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**Spatial and dynamic model**

We developed a novel model of compartmentalized intra-host viral population dynamics that incorporates the impact of drug treatment[1-3], the evolution of drug resistance[4-9]; and a binary spatial structure[10,11]. The spatial and dynamic model of HIV-1 spread within patients takes into account the infection of CD4+ T-cells, deposition of free virus particles onto follicular dendritic cells, the movement of virus particles or cells between two compartments, and the impact of drug therapy. We assume differences in size of the two compartments – the main compartment ($i = 0$) and the sanctuary site ($i = 1$). A fraction ($u_i$) of the body is assumed to comprise of each compartment ($u_0 + u_1 = 1$), and the production rate of uninfected CD4+ T cells in the body ($\Lambda$) is apportioned between the main compartment ($\Lambda u_0$) and the sanctuary site ($\Lambda u_1$).

Viral strains are either drug-sensitive ($j = 0$) or drug-resistant ($j = 1$), and the free virus particles are either infectious ($k = 0$) or not ($k = 1$). Virus particles in compartment i with resistance strain $j$ and infectiousness $k$ ($V_{ijk}$) are cleared at rate $c$. In the absence of drug, the rate at which an uninfected cell in compartment $i$ becomes infected with strain $j$ is proportional to the density of infectious virus particles of that strain within its compartment ($V_{ij0}/u_i$) and scaled by the transmission coefficient $\beta$.

A fraction of the cells infected with HIV-1 become long-lived ($\theta$) with clearance rate $\mu_Y$ or short-lived ($1 - \theta$) with clearance rate $\mu_Q$. Long-lived and short-lived infected cells within compartment $i$ infected with resistance strain $j$ are denoted as $Y_{ij}$ and $Q_{ij}$, respectively. Long-lived infected cells become short-lived infected cells at rate $\gamma$. Cells infected with the drug-sensitive strain produce virus particles at rate $\rho$ per cell; drug

resistance imposes a fitness cost that reduces production by a factor $\pi$. A fraction $r$ of all new cell infections opposes the genotype (drug-resistant or drug-sensitive) of the infecting virus particle. We purposefully neglect the latent reservoir that might undergo proliferative renewal through homeostatic proliferation.

To distinguish the sanctuary site from the main compartment, we allow a difference in the effectiveness of the drugs. As we compare our model to measurements of productively infected cells, one drug class is assumed to reduce the infection of cells in compartment $i$ through contact with drug-sensitive virus particles by a factor $z_i$. Another drug class is modeled as making a fraction of virus particles $\tilde{z}_i$ produced by cells in compartment $i$ infected with the drug-sensitive strain noninfectious. For simplicity, we assume that the drug-resistant strain is resistant to both drug classes. Drug resistance reduces the effect of the first class of drug by a factor $w$ and the second class of drug by a factor $\tilde{w}$. The model accounts for the movement of cells and virus particles between compartments. The rate of movement from compartment $i$ to the other compartment is $\tau_i$ per cell or per virus particle. Accounting for the inequity of their sizes this parameter also differs between the main compartment and the sanctuary site ($\tau_0 u_0 = \tau_1 u_1$).

The relationship of the decay of virus in the follicular dendritic cell pool is complex compared to plasma viral RNA because of the changes of avidity of HIV-1 binding to complement receptors on the follicular dendritic cell network and the small fraction of viral RNA in blood as opposed to the lymphoid tissue reservoir. As virus is released from the follicular dendritic cell pool, the remaining virus binds more tightly to complement receptors and is released more slowly. To simplify the model, we streamlined the loading of free virus particles onto the surface of the follicular dendritic cell network of B cell

follicles by ignoring the competition for complement receptors and considering only virus particles bound by few receptors ($F_{ijk}$) or by many ($G_{ijk}$). Free virus particles are bound to follicular dendritic cells at rate $\phi$, and virus particles bound by few receptors can become bound by many receptors at rate $\tilde{\phi}$. Conversely, virus particles bound by many receptors can revert to being bound by few receptors at rate $\tilde{\psi}$ and virus particles bound by few receptors can be released back into free form at rate $\psi$. Our simplified model reproduces previous findings that early on during treatment, virus stored in immune complexes on the surface of the follicular dendritic cell network can decline in more than one phase and at a different rate than the decline of viral RNA in plasma[12].

