MAJOR ARTICLE

Antiretroviral Activity of the Anti-CD4 Monoclonal Antibody TNX-355 in Patients Infected with HIV Type 1

Daniel R. Kuritzkes,[1,a] Jeffrey Jacobson,[2,a] William G. Powderly,[3] Eliot Godofsky,[4] Edwin DeJesus,[5] Frances Haas,[6] Keith A. Reimann,[7] Jeffrey L. Larson,[8] Patrice O. Yarbough,[8] Valentin Curt,[8] and William R. Shanahan, Jr.[8]

[1]Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver; [2]Division of Infectious Diseases, Mount Sinai School of Medicine, New York, New York; [3]Division of Infectious Diseases, Washington University, St. Louis, Missouri; [4]Bach & Godofsky, Bradenton, and [5]IDC Research Initiative, Altamonte Springs, Florida; [6]Associates in Medical and Mental Health, Tulsa, Oklahoma; [7]Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center, Boston, Massachusetts; [8]Tanox, Houston, Texas

Background. We wished to determine the safety and anti–human immunodeficiency virus (HIV) type 1 activity of single doses of TNX-355, a humanized IgG4 anti-CD4 monoclonal antibody with potent activity against HIV-1 in vitro, in HIV-infected subjects.

Methods. Sequential cohorts of 6 HIV-1–infected subjects each received infusions of TNX-355. Data included plasma HIV-1 RNA level, $CD4^+$ T cell count, TNX-355 coating of $CD4^+$ T cells, and serum TNX-355 levels.

Results. Dose-related reductions in plasma HIV-1 RNA loads correlated with complete $CD4^+$ T cell coating by TNX-355. Peak median decreases in plasma HIV-1 RNA loads were 0.56, 1.33, and 1.11 $\log_{10}$ copies/mL and occurred on days 4–7, 14, and 21 for the 3.0, 10, and 25 mg/kg doses, respectively. Dose-dependent increases in $CD4^+$ T cell count occurred within 24 h of dosing.

Conclusions. Single doses of TNX-355 reduced plasma HIV-1 RNA loads and increased $CD4^+$ T cell counts in HIV-infected subjects. The further assessment of therapeutic potential awaits data from longer-duration trials.

The treatment of HIV type 1 typically involves combinations of drugs that inhibit the viral reverse transcriptase or protease. Failure of the currently available antiretroviral agents leads to the development of HIV-1 drug resistance. The prevalence of drug-resistant HIV-1 among newly infected persons increased from 3.4% during the period 1995–1998 to 12.4% during the period 1999–2000 [1]. Among patients who receive care for HIV-1 infection, the prevalence of antiretroviral drug resistance is ~50% [2]. Clearly, new approaches to control viral replication are needed.

Virus entry provides a novel target for HIV-1 inhibition. Entry is a complex multistep process that involves attachment, coreceptor binding, and membrane fusion [3]. Drugs that target each of these steps are currently in clinical development. Enfuvirtide, a 36-aa peptide that blocks HIV-1 entry by preventing fusion, is approved for use in patients failing currently available antiretroviral therapies. Randomized clinical trials have shown the potent antiviral activity of enfuvirtide when it is administered together with antiretroviral regimens that have been optimized by the use of drug-resistance testing [4, 5]. These results provide evidence that therapeutic intervention at the stage of virus entry may be effective in the treatment of HIV-1 infection.

The monoclonal antibody (MAb) TNX-355 (formerly known as Hu5A8) is a humanized IgG4 MAb that inhibits HIV-1 entry by a unique mechanism. TNX-355 was engineered from its murine progenitor by grafting the murine complementarity-determining region (CDR) onto a human IgG4 construct and then

Received 13 June 2003; accepted 24 July 2003; electronically published 14 January 2004.

Presented in part: 10th Conference on Retroviruses and Opportunistic Infections, Boston, 9–14 February 2003 (abstract 13).

Potential conflict of interest: J.L.L., P.O.Y, V.C., and W.R.S. are current or past employees of Tanox and may hold stock or stock options in the company.

Financial support: Tanox; National Institutes of Health (grants RR00036, RR00051, and RR16482).

