

Research Letters

AIDS 2008, 22:307–313

HIV dynamics and immunosenescence

Wayne M. Getz

Three differential equations modelling HIV virion, and infected and uninfected immune-cell densities during acute and chronic phases of infection underlie in-vivo HIV viral dynamics models. Adding a fourth equation that simulates immunosenescence through the drawdown of a stem-cell reservoir permits the phase of full-blown AIDS to be incorporated in the model. This greatly enhances the utility of the model in designing interventions and explains why time-to-full-blown AIDS decreases with age at infection.

A simple but effective in-vivo virion/immune-cell model underlying analyses of the progression and treatment of HIV in infected individuals is based on a system of three ordinary differential equations describing the dynamics of uninfected immune system cells (x), infected immune system cells (y), and free virions (v) [1,2]. The model is based on immune cells (often interpreted as CD4 cells) that are produced at a constant rate but die at a rate proportional to the density of these cells in the blood. On infection of one of these immune cells by an HIV virion, the model assumes that the infected cell will die at a characteristic rate releasing a specified 'burst-size' number of new virions. These virions then go on to infect uninfected immune cells at a rate proportional to the densities of virions and uninfected immune cells in the blood. With an appropriate set of rate and burst-size parameters, this model is able to capture virion and cell densities during the initial acute and later chronic stages of the infection (Fig. 1a). With the addition of a fourth differential equation that, as described below, can be interpreted as modelling the drawdown of a reservoir of stem cells, also interpreted as a process of immunosenescence [3], in which the size of the reservoir is set either by a tolerance to an accumulation of genetic errors [4] or the loss of telomere function [5], the final full-blown AIDS stage of the infection can also be captured by the model.

In particular, in the three differential equations that underlie many current in-vivo HIV immune cell models, uninfected cells are produced at a constant rate λ , infected cells at a rate βxv (transmission process with rate parameter β), and virions at a rate ky , whereas δx , ay , and uv are, respectively, the rates at which uninfected cells, infected cells and virions are removed from the system (death processes): k/a represents the burst size. This basic model and its elaborations do not account for immune

cell production limitations caused by accumulated genetic errors [4] and the loss of telomere function [5] that ultimately results in immune senescence [3]. These factors can be modelled by assuming a finite reservoir of hematopoietic stem cells [6] represented by the variable $z(t)$ that decreases steadily with time t . A simple elaboration to the basic model that accounts for this is:

$$\begin{aligned}\frac{dx}{dt} &= \lambda(x, z) - \delta x - \beta xv, & \frac{dy}{dt} &= \beta xv - ay, \\ \frac{dv}{dt} &= ky - u(z)v, & \frac{dz}{dt} &= -c\lambda(x, z).\end{aligned}$$

The basic model [1,2] is the first three equations with constant λ and u . The additional reservoir equation implies a drawdown rate c per uninfected cell production unit λ that we now assume depends on the size of the stem-cell reservoir $z(t)$ and the level $x(t)$ of uninfected cells. We will also assume now that the virion clearance rate u depends on $z(t)$. These dependencies can be phenomenologically described as follows. First, we expect $\lambda(x, z)$ to be a decreasing function of x because production should increase when uninfected cell counts are low. Initially, for non-depleted values of z , there should be little effect on either the maximum rates λ_0 or u_0 until the reservoir $z(t)$ approaches exhaustion, at which point $\lambda(x, z)$ reduces to 0 and $u(z)$ to its background rate γu_0 ($0 < \gamma < 1$ to account for the loss of macrophage activity in removing virions). The extent to which z must be reduced for these rates to be at half their maximum values depends on many factors. Simple forms appropriate to the resolution of the model are:

$$\begin{aligned}\lambda(x, z) &= \frac{\lambda_0(x)z}{b_\lambda + z} \text{ and } u(z) = \frac{u_0(\gamma b_u + z)}{b_u + z}, \\ \text{where } \lambda_0(x) &= \frac{\bar{\lambda}_0}{1 + (x/b_x)}\end{aligned}$$

accounts for increasing production rates as the uninfected cell population drops.

