Supplementary Note 2: Molecular Mechanism of Action (module II)

In this note we briefly summarize the utilized molecular mechanism of action (MMOA) model from [1] and explain its parameterizations. We will use this model to estimate inhibition of reverse transcription by NRTIs (denoted ε) and inhibition of cell infection (denoted by η), taking intracellularly active NRTI-TP concentrations as input (module I, Supplementary Note 1). Parameter η will be used to assess inhibition of systemic infection after virus exposure, as outlined in **Supplementary Note 3** (module III). Furthermore, we explain how to infer the effect of NRTI combinations and the fitness of mutant viruses.

SN2 Molecular Mechanism of Action (MMOA) for NRTIs

Activated NRTIs-triphosphates inhibit the crucial step of converting viral genomic RNA into double stranded DNA, before the latter is integrated into the host cellular DNA. Briefly, NRTI-TPs are analogs of endogenous deoxynucleotide triphosphates (dNTPs), which compete with the latter for incorporation into nascent DNA during reverse transcriptase mediated viral DNA polymerization. Because they lack a hydroxyl group, they act as 'chain terminators' after becoming incorporated into the primer, preventing its elongation by the next incoming nucleotide. Thus, NRTI-TP incorporation brings reverse transcription to a halt, unless the inhibitor becomes excised from the terminated primer. The MMOA model [1] regards the direct effect of NRTI-TPs in terms of a prolongation of the time required to form a reverse transcript. This 'time' can then be used to compute the IC_{50} value for inhibition of host cell infection. This is because the propensity that essential components of the virus become degraded intracellularly increases with the time that the virus resides in its fragile state before proviral integration.

SN2.1 Single NRTIs

The residual reverse transcription in the presence of activated (tri-phosphorylated) nucleoside analogs ($1-\varepsilon(I)$) is expressed as [1]:

$$1-\varepsilon(I)=\frac{T_{0\to \mathrm{N}}(\emptyset)}{T_{0\to \mathrm{N}}(I)} \qquad \text{(inhibition by NRTI-TP)}, \tag{SN2.1}$$

where $T_{0\to \mathrm{N}}(\emptyset)$ and $T_{0\to \mathrm{N}}(I)$ denote the expected time to finalize reverse transcription in the absence of drugs '$\emptyset$' and in the presence of active nucleoside analogs triphosphates 'I' respectively. It has been shown in [2] that the time required for DNA synthesis/polymerization exceeds the other processes involved in reverse transcription (e.g. strand transfer and initiation). Furthermore, there is an excess of RT enzymes (≈ 250 [3,4]) in comparison to RNA template (typically a single dimeric RNA), such that dissociation and association of RT to the template:primer complex can be neglected. All in all this means that $T_{0\to \mathrm{N}}$ approximates the time required for the DNA polymerization process (i.e. the time required for going from the 'empty' primer to the full polymerization product $0 \to$ N). In order to assess RT inhibition by NRTI-TPs, we will therefore focus on RT-induced polymerization in the presence and absence of inhibitors.
Note, that one can also assess the inhibition of a mutant virus 'mut' by the drug I, and the fitness f of a mutant (in the absence of drugs), relative to the wild type 'wt'.

$$1-\varepsilon(I,\mathrm{mut})=\frac{T_{0\to \mathrm{N}}(\emptyset,\mathrm{mut})}{T_{0\to \mathrm{N}}(I,\mathrm{mut})} \qquad \text{(inhibition of mutant)}, \tag{SN2.2}$$

$$\nu(\mathrm{mut})=\frac{T_{0\to \mathrm{N}}(\emptyset,\mathrm{wt})}{T_{0\to \mathrm{N}}(\emptyset,\mathrm{mut})} \qquad \text{(fitness of mutant)}. \tag{SN2.3}$$

In order to compute $T_{0\to \mathrm{N}}$, we interpret the polymerization process as a Markov Jump Process, where we would like to compute the *mean first hitting times*, $T_{0\to \mathrm{N}}$, i.e. the time from initialization of the polymerization process '0' to full length viral DNA 'N'. We can write (linearity of the expectation value)

$$T_{0\to \mathrm{N}}=\sum_{i=0}^{N-1} T_{i\to i+1}. \tag{SN2.4}$$

where i refers to the length of the primer and $T_{i\to i+1}$ denotes the expected time to extend the primer by one nucleotide. We consider four basic reactions: (i) The primer may be shortened by one nucleotide during the pyrophosphorolysis reaction RT/T:P^i $\to$ RT/T:P^{i-1}. (ii) The primer may be extended by one base during the polymerase reaction RT/T:P^i $\to$ RT/T:P^{i+1}, (iii) the NRTI-TP may be incorporated and the primer blocked RT/T:P^i $\to$ RT/T:$\tilde{P}^{i+1}$ and (iv) the NRTI may be excised from the blocked primer RT/T:$\tilde{P}^{i+1}$ $\to$RT/T:P^i. Therefore (see [1] for derivation) we have