[a] Present affiliations: Section of Retroviral Therapeutics, Brigham and Women's Hospital, and Division of AIDS, Harvard Medical School, Boston, Massachusetts (D.R.K.); Division of Infectious Diseases, Beth Israel Medical Center, New York, New York (J.J.).

Reprints or correspondence: Dr. Daniel Kuritzkes, Partners AIDS Research Center, Brigham and Women's Hospital, 65 Landsdowne St., Rm. 449, Cambridge, MA 02139 (dkuritzkes@partners.org).

The Journal of Infectious Diseases 2004; 189:286–91

0022-1899/2004/18902-0016$15.00

mutating amino acids in the framework region of the CDR to produce a framework that is >95% human. The IgG4 isotype was chosen to lessen the chances for $CD4^+$ T cell depletion by antibody- and complement-mediated cytotoxicity. This MAb, like its murine progenitor (Mu5A8), binds to extracellular domain 2 of rhesus and human CD4, thereby preventing post-binding entry of the virus into $CD4^+$ cells [6–10]. Human and murine MAbs inhibit the in vitro infectivity of diverse primary isolates of HIV-1, with IC_{50} values of 0.0004–0.152 μg/mL (Tanox, data on file). TNX-355 transiently reduces plasma viremia in rhesus monkeys infected with simian immunodeficiency virus (SIV_{mac}) [11]. The antibody binding site on CD4 is distinct from the site required for the binding of HIV-1 envelope gp120 and is distinct from the site needed for interaction with major histocompatibility complex proteins. Unlike anti-CD4 antibodies that target domain 1, Mu5A8 and TNX-355 do not interfere with immunological functions that involve antigen presentation [10, 12]. Therapeutic immunoglobulin molecules typically have long half-lives, which permits relatively infrequent dosing. We therefore undertook a proof-of-concept study to determine the safety, pharmacokinetics, and anti-HIV activity of single doses of TNX-355 in HIV-1–infected patients with uncontrolled viral replication.

PATIENTS AND METHODS

Study design. This was a multicenter, open-label, dose-ranging study of TNX-355 in patients infected with HIV-1. Qualifying subjects were enrolled into 5 sequential dose cohorts of 6 patients each. Subjects were admitted overnight to an inpatient General Clinical Research Center or pharmacokinetics unit for study drug administration and for monitoring during the first 24 h after dosing. The initial cohort received a single intravenous infusion of TNX-355 at 0.3 mg/kg; successive cohorts received increasing TNX-355 doses of 1.0, 3.0, 10, or 25 mg/kg. To accommodate a wide range of doses, TNX-355 was supplied at a concentration of 5 mg/mL and, after removal of the appropriate volume, added to 100-mL bags of sterile saline for infusion at a rate of 200 mL/h. The volume of infusion was, therefore, 100 mL for the 0.3, 1.0, and 3.0 mg/kg doses; 200 mL for the 10 mg/kg dose; and 400–500 mL for the 25 mg/kg dose. All subjects in a cohort completed the day 14 safety evaluation before enrollment at the next higher dose began.

Patient population. Eligible subjects were HIV-1 seropositive, aged ⩾18 years, and had $CD4^+$ T cell counts >100 cells/mm^3 and plasma HIV-1 RNA levels of at least 5000 copies/mL obtained at 2 consecutive visits within 30 days of study entry but at least 48 h apart. The difference between plasma HIV-1 RNA levels at the 2 screening visits could not be >0.5 log_{10} copies/mL. Subjects who did or did not receive antiretroviral therapy were eligible if the treatment regimen had been unchanged for at least 8 weeks prior to enrollment and if they were willing to remain on the same regimen (or on no antiretroviral therapy) for the first 28 days of the study. Exclusion criteria included the presence of another active infection requiring therapy, the use of immunomodulating drugs or systemic chemotherapy, receipt of investigational immunomodulatory or antiretroviral therapy within 12 weeks before enrollment, prior participation in an HIV vaccine trial, pregnancy, and breast-feeding. Female subjects with childbearing potential and heterosexually active male subjects were required to use effective contraception during the study. The present study conformed to human experimentation guidelines of the US Department of Health and Human Services and those of the authors' institutions. Signed, informed consent was obtained from all subjects.

