



Short Communication

The efficacy of an anti-CD4 monoclonal antibody for HIV-1 treatment

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ABSTRACT

The availability of 24 antiretroviral (ARV) drugs within six distinct drug classes has transformed HIV-1 infection (AIDS) into a treatable chronic disease. However, the ability of HIV-1 to develop resistance to multiple classes continues to present challenges to the treatment of many ARV treatment-experienced patients. In this case report, we describe the response to ibalizumab, an investigational CD4-binding monoclonal antibody (mAb), in a patient with advanced immunodeficiency and high-level five-class antiretroviral resistance. After starting an ibalizumab-based salvage regimen, the patient had an approximately 4.0 log₁₀ reduction in viral load. An inadvertently missed infusion at week 32 led to the rapid loss of virologic response and decreased susceptibility to the remainder of the patient's salvage therapy regimen. Following the reinstatement of ibalizumab, phenotypic and genotypic resistance to ibalizumab was detected. Nonetheless, plasma HIV-1 RNA levels stabilized at ~2.0 log₁₀ copies/ml below pre-ibalizumab levels. Continued ARV drug development may yield additional clinical and public health benefits. This report illustrates the promise of mAbs for HIV-1 therapy in highly treatment-experienced patients. Therapeutic mAbs may also have a role in pre-exposure prophylaxis in high-risk uninfected populations and may facilitate directly observed therapy (DOT) if two or more synergistic long acting agents become available.

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We describe the course of a 56 year-old HIV-1-infected man that underwent treatment with 20 antiretroviral (ARV) drugs between 1991 and 2009 but who nonetheless had persistent HIV-1 viremia. Since 1997, the patient had plasma HIV-1 RNA levels usually exceeding 500,000 copies/ml and advanced immunosuppression, with most CD4 lymphocyte counts below 100 cells/mm³ (Fig. 1A). During this time, the patient experienced three transient virologic responses to ARV therapy characterized by reductions in plasma HIV-1 RNA levels of ≥ 1.0 log₁₀ following the administration of a new ARV. The first response was a 2.5 log₁₀ reduction in plasma HIV-1 RNA levels in 1997 after administration of an efavirenz-containing regimen, the second was a 1.0 log₁₀ reduction in 2006 after administration of an enfuvirtide-containing regimen, and the third was a 1.9 log₁₀ reduction in 2007 after administration of a regimen containing darunavir and raltegravir (Fig. 1A). However, by 2009, the patient's virus had acquired extensive resistance to ARVs belonging to each of the six drug classes based on the re-

sults of both genotypic and phenotypic drug resistance assays (Table 1). **Supplementary Table 1** summarizes the results of 11 genotypic resistance tests performed between June 1998 and October 2010.

In June 2009, the patient enrolled in a double-blinded phase IIb dose-response clinical trial entitled "Dose-response study of ibalizumab (TaiMed Biologics Inc., Irvine, CA) plus optimized background regimen in patients with HIV-1 (TMB-202; NCT00784147)." This blinded investigation compared monthly infusions of ibalizumab (formerly designated as TNX-355) 2000 mg IV every 4 weeks with 800 mg IV every 2 weeks. Because baseline drug resistance testing demonstrated enfuvirtide susceptibility (despite a 152-fold reduction in susceptibility observed in 2007) and susceptibility to etravirine, the patient's background regimen was optimized to comprise these two ARVs. Two weeks following the start of study treatment, the patient's plasma virus level decreased by >2.5 log₁₀ (Fig. 1B). Within 24 weeks, consecutive plasma HIV-1 RNA levels were <75 copies/ml – representing the approved lower limit of quantification for the Versant HIV RNA 3.0 assay ("bDNA"; Siemens Healthcare Diagnostics, Berkeley, CA). Despite this favorable virologic response, the patient's CD4 lymphocyte count remained below 20 cells/mm³.