A set of 34 coupled ordinary differential equations define the rate at which the total number of uninfected cells ($X_i$), infected cells ($Y_{ij}$ and $Q_{ij}$), free virus particles ($V_{ijk}$) and follicular dendritic cell-associated virus particles ($F_{ijk}$ and $G_{ijk}$) in the body change with time.

For $i=0,1$

$$dX_i/dt = \Lambda u_i - \left((1-z_i)V_{i00} + (1-wz_i)V_{i10}\right)\beta X_i/u_i - (\mu_X + \tau_i)X_i + \tau_{\hat{i}}X_{\hat{i}}$$

$$dY_{i0}/dt = \left((1-r)(1-z_i)V_{i00} + r(1-wz_i)V_{i10}\right)\beta\theta X_i/u_i - (\gamma + \mu_Y + \tau_i)Y_{i0} + \tau_{\hat{i}}Y_{\hat{i}0}$$

$$dY_{i1}/dt = \left(r(1-z_i)V_{i00} + (1-r)(1-wz_i)V_{i10}\right)\beta\theta X_i/u_i - (\gamma + \mu_Y + \tau_i)Y_{i1} + \tau_{\hat{i}}Y_{\hat{i}1}$$

$$dQ_{i0}/dt = \left((1-r)(1-z_i)V_{i00} + r(1-wz_i)V_{i10}\right)\beta(1-\theta)X_i/u_i + \gamma Y_{i0} - \left(\mu_Q + \tau_i\right)Q_{i0} + \tau_{\hat{i}}Q_{\hat{i}0}$$

$$dQ_{i1}/dt = \left(r(1-z_i)V_{i00} + (1-r)(1-wz_i)V_{i10}\right)\beta(1-\theta)X_i/u_i + \gamma Y_{i1} - \left(\mu_Q + \tau_i\right)Q_{i1} + \tau_{\hat{i}}Q_{\hat{i}1}$$

$$dV_{i00}/dt = \rho(1-\tilde{z}_i)(Y_{i0} + Q_{i0}) - (c + \phi + \tau_i)V_{i00} + \psi F_{i00} + \tau_{\hat{i}}V_{\hat{i}00}$$

$$dV_{i10}/dt = \rho\pi(1-\tilde{w}\tilde{z}_i)(Y_{i1} + Q_{i1}) - (c + \phi + \tau_i)V_{i10} + \psi F_{i10} + \tau_{\hat{i}}V_{\hat{i}10}$$

$$dV_{i01}/dt = \rho\tilde{z}_i(Y_{i0} + Q_{i0}) - (c + \phi + \tau_i)V_{i01} + \psi F_{i01} + \tau_{\hat{i}}V_{\hat{i}01}$$

$$dV_{i11}/dt = \rho\pi\tilde{w}\tilde{z}_i(Y_{i1} + Q_{i1}) - (c + \phi + \tau_i)V_{i11} + \psi F_{i11} + \tau_{\hat{i}}V_{\hat{i}11}$$

For *i=0,1; j=0,1; k=0,1*

$$dF_{ijk}/dt = \phi V_{ijk} - (\psi + \tilde{\phi})F_{ijk} + \tilde{\psi}G_{ijk}$$

$$dG_{ijk}/dt = \tilde{\phi}F_{ijk} - \tilde{\psi}G_{ijk}$$

where the circumflex represents the other compartment: $\hat{\imath} = 1$ if $i = 0$ and vice versa. Supplementary Table 1 lists the model parameter estimates.