Assessment and follow-up. All subjects were assessed at preentry, entry (day 0), and days 1, 2, 3, 4, 7, 14, and 28 after dosing; subjects in the 10 and 25 mg/kg cohorts underwent additional assessments on day 21. Study visits included clinical assessment, safety tests, and the determination of plasma HIV-1 RNA level, $CD4^+$ and $CD8^+$ T cell counts, the percentage of activated $CD4^+$ T cells ($CD4^+CD25^+$ cells), and the extent of $CD4^+$ T cell coating by TNX-355. An additional $CD4^+$ T cell count was done 3 h after the administration of TNX-355. Serum samples for pharmacokinetic analysis were obtained on day 0 before dosing, 0.5, 1, 3, 6, and 12 h after the start of infusion, and at each subsequent study visit. A final serum drug level was obtained 90 days after dosing. The immunogenicity of TNX-355 was assessed by measuring serum concentrations of anti–TNX-355 antibodies on days 14, 28, and 90. All laboratory assessments were conducted by a central laboratory (ICON Laboratories), with the exception of drug levels and anti-drug antibody levels, which were measured at Tanox.

Plasma HIV-1 RNA levels were determined by the Amplicor HIV-1 Monitor Assay (version 1.0; Roche Molecular Systems). The TNX-355 coating of $CD3^+CD4^+$ cells was determined by a flow cytometry–based competitive binding assay, developed for the trial and performed at ICON, in which phycoerythrin-labeled TNX-355 was added to subjects' blood specimens, to assess whether unoccupied binding sites remained on $CD3^+CD4^+$ lymphocytes. Fluorescence intensity limits corresponding to uncoated or completely coated cells were determined using blood from healthy seronegative donors that was left untreated or preincubated with 100 μg/mL of unlabeled TNX-355, respectively. Subject specimens were determined to be uncoated or completely coated when ⩾95% of cells showed fluorescence intensity above or below these limits, respectively. Subject specimens in which >5% of cells showed intermediate fluorescence intensity were described as partially coated. The titer of anti–TNX-355 antibodies was determined by serum ELISA. Samples were considered to have a positive result if both of the following conditions were met: (1) the sample

bound an amount of TNX-355 greater than that bound by 26.5 ng/mL of anti-idiotype antibody to TNX-355 and (2) the titer in postdose samples was at least 2-fold greater than that in the predose sample. If both criteria were met, a binding inhibition test with soluble drug was done to confirm that the response was drug specific.

Pharmacokinetic analysis. Pharmacokinetic analysis was conducted by Dr. William Petros (West Virginia University) and one of us (J.A.L.). Pharmacokinetic parameters were determined using noncompartmental methods (model independent) with WinNonlin Professional software (version 3.2; Pharsight) and were based on individual subject serum concentration–time data. The area under the serum concentration time curve to the last collection time ($AUC_{(0\text{-}LAST)}$) was calculated by the linear trapezoidal rule. The mean residence time (MRT) was calculated as the area under the moment curve divided by $AUC_{(0\text{-}LAST)}$. The volume of distribution (V_d) was calculated by MRT × CL (drug clearance). The terminal elimination phase half-life ($T_{1/2}$) was determined using a uniform weighting of the serum concentrations. Summary descriptive statistics of the pharmacokinetic parameters were also determined using WinNonlin software.

Data analysis. Plasma HIV-1 RNA levels were $\log_{10}$ transformed before analysis. Baseline plasma HIV-1 RNA and $CD4^+$ T cell count were defined as the mean of the second screening value and the day 0 (preinfusion) value. Data are presented as the median change from baseline.

RESULTS

Study population and baseline characteristics. Thirty subjects were enrolled at 6 centers between October 2001 and August 2002; all subjects completed the study. Table 1 shows the baseline characteristics of the entire study population. Baseline characteristics were similar in each of the dose cohorts (data not shown). All subjects had previously received antiretroviral therapy; 19 were on a failing regimen at the time of enrollment.

