIA was done to evaluate any potential anti-HIV activity of the antibody.

We recently reported that substantial serum levels of this antibody have been maintained in our patients [24]. Mean serum half-life of the antibody (8–16 days) was much longer than those of murine MAbs (~15 h [37]) but shorter than that of normal IgG (21 days [38]). Anti-antibody responses to the chimeric antibody were found only transiently in 3 patients, and they were not associated with any clinical symptoms or signs of adverse reactions to the antibody. A whole-body exanthema in patient K was likely due to a toxic reaction to amoxicillin-clavulanate, which is a well-known effect of these drugs in AIDS patients [34, 35]. Patient E complained of progressing tiredness after each infusion. This finding was probably a sign of a progressing HIV-CMV encephalitis and was not considered a reaction to the study drug. In summary, the results of this phase I/IIA study in patients with advanced HIV disease demonstrated that the antibody was well tolerated and safe and showed low, if any, and transient immunogenicity, which was clinically not relevant. High serum levels of the antibody could be maintained.

From an ethical point of view, the withdrawal of zidovudine was problematic because at that time it was the only registered antiretroviral drug known to prolong survival in AIDS patients [39]. Patients were informed about this clearly. Of interest, however, no statistically significant survival differences among the 12 study patients (off zidovudine for 7 months) and 15 patients who participated in the pretrial selection phase (off zidovudine for 1 month) were observed. The small number of patients, however, precludes an analysis of efficacy. At best, we could speculate that withdrawal of zidovudine did not influence survival. The hematologic data from the 12 patients strongly suggest that they did not secretly take zidovudine during the trial. During zidovudine treatment, all 12 patients had a moderate macrocytic anemia, a frequent side effect of zidovudine [40, 41]. Discontinuation of zidovudine was followed by normalization of the mean corpuscular volume, a rapid increase of red blood cell counts, and, somewhat less pronounced, an increase in hemoglobin level in all patients. The opposite effects were observed when zidovudine treatment was resumed.

Three patients were still alive in group 3 (strongly capture positive) 2-1/2 years after the first infusion, compared with only 1 patient each in groups 1 and 2. Although interesting, this difference cannot be interpreted to indicate a dose-dependent therapeutic effect on survival for the following reasons: The number of patients in each group is too small to do a multivariate analysis to detect the relevant factors for the difference in survival between groups; group 3 patients had the highest pretrial mean $CD4^+$ lymphocyte counts; and patients were not randomly assigned to the 3 treatment groups but selected according to their gp120 capture ratio.

Some antibodies to the gp120 envelope protein of HIV-1 may enhance HIV infection in vitro [40–44]. In addition, a correlation of antibody-dependent enhancement of infection with disease progression in HIV-infected persons has been reported [45]. A recent study showed that neutralizing and enhancing antibodies can be induced by different epitopes on gp120 [46]. The antibody has been shown not to enhance HIV-1 infection in vitro (data not shown) and in hu-PBL-SCID mice studies [18]. Another recently chimerized mouse-human MAb specific to the PND of gp120 showed anti-HIV activities similar to those reported for the antibody [47]. It exhibited no enhancement in vitro and the authors suggested that neutralizing antibodies to PND neutralize but do not enhance HIV infection. In agreement with these in vitro observations, the lack of any clinical evidence of a faster progression to death in our patients compared with the 15 patients without the chimeric antibody and the absence of a dose-related p24 increase after the infusions suggest that there was no antibody-dependent enhancement of HIV-1 infection.

In this study, considerable effort was made to measure virus load in the treated patients. It was known that in vitro the antibody was able to neutralize effectively the infectivity of HIV-1_{IIIB} and weakly that of HIV-1_{MN} [13, 14]. HIV-1_{MN}–related strains were reported to be prevalent in HIV-1–infected persons [48–50]. We therefore developed a gp120 capture assay to select patients infected with the antibody-reactive HIV-1 strains. Eight of 17 patients who showed sufficient virus growth also showed gp120 capture. We administered the highest dose to patients with the highest gp120 reactivity, thereby increasing the likelihood of observing possible antiviral activity of the antibody yet fully aware of the negative consequences on the statistical analysis of the outcome.