The first of these equations, for example, is formulated by showing that the rate of change of uninfected cells in compartment *i* $(dX_i/dt)$ is equal to the rate at which uninfected cells are produced, $(\Lambda u_i)$ plus the rate of inflow of uninfected cells from the other compartment $(\tau_i X_i)$, minus the rate at which they become infected $(((1 - z_i)V_{i00} + (1 - wz_i)V_{i10})\beta X_i/u_i)$, move to the other compartment $(\tau_i X_i)$ or are lost through clearance $(\mu_X X_i)$.

The spatial and dynamic model was fitted by least squares to data relating to the density of RNA in plasma, CD4+ cells infected with HIV-1 in lymphatic tissue, and virus particles bound to the follicular dendritic cell network between day 0 and month 6 of treatment. Averaged across twelve patients for robustness, the data includes three patients described in detail here and an additional nine patients described elsewhere[13].

To compare the model to data, we assume that the sanctuary site resides in a small part of the lymphoid tissue. The assumption that only part – not all – of the lymphoid tissue makes up the sanctuary site is based upon the observation that the density of infected cells in rectum, ileum and lymph node biopsies all decline by at least an order of magnitude over the first 6 months of therapy (Extended Data Figure 1). These data indicate that a notable proportion of virus replication in each of these regions is prevented by the action of drugs. Traditional mathematical models with homogeneous mixing

struggle to accommodate such large drops in the density of infected cells without predicting full clearance.

We recognize that mechanisms not fully represented by traditional models could be acting to sustain *de novo* infection despite a fairly sizable reduction in HIV-1-infected cell measures. Notably, there may be spatial heterogeneity in the spread of HIV-1 infection such that even within a lymph node (or other lymphoid tissue type), there could be some spatially localized patches of ongoing replication sustaining high-infection densities, whilst in other patches there is no viral replication and infection densities are lower. The observed variability between assays (both by time and patient) in measurements of infected cells supports this idea. Although we do not model it here, it is noteworthy that cell-to-cell transmission could be a contributing factor in spatially heterogeneous transmission patterns[14].

We assume that viral replication can occur in small spatial patches (sanctuary sites) dotted around the lymphatic tissue and potentially also distributed elsewhere throughout the body (Fig. 2). For simplicity, however, we model the patches as a single compartment situated in the lymphatic tissue that harbors a circumscribed focus of infection in space (small numbers of CD4+ T cells, in addition to localized extracellular fluid and follicular dendritic cell network, in which virus particles can exist). We model the remainder of the lymphatic tissue and extracellular fluid distributed in the body in a different, main compartment.

Because the data are density measures (RNA copies/ml, RNA positive cells/g, or RNA copies/g), we first scaled up the numbers to amounts appropriate to a hypothetical 70 kg man for the model-based analysis. We assume that the 700 g of lymphoid tissue is

distributed among lymph nodes (10%), ileum (45%), and rectum (45%). To approximate the total average number of follicular dendritic cell-associated virus particles in the body, we estimated the median (across patients) of the RNA density in each of the three lymphoid tissue types and then scaled these densities according to the above assumptions about the makeup and size of lymphoid tissue. Each virus particle was also assumed to contain 2 copies of RNA. Model-derived estimates of the total number of follicular dendritic cell-associated virus particles in the body were fitted to data-derived estimates by calculating the sum across both the main compartment and the lymphoid tissue sanctuary of the model ($\sum_i^{0,1}\sum_j^{0,1}\sum_k^{0,1}(F_{ijk}+G_{ijk})$)

Estimates of the total average number of infected cells in the body were complicated by the fact that in many assays, infected cell counts dipped below the limits of detection that varied across tests. To account for these complexities, we first calculated an upper bound on the median (across the 12 patients) of the infected cell density at each time point and for each of the three tissue types by assuming all undetectable measures contribute a value equal to the limit of detection. A lower bound on the median was similarly calculated by assuming that undetectable measures contribute a value of zero. By accounting for total body size and the estimated makeup of lymphoid tissue, as above, a scaled upper limit was then calculated from three tissue-specific upper limits. A scaled lower limit was similarly determined. When the scaled lower limit was positive (detectable), the model was fitted to the mean of the scaled upper and lower limits. When the scaled upper limit was zero (undetectable), the mean was not calculated and the model was fitted below the scaled upper limit. Model-derived estimates of the total number of infected CD4+ T-cells in the body ($\sum_i^{0,1}\sum_j^{0,1}(Y_{ij}+Q_{ij})$) were fitted to these data-derived estimates.