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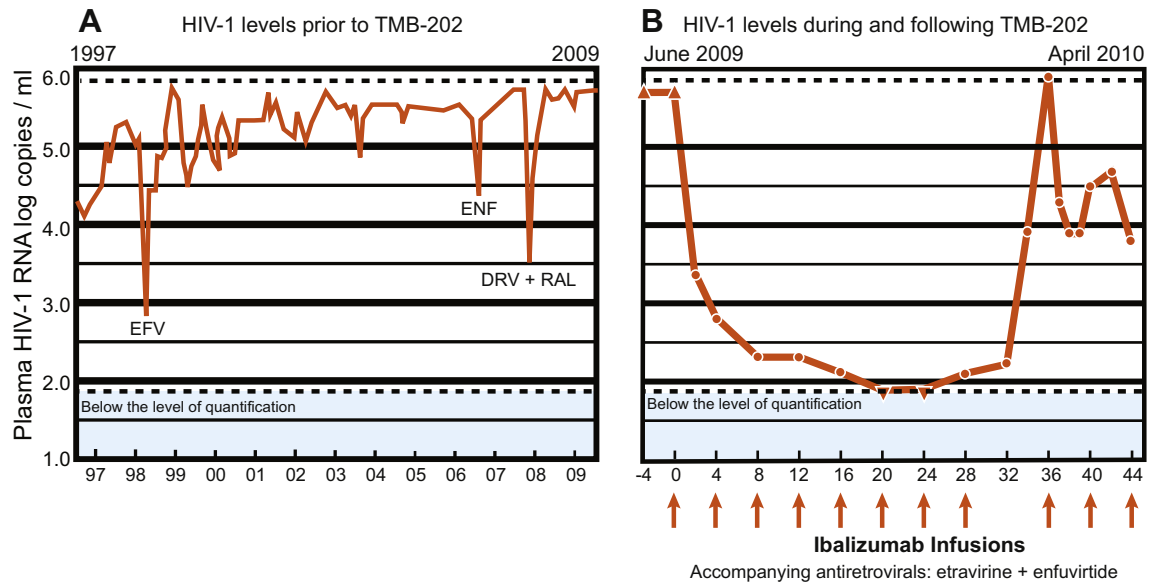


Fig. 1. The patient's plasma HIV-1 RNA levels (log copies/ml) during the 13 years prior to enrollment into TMB-202 (A, left panel) and during the 44 weeks following enrollment into TMB-202 (B, right panel). The mAb ibalizumab was administered as part of a dose-response trial for 24 weeks and open-label thereafter. The orange arrows indicate monthly intravenous infusions of 2000 mg of ibalizumab. In December 2009, it was discovered that the patient had been randomized to receive ibalizumab. However, a drug labeling error led to receipt of placebo rather than ibalizumab at week 32. Between 1997 and 2009, the patient experienced three transient decreases in plasma HIV-1 RNA levels coincident with the administration of new ARV regimens containing efavirenz (EFV, 1998), enfuvirtide (2006), and darunavir plus raltegravir (DRV + RAL, 2007). The ▲ symbol indicates plasma HIV-1 RNA levels above the limit of quantification (500,000 copies/ml). The ▼ symbol indicates plasma HIV-1 RNA levels below the limit of quantification (75 copies/ml). The week 36 plasma level measurement was performed on a diluted sample and determined to be approximately 5.0 million copies/ml. The results of six-class phenotypic susceptibility testing prior to study enrollment (June 2009) and following virologic rebound (March 2010) are shown in Table 1.

Table 1

PhenoSense susceptibility results for each of the six antiretroviral drug classes prior to the start of ibalizumab and following virologic rebound after a missed ibalizumab infusion.