Change in plasma HIV-1 RNA levels. Figure 1 shows the median change in plasma HIV-1 RNA levels over time in each dose cohort. Minimal effects were observed in the 0.3 and 1.0 mg/kg dose groups. By contrast, substantial reductions in plasma HIV-1 RNA levels were evident after single infusions of TNX-355 doses of 3.0, 10, and 25 mg/kg. Peak median reductions in plasma HIV-1 RNA levels were 0.56 $\log_{10}$ on days 4–7 in the 3.0 mg/kg dose cohort, 1.33 $\log_{10}$ on day 14 in the 10.0 mg/kg dose cohort, and 1.11 $\log_{10}$ on day 21 in the 25.0 mg/kg dose cohort. Five of 6 subjects in both the 10 and 25 mg/kg dose groups had a decrease in plasma HIV-1 RNA levels of at least 1.0 $\log_{10}$ during the 28-day study. The remaining subject in the 10 mg/kg group had a peak decrease of 0.98 $\log_{10}$ on day 14, and the remaining subject in the 25 mg/kg group had a peak decrease of 0.90 $\log_{10}$ on day 7.

Table 1. Baseline characteristics of the study population.

Characteristic	Value
Age, mean (range), years	42.3 (28–57)
Sex	
Male	27 (90)
Female	3 (10)
Ethnicity	
White	24 (80)
Black	2 (6.7)
Hispanic	3 (10)
Other	1 (3.3)
Weight, mean (range), kg	72 (50–87)
$CD4^+$ T cell count, median (range), cells/mm^3	282 (165–902)
Baseline plasma HIV-1 RNA levels, median (range), $\log_{10}$ copies/mL	4.72 (3.57–5.67)

NOTE. Data are no. (%) of subjects unless otherwise specified.

The extent and duration of virus suppression correlated with dose and with the extent and duration of $CD4^+$ T cell coating by TNX-355 (table 2). Partial coating of $CD4^+$ T cells through day 28 was observed in subjects in the 0.3 and 1.0 mg/kg dose cohorts. Full coating of $CD4^+$ T cells was not observed for any subjects in the 0.3 mg/kg dose group but was observed in at least some subjects in all other dose groups. Complete coating lasted for 4–6 days in the 3.0 mg/kg dose cohort (4/6 subjects), 8–20 days in the 10 mg/kg cohort (6/6 subjects), and 15–34 days in the 25 mg/kg cohort (6/6 subjects).

Lymphocyte subset analysis. Figure 2 shows the median change in $CD4^+$ T cell count over time for each dose cohort. Peak median increases in $CD4^+$ T cell counts of 23, 37, 148, 131, and 244 cells/mm^3 were observed in the 0.3, 1.0, 3.0, 10, and 25 mg/kg dose groups, respectively. Peak increases occurred 1 day after infusion and were sustained longer in subjects in the higher dose cohorts. Changes in the percentage of $CD4^+$ T cells expressing CD25 and in the number of $CD8^+$ T cells were minimal and did not differ substantially among dosing groups (data not shown).

Pharmacokinetics. The systemic exposures to TNX-355 were observed at all dose levels (table 2). Maximum drug concentrations in serum generally were observed within the first hour after dosing, although the time to peak concentration (T_{max}) was prolonged at the higher dose levels. Peak serum drug concentrations (C_{max}) and AUC were dose dependent but increased disproportionately to dose, especially at the lower dose levels. V_d approximated the plasma volume. The MRT for TNX-355 in the blood, CL, and $T_{1/2}$ increased with increases in dose, which suggests that the elimination of TNX-355 is a saturable process. There were no apparent correlations between the phar-