To fit the model to the observed levels of plasma HIV-1 RNA, we began by estimating the total number of free virus particles in all extracellular fluid of the body, including the blood, but not residing in the sanctuary sites. We assume that the density of virus in plasma was representative of the density of virus in the extracellular fluid of non-sanctuary regions. The density of virus in sanctuary regions was assumed to be higher. We then scaled the median density of virus in plasma to account for the assumptions that two RNA copies exist in each virus particle and a total 15 liters of extracellular fluid. Model predictions of the total number of free virus particles in the main compartment of the body ($\sum_k^{0,1} \sum_j^{0,1} V_{0jk}$) were fitted to these data. When the median plasma HIV-1 RNA elicited an undetectable reading, these model predictions were fitted below the scaled limit of detection. At the long-term equilibrium (360 weeks after therapy[15]), the model was similarly fitted to numbers of free virus particles inferred from RNA density measured in plasma using a quantitative reverse transcription PCR single copy assay[15].

Beyond month 6 of antiretroviral therapy, the model was fitted to data of infected cells and follicular dendritic cell-associated virus particles in lymphoid tissue under the assumption that there is no further decline in these values compared to the observations at month 6. Thus, ongoing replication is assumed to sustain infection levels in the lymphoid tissue at these levels. The size of the drug sanctuary that we estimate, therefore, represents an upper limit and the rate of mixing between the drug sanctuary and the main compartment represents a lower limit. All of our model predictions assume that the within-host basic reproductive number, $R_0$, is equal to 8[16]. The model fits the data well (Extended Data Fig. 5).

**Estimating the wait time for generating an HIV-1 strain with a triple mutation during antiretroviral therapy**

Can virus replication be ongoing in a sanctuary site without the generation and spread of drug-resistant strains throughout the body? To address this question it is important to understand whether fully drug-resistant strains capable of replication in areas of high effective drug concentrations (Extended Data Fig. 4b), such as the blood, will be generated by random mutation as a result of ongoing replication in sanctuary sites. If they are generated, our model predicts that they will spread to the main compartment of the body where they will thrive.

In addressing this question we begin by highlighting two points: first, antiretroviral therapy is highly effective because it combines multiple (typically three) drugs; and second, viral mutations can impose a fitness cost on the virus. Any drug-resistant strain capable of replicating at high effective drug concentrations therefore probably possesses at least three mutations, one for each drug. More than three mutations may be necessary if additional fitness compensatory mutations are also required. Viral strains with only one or two drug-resistant mutations would probably have some degree of resistance to triple therapy, but not enough to replicate at high effective drug concentrations. We assume that these partially drug-resistant strains would be outcompeted by sensitive strains in areas of low-effective drug concentrations because of the fitness cost of drug resistance.

We adapted our model to estimate the expected duration of antiretroviral therapy required before generation of a viral strain with substitutions at three particular positions. In this adaptation, only the action of a single drug class (the reverse transcriptase inhibitor) was considered for simplicity; however, we modeled a total of eight strains spanning all