This extended model, in addition to replicating the acute and chronic infection stages of a typical HIV infection profile, captures the senescing immune system state at the onset of full-blown AIDS (Fig. 1) [5]. The parameters used in Figure 1 are illustrative, because considerable variability exists among individuals in the intensity of infection at different stages of infection and in the time from infection to the onset of full-blown AIDS; the latter, for example, is known to decrease with age, with estimates [7,8] indicating a mean of 14–15 years at approximately the age of 10 years to about 8 years at

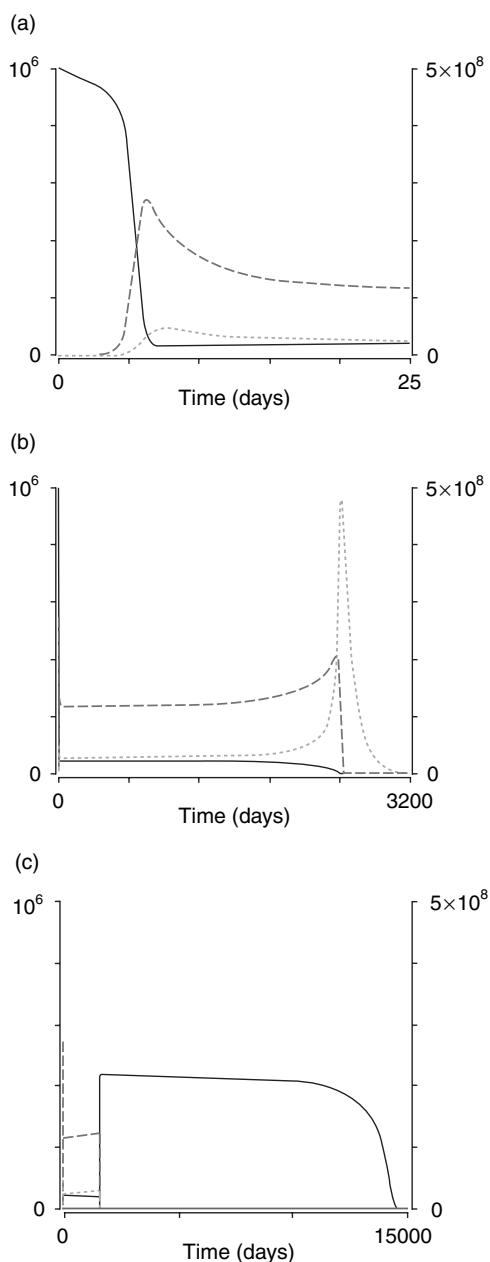


Fig. 1. Solution to the model for parameter values $\bar{\lambda}_0 = 2 \times 10^6$, $\delta = 0.02$, $a = 0.5$, $k = 100$, $u_0 = 1$, $\beta = 4 \times 10^{-8}$ (cf. 1) and initial conditions $x(0) = 10^6$, $y(0) = 0$, and $v(0) = 100$. Trajectories (numbers per millilitre of blood): black lines (—) are healthy cells (x , left axis), dashed lines (---) are infected cells (y , left axis), and dotted lines (----) are virions (v , right axis). (a) A 2-week transient spike relaxes in the presence of an inexhaustible stem cell reservoir (infinite z) to the set point equilibrium $x = 1.1 \times 10^4$, $y = 2.9 \times 10^5$, and $v = 3.2 \times 10^7$. (b) For a finite stem-cell reservoir, initial condition $z(0) = 1000$ (scale depends on units) and parameter values $c = 2.4 \times 10^{-6}$, $b_\lambda = 20$, $b_x = 10\,000$, $b_u = 100$ and $\gamma = 1/100$, the immune system collapses in uninfected individual ($v(0) = 0$, solution not shown) in approximately 72 years for the given initial condition.

approximately the age of 50 years. This decrease is expected under the assumption of a continuously drawdown hematopoietic stem-cell reservoir, because the reservoir equation implies $z(0)$ decreases with the individual's age at the start of infection. It is also expected that the progression rate to full-blown AIDS is increased by factors that draw down the reservoir, such as previous parasite loads, chronic disease, and co-infections [3].

The first three equations in the four equation model presented here are too simple to capture many subtle phenomena associated with the acute and chronic phases of HIV infection, such as the persistence of low levels of plasma virus during potent antiretroviral therapy or the accumulation of mutations in immune cells over time. Multicell-type models employing additional differential equations are needed to investigate such phenomena [4,9]. Despite numerous elaborations to acute/chronic phase models represented by the first three equations, however, a basic model that captures all three in-vivo phases of HIV/immune cell dynamics (acute, chronic, collapse) using a single set of relatively simple differential equations has yet to be proposed. The four equation model presented here fulfils this role, although it will also need to be elaborated when used to address questions regarding the most effective design of HAART regimens or issues relating to the emergence of strains resistant to such interventions [9–11]. In addition, the fourth equation stresses our need to investigate and characterize more fully the exhaustibility of an individual's immunological system, particularly with regard to assessing its state as a hematopoietic stem-cell reservoir as a result of changes in lymphatic organs and bone marrow structure with age and history of infections. Finally, if immune system senescence is caused by the kind of extractive process modelled here, the results presented in Figure 1c have a profound bearing on the appropriate time to start antiretroviral treatment in HIV-positive individuals, particularly in individuals with co-infections such as *Mycobacterium tuberculosis*.