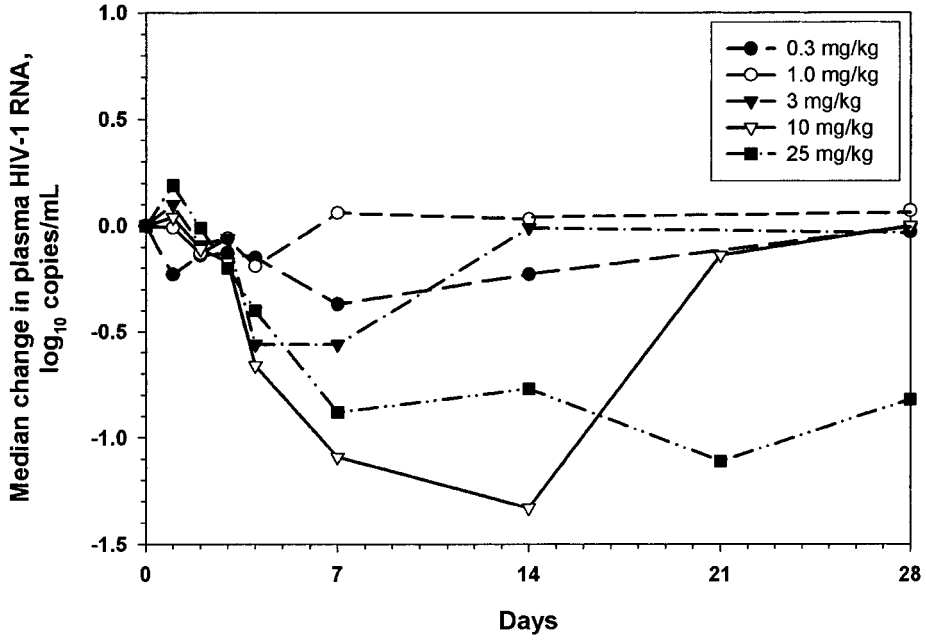

Figure 1. Median change in plasma HIV type 1 RNA levels over time for the various dosing groups

macokinetics of the drug and subject body weight or body surface area.

Safety. Twenty subjects (66.7%) experienced ⩾1 adverse event after receiving the study drug. No serious adverse events occurred, and there were no discontinuations of study drug infusion caused by them. Adverse events that occurred in ⩾3 subjects were headache (13.3%) and rash (10.0%). Pruritus, urticaria, and nasal congestion each occurred in 2 subjects (6.7%) each. There was no evidence of an increased incidence of infection. No study drug–related laboratory abnormalities were reported, nor was there any evidence of anti–TNX-355 antibody formation.

DISCUSSION

Virus entry inhibitors provide several theoretical advantages over currently available antiretroviral agents targeted against HIV-1 reverse transcriptase or protease. Because these inhibitors act extracellularly, they do not require transport across a cell membrane or intracellular activation. In addition, inhibitors can be targeted against host cell receptors, which are not subject to rapid mutation.

In the present proof-of-concept study, single intravenous infusions of TNX-355 reduced plasma HIV-1 RNA levels in a dose-responsive fashion in antiretroviral therapy–experienced subjects. Median reductions in plasma viremia were >1 $\log_{10}$ at the 10 and 25 mg/kg dose levels. The duration of complete $CD4^+$ T cell coating by TNX-355 correlated with dose, as did the duration and extent of virus suppression. Nadirs in plasma HIV-1 RNA levels occurred on day 14 for the 10 mg/kg dose and on day 21 for the 25 mg/kg dose. By contrast, peak increases in $CD4^+$ T cell count occurred within 24 h of TNX-355 infusion. Rapid dose-dependent increases in $CD4^+$ T cell counts were observed before substantial reductions in plasma viremia but returned to baseline before the nadir in plasma HIV-1 RNA levels. Similar increases in $CD4^+$ T cells were seen in healthy nonhuman primates after the administration TNX-355 [10], which suggests that TNX-355 binding to CD4 may result in a redistribution of $CD4^+$ T cells from lymphoid tissues to the peripheral blood. Whether this redistribution is evidence of a perturbation of immune homeostasis requires further exploration. Mathematical modeling of the relationship among CD4 cell coating, viral clearance, and $CD4^+$ cell turnover may provide insights into the mechanisms by which TNX-355 exerts its effects in vivo.

Single infusions of TNX-355 were well tolerated. There was no clinical evidence of additional immunosuppression caused by the administration of TNX-355, nor was there any evidence of inappropriate immune activation. No anti–TNX-355 antibodies developed, which suggests that the immunogenicity observed in macaques might be avoided in humans. Longer studies of long-term dosing with TNX-355 are needed to assess more completely the safety and immunogenicity of this molecule.

Entry of HIV-1 into the cell is a complex, multistep process that offers several points for potential intervention [3]. TNX-355 blocks HIV-1 entry by a novel mechanism that is distinct from other entry inhibitors that prevent attachment, coreceptor binding, or fusion. TNX-355 recognizes an epitope in domain

Table 2. Pharmacokinetics of TNX-355, by dose group.