strains with either a drug-sensitive or drug-resistant option at each of three positions. Each mutated strain was assumed to be a fraction, $\pi = 0.9$, as fit as the wildtype sequence in the absence of therapy. Furthermore, each single or double mutant was assumed to reduce the impact of the drug by the same fraction, $w = 0.92$. Under these parameters, strains with single and double mutations would be only partially resistant to the full complement of drugs taken during antiretroviral therapy. Thus, they would not be capable of continuous replication at high effective drug concentrations and could only exist within the lymphoid tissue sanctuary (Fig 3). Even in the lymphoid tissue sanctuary, however, these viruses can only grow to very low-levels because their replication is suppressed through competition with drug-sensitive strains (Fig 4a). Based upon a recent review of HIV-1 mutation rates[17], the probability of mutation at each generation, at each site between the drug-sensitive and drug-resistant option is assumed to equal $r = 2.4 \times 10^{-5}$. At each round of infection, the probability of mutation (in either direction) at no sites, one particular site only, two particular sites only, and all sites is $(1-r)^3$, $(1-r)^2 r$, $(1-r)r^2$ and $r^3$, respectively.

Using this model, we first calculated the expected number of cells infected with a strain with none, one, two or three particular mutations at the untreated equilibrium (Supplementary Table 2, row 1). Our findings agree with previous calculations that each single mutant would be expected to exist multiple times ( $2.2 \times 10^4$ cells) at the instant that antiretroviral therapy is started [7,14]. Each particular double mutant occurs less often, but is nevertheless also expected to be present at the start of therapy (10 cells). By contrast, each particular triple mutant occurs sufficiently rarely that it would be very unlikely to be present at the start of treatment (expected number $= 6.8 \times 10^{-3}$ cells).

Based upon the premise that any particular triple mutant is unlikely to be present at the start of treatment, we next ran model simulations to estimate the duration on antiretroviral therapy that it would take until the first cell is infected with a particular triple mutant. In any infection generation, this could occur by three mechanisms: three mutations could arise from the wild-type sequence; two mutations could arise from an existing single-mutant strain; or one mutation could arise from an existing double-mutant strain. We numerically integrated the model outputs to find the cumulative number of cells infected with a triple mutant through each of these mechanisms (Supplementary Table 2, row 2) during the first year of antiretroviral therapy. Summing across these three mechanisms gives a total rate of acquiring a triple mutant as $7.9 \times 10^{-4}$ cells per year (Supplementary Table 2, row 3). The expected duration of treatment needed before a triple mutant would be observed (assuming it is not present at the start of antiretroviral therapy) is estimated from this rate to be 1300 years (Supplementary Table 2). Thus, it would occur sufficiently rarely that it would not be expected to occur over the duration of antiretroviral therapy. This supports our assumption that drug-resistant strains capable of persistent cycles of *de novo* infection are not typically generated during the course of treatment with low-level ongoing replication. For completeness, the rate of acquiring single mutants and double mutants was also calculated from model simulations (Supplementary Table 2, rows 3).

We acknowledge that our estimate for the waiting time for a particular triple mutant is dependent upon a number of assumptions that we make. These include the mutation rate and the fitness cost of single and double mutants in the absence of antiretroviral therapy. More than one particular set of triple mutants could also be fully resistant to the complement of drugs taken or more than three mutations could be necessary. However, we note that each of these assumptions also has an equivalent impact upon the number of

cells with the fully drug-resistant strain that would be expected to exist at the start of antiretroviral therapy. The fact is that fully resistant strains do not typically exist at the start of treatment because if they did, they would inevitably be selected for once antiretroviral therapy starts and treatment would therefore fail. Our calculations indicate that the probability of a fully drug-resistant strain existing at the start of antiretroviral therapy is equal to the probability of one being generated over nine years of treatment by ongoing replication in a drug sanctuary. The observation that the existence of a triple mutant prior to antiretroviral therapy is rare therefore indicates that the generation of a triple mutant during therapy will also be rare.

