Supplementary Note 3: Infection probability after virus exposure (module III)

In this note, we derive the equations describing *systemic* infection probabilities after a single exposure with n viruses in untreated- or PrEP-treated individuals. For estimating the latter, input from module II (MMOA) is required (inhibition of target cell infection η). We also explain how the effect of PrEP on inhibiting *systemic* infection, following exposure to $i = 1, \ldots, \infty$ viruses can be inferred.

SN3 Probability of infection after virus exposure

Unprotected intercourse between an HIV-1 infected person (potential transmitter) and an uninfected person (potential recipient) does not always lead to HIV-1 infection of the recipient, suggesting that HIV-1 infection is a *stochastic* process. The mean infection probability per unprotected intercourse is reported to be very low (less than 1 % per heterosexual contact and less than 10 % per homosexual contact [1]).

After intercourse, viruses need to reach a target cell environment receptive for viral replication in order to establish an infection in the recipient. This process necessitates the virus to overcome several physiological barriers (i.e. the mucosal barrier). There is evidence that only very few founder viruses establish an infection [2,3], which argues that very few viral particles enter a target cell environment after transmission. However, even after reaching such an environment, infection will not always take place: Viral replication comprises various steps and viruses can be eliminated before producing any progeny. However, if a single virus succeeds in completing its replication cycle, a multiple of viral progeny (around 1000) is being produced that renders viral extinction in consecutive replication cycles unlikely. Thus, if a virus reaches the final stage of its replication cycle (virus release), it can be considered as a '*point of no return*'. Consequently, the probability of completion of the replication cycle by a single virus in a target cell environment can be considered as a good approximation to the probability of a transmitted virus establishing an infection.

SN3.1 Infection probability after exposure to a single transmitted virus

In order to compute the infection probability, we adapt and simplify a viral dynamic model reported in a previous work [4]. In the current work, we are primarily concerned with the initial phase of infection, which exhibits intrinsic stochasticity (see e.g. [5,6]). To properly deal with the stochasticity of the infection process, we derived a *chemical master equation* (CME) from the reaction rate equations of the viral dynamics model presented in [4]. Furthermore, we simplified it, ignoring the macrophage compartment since it does not contribute to the early infection events in our model simulation (data not shown). This is also in line with the observations by Ping et al. and Isaacman-Beck et al. [7,8], which show that that the transmitter/founder viruses are exclusively T cell-tropic.

Figure SN3.1 illustrates the viral dynamic model used. The model considers five possible states of the virus: V, T_1, T_2, Pro and $\varnothing$. The state V represents a free virion in imminent proximity of its target cells. In state T_1 the virus has successfully entered a T cell and reverse-transcribed its genome. In state T_2, the virus has successful integrated its reverse-transcribed genome and the cell starts producing viral building blocks. State Pro denotes the proliferative step, i.e. the virus succeeded to produce progeny. Since a single late infected cell T_2 produces a multitude of viruses (1000 on average [9]), it is very likely that the infection is established once state Pro is reached. Finally, $\varnothing$ denotes the clearance of infection before entering state Pro. As can be seen in Figure SN3.1, Pro and $\varnothing$ are absorbing states.

The *chemical master equation* modelling the events that occur after a challenge by a single virus is defined by:

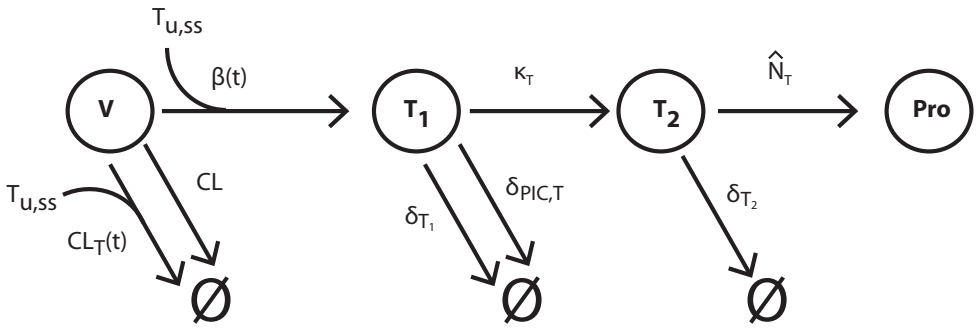

Figure SN3.1: **Schematic representation of the viral replication cycle:** Free virus V can be cleared with rate constant CL by the immune system or can be cleared by an unsuccessful attempt to infect a T cell with rate $\mathrm{CL_T}$. Free virus can also succeed infecting a T cell at rate β_T(t) and advance to state T_1 (early infected T-cell), which denotes the state where the virus has successfully reverse-transcribed its genome. Early infected T cells can be cleared with rate constant δ_{T1} or due to the degradation of the viral pre-integration complex with rate constant $\delta_{\mathrm{PIC,T}}$. The virus at the T_1 state can advance to the late infected state T_2 (viral genome has been successfully integrated into the host cell, which starts producing viral building blocks) with rate constant k_T. During state T_2 the infected T cell gets either cleared with the rate δ_{T2}, or it produces viral progeny (state Pro) with rate $\widehat{N}_\mathrm{T}$.

$$\frac{dP_t(V)}{dt} = -\left(\mathrm{CL} + \mathrm{CL_T}(t)\cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(t)\cdot \mathrm{T_{u,SS}}\right)\cdot P_t(V) \tag{SN3.1}$$

$$\frac{dP_t(T_1)}{dt} = \beta_\mathrm{T}(t)\cdot \mathrm{T_{u,SS}}\cdot P_t(V) - \left(\delta_{\mathrm{PIC,T}} + \delta_{\mathrm{T1}} + k_\mathrm{T}\right)\cdot P_t(T_1) \tag{SN3.2}$$

$$\frac{dP_t(T_2)}{dt} = k_\mathrm{T}\cdot P_t(T_1) - \left(\delta_{\mathrm{T2}} + \widehat{N}_\mathrm{T}\right)\cdot P_t(T_2) \tag{SN3.3}$$

$$\frac{dP_t(\mathrm{Pro})}{dt} = \widehat{N}_\mathrm{T}\cdot P_t(T_2) \tag{SN3.4}$$

where the term $P_t(*)$ denotes the probability of state $* \in \{V, T_1, T_2, \mathrm{Pro}\}$ at the time t. A viral challenge is simulated by numerically integrating the equations above with initial condition $P(V,t) = 1$, while the probability of all other states is zero initially. Note that we assumed that the number of T-cells $\mathrm{T_{u,SS}}$ in the recipient equals the steady state value in the absence of virus, i.e. $\mathrm{T_{u,SS}} = \lambda_\mathrm{T}/\delta_\mathrm{T}$ where the terms λ_T and δ_T denote the production and death rate constants of native T cells respectively.

From the conservation of probability, we can write the probability of the extinction of a virus as

$$P_t(\varnothing) = 1 - P_t(V) - P_t(T_1) - P_t(T_2) - P_t(\mathrm{Pro}). \tag{SN3.5}$$

Further, for $t \to \infty$, the probabilities of states V, T_1, T_2 tend to zero and the stationary values of the probability of $P(\mathrm{Pro})$ and $P(\varnothing)$ become complementary to one another:

$$P_\infty(\varnothing) = 1 - P_\infty(\mathrm{Pro}). \tag{SN3.6}$$

SN3.2 Effect of NRTIs

We have previously shown that the influence of NRTIs on the viral replication cycle can be considered in two ways[4,10,11]: Since activated intracellular NRTI-triphosphates inhibit reverse transcription, in the model (Fig. SN3.1) they reduce the rate of transition from the state V to T_1:

$$\beta_\mathrm{T}(\mathrm{t}) = (1 - \eta(t))\cdot \beta_T(\emptyset), \tag{SN3.7}$$