Fig. 1. (Continued).

The solution indicates that this initial condition corresponds to a partly depleted system, given the relatively fast progression to full-blown AIDS approximately 2600 days, after which the viral count rises by an order of magnitude before collapsing to 0 when the individual dies from a collapsed immune system somewhere between 3000 and 3500 days. (c) For the above parameter values, the application of an antiretroviral treatment at day 1500, if it has the effect of reducing k by 97% (i.e. $k = 3$, $t > 1500$), the infection is effectively eliminated, but the immune system still collapses at approximately day 16000. If treatment is delayed 500 days (starts day 2000), the immune system fails at approximately day 12 200 (not shown), reducing the individual's effective life by more than 10 years.

Acknowledgements

This work was funded by a Grant from NIH. I would like to thank John Hargrove, Brian Williams, Travis Porco, Maria Sanchez, and Russell Vance for comments that have improved this Research Letter.

Department of Environmental Science, Policy and Management, University of California, Berkeley, California, USA.

Received: 14 August 2007; revised: 24 October 2007; accepted: 31 October 2007.

References

- Herz AVM, Bonhoeffer S, Anderson RM, May RM, Nowak MA. **Viral dynamics in vivo: limitations on estimates on intracellular delay and virus decay.** *Proc Natl Acad Sci USA* 1995; **93**:7247–7251.
- Nowak MA, May RM. **Virus dynamics: mathematical principles of immunology and virology.** Oxford, UK: Oxford University Press; 2000.
- Appay V, Almeida JR, Sauce D, Autran B, Papagno L. **Accelerated immune senescence and HIV-1 infection.** *Exp Gerontol* 2007; **42**:432–437.
- Galvani AP. **The role of mutation accumulation in HIV progression.** *Proc Royal Soc B* 2005; **272**:1851–1858.
- Harrington L. **Does the reservoir for self-renewal stem from the ends?** *Oncogene* 2004; **23**:7283–7289.
- Scadden DT. **Stem cells and immune reconstitution in AIDS.** *Blood Rev* 2003; **17**:227–231.
- Belanger F, Meyer L, Carré N, Coutellier A, Deveau C, The SeroCo Study Group. **Influence of age at infectivity on human immunodeficiency virus disease progression to different clinical endpoints: The SEROCO cohort (1988–1994).** *Int J Epidemiol* 1997; **26**:1340–1345.
- Williams BG, Granich R, Chauhan LS, Dharmshaktu NS, Dye C. **The impact of HIV/AIDS on the control of tuberculosis in India.** *Proc Natl Acad Sci USA* 2005; **102**:9619–9624.
- Kim H, Perelson AS. **Viral and latent reservoir persistence in HIV-1-infected patients on therapy.** *PLoS Comput Biol* 2006; **2**:1232–1247; e135 DOI10.1371/journal.pcbi.0020135.
- Bonhoeffer S, May RM, Shaw GM, Nowak MA. **Virus dynamics and drug therapy.** *Proc Natl Acad Sci USA* 1997; **94**:6971–6976.
- Ramkissoon S. Modelling the interaction between human immunodeficiency virus, *Mycobacterium tuberculosis*, and the human immune system, including the effects of drug therapy. MSc dissertation. School of Physics, University of KwaZulu-Natal: Kwa-Zulu, Natal, South Africa; February 2007.

Combination antiretroviral therapy failure and HIV super-infection

Daniela Bezemer^a, Ard van Sighem^a, Frank de Wolf^{a,b}, Marion Cornelissen^c, Antoinette C. van der Kuyl^c, Suzanne Jurriaans^c, Lia van der Hoek^c, Maria Prins^{d,e}, Roel A. Coutinho^{e,f} and Vladimir V. Lukashov^c

In addition to development or selection of resistance, failure to continuously suppress HIV-1 production while still using initially effective combination antiretroviral therapy (cART) may result from super-infection with a drug-resistant

strain. Both transmission of drug resistant HIV and super-infection have been demonstrated. We analysed HIV *pol* genes obtained before start of initially successful cART and during failure while still on cART in 101 patients. Difference in precART and cART failure sequences were explained by evolution and not by super-infection.

A considerable proportion of new HIV-1 infections are caused by viruses carrying antiretroviral drug-resistant mutations [1–3]. In The Netherlands, 6% of recent infections were found to contain mutations associated with resistance [4]. Although rare, super-infection with another and even drug resistant HIV-1 strain has been demonstrated [5–13]. Given the popularity of serosorting amongst HIV-1-infected males having sex with males (MSM) [14–20], we therefore investigated to what extent super-infection of initially successfully cART-treated individuals could explain treatment failure.