All patients showed considerable viremia confirmed by different methods: p24-UD, p24-HD, qrPCR, bDNA signal amplification, and TCID. qrPCR and TCID showed wide intrapatient variations and were not sensitive enough to detect slight changes in virus load. However, bDNA and p24-HD analyses provided results that allowed measurement of changes in virus load. The different slopes of these parameters suggest that some p24 antigen is not associated with virus particles (data not shown).

bDNA and p24-HD analyses indicated a significant antiviral effect in patients B, F, G, I, and M. In addition, significant initial reductions in either bDNA or p24-HD values were observed in patients A and E. In patients H and K, one or both parameters remained stable compared with the predose (day zero) level for several months before significant increases in virus load occurred. Reductions were generally <1 log, but were frequently most of the circulating virus and, thus, may be of biologic significance.

In contrast to our expectations, the antiviral effects were evenly distributed between all 3 treatment groups. There is no correlation between the demonstrated anti–HIV-1 effect and the gp120 reactivity with the antibody or doses used. This may be attributed to the fact that the cultured virus used for the gp120 capture assay was not representative of the circulating virus predominant in vivo: The procedure used to isolate virus might select a fraction of the clinical viruses with rapid and high replication capacities.

The antibody may have several mechanisms of antiviral action. It may neutralize in vivo the infectivity of free virus. Alternatively, the transient initial increase in particle-associated RNA (figure 4) may indicate the lysis of HIV-1–infected cells by antibody–dependent cellular cytotoxicity [15] or complement-mediated lysis [51, 52]. The fact that treatment with the neutralizing antibody resulted in reductions in the virus load in some patients is encouraging and indicates that additional clinical studies with this chimeric antibody are warranted. In addition, since it has been shown that combinations of MAbs may have synergistic effects in vitro [53, 54], and to prevent the possible emergence of escape mutants, cocktails of antibodies recognizing different neutralizing epitopes also should be considered for further studies.

Acknowledgments

We thank M. Vogt for his contribution in patient selection; J. Gelzer and M. Wilhelm for their support; T. W. Chang, R. M. Krause, and R. Zinkernagel for advice; M. Winiger and K. Hodapp for their diligent attention to the patients; V. Nadai, C. Probst, and D. Pontelli for technical assistance; W. Kremers for statistical support; and especially D. Ho and Y. Cao for assaying the samples with the bDNA amplification method.

References

1. Clark SJ, Saag MS, Decker WD, et al. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-infection. N Engl J Med 1991;324:954–60.
2. Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. N Engl J Med 1991;324:361–4.
3. Iwarson S, Ahlmén J, Eriksson E, et al. Hepatitis B globulin in prevention of hepatitis B among hospital staff members. J Infect Dis 1977;135:473–7.
4. Seeff LB, Wright EC, Zimmermann HJ, et al. Type B hepatitis after

needle-stick exposure: prevention with hepatitis B immune globulin. Ann Intern Med **1978**;88:285–93.