Drug class	June 2009 (fold-resistant)	June 2009 interpretation	March 2010 (fold-resistant)	March 2010 interpretation	Clinical cutoffs
<i>Nucleoside RT inhibitors (NRTIs)</i>					
Abacavir	20	R	12	R	4.5–6.5
Didanosine	4.9	R	4.1	R	1.3–2.2
Emtricitabine	>200	R	8.7	R	>3.5
Lamivudine	>200	R	6.3	R	>3.5
Stavudine	>200	R	13	R	>1.7
Zidovudine	>200	R	444	R	>1.9
Tenofovir	8.4	R	11	R	1.4–4.0
<i>Non-nucleoside RT inhibitors (NNRTIs)</i>					
Efavirenz	5.9	R	>200	R	>3.0
Etravirine	0.7	S	5.7	I	2.9–10
Nevirapine	72	R	>200	R	>4.5
<i>Protease inhibitors (PIs)</i>					
Atazanavir	107	R	58	R	>5.2
Darunavir	151	R	155	R	10–90
Fosamprenavir	143	R	>200	R	4–11
Indinavir	28	R	26	R	>10
Lopinavir	65	R	126	R	9–55
Nelfinavir	45	R	43	R	>3.0
Saquinavir	35	R	31	R	2.3–12
Tipranavir	12	R	9.8	R	2.0–8.0
<i>Integrase inhibitors</i>					
Raltegravir	>200	R	9.9	R	NA
<i>Fusion inhibitors</i>					
Enfuvirtide	2.1	S	104	R	>9.0
<i>CCR5 inhibitors</i>					
Maraviroc	R5+/X4+	R	R5+/X4+	R	R5+/X4+

Genotypic susceptibility data for the NRTIs, NNRTIs, and PIs can be found in Table 1 of the Supplementary materials. Integrase inhibitor susceptibility testing in 2008 and 2009 demonstrated four Raltegravir-resistance mutations: L74 M, T97A, Y143C, and S230R. Phenotypic susceptibility testing in 2007 revealed 152-fold decreased Enfuvirtide susceptibility. The presence of CXCR4 tropic virus (X4+) indicates intrinsic nonresponsiveness to Maraviroc and other CCR5 inhibitors.

Following completion of the blinded study in December 2009, the patient was identified as a monthly ibalizumab recipient. At week 32, the patient was inadvertently administered an infusion of placebo in place of ibalizumab. By week 36, the patient's plasma HIV-1 RNA had risen to >500,000 copies/ml by standard testing and was estimated at approximately 5 million plasma HIV-1 RNA copies/ml based on viral load testing of a diluted plasma sample. A repeat phenotypic susceptibility test revealed the emergence of a variant that displayed 6-fold and 40-fold resistance to etravirine and enfuvirtide, respectively (Table 1). Reinstitution of ibalizumab resulted in an approximately 2.7 log₁₀ reduction in plasma HIV-1 RNA levels within 2 weeks, but further reductions in virus levels were not observed. Despite the re-administration of various additional ARVs in combination with ibalizumab, enfuvirtide, and etravirine, the patient's plasma HIV-1 RNA levels have persisted at approximately 4.0 log₁₀ copies/ml through February 2011.

Ibalizumab susceptibility testing was performed prior to the start of TMB-202 and 7 months after the reinstatement of ibalizumab following the unintended week 32 interruption (Fig. 2). The dose–response curve of the June 2009 virus exhibited a classic sigmoidal shape with a maximum percent inhibition (MPI) approaching 100% and an IC₅₀ value within the normal range for ibalizumab treatment-naïve viruses. In contrast, the dose response curve of the October 2010 virus indicated that only 50% of virus infection was susceptible to ibalizumab inhibition (MPI = 50%). No significant differences in the IC_{half max} values were noted (defined as the concentration of drug required to inhibit 50% of the MPI). Sequencing the HIV-1 envelope *gp120* region from the June 2009, July 2010, and October 2010 viruses demonstrated the acquisition of a T (Thr) to I (Ile) mutation in the July 2010 isolate and a T (Thr) to I (Ile) or L (Leu) mutation in the October 2010 isolate which resulted in the disruption of a potential N-linked glycosylation site (N-X-S/T-X (PNGS)) in the HIV-1 envelope V5 loop (Supplementary Fig. 1). Informed consent was obtained for the clinical trial and human subjects' approval was obtained for the additional tests performed for this study.