We analyzed HIV-1 *pol* sequences obtained from patients before starting cART and during failure, while still on cART. The clinical, virological and immunological data of patients were collected within the framework of the ATHENA national observational cohort [21]. Failure was defined by a detectable HIV-1 RNA load while on cART, after at least one successfully suppressed plasma sample taken following start of cART. In total, 9390 patients started cART and experienced initial success. There were 22 395 person-years of follow-up from the first successful load measurement until the earliest of cART failure and the last available load measurement, with a rate of failure of 0.25 per person-year. Three thousand seven hundred and twelve patients with a median follow-up of 2.5 years since the first suppressed measurement [interquartile range (IQR) = 0.8–5.3 years] did not fail cART. Five thousand six hundred and seventy-eight (60%) failed within a median of 0.9 years (IQR = 0.3–2.2 years). Of these patients, 32% were pretreated before start of cART, which was significantly larger compared to the group of patients that did not fail (13%, $P < 0.001$). HIV-1 polymerase gene (*pol*) sequences, both before starting cART and during virological failure while still on cART, were available for 101 patients older than 16 years. Population-based nucleotide sequencing of the HIV-1 *pol* gene was performed as described previously [4], and sample contamination was checked for at the respective sequencing sites. Multiple sequence alignment was performed using the default parameters of ClustalX, release 1.83. From 55 patients, more than two sequences were available, which allowed for analysis of 338 sequences in total. The median percentage of ambiguous sites among the total 338 sequences was 0.6% (IQR = 0.2–1.0%). Pairwise sequence distances were calculated taking into account ambiguous sites by the mixed weighted distance method, as previously described by Gonzales *et al.* [12]. Phylogenetic analysis was performed using the MEGA program, Neighbour-joining model with

Table 1. Median pairwise nucleotide sequence distances, according to the mixed weighted distance method as described in Gonzales *et al.* [12].

Median percentage pairwise nucleotide sequence distance between	At synonymous sites	At nonsynonymous sites	Total distance
All subtype B sequences obtained from the first sample taken during cART failure at PR	13.2 (IQR = 10.1–18.6)	4.7 (IQR = 3.5–6.0)	6.7 (IQR = 5.4–8.6)
All subtype B sequences obtained from the first sample taken during cART failure at RT	17.4 (IQR = 14.7–20.8)	3.2 (IQR = 2.5–3.9)	6.0 (IQR = 5.2–7.1)
The last sample taken before cART and the first sample taken during failure at PR	2.2 (range = 0–13.7)	0.9 (range = 0.0–5.7)	1.2 (range = 0.0–6.1)
The last sample taken before cART and the first sample taken during failure at RT	3.6 (range = 0.0–11.0)	0.8 (range = 0.0–3.2)	1.4 (range = 0.0–4.7)
The last sample taken before cART and the first sample taken during failure in rates per year at PR	0.8 (range = 0–18.4)	0.3 (range = 0.0–9.2)	0.4 (range = 0.0–11.3)
The last sample taken before cART and the first sample taken during failure in rates per year at RT	1.3 (range = 0.0–15.3)	0.3 (range = 0.0–1.8)	0.5 (range = 0.0–4.6)
Intra-patient sequence pairs PR (<i>n</i> = 1094)	2.1 (range = 0.0–13.7)	1.1 (range = 0.0–6.6)	1.5 (range = 0.0–6.6)
Intra-patient sequence pairs RT (<i>n</i> = 1040)	2.9 (range = 0.0–11.3)	0.8 (range = 0.0–3.4)	1.3 (range = 0.0–4.7)

cART, Combination antiretroviral therapy; IQR, interquartile range; PR, protease; RT, reverse transcriptase.

Kimura two-parameter distances and bootstrap analysis (1000 replications), ignoring the ambiguous sites. Both the protease (PR) and reverse transcriptase (RT) region could be included in the analysis for 98 patients, and, either PR or RT was available for three patients. Therefore, two phylogenetic analyses were performed: one for the sequence set sharing at least RT, the other for sequences sharing at least PR. Bootstrap values higher than 80 were considered to be significant. Sequences were screened for resistance-conferring mutations at the amino acid positions described by the International AIDS Society (USA) [22], these sites were not ignored in the analysis.