5. Loofbourow JC, Cabasso VJ, Roby RE, Anuskiewicz W. Rabies immune globulin (human)-clinical trials and dose determination. JAMA **1971**;217:1825–31.
6. Groothuis JR, Levin MJ, Rodriguez W, et al. Use of intravenous gamma globulin to immunize high risk children against respiratory syncytial virus: safety and pharmacokinetics. Antimicrob Agents Chemother **1991**;35:1469–73.
7. Jackson GG, Perkins JT, Rubenis M, et al. Passive immunoneutralization of human immunodeficiency virus in patients with advanced AIDS. Lancet **1988**;2:647–52.
8. Karpas A, Hill F, Youle M, et al. Effects of passive-immunization in patients with acquired immunodeficiency syndrome–related complex and acquired immunodeficiency syndrome. Proc Natl Acad Sci USA **1988**;85:9234–7.
9. Karpas A, Hewlett IK, Hill F, et al. Polymerase chain reaction evidence for human immunodeficiency virus I neutralization by passive immunization in patients with AIDS and AIDS-related complex. Proc Natl Acad Sci USA **1990**;87:7613–7.
10. Vittecoq D, Mattlinger B, Barré-Sinoussi F, et al. Passive immunotherapy in AIDS: a randomized trial of serial human immunodeficiency virus–positive transfusions of plasma rich in p24 antibodies versus transfusions of seronegative plasma. J Infect Dis **1992**;165:364–8.
11. Emini EA, Schleif WA, Nunberg JH, et al. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature **1992**;355:728–30.
12. Putkonen P, Thorstensson R, Ghavamzadeh L, et al. Prevention of HIV-2 and SIV_{sm} infection by passive immunization in cynomolgus monkeys. Nature **1991**;352:376–7.
13. Fung MSC, Sun C, Sun NC, Chang NT, Chang TW. Monoclonal antibodies that neutralize HIV-1 virions and inhibit syncytium formation by infected cells. Biotechnology **1987**;5:940–6.
14. Kim YW, Fung MS, Sun NC, Sun CR, Chang NT, Chang TW. Immunoconjugates that neutralize HIV virions kill T cells infected with diverse strains of HIV-1. J Immunol **1990**;144:1257–62.
15. Liou RS, Rosen EM, Fung MSC, et al. A chimeric mouse-human antibody that retains specificity for HIV gp120 and mediates the lysis of HIV-infected cells. J Immunol **1989**;143:3967–75.
16. Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of functional human immune system to mice with severe combined immunodeficiency. Nature **1988**;335:256–9.
17. Mosier DE, Gulizia RJ, Baird SM, Wilson DB, Spector DH, Spector SA. Human immunodeficiency virus infection of human-PBL-SCID mice. Science **1991**;251:791–4.
18. Safrit JT, Fung MSC, Andrews CA, et al. hu-PBL-SCID mice can be protected from HIV-1 infection by passive transfer of monoclonal antibody to the principal neutralizing determinant of envelope gp 120. AIDS **1993**;7:15–21.
19. Braun DG, Mc Kinney S, Gordon W, et al. A mouse/human chimeric monoclonal antibody to HIV-1 qualifying for a phase I trial in patients with AIDS. In: Abraham NG, Marks P, Konwalinka G, Sachs L, Tawassoli M, eds. Molecular biology of haematopoiesis. Vol 2. Andover, UK: Intercept, **1992**:437–48.
20. Liou RS, Fung MSC, Zühlke U, et al. Mouse/human chimeric anti–HIV-1 gp120 antibody to the principal neutralizing determinant: tolerability and pharmacokinetics in cynomolgus monkeys, *Macaca fascicularis*. Biotherapy **1992**;5:291–9.
21. Günthard H, Opravil M, Ledergerber B, et al. Prognostic value of various patterns of change in CD4-lymphocyte count in 420 asymptomatic HIV-1–infected patients. Dtsch Med Wochenschr **1993**;118:737–45.
22. Classification system for human T-lymphotropic virus type III/lymphadenopathy–associated virus infections. MMWR **1986**; 35:334–9.
23. Centers for Disease Control. Revision of the CDC surveillance case definition for AIDS. MMWR **1987**;35:3–15.
24. Schüpbach J, Günthard H, Fung MSC, et al. Pharmacokinetics of an HIV-1 gp120-specific chimeric antibody in patients with HIV-1 disease. Biotherapy **1993**;6:205–15.
25. Sun NC, Ho DD, Sun CRY, et al. Generation and characterization of monoclonal antibodies to the putative CD4-binding domain of human immunodeficiency virus type 1 gp120. J Virol **1989**;63:3579–85.
26. Schüpbach J, Böni J. Quantitative and sensitive detection of immunecomplexed and free HIV antigen after boiling of serum. J Virol Methods **1993**;43:247–56.
27. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med **1990**;321:1621–5.
28. Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA **1980**;77:7415–9.
29. Sambrook J, Fritsch E, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, **1989**.
30. Krone WJA, Sninsky JJ, Goudsmit J. Detection and characterization of HIV-1 by polymerase chain reaction. J Acquir Immune Defic Syndr **1990**;3:517–24.
31. Pachl C, Lindquist C, Kern D, et al. Quantitation of HIV-1 RNA in plasma using a signal amplification branched DNA (bDNA) assay [abstract WS-A24-1]. In: Program and abstracts of the IX International Conference on AIDS/IV STD World Congress (Berlin). London: Wellcome Foundation, **1993**.
32. Urdea M. Synthesis and characterization of branched DNA (bDNA) for the direct and quantitative detection of CMV, HBV, HCV and HIV. Clin Chem **1993**;39:725–6.
33. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc **1958**;53:457–81.
34. Coopman SA, Johnson RA, Platt R, Stern RS. Cutaneous disease and drug reactions in HIV infection. N Engl J Med **1993**;328:1670–4.
35. Battegay M, Opravil M, Wüthrich B, Lüthy R. Rash with amoxycillin-clavulanate therapy in HIV-infected patients [letter]. Lancet **1989**;2:1100.
36. Piatak MJ, Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science **1993**;259:1749–54.
37. Wahren B, Bratt G, Hinkula J, et al. Monoclonal antibodies given as passive treatment to HIV-infected individuals. In: Girard M, Valette L, eds. Retroviruses of human AIDS and related animal diseases. 5e colloque des "Cent Gardes." Paris: Pasteur Mérieux **1990**:263–7.
38. Morell A, Terry WD, Waldmann TA. Metabolic properties of IgG subclasses in man. J Clin Invest **1970**;49:673–80.
39. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N Engl J Med **1987**;317:185–91.
40. Richman DD, Fischl MA, Grieco MH, et al. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N Engl J Med **1987**;317:192–7.
41. Koch MA, Volberding PA, Lagakos SW, Booth DK, Pettinelli C, Myers MW. Toxic effects of zidovudine in asymptomatic human immunodeficiency virus–infected individuals with $CD4^+$ cell counts of 0.50 $\times 10^9$/L or less. Detailed and updated results from protocol 019 of the AIDS Clinical Trials Group. Arch Intern Med **1992**;152:2286–92.
42. Robinson WE, Montefiori DC, Mitchell WM. Antibody dependent