Two humanized mAbs targeting host receptors are in phase II clinical development: ibalizumab binds domain 2 of the CD4 receptor and PRO140 (Progenics Pharmaceuticals, Tarrytown, NY) attaches to the ligand-binding site of the CCR5 coreceptor (Huber

et al., 2008). Ibalizumab binding does not inhibit HIV-1 gp120 attachment to CD4 domain 1, but rather inhibits a post-attachment step required for cell entry (Burkly et al., 1992; Freeman et al., 2010; Moore et al., 1992; Song et al., 2010). In contrast to mAbs that bind CD4 domain 1, ibalizumab does not deplete CD4+ lymphocytes or interfere with MHC Class II immune function (Boon et al., 2002; Jacobson et al., 2009; Kuritzkes et al., 2004). Both PRO140 and ibalizumab are potentially amenable to subcutaneous administration (Jacobson et al., 2009, 2010) and contain an IgG4 Fc domain that does not trigger antibody- and complement-dependent cytotoxicity (Burkly et al., 1992; Jacobson et al., 2010; Reimann et al., 1997).

This case report provides insight into the antiretroviral potency of ibalizumab as illustrated by the initial response to therapy (~4.0 log₁₀ reduction in viral load) in combination with etravirine and re-use of enfuvirtide, the rapid loss of that initial response following an inadvertently missed infusion, and the sustained stabilization of plasma HIV-1 RNA levels at ~2.0 log₁₀ copies/ml below pre-ibalizumab levels, despite notable reductions in susceptibility to ibalizumab and the optimized background ARVs. The magnitude of the virologic response reported in this study is greater than responses observed in earlier clinical trials of ibalizumab. This discrepancy may be explained by synergistic effects related to low CD4 counts in this patient coupled with the prevention of new infections of CD4+ lymphocytes by ibalizumab, or synergy between ibalizumab and the fusion inhibitor enfuvirtide, as previously reported (Zhang et al., 2006).

It is possible that the increased number of clinic visits during study period contributed to increased adherence and the resulting virological response. The patient, however, had high-levels of self-reported adherence before the 24 week study period (which was reflected by the history of transient virological responses to earlier salvage therapy regimens) and during the year following the study. Although the inadvertently missed infusion led to a rapid loss of virological response, the possibility that virological failure would have eventually occurred even without the missed infusion cannot be excluded. Indeed despite having plasma HIV-1 RNA levels below the level of detection at weeks 20 and 24, the plasma HIV-1 RNA level at week 28 was approximately 120 copies/ml. Nonetheless, the rapid loss of response to therapy and subsequent development