where the term $\beta_T(\emptyset)$ is the rate of successful penetration of the target cell, release of viral contents and subsequent reverse transcription in absence of drugs. The term $(1 - \eta(t))$ is the fraction of activity in the presence of intracellularly active NRTI-triphosphates, obtained from module II (**Supplementary Note 2**). Secondly, NRTIs increase the rate of clearance of virus due to unsuccessful infection:

$$\mathrm{CL_T}(t) = \left(\frac{1}{\rho} - (1 - \eta(t))\right)\cdot \beta_T(\emptyset) \tag{SN3.8}$$

where the term ρ is the probability that the transition is successful in absence of drugs.

The influence of NRTIs is implemented using the Emax-model with hill coefficient 1 [12]:

$$(1 - \eta(t)) = \frac{\mathrm{IC}_{50}}{\mathrm{IC}_{50} + C_{\mathrm{cell}}(t)} \tag{SN3.9}$$

where the term $C_{\mathrm{cell}}(t)$ is the intracellular concentration of active NRTI-triphosphate at time t and the term IC_{50} denotes the intracellular concentration where the target process is inhibited by 50% (see [10] for more details) in units μM, which can be determined by fitting the above equation to the output of module II (Supplementary Note 2). Thus, NRTIs alter the infection probability after viral challenge (eqs. (SN3.1)-(SN3.4)) in a time-dependent manner by affecting the terms $\beta_{\mathrm{T}}(\mathrm{t})$ and $\mathrm{CL}_{\mathrm{T}}(t)$.

SN3.3 Steady state intracellular concentration of active anabolites

For generating Fig. 3 (main manuscript) we assumed steady state intracellular concentration ranges of 0.03-1.44 μM TFV-DP [13,14,15,16], 10-66.66 μM FTC-TP [16,17,14], 2.78-55.56 μM 3TC-TP [18,19,20], 0.44-0.88 μM for ABC-TP [21], 0.0056-0.056 μM for AZT-TP [22] and 0.034-0.56 μM D4T-TP [23]. The unit conversion was performed assuming an average cell volume of 180 μm^3 for resting CD4$^+$ T-cells [24]. Note that the measurement of intracellular NRTI-TP may depend on sample processing (whether a cell homogenate, e.g. peripheral blood mononuclear cells (PBMC) is analyzed and whether cells are viable vs. freshly lysed). Furthermore concentrations ranges may depend on the sampling design (e.g. first dose vs. steady-state pharmacokinetics; cells derived from HIV-infected individuals vs. HIV-negative volunteers). The ranges indicates should thus only provide a rough guidance.

SN3.4 Infection probability after exposure to a single virus assuming constant drug effect $(1 - \eta)$

The set of equations (SN3.1)-(SN3.4) can easily incorporate the effect of a time varying inhibition by NRTIs. However, under certain circumstances the term $(1-\eta(t))$ may be approximated by a constant. Obviously, in absence of drugs the term $(1 - \eta(t)) = 1$ is constant. Similarly, when NRTIs are administered regularly, concentration changes will be very small on the time scales of interest and therefore the change in the magnitude of $(1-\eta(t))$ will be negligibly small, i.e. $(1 - \eta(t)) \approx (1 - \eta)\ \forall\ t$. In case of time-invariant $(1 - \eta)$, the stationary probabilities of viral extinction can be solved analytically. We will make use of this when computing the concentration response as shown in Figure 3 (main manuscript). When $(1 - \eta)$ is constant, we can interpret the viral replication cycle (Figure SN3.1) as a multi-stage *branching process* and can compute the stationary probabilities of extinction and proliferation analytically. At an intermediate stage, the virus can either advance to the next stage in the replication cycle or it can be cleared, terminating the cycle. If a virus reaches the productive (final) stage, the virus can produce progeny, whereas at intermediate stages the virus cannot. Of particular interest is the probability that a single virus becomes cleared during its replication cycle before it can produce any progeny $P_\infty(\varnothing|V_0 = 1)$. Since the virus can be cleared at different stages of the replication cycle $P_\infty(\varnothing|V_0 = 1)$ can be decomposed:

$$\begin{aligned} P_\infty(\varnothing|V_0 = 1) &= p(V \to \varnothing) \\ &+ p(V \to T_1 \to \varnothing) \\ &+ p(V \to T_1 \to T_2 \to \varnothing) \end{aligned} \tag{SN3.10}$$

where $p(V \to \varnothing)$ denotes the probability that the free virus is cleared. The term $p(V \to T_1 \to \varnothing)$ denotes the joint probability that the free virus advances to stage T_1 and is then cleared. Similarly, the term $p(V \to T_1 \to T_2 \to \varnothing)$ denotes the joint probability that the free virus advances to T_1 and then to T_2 where it is cleared before producing any progeny.

When the effect of NRTIs is considered, we derive a compact formula (see subsection SN3.5 for derivation):

$$P_\infty(\varnothing|V_0 = 1) = 1 - (1 - \eta) \cdot \alpha \tag{SN3.11}$$

with

$$\alpha := \frac{\beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}}{\mathrm{CL} + \frac{\beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}}{\rho}} \cdot \Big(1 - p(T_1 \to \varnothing|V \to T_1) - p(T_1 \to T_2|V \to T_1) \cdot p(T_2 \to \varnothing|T_1 \to T_2)\Big), \tag{SN3.12}$$

where $p(T_1 \to \varnothing | V \to T_1)$ denotes the conditional probability of viral elimination at stage T_1, given that T_1 has been reached and $p(T_1 \to T_2 | V \to T_1) \cdot p(T_2 \to \varnothing | T_1 \to T_2) = p(T_2 \to \varnothing | V \to T_1 \to T_2)$ denotes the probability of viral clearance in stage T_2, conditioned T_2 has been reached.
In the absence of drug, we have $(1 - \eta) = 1$, thus from the Eqn (SN3.11) it follows that $\alpha = 1 - P_\infty(\varnothing | V = 1)$. Thus, the term α can be interpreted as the probability that a single virus can complete the replication cycle and produce viral progeny in the absence of NRTIs. Of particular interest is the relation revealed by the eq. (SN3.11), which highlights that NRTIs reduce the probability of proliferation (infection) by a factor $(1 - \eta)$ corresponding to the efficacy against their targeted process.

SN3.4.1 Infection probability after exposure to n viruses (*per challenge*)

Up to now, we have discussed the probability of infection considering that a single virus has reached a target cell environment. The infection event, given $n = 0 \ldots \infty$ transmitted viruses, can be thought of as a *Bernoulli chain*. Under the assumption of independence, the probability that all n viruses fail to produce progeny is given by:

$$P_t(\varnothing | V_0 = n) = \left(P_t(\varnothing | V_0 = 1)\right)^n \tag{SN3.13}$$

Thus, the infection probability (any of the n viruses succeeds in producing progeny and $t \to \infty$) is given by

$$P(\text{inf} | V_0 = n) = 1 - \left(P_\infty(\varnothing | V_0 = 1)\right)^n = 1 - \left(1 - P_\infty(\text{Pro} | V_0 = 1)\right)^n \tag{SN3.14}$$

SN3.4.2 Efficacy of PrEP *per challenge*

From here it is straightforward to compute the efficacy of PrEP *per challenge* with $i = 1, \ldots, \infty$ viruses (e.g. after coitus with an infected individual),

$$\varphi = 1 - \frac{P_S(\text{inf} | V_0 = i)}{P_\emptyset(\text{inf} | V_0 = i)}. \tag{SN3.15}$$

Where $P_S(\text{inf} | V_0 = i)$ and $P_S(\text{inf} | V_0 = i)$ denote the probabilities of infection after exposure to i viruses when a PrEP strategy S was applied vs. PrEP was not applied $\emptyset$. Note that φ is not defined in the case that no virus is being transmitted. The PrEP efficacy *per typical virus challenge* ψ is then defined by,

$$\boxed{\psi = 1 - \sum_{i=1}^{\infty} P(V_0 = i | n > 0)(1 - \varphi)} \tag{SN3.16}$$

In the equation above, $P(V_0 = i | n > 0) = P(V_0 = i)/(1 - P(V_0 = 0))$ is the conditional probability that $i = 1, \ldots, \infty$ viruses reach a target-site compartment after exposure (e.g. coitus) among all cases were there was an actual exposure $n > 0$. The exposure probabilities are detailed in **Supplementary Note 4**.