The study group included 85 men and 16 women. Transmission risk groups were MSM (*n* = 68), heterosexual transmission (*n* = 21), injecting drug use (IDUs, *n* = 6), and blood transfusion (*n* = 2). For four patients the route of transmission was unknown. Median CD4 cell count at cART initiation was 200×10^6 cells/ μ l (IQR = 70–310 cells/ μ l). Median time between the start of cART and viral suppression was 4.1 months (IQR = 1.5–7.1 months). Initial viral suppression lasted 4.9 months (IQR = 2.5–9.6 months). Median time between the start of failure while still on cART and the first blood sample used for HIV isolation and sequencing was 3.3 months (IQR = 0.2–20.9 months). Half of the patients (*n* = 51) were antiretroviral treatment naïve at the start of cART, of whom 23% (*n* = 11) presented with resistance-conferring mutations before cART. The other half of the patients (*n* = 50) had experienced antiretroviral treatment before, and 72% (*n* = 36) presented with drug-resistant mutations before start of cART. Sequences obtained after initial cART failure showed in 83 (81%) of 101 patients drug-resistant mutations and in all sequences obtained thereafter.

Phylogenetic analysis showed 85 patients being infected with HIV-1 subtype B viruses. For 101 patients, the sequences obtained before start and during failure of

cART clustered together with bootstrap values above 90. Two pairs of patients had sequences that clustered together with bootstrap values of 99 but, within those clusters, the sequence clusters from the respective patients did not intermix.

Table 1 shows median pairwise nucleotide sequence distances, which were significantly smaller at intra- than at inter-patient level ($P < 0.001$). The highest absolute distances between the last sample taken before cART and the first sample taken during failure become smaller after correction for time between sequences, whereas the distance corresponding to the shortest time interval (0.4 years) became highest when extrapolated to a yearly rate. Positive selection between the last sample taken before cART and the first sample taken during failure at PR (nonsynonymous mutations per nonsynonymous site/synonymous mutations per synonymous site > 1) was found in 14 patients, 12 at PR and two at RT. Those intra-patient sequence pairs with a nucleotide distance $> 4.5\%$ in PR (seven patients) or $> 3.0\%$ in RT (15 patients) revealed no signs of recombination with a different strain at the amino acid level because the distance was only due to single (several ambiguous) substitutions, many at known resistance conferring sites.

In conclusion, in this selected subgroup of patients who experienced virological failure while still on initially successful cART, no evidence for super-infection with resistant HIV-1 was observed. Transmission risk behaviour around cART failure was reported in this small study group. Three IDUs reported risk behaviour between the cART start date and the date of cART failure: injecting drugs in two, one including needle sharing, and unprotected sex with a steady HIV-1 positive partner in the third. Four MSM reported unprotected anal sex between the date of starting cART and that of virological failure. When HIV is transmitted from a donor in a tight and limited transmission network (e.g. the originally infecting or infected partner),

detecting super-infection is almost impossible. Different treatment regimens for sero-concordant couples might be protective, but substantial cross-resistance between drugs should be considered [22].

^aHIV Monitoring Foundation, Academic Medical Centre, University of Amsterdam, the Netherlands;

^bDepartment of Infectious Disease Epidemiology, Imperial College London, UK; ^cLaboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands; ^dDepartment of Internal Medicine, Academic Medical Center, University of Amsterdam, the Netherlands; ^eDepartment of Infectious Diseases, Health Service Amsterdam, the Netherlands; and ^fCenter for Infectious Disease Control, National Institute of Public Health and the Environment, Bilthoven, the Netherlands.

Sponsorship: DB was supported by grant 7014 from AIDS Fund Netherlands.

Received: 11 October 2006; accepted: 17 October 2007.