Dose, mg/kg	T_{max}, h	C_{max}, μg/mL	$AUC_{(0-LAST)}$, μg/h/mL	V_d, mL/kg	MRT, h	$T_{1/2}$, h	CL, mL/min
0.3	0.8 (0.3)	5.4 (0.8)	29.3 (5.9)	44.6 (8.1)	5.1 (2.7)	3.4 (2.5)	699.6 (165.0)
1.0	0.8 (0.3)	21.0 (3.4)	277.9 (129.4)	43.7 (6.9)	9.7 (3.1)	8.1 (2.0)	298.6 (98.2)
3.0	0.8 (0.3)	122.9 (12.3)	2938.6 (63.1)	25.5 (7.3)	23.0 (4.4)	14.1 (2.1)	77.7 (27.6)
10.0	2.2 (2.6)	328.0 (38.8)	20,644.2 (376.1)	29.5 (5.6)	59.5 (10.1)	28.7 (6.6)	37.1 (6.2)
25.0	4.6 (4.5)	750.2 (233.1)	73,024.8 (1886.3)	42.7 (10.8)	113.3 (9.3)	57.2 (20.5)	26.2 (5.7)

NOTE. Data are mean (SD). $AUC_{(0-LAST)}$, area under the serum concentration time curve to the last collection time; C_{max}, maximum serum concentration; CL, drug clearance; MRT, mean residence time; T_{max}, time to maximum serum drug concentration; $T_{1/2}$, terminal elimination phase half-life; V_d, volume of distribution.

2 of CD4 and does not prevent the virus from binding to CD4 (at domain 1). Although the precise molecular mechanism of the action of TNX-355 has not been ascertained, CD4 binding by the original murine MAb (Mu5A8) prevents the exposure of gp41 epitopes that are normally revealed on gp120 binding to CD4 [8]. The antibody could block virus entry by preventing the close approximation of the ⩾2 CD4 molecules on the cell surface that might be needed to fully engage the gp120/gp41 trimeric complex. However, the results of other studies have suggested that the stoichiometry of CD4 receptor binding to trimeric gp140 of SIV is 1:1 (and not 2:1 or 3:1). The same stoichiometry is likely for HIV [13]. Moreover, CD4 dimerization is required for CD4 immune function [14]. The preservation of CD4 immune function in the presence of TNX-355 in rhesus monkeys [9] and in human peripheral blood mononuclear cells in vitro (Tanox, unpublished results) argues against this mechanism of TNX-355 action.

Alternatively, TNX-355 could block the postattachment conformational changes in CD4 and/or gp120/gp41 that are needed for binding to the chemokine coreceptor or for the deployment of the fusion machinery housed on the ectodomain of gp41. It is interesting to note that residues 121–124 and 127–134 in domain 2 of CD4 are critical for TNX-355 binding and that these residues are in the region of the disulfide bond in domain 2 between cysteines (at positions 130 and 159) critical to HIV entry [6, 15]. These cysteines are in equilibrium between the oxidized (disulfide) and reduced (dithiol) state on the cell surface, and the oxidized state is required for HIV entry. Therefore, another potential mechanism of action is a TNX-355–induced shift in equilibrium in favor of the reduced state of these cysteine residues.