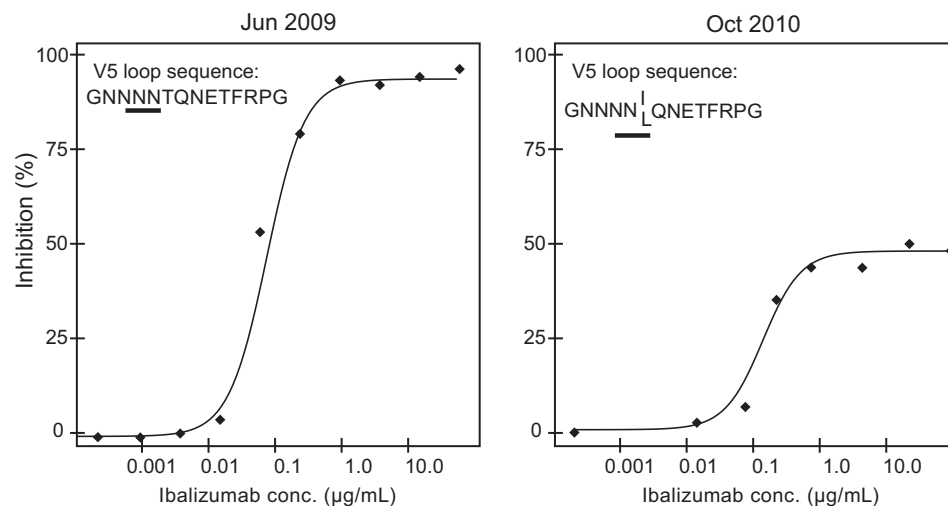


Fig. 2. Ibalizumab susceptibility prior to TMB-202 (June 2009) and 7 months following re-institution of ibalizumab following the week 32 unintended interruption (October 2010). The dose–response curve of the June 2009 virus exhibited a classic sigmoidal shape with a maximum percent inhibition (MPI) approaching 100% and an IC₅₀ value within the normal range for ibalizumab treatment-naïve viruses. In contrast, the dose response curve of the October 2010 virus indicated that only 50% of virus infection was susceptible to ibalizumab inhibition (MPI = 50%). Sequencing the HIV-1 envelope *gp120* region from the June 2009, July 2010, and October 2010 viruses demonstrated the acquisition of a T (Thr) to I (Ile) mutation in the July 2010 isolate and a T (Thr) to I (Ile) or L (Leu) mutation in the October 2010 isolate which resulted in the disruption of a potential N-linked glycosylation site (N-X-S/T-X (PNGS)) in the HIV-1 envelope V5 loop.

of resistance to all three components of the salvage therapy regimen underscores the potential grave consequences of missing a single dose of an infrequently administered potent ARV.

This case report represents the second description of clinical resistance to ibalizumab. Toma et al. recently elucidated a genotypic and phenotypic mechanism of ibalizumab resistance (Toma et al., 2011). First, they demonstrated that HIV-1 can acquire reductions in ibalizumab susceptibility via mutations that disrupt potential N-linked glycosylation sites (PNGS) in the V5 loop of HIV-1. Second, they demonstrated that reductions in susceptibility to ibalizumab manifest phenotypically as reductions in maximal percent inhibition of virus replication (MPI) at saturating drug concentrations – a characteristic shared with agents that bind to host cell targets and exhibit non-competitive, allosteric inhibition, such as CCR5 inhibitors (Westby et al., 2007) – vs. the resistant phenotypes of competitive inhibitors that manifest as increasing IC₅₀ values (rightward shifts of sigmoidal dose response curves that reach 100% inhibition at saturating drug concentrations). The post-ibalizumab treatment *gp120* sequences from July 2010 and October 2010 exhibited 8 and 13 amino acid differences, respectively, from the pre-ibalizumab treatment sequences from June 2009 (GenBank Accession Nos.: JF701703 to JF701706). Notably, one of the differences shared by both of the post treatment sequences resulted in the disruption of one of the previously described potential N-linked glycosylation sites in V5 associated with reductions in ibalizumab susceptibility (Toma et al., 2011).

Given the availability of six drug classes of small molecule medications in most industrialized countries it is not surprising that mAbs have not contributed to our current antiretroviral armamentarium. Manufacturing of biologic therapeutics (e.g. mAb) is expensive compared to chemical agents. Consequently, small molecule inhibitors of virus and host cell targets have been the focus of antiretroviral drug development from the onset. This is best illustrated by the fact that the murine precursor of ibalizumab (5A8) was identified 17 years prior to the use of the humanized mAb for deep salvage therapy in the patient we describe in this report (Burkly et al., 1992). However, mAbs may fill several important niches in HIV-1 treatment and prevention. In addition to an advanced-stage treatment option, therapeutic mAbs may be used for pre-exposure prophylaxis in high-risk uninfected populations. Moreover, if additional synergistic long acting antiretroviral agents are approved, it may become possible to create effective long-acting regimens that could be administered no more than once weekly. Such regimens would make it possible to treat nonadherent patient populations with directly observed therapy (DOT).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.antiviral.2011.09.010.

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