References

- de Ronde A, van Dooren M, van der Hoek L, Bouwhuis D, de Rooij E, van Gemen B, et al. **Establishment of new transmissible and drug-sensitive human immunodeficiency virus type 1 wild types due to transmission of nucleoside analogue-resistant virus.** *J Virol* 2001; **75**:595–602.
- Lukashov VV, de Ronde A, de Jong JJ, Goudsmit J. **Epidemiology of HIV-1 and emerging problems.** *Int J Antimicrob Agents* 2000; **16**:463–466.
- Vella S, Palmisano L. **The global status of resistance to antiretroviral drugs.** *Clin Infect Dis* 2005; **41** (Suppl 4):S239–S246.
- Bezemer D, Jurriaans S, Prins M, van der Hoek L, Prins JM, de Wolf F, et al. **Declining trend in transmission of drug-resistant HIV-1 in Amsterdam.** *AIDS* 2004; **18**:1571–1577.
- van der Kuyl AC, Kozaczynska K, van den Burg R, Zorgdrager F, Back N, Jurriaans S, et al. **Triple HIV-1 infection.** *N Engl J Med* 2005; **352**:2557–2559.
- Cornelissen M, Jurriaans S, Kozaczynska K, Prins JM, Hamidjaja RA, Zorgdrager F, et al. **Routine HIV-1 genotyping as a tool to identify dual infections.** *AIDS* 2007; **21**:807–811.
- Allen TM, Altfield M. **HIV-1 superinfection.** *J Allergy Clin Immunol* 2003; **112**:829–835.
- Pernas M, Casado C, Fuentes R, Perez-Elias MJ, Lopez-Galindez C. **A dual superinfection and recombination within HIV-1 subtype B 12 years after primoinfection.** *J Acquir Immune Defic Syndr* 2006; **42**:12–18.
- Smith DM, Wong JK, Hightower GK, Ignacio CC, Koelsch KK, Petropoulos CJ, et al. **HIV drug resistance acquired through superinfection.** *AIDS* 2005; **19**:1251–1256.
- Brenner B, Routy J, Quan YD, Moisi D, Oliveira M, Turner D, et al. **Persistence of multidrug-resistant HIV-1 in primary infection leading to superinfection.** *AIDS* 2004; **18**:1653–1660.
- Diaz RS, Pardini R, Catroxo M, Operskalski EA, Mosley JW, Busch MP. **HIV-1 superinfection is not a common event.** *J Clin Virol* 2005; **33**:328–330.
- Gonzales MJ, Delwart E, Rhee SY, Tsui R, Zolopa AR, Taylor J, et al. **Lack of detectable human immunodeficiency virus type 1 superinfection during 1072 person-years of observation.** *J Infect Dis* 2003; **188**:397–405.
- Fultz PN. **HIV-1 superinfections: omens for vaccine efficacy?** *AIDS* 2004; **18**:115–119.
- Truong HHM, Kellogg T, Klausner JD, Katz MH, Dilley J, Knapper K, et al. **Increases in sexually transmitted infections and sexual risk behaviour without a concurrent increase in HIV incidence among men who have sex with men in San Francisco: a suggestion of HIV serosorting?** *Sex Transm Infect* 2006; **82**:461–466.
- Stolte IG, Dukers NHTM, Geskus RB, Coutinho RA, De Wit JBR. **Homosexual men change to risky sex when perceiving less threat of HIV/AIDS since availability of highly active antiretroviral therapy: a longitudinal study.** *AIDS* 2004; **18**:303–309.
- Stolte IG, Dukers NH, de Wit JB, Fennema JS, Coutinho RA. **Increase in sexually transmitted infections among homosexual men in Amsterdam in relation to HAART.** *Sex Transm Infect* 2001; **77**:184–186.
- Cox J, Beauchemin J, Allard R. **HIV status of sexual partners is more important than antiretroviral treatment related perceptions for risk taking by HIV positive MSM in Montreal, Canada.** *Sex Transm Infect* 2004; **80**:518–523.
- Elford J. **Changing patterns of sexual behaviour in the era of highly active antiretroviral therapy.** *Curr Opin Infect Dis* 2006; **19**:26–32.
- Stolte IG, De Wit JBF, van Eeden A, Coutinho RA, Dukers NHTM. **Perceived viral load, but not actual HIV-1-RNA load, is associated with sexual risk behaviour among HIV infected homosexual men.** *AIDS* 2004; **18**:1943–1949.
- van der Bij AK, Kolader ME, de Vries HJ, Prins M, Coutinho RA, Dukers NH. **Condom use rather than serosorting explains differences in HIV incidence among men who have sex with men.** *J Acquir Immune Defic Syndr* 2007; **45**:574–580.
- van Sighem AI, van de Wiel MA, Ghani AC, Jambroes M, Reiss P, Gyssens IC, et al. **Mortality and progression to AIDS after starting highly active antiretroviral therapy.** *AIDS* 2003; **17**:2227–2236.
- Johnson VA, Brun-Vezinet F, Clotet B, Kuritzkes DR, Pillay D, Schapiro JM, et al. **Update of the drug resistance mutations in HIV-1: Fall 2006.** *Top HIV Med* 2006; **14**:125–130.

Prevalence and impact of HIV-1 protease mutation L76V on lopinavir resistance

Carmen de Mendoza, Carolina Garrido, Angélica Corral, Natalia Zahonero and Vincent Soriano

Besides I47A, mutation L76V at the HIV protease gene has recently been proposed to cause lopinavir resistance. This change was present in 37 (2.7%) out of 1376 patients failing protease inhibitor containing regimens. Although 26 (70%) were on lopinavir, most had previously failed other protease inhibitors and carried multiple protease inhibitor resistance mutations. Therefore, L76V does not appear to be a primary lopinavir resistance change when the drug is used in combination therapy.