Our analysis is important for explaining why, despite ongoing replication in a lymphoid tissue sanctuary, drug-resistant strains are not typically generated during antiretroviral therapy through a stepwise process. Low drug concentrations in a sanctuary are important for ensuring that a partially drug-resistant strain, that is, a single or double mutant, does not dominate in the sanctuary site. If they did dominate, given the observed dynamics and estimated size of the sanctuary site, stepwise addition of mutations would occur rapidly. This can be understood by noting that our model estimates that an average of $2.8\times10^7$ new infections of cells would occur in the lymphoid tissue sanctuary during each year of potent antiretroviral therapy. Almost all of these infections would be of the drug-sensitive strain (Fig 4a). If, however, partially drug-resistant strains could thrive in the sanctuary site, this rate of viral replication would be sufficient to generate a higher order mutation (+ 1 mutation) more than once per day $\approx r\times2.8\times10^7/365=2$ per day). Stepwise accumulation of three mutations would therefore be expected to occur very rapidly indeed. Each stepwise addition would only take one day plus some time for the more drug-resistant strain to outgrow the other. By accounting for competition from drug-

sensitive strains, our model predicts that partially drug-resistant strains replicate orders of magnitude less frequently than this, meaning that stepwise accumulation of a triple mutant is much less likely to occur. Competition between drug-sensitive and partially drug-resistant strains within the lymphoid sanctuary site is therefore crucial for understanding why stepwise accumulation of mutations is not seen despite ongoing replication.

Our model has shown that the emergence of drug resistance mutations is not necessary to demonstrate ongoing viral replication in treated patients; any clear acquisition of HIV-1 sequence changes is sufficient. Finding compelling evidence of sequence changes over shorter time frames is more challenging than over longer time frames, but we have nevertheless revealed clear evidence of such changes over the first six months of therapy. Our model argues that the mutated sequences do not harbor drug resistance mutations owing to the low levels of drug at the sites of replication (and lack of selection pressure).

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| Parameter | Description | Value used | Source |
|---|---|---|---|
| $\rho$ | The production rate of virus particles | 72 day$^{-1}$ cell$^{-1}$ | Fitted |
| $r$ | Probability of mutation per cell infection | 2.4x10$^{-5}$ | Sanjuan, et al. 2010[17] |
| $w$ | The relative impact of the RT inhibitor against the partially drug-resistant strain | 0.92 | Assumed to demonstrate three outcomes to changes in the drug effectiveness in the sanctuary |
| $\tilde{w}$ | The relative impact of the protease inhibitor against the partially drug-resistant strain | 0.92 | |
| $\mu_X$ | Clearance rate of uninfected cells | 3.5x10$^{-3}$ day$^{-1}$ cell$^{-1}$ | Michie, et al. 1992[18] |
| $\mu_Y$ | Clearance rate of long-lived infected cells | 6.7x10$^{-3}$ day$^{-1}$ cell$^{-1}$ | Fitted |
| $\mu_Q$ | Clearance rate of short-lived infected cells | 1 day$^{-1}$ cell$^{-1}$ | Markowitz, et al. 2003[19] |
| $c$ | Clearance rate of free virus | 134 day$^{-1}$ particle$^{-1}$ | Fitted |
| $\gamma$ | The rate at which long lived infected cells becomes short lived infected cells | 6.7x10$^{-3}$ day$^{-1}$ cell$^{-1}$ | Assumed to equal the clearance rate of long-lived infected cells $\mu_Y$ |
| $\theta$ | The fraction of infected cells that become long-lived cells | 1.46x10$^{-4}$ | Fitted |
| $u_i$ | The fraction of the body in compartment *i* | $u_0 = 1 - u_1$<br>$u_1 = 4.3\text{x}10^{-5}$ | Fitted ($u_1$ represents upper limit) |
| $z_0$ | Effectiveness in the main compartment of the RT inhibitor in preventing the infection of cells. | 1 | Assumed to model a main compartment where drugs penetrate effectively |
| $\tilde{z}_0$ | Effectiveness in the main compartment of the protease inhibitor in making virus particles that are produced non-infectious. | 1 | |
| $z_1$ | Effectiveness in the sanctuary site of the RT inhibitor in preventing the infection of cells | a) 0.3<br>b) 0.6<br>c) 1 | Assumed to demonstrate three outcomes |
| $\tilde{z}_1$ | Effectiveness in the sanctuary site of the protease inhibitor in making virus particles that are produced non-infectious. | a) 0.3<br>b) 0.6<br>c) 1 | |
| $\beta$ | Transmission coefficient of sensitive virus in the main compartment | 5.0x10$^{-10}$ day$^{-1}$ cell$^{-1}$ particle$^{-1}$ | Fitted |
| $\pi$ | The relative rate of virus production by cells infected with the resistant strain compared to those infected with the sensitive strain | 0.9 | Assumed to demonstrate three outcomes to changes in the drug effectiveness in the sanctuary |
| $\Lambda$ | Birth rate of uninfected cells | 1.04x10$^{8}$ | Fitted |
| $\tau_i$ | Rate of movement of cells and free virus from compartment *i* to the other compartment | $\tau_1 = 0.2$ day$^{-1}$ cell$^{-1}$<br>$\tau_0 = \tau_1 u_1/u_0$ day$^{-1}$ cell$^{-1}$<br>(the unit is day$^{-1}$ particle$^{-1}$ when it applies to free virus) | Fitted (represents lower limit) |
| $\phi$ | Rate at which free virus particles are bound to follicular dendritic cells | 82.25 day$^{-1}$ particle$^{-1}$ | Fitted. |
| $\tilde{\phi}$ | Rate at which virus particles bound by few receptors to follicular dendritic cells become bound by many receptors | 0.0221 day$^{-1}$ particle$^{-1}$ | Fitted |
| $\psi$ | Rate at which virus particles bound by few receptors to follicular dendritic cells are released into plasma | 0.67 day$^{-1}$ particle$^{-1}$ | Fitted |
| $\tilde{\psi}$ | Rate at which virus particles bound by many receptors to follicular dendritic cells become bound by few receptors | 0.0599 day$^{-1}$ particle$^{-1}$ | Fitted |