The relatively short apparent serum half-life of TNX-355 and the high doses needed to achieve sustained $CD4^+$ T cell coating and virus suppression were somewhat surprising findings. By comparison, doses in the range of 1–5 mg/kg are commonly recommended for MAbs approved for therapeutic use in other diseases. Under the assumption that there are 2.5×10^{11} $CD4^+$ T cells in the body [16] and a CD4 receptor density of 6×10^4 per T cell [17], a dose of 3.75 mg, or <0.1 mg/kg, would suffice for complete $CD4^+$ T cell coating if each TNX-355 molecule engages only 1 CD4 molecule. This simplistic calculation considers neither other $CD4^+$ cell types (e.g., monocytes, macrophages, and dendritic cells) nor the excess concentration of antibody needed to favor CD4 binding and to penetrate the liquid film surrounding the T cell. However, with a binding affinity of 8×10^{-11} mol/L (12 ng/mL) to CD4 (Tanox, unpublished results), the mean blood concentrations achieved at even the lowest dosage investigated in the present study would exceed by almost 500-fold the concentrations of TNX-355 needed to favor CD4 binding. The apparent rapid clearance of TNX-355 and the high dose requirement observed in the present study could be explained by, among other factors, the development of anti-drug antibodies, the rapid internalization of TNX-355–CD4 receptor complexes, and/or a large, unrecognized pool of CD4 molecules expressed on other cell types. There was no evidence of the development of anti–TNX-355 antibodies, and furthermore, under the assumption of successful primary immunization, such antibodies would not be expected to be expressed at levels adequate to reduce drug levels or to neutralize activity for 1–2 weeks. It has been reported that cultured adipocytes express CD4, CCR5, and CXCR4 receptors and that preadipocytes can become infected with HIV in vitro [18]. Even if adipocytes express CD4 and may become infected with HIV, the volume of distribution for TNX-355 observed in the present study is similar to plasma volume, which suggests that extravascular distribution does not play a major role in the pharmacokinetic behavior of TNX-355. Rapid internalization with subsequent degradation and release has been observed for other anti-CD4 antibodies when CD4 molecules bind to the cell surface [19]. Therefore, this mechanism would appear to be the most likely explanation for our results. Nevertheless, our results suggest that doses in the range of 10–40 mg/kg/month may be effective in maintaining virus suppression, and a multiple-dose, phase 1b trial is under way.

In summary, the results of the present proof-of-concept study demonstrate the feasibility of inhibiting HIV-1 in vivo by a CD4-specific MAb and suggest that TNX-355 is a promising new candidate for the treatment of HIV-1 infection. A MAb that suppresses HIV-1 replication for 2–3 weeks after administration could offer significant clinical benefits in the

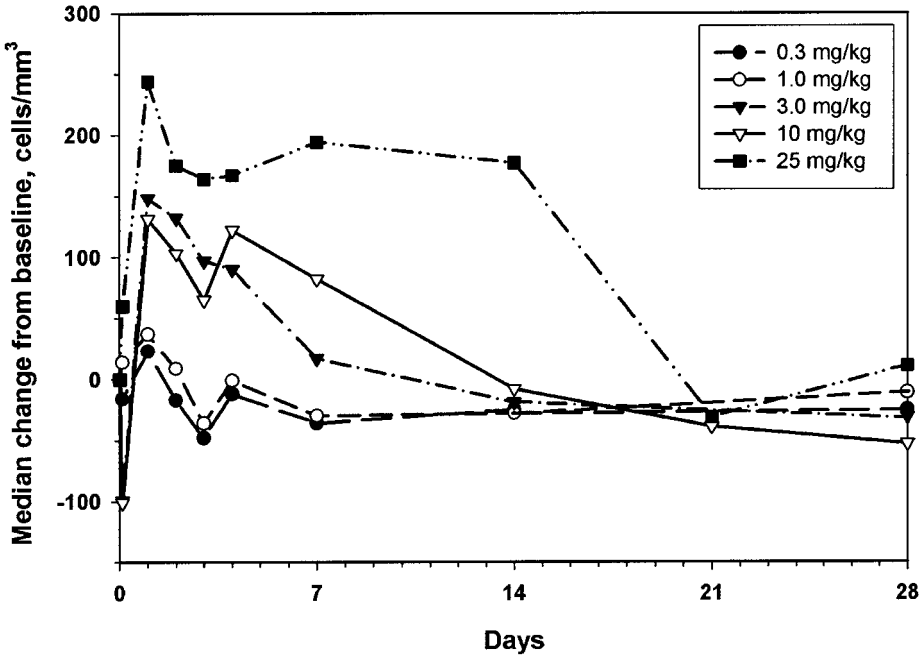

Figure 2. Median change in $CD4^+$ T cell counts over time for the various dosing groups

treatment of drug-resistant HIV-1 infection. Combined use with other entry blockers could lead to a potent synergistic inhibition of HIV-1 replication. Other potential uses for TNX-355 include postexposure prophylaxis and the prevention of mother-to-child transmission of HIV-1. A more definitive assessment of the safety and therapeutic potential of TNX-355 awaits data from controlled clinical trials.