Virological failure in drug-naïve HIV-1-infected patients treated with lopinavir/ritonavir-based regimens has rarely been associated with selection of resistance mutations at the protease gene, and mainly in individuals with low drug compliance [1]. Selection of the protease change I47A has been the most frequent mutation found in this situation [1,2], although other substitutions, including V32I, have also been associated with high-level lopinavir resistance in prior drug-naïve individuals [1,2]. Mutation I47A may cause a more than 100-fold loss of susceptibility to lopinavir [2,3].

Interestingly, HIV-2 may be prone to select for 47A because this virus only requires a single nucleotide substitution for a shift to arginine (GCA) at this position. Although HIV-2 naturally has a valine (GTA) at codon 47 [4], the wild-type HIV-1 has isoleucine at this position encoded by AUA, and therefore two nucleotide substitutions are required for the occurrence of I47A.

At the last Drug Resistance Workshop held in Barbados in June 2007, Nikhuis *et al.* [5] reported three HIV-1 drug-naïve individuals who failed a first-line regimen including lopinavir/ritonavir, all of whom selected a L76V change at the viral protease that apparently was responsible for lopinavir resistance. In all instances, L76V was accompanied by the M46I substitution. Site-directed mutagenesis experiments demonstrated that the single L76V mutation significantly reduced lopinavir susceptibility (by 12-fold on average), although with a relatively high cost in diminished replication capacity, which was compensated by the accumulation of M46I that did not confer any lopinavir resistance on its own [5]. Interestingly, L76V caused cross-resistance to amprenavir (five-fold loss of susceptibility) and, conversely, produced hypersusceptibility to atazanavir, saquinavir and tipranavir.

Given that lopinavir/ritonavir is one of the most widely used protease inhibitors [6], we examined the prevalence of mutation L76V in a relatively large clinical database of resistance genotypes derived from antiretroviral-experienced patients in Spain. From a total of 4457 genotypes from HIV-1-infected individuals tested since January 1999 to June 2007, 1376 belonged to patients failing protease inhibitors. Overall, 37 patients harboured viruses with the protease change L76V (prevalence of 2.7%). Of note, 26 of them (70%) were taken lopinavir/ritonavir at the time of failure, whereas the remainder were under other distinct protease inhibitors. Focusing exclusively on genotypes belonging to patients failing lopinavir/ritonavir, L76V was found in 26 out of 510 specimens (5.1%). Figure 1 shows the prevalence of mutations so far reported to be associated with significant lopinavir

resistance [7]. Interestingly, the only change that was significantly more prevalent in patients failing lopinavir compared to patients failing other protease inhibitors was L76V (5.1% versus 2.7%, $P < 0.01$).

It is noteworthy that 33 out of the 37 patients harboring L76V viruses had codon 46 changes (46I in thirty and 46L in three), which are well known protease inhibitor resistance substitutions [8]. However, other primary resistance mutations at the protease were seen in almost all these patients, including I54L/M/V ($n = 27$), L90M ($n = 22$), V82A/F/S/T ($n = 17$), I84V ($n = 15$) and I47V ($n = 1$). Moreover, all patients with L76V viruses had previously failed other protease inhibitors before the current failure on lopinavir. When the estimated impact of L76V on lopinavir resistance was assessed using the Stanford drug resistance rules in our series of patients, the presence of L76V only contributed to slightly increase lopinavir resistance because the accompanying mutations already accounted for intermediate or high-level resistance to the drug in all cases. It should be noted that all patients with L76V identified in our database were infected by HIV-1 subtype B strains, despite 9% of the whole database study population carrying non-B subtypes.

None of the subjects carrying L76V viruses harboured I47A, which was present in only four patients in the whole study population, suggesting that I47A and L76V may represent divergent pathways for lopinavir resistance, a hypothesis which is supported by structural models [3]. However, our results suggest that only I47A may be selected in the absence of other protease inhibitor resistance mutations and be responsible for clinically relevant lopinavir resistance. By contrast, selection of L76V generally occurs in patients who already have failed other protease inhibitors and have accumulated other resistance mutations at the protease.

Our study, however, should be interpreted with caution. In all studies conducted so far in which L76V has been recognised in patients failing lopinavir, the drug was taken as monotherapy, and this strategy has never been used in our institution. As the selection of resistance mutations may differ in patients under mono- or combination therapy, our results may only apply to those patients taking lopinavir along with at least another two antiretroviral drugs. It may be the case that the minimal impact on lopinavir susceptibility caused by L76V may explain its selection in those patients only treated with lopinavir monotherapy in whom low exposure to the drug may occur in some body compartments.