SN3.5 Derivation of results in section SN3.4 and parametrization

The probability of extinction in eq. (SN3.10) consists of the joint probabilities which can be decomposed into conditional probabilities which directly relate to model parameters. For example, the term $p(V \to T_1 \to \varnothing)$ is equal to the probability that a virus V reaches the T_1 stage $p(V \to T_1)$ times the conditional probability that the virus is cleared in the T_1 stage when it is there $p(T_1 \to \varnothing | V \to T_1)$. Equation (SN3.10) can thus be rewritten

$$\begin{aligned} P_\infty(\varnothing|V_0 = 1) &= p(V \to \varnothing) \\ &+ p(V \to T_1) \cdot p(T_1 \to \varnothing | V \to T_1) \\ &+ p(V \to T_1) \cdot p(T_1 \to T_2 | V \to T_1) \cdot p(T_2 \to \varnothing | T_1 \to T_2) \end{aligned} \tag{SN3.17}$$

Note that in the above equation all terms denote the probability that a particular reaction happens next, for example $p(T_1 \to \varnothing | V \to T_1)$ denotes the probability that reaction $T_1 \to \varnothing$ happens next when the virus is in state T_1.

Given a single free virus, the probabilities of different reactions to fire next can be written as follows:

$$p(V \to \varnothing) = \frac{\mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}}}{\mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}}} \tag{SN3.18}$$

$$p(V \to T_1) = \frac{\beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}}}{\mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}}} \tag{SN3.19}$$

$$p(T_1 \to \varnothing | V \to T_1) = \frac{\delta_{\mathrm{PIC,T}} + \delta_{\mathrm{T1}}}{\delta_{\mathrm{PIC,T}} + \delta_{\mathrm{T1}} + k_\mathrm{T}} \tag{SN3.20}$$

$$p(T_1 \to T_2 | V \to T_1) = \frac{k_\mathrm{T}}{\delta_{\mathrm{PIC,T}} + \delta_{\mathrm{T1}} + k_\mathrm{T}} \tag{SN3.21}$$

$$p(T_2 \to \varnothing | T_1 \to T_2) = \frac{\delta_{\mathrm{T2}}}{\delta_{\mathrm{T2}} + \widehat{N}_\mathrm{T}} \tag{SN3.22}$$

Substitution of (SN3.18) and (SN3.19) in (SN3.17) gives following equation:

$$P_\infty(\varnothing|V_0 = 1) = \frac{\mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}} \cdot (\mathrm{pT_1} + \mathrm{pT_2})}{\mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}}} \tag{SN3.23}$$

where, for ease of readability we used the following shorthand

$$\begin{aligned} \mathrm{pT_1} &:= p(T_1 \to \varnothing | V \to T_1) \\ \mathrm{pT_2} &:= p(T_1 \to T_2 | V \to T_1) \cdot p(T_2 \to \varnothing | T_1 \to T_2) \end{aligned} \tag{SN3.24}$$

The denominator of eq. (SN3.23) can be simplified by substituting eq. (SN3.7) and eq. (SN3.8) as shown below:

$$\begin{aligned} & \mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}} \\ = \; & \mathrm{CL} + \left(\frac{1}{\rho} - (1-\eta)\right) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} + (1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} \\ = \; & \mathrm{CL} + \frac{\beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}}{\rho} - \cancel{(1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}} + \cancel{(1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}} \\ = \; & \mathrm{CL} + \frac{\beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}}{\rho} \end{aligned} \tag{SN3.25}$$