References

1. Little SJ, Holte S, Routy JP, et al. Antiretroviral-drug resistance among patients recently infected with HIV. N Engl J Med **2002**; 347:385–94.
2. Richman DD, Bosette SA, Morton SC, et al. The prevalence of antiretroviral drug resistance in the US (abstract LB-17). In: Program and abstracts of the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago). Washington, DC: American Society for Microbiology, **2001**:14.
3. Doms RW, Trono D. The plasma membrane as a combat zone in the HIV battlefield. Genes Dev **2000**; 14:2677–88.
4. Lalezari JP, Henry K, O'Hearn M, et al. Enfuvirtide, an HIV-1 fusion inhibitor, for drug-resistant HIV infection in North and South America. N Engl J Med **2003**; 348:2175–85.
5. Lazzarin A, Clotet B, Cooper D, et al. Efficacy of enfurvitide in patients infected with drug-resistant HIV-1 in Europe and Australia. N Engl J Med **2003**; 348:2186–95.
6. Burkly LC, Olson D, Shapiro R, et al. Inhibition of HIV infection by a novel CD4 domain 2–specific monoclonal antibody. J Immunol **1992**; 149:1779–87.
7. Burkly LC, Mulrey N, Blumenthal R, Dimitrov DS. Synergistic inhibition of human immunodeficiency virus type 1 envelope glycoprotein-mediated cell fusion and infection by an antibody to CD4 domain 2 in combination with anti-gp120 antibodies. J Virol **1995**; 69:4267–73.
8. Moore JP, Sattentau GQ, Klasse PJ, Burkly LC. A monoclonal antibody to CD4 domain 2 blocks soluble CD4-induced conformation changes in the envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1) and HIV-1 infection of $CD4^+$ cells. J Virol **1992**; 66:4784–93.
9. Reimann K, Burkly LC, Burrus B, Waite BCD, Lord CI, Letvin N. In vivo administration to rhesus monkeys of a CD4-specific monoclonal antibody capable of blocking AIDS virus replication. AIDS Res Hum Retroviruses **1993**; 9:199–207.
10. Reimann K, Lin W, Bixler S, et al. A humanized form of a CD4-specific monoclonal antibody exhibits decreased antigenicity and prolonged plasma half-life in rhesus monkeys while retaining its unique biological and antiviral properties. AIDS Res Hum Retroviruses **1997**; 13:933–43.
11. Reimann KA, Khunkhun R, Lin W, Gordon W, Fung M. A humanized, nondepleting anti–CD-4 antibody that blocks virus entry inhibits virus replication in rhesus monkeys chronically-infected with simian immunodeficiency virus. AIDS Res Hum Retroviruses **2002**; 18:747–55.
12. Boon L, Holland B, Gordon W, et al. Development of anti-CD4 MAb hu5A8 for treatment of HIV-1 infection: preclinical assessment in nonhuman primates. Toxicology **2002**; 172:191–203.
13. Kim M, Chen B, Hussey RE, et al. The stoichiometry of trimeric SIV glycoprotein interaction with CD4 differs from that of anti-envelope antibody Fab fragments. J Biol Chem **2001**; 276: 42667–76.
14. Moldavan M-C, Yachou A, Levesque K, et al. CD4 dimers constitute the functional component required for T cell activation. J Immunol **2002**; 169:6261–8.
15. Matthias LJ, Yam PTW, Jiang XM, et al. Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. Nat Immunol **2002**; 3:727–32.
16. Clark DR, de Boer RJ, Wolthers KC, Miedema F. T cell dynamics in HIV-1 infection. Adv Immunol **1999**; 73:301–27.
17. Lee B, Sharron M, Montaner LJ, Weissman D, Doms RW. Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages. Proc Natl Acad Sci USA **1999**; 96:5215–20.
18. Hazan U, Romero IA, Cancello R, et al. Human adipose cells express CD4, CXCR4, and CCR5 [corrected] receptors: a new target cell type for the immunodeficiency virus-1? FASEB J **2002**; 16:1254–6.
19. Morel P, Vincent C, Wijdenes J, Revillard JP. Internalization and degradation of anti-CD4 monoclonal antibodies bound to human peripheral blood lymphocytes. Mol Immunol **1993**; 30:649–57.