Overall, our results suggest that although drugs such as atazanavir and saquinavir may select for unique distinct protease resistance changes, mutations at codons 32, 47 and now L76V appear to be shared in the resistance pathways for fosamprenavir, lopinavir and darunavir

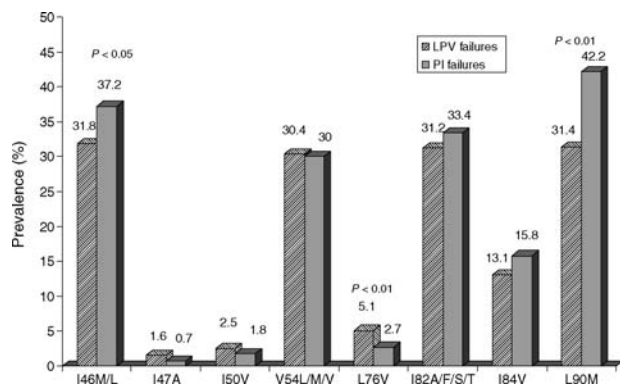


Fig. 1. Prevalence of mutations at the HIV-1 protease associated with lopinavir resistance.

[9–11]. Therefore, it may be worth exploring the best way to sequence protease inhibitors in order to minimise the impact of cross-resistance mutations within this family.

Acknowledgements

This work was supported in part by grants from Fundación Investigación y Educación en SIDA (IES), Red de Investigación en SIDA (RIS, ISCIII-RETIC RD06), Fondo de Investigaciones Sanitarias (FIS) Project CP06/00284 and Project PI06/1826 and Agencia Laín Entralgo.

Department of Infectious Diseases, Hospital Carlos III, Madrid, Spain.

Received: 26 June 2007; revised: 24 October 2007; accepted: 2 November 2007.

References

1. Friend J, Parkin N, Liegler T, Martin J, Deeks S. **Isolated lopinavir resistance after virological rebound of a ritonavir/lopinavir-based regimen.** *AIDS* 2004; **18**:1965–1966.
2. De Mendoza C, Valer L, Bachelier L, Pattery T, Corral A, Soriano V. **Prevalence of the HIV-1 protease mutation I47A in clinical practice and association with lopinavir resistance.** *AIDS* 2006; **20**:1071–1073.
3. Kagan R, Shenderovich M, Heseltine P, Ramnarayan K, Heseltine P. **Structural analysis of an HIV-1 protease I47A mutant resistant to the protease inhibitor lopinavir.** *Protein Sci* 2005; **14**:1870–1878.
4. Rodes B, Toro C, Sheldon J, Jimenez V, Mansinho K, Soriano V. **High rate of proV47A selection in HIV-2 patients failing lopinavir-based HAART.** *AIDS* 2006; **20**:127–129.
5. Nijhuis M, Wensing A-M, Bierman W, de Jong D, van Rooyen W, Kagan R, *et al.* **A novel genetic pathway involving L76V and M46I leading to lopinavir resistance.** *Antivir Ther* 2007; **12** (suppl):140.
6. Hammer S, Saag M, Schechter M, Montaner J, Schooley R, Jacobsen D, *et al.* **Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society – USA panel.** *JAMA* 2006; **296**:827–843.
7. Rhee S, Taylor J, Wadhwa G, Ben-Hur A, Brutlag D, Shafer R. **Genotypic predictors of HIV-1 drug resistance.** *Proc Natl Acad Sci USA* 2006; **103**:17355–17360.
8. Johnson V, Brun-Vezinet F, Clotet B, Gunthard H, Kuritzkes D, Pillay D, *et al.* **Update of the drug resistance mutations in HIV-1: Fall 2007.** *Top HIV Med* 2007; **15**:119–125.
9. Delaugerre C, Mathez D, Peytavin G, Berthé H, Long K, Galperine T, *et al.* **Key amprenavir resistance mutations counteract dramatic efficacy of darunavir in highly experienced patients.** *AIDS* 2007; **21**:1210–1213.
10. Kovalevsky A, Liu F, Leshchenko S, Ghosh A, Louis J, Harrison R, *et al.* **Ultra-high resolution crystal structure of HIV-1 protease mutant reveals two binding sites for clinical inhibitor TMC114.** *J Mol Biol* 2006; **363**:161–173.
11. Poveda E, De Mendoza C, Martin-Carbonero L, Corral A, Briz V, Soriano V. **Prevalence of darunavir-associated resistance mutations in HIV-infected patients mailing other protease inhibitors.** *J Antimicrob Agents* 2007; In press.