enhancement of human immunodeficiency virus type 1 infection. Lancet **1988**;1:790–4.

43. Homsy J, Tateno M, Levy JA. Antibody-dependent enhancement of HIV infection [letter]. Lancet **1988**;1:1285–6.
44. Takeda A, Tuazon CU, Ennis FA. Antibody-enhanced infection by HIV-1 via Fc receptor–mediated entry. Science **1988**;242:580–3.
45. Homsy J, Meyer M, Levy JA. Serum enhancement of human immunodeficiency virus (HIV) infection correlates with disease in HIV-infected individuals. J Virol **1990**;64:1437–40.
46. Takeda A, Robinson JE, Ho DD, Debouck Ch, Haigwood NL, Ennis FA. Distinction of human immunodeficiency virus type 1 neutralization and infection enhancement by human monoclonal antibodies to glycoprotein 120. J Clin Invest **1992**;89:1952–7.
47. Matsushita S, Maeda H, Kimachi K, et al. Characterization of a mouse/human chimeric monoclonal antibody (C beta 1) to a principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. AIDS Res Hum Retroviruses **1992**;8:1107–15.
48. LaRosa GJ, Davide JP, Weinhold K, et al. Conserved sequence and structural elements in the HIV-1 principal neutralizing determinant. Science **1990**;249:932–5.
49. Zwart G, De Jong JJ, Wolfs T, et al. Predominance of HIV-1 serotype distinct from LAV-1/HTLV-IIIB [letter]. Lancet **1990**;335:474.
50. Carrow EW, Vujcic LK, Glass WL, et al. High prevalence of antibodies to the gp120 V3 region principal neutralizing determinant of HIV-1_{MN} in sera from Africa and the Americas. AIDS Res and Hum Retroviruses **1991**;7:831–8.
51. Spear GT, Takefman DM, Sullivan BL, Landay AL, Zolla-Pazner S. Complement activation by human monoclonal antibodies to human immunodeficiency virus. J Virol **1993**;67:53–9.
52. Nakamura M, Sasaki H, Terada M, Ohno T. Complement-dependent virolysis of HIV-1 with monoclonal antibody NM-01. AIDS Res and Hum Retroviruses **1993**;9:619–26.
53. Thali M, Furman C, Wahren B, et al. Cooperativity of neutralizing antibodies directed against the V3 and CD4 binding regions of the human immunodeficiency virus gp120 envelope glycoprotein. J Acquir Immune Defic Syndr **1992**;5:591–9.
54. Buchbinder A, Karwowska S, Gorny MK, Burda ST, Zolla-Pazner S. Synergy between human monoclonal antibodies to HIV extends their effective biologic activity against homologous and divergent strains. AIDS Res and Hum Retroviruses **1992**;8:425–7.