Similarly, the numerator of eq. (SN3.23) can be also simplified

$$\begin{aligned} & \mathrm{CL} + \mathrm{CL}_T(t) \cdot \mathrm{T_{u,SS}} + \beta_\mathrm{T}(\mathrm{t}) \cdot \mathrm{T_{u,SS}} \cdot (\mathrm{pT_1} + \mathrm{pT_2}) \\ = \; & \mathrm{CL} + \left(\frac{1}{\rho} - (1-\eta)\right) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} + (1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} \cdot (\mathrm{pT_1} + \mathrm{pT_2}) \\ = \; & \mathrm{CL} + \frac{\beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}}{\rho} - (1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} + (1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} \cdot (\mathrm{pT_1} + \mathrm{pT_2}) \\ = \; & \mathrm{CL} + \frac{\beta_T(\emptyset) \cdot \mathrm{T_{u,SS}}}{\rho} - (1-\eta) \cdot \beta_T(\emptyset) \cdot \mathrm{T_{u,SS}} \cdot (1 - (\mathrm{pT_1} + \mathrm{pT_2})) \end{aligned} \tag{SN3.26}$$

Substituting eqs. (SN3.25)-(SN3.26) into eq. (SN3.23) gives

$$\begin{aligned} P_\infty(\varnothing|V_0=1) &= 1-\frac{(1-\eta)\cdot\beta_T(\emptyset)\cdot \mathrm{T_{u,SS}}\cdot(1-(\mathrm{pT_1}+\mathrm{pT_2}))}{\mathrm{CL}+\frac{\beta_T(\emptyset)\cdot \mathrm{T_{u,SS}}}{\rho}} \\ &= 1-(1-\eta)\cdot\left(\frac{\beta_T(\emptyset)\cdot \mathrm{T_{u,SS}}\cdot(1-(\mathrm{pT_1}+\mathrm{pT_2}))}{\mathrm{CL}+\frac{\beta_T(\emptyset)\cdot \mathrm{T_{u,SS}}}{\rho}}\right) \qquad \text{(SN3.27)} \\ &= 1-(1-\eta)\cdot\alpha \qquad \text{(SN3.28)} \end{aligned}$$

where

$$\alpha := \frac{\beta_T(\emptyset)\cdot \mathrm{T_{u,SS}}\cdot(1-(\mathrm{pT_1}+\mathrm{pT_2}))}{\mathrm{CL}+\frac{\beta_T(\emptyset)\cdot \mathrm{T_{u,SS}}}{\rho}} \qquad \text{(SN3.29)}$$

In absence of drug, we have $(1-\eta)=1$, thus from eq. (SN3.28) it follows that $\alpha = 1-P(\varnothing|V_0=1)$ and the term α can be interpreted as the probability that a single virus succeeds to produce progeny in absence of NRTIs.

The rate constants for the viral dynamics model are summarized in[4,10] and are also reported in Table SN3.1 below. The term α is computed after substituting all rate constants in eq. (SN3.29) and was found to be 0.0996, i.e. the probability of infection when a single virus is in a target cell compartment is roughly 10%.

Parameter	Value	Reference
λ_T	$2\cdot10^9$	[25]
$\delta_\mathrm{T}, \delta_{T_1}$	0.02	[9]
δ_{T_2}	1	[26]
$\delta_\mathrm{PIC,T}$	0.35	[27,28]
k_T	0.35	[28]
$\beta_\mathrm{T}(\emptyset)$	$8\cdot10^{-12}$	[29]
$\widehat{N}_\mathrm{T}$	1000	[9]
CL(naive)	2.3	[30,6]

Table SN3.1: **Parameters used for the viral dynamics model** Excerpt from[4], expect for CL(naive), which assumed that virus clearance is smaller in virus-naive individuals compared to infected individuals, in line with[31,32]. All parameters refer to the absence of drug treatment $\emptyset$. All parameters in units [1/day].)

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