**Supplementary Table 1. Best-fit parameter estimates for the spatial and dynamic model.** Parameter values obtained with published data are listed.

| Row | Number of substitutions in strain (n) | n=0 | n=1 | n=2 | n=3 |
|---|---|---|---|---|---|
| 1 | Average number of cells infected with each particular n-substitution strain at the start of therapy | $9.2 \times 10^{7}$ | $2.2 \times 10^{4}$ | 10 | $6.8 \times 10^{-3}$ |
| 2 | Number of new infections of cells with the triple mutant strain caused by stepwise mutation from all of n-substitution strains during the first year of therapy | $3.9 \times 10^{-7}$ | $7.6 \times 10^{-4}$ | $3.2 \times 10^{-5}$ | |
| 3 | Total number of new infections of cells with each particular n-substitution strain during the first year of therapy | $2.8 \times 10^{7}$ | $1.9 \times 10^{4}$ | 11 | $7.9 \times 10^{-4}$ |
| 4 | Average waiting time on therapy to the first cell infected with the mutant if not present at the start of therapy | Present at the start of therapy | Present at the start of therapy | Present at the start of therapy | 1300 years |

**Supplementary Table 2**. **Estimating the wait time for the generation of an HIV-1 strain with a particular triple mutation during antiretroviral therapy.** Here we provide a breakdown of the calculations used to estimate the wait time for a particular single, double or triple mutant to occur through ongoing replication in a sanctuary site. We estimate that a triple mutation takes on average 1300 person years to appear, indicating that triple mutations would be very unlikely to occur within the lifetime of an HIV-1 infected patient under treatment. This calculation supports our argument that strains, which are fully resistant to the full complement of drugs provided during treatment, would not typically be generated during antiretroviral therapy. Only partially resistant strains, that is, those with one, or more rarely, two mutations would normally appear. For all calculations the single point mutation rate estimate ($2.4 \times 10^{-5}$ cell infections$^{-1}$) is used[17].