$$T_{i\to i+1} = \left(\tau_{\widetilde{i+1}} \cdot \rho_{i\to\widetilde{i+1}} + \tau_i + \rho_{i\to i-1} T_{i-1\to i}\right)\frac{1}{\rho_{i\to i+1}}. \tag{SN2.5}$$

where $\tau_i, \tau_{\widetilde{i+1}}$ are the waiting times in states i and $\widetilde{i+1}$ (the NRTI-blocked state) respectively and $\rho_{i\to i+1}, \rho_{i\to i-1}$ are the probabilities to jump from state i to state $i+1$ and to state $i-1$ respectively. The parameter $\rho_{i\to\widetilde{i+1}}$ denotes the probability that the chain of length i gets terminated by incorporation of a nucleoside analog (state $\widetilde{i+1}$). The waiting times τ and jump-probabilities ρ are defined as follows:

$$\tau_i = \frac{1}{r_{\text{pol}}(i+1) + r_{\text{pyro}}(i) + r_{\text{term}}(i+1)}, \quad \tau_{\widetilde{i+1}} = \frac{1}{r_{\text{exc}}(i+1)},$$
$$\rho_{i\to i+1} = r_{\text{pol}}(i+1)\cdot\tau_i, \quad \rho_{i\to i-1} = r_{\text{pyro}}(i)\cdot\tau_i, \quad \rho_{i\to\widetilde{i+1}} = r_{\text{term}}(i+1)\cdot\tau_i, \tag{SN2.6}$$

where $r_{\text{pol}}(i+1)$ and $r_{\text{term}}(i+1)$ denote the polymerase- and chain terminating reactions (attachment of the next incoming nucleoside or its analog), which depend on the efficacy of incorporation of the respective types of nucleotides (deoxyadenosine, -thymidine, -guanine or -cytosine triphosphate) or their respective analogs at position $i+1$ in the primer. The parameter $r_{\text{exc}}(i+1)$ denotes the rate of release (excision reaction) of a primer that has been terminated at position $i+1$ by an NRTI-TP. The parameter $r_{\text{pyro}}(i)$ denotes the pyrophosphorolysis reaction, i.e. the rate at which a nucleoside is removed from the end of the primer. Note, that τ and ρ depend on the sequence context because the rates of nucleoside attachment and -removal depend on the types of nucleotides (and -analogs) to be incorporated and -removed respectively. Using eq. (SN2.4), one can compute $T_{0\to\text{N}}$ recursively given recursion start

$$T_{0\to 1} = \left(\tau_{\tilde{1}} \cdot \rho_{0\to\tilde{1}} + \tau_0\right)\frac{1}{\rho_{0\to 1}}, \tag{SN2.7}$$

with $\tau_0 = \frac{1}{r_{\text{pol}}(1)+r_{\text{term}}(1)}, \tau_{\tilde{1}} = \frac{1}{r_{\text{exc}}(1)}$ and $\rho_{0\to 1} = r_{\text{pol}}(1)\cdot\tau_0, \rho_{0\to\tilde{1}} = r_{\text{term}}(1)\cdot\tau_0$. The polymerization rates $r_{\text{pol}}, r_{\text{term}}$ are defined in terms of mass-action kinetics. I.e. in the presence (left) and absence (right) of inhibitors we have

$$r_{\text{term}}(I) = \frac{k_{\text{term}}\cdot[I]}{K_{\text{D},I}\left(1+\frac{[\text{dNTP}]}{K_{\text{D,dNTP}}}\right)+[I]}, \qquad r_{\text{term}}(\emptyset) = 0, \tag{SN2.8}$$

$$r_{\text{pol}}(I) = \frac{k_{\text{pol}}\cdot[\text{dNTP}]}{K_{\text{DdNTP}}\left(1+\frac{[I]}{K_{\text{D},I}}\right)+[\text{dNTP}]}, \qquad r_{\text{pol}}(\emptyset) = \frac{k_{\text{pol}}\cdot[\text{dNTP}]}{K_{\text{D,dNTP}}+[\text{dNTP}]}. \tag{SN2.9}$$

Where k_{term}, k_{pol}, $K_{\text{D},I}$ and $K_{\text{D,dNTP}}$ denote the catalytic rate constants for incorporation of the NRTI-TP vs. the dNTP and the respective dissociation constants of the NRTI-TP and dNTP to the reverse transcriptase respectively, which are compiled in Table SN2.1. Concentrations of endogenous dNTPs in HIV-1 target cells (here we modelled resting CD4$^+$ T-cells, which make up the majority of HIV-1 target cells) are stated in the caption of Table SN2.1. The rate of NRTI excision was set to $r_{\text{exc}} = 0.0016$ (s^{-1}) for thymidine and adenosine analogs (AZT, D4T, TDF) and to $r_{\text{exc}} = 0.00053$ (s^{-1}) for guanine and cytosine analogs (ABC, 3TC & FTC-TP) respectively and $r_{\text{pyro}} = 0.000898$ (s^{-1}) for all incorporated endogenous dNTPs [1].

The dose response curve for eq. (SN2.1) has the shape of the standard Emax-model with slope coefficient 1 (see von Kleist et al. [1]), which is in line with observations by Shen et al. [5] for this inhibitor class.

SN2.1.1 Residual cell infection

If the virus does not succeed to reverse-transcribe its genome in time, the virus will eventually be cleared intracellularly. In the following, we want to assess the relative probability that reverse transcription finishes in the presence

of NRTIs, before the virus is cleared. The derivations and scaling is outlined in von Kleist et al.[1], Supplementary Text S1. We get

$$1 - \eta(I) = \frac{1}{\rho_{\emptyset,RT} + \frac{1-\rho_{\emptyset,RT}}{1-\varepsilon(I)}}, \tag{SN2.10}$$

with $1 - \eta(I) = \rho_{I,RT}/\rho_{\emptyset,RT}$ denotes the relative probability to succeed with reverse transcription before the virus is cleared in the presence of inhibitors I and $\rho_{\emptyset,RT} = 0.5^6$ is the probability to succeed with reverse transcription in the absence of inhibitors.

The fitness of the mutant with regard to target cell infection in the absence of treatment can be computed accordingly:

$$f(\text{mut}) = \frac{1}{\rho_{\emptyset,RT} + \frac{1-\rho_{\emptyset,RT}}{\nu(\text{mut})}}, \tag{SN2.11}$$

SN2.1.2 MMOA parameterization

The MMOA model requires kinetic parameters for the incorporation and removal of NRTI-TP and dNTP respectively, as well as typical concentrations of dNTPs in HIV-1 target cells. The kinetic parameters can be derived from pre-steady state kinetic *in vitro* experiments and are compiled below. For all simulations we used parameters for DNA-dependent polymerization of a heteromeric random template of length 10000nt. For all simulations we used dNTP concentrations in resting $CD4^+$ T-cells, since they represent the most relevant HIV-1 target cell compartment. Concentrations of endogenous dNTPs in other HIV-1 target cells can be measured *ex vivo* and are compiled in e.g. Smith et al[7].

	WT			M184V		K65R		K65R+M184V		
				fold change w.r.t wild type						
	K_D [μM]	k_{pol} [s^{-1}]	ref.	K_D	k_{pol}	K_D	k_{pol}	K_D	k_{pol}	ref
dATP	7.8	44.8	[8,9,10]	2.4	0.66	0.93	0.23	4.9	0.4	[11,9,12,13]
dTTP	15.3	15.6	[9,10,14,15]	3.5	1.08	0.57	0.33	1.76	0.77	[11,9,13,15]
dCTP	18.25	10.2	[10,16,17,18]	2.66	1.3	1.5	0.62	2.4	0.78	[11,9,12,13,18]
dGTP	10.5	20	[19,20]	1.75	1.5	1.65	0.37	4.7	1.1	[11,19,21,22,23]
3TC-TP	26.83	0.038	[16,18,11]	35	0.55	0.77	0.081	17.9	0.035	[11,18]
FTC-TP	19	0.0563	[18,24]	35*	0.55*	1.59	0.128	1	0.016	[18]
TFV-DP	40.5	28	[8,11]	1.3	0.98	0.78	0.046	2.87	0.14	[11,12]
ABC-TP	21	1	[21]	-	-	-	-	-	-	-
AZT-TP	7.15	1.9	[10,14]	-	-	-	-	-	-	-
D4T-TP	34.5	13.4	[15,25]	-	-	-	-	-	-	-

Table SN2.1: **Micro-kinetic parameters.** Parameters related to the wild type virus are stated in absolute units, whereas parameters related to mutant viruses are stated in terms of a fold change with respect to the wild type parameter. The reported values are for DNA-dependent polymerization. r_{exc} in resting $CD4^+$ T-cells for adenosine and thymidine analogs was set to 0.0016 [1/s], whereas the value for guanine and cytosine analogs was fixed to 0.00053 [1/s]. The intracellular concentration for dATP, dTTP, dCTP and dGTP in the resting $CD4^+$ T-cells are 1.7- , 1.5- , 1.9- and 1.7- μM respectively[7]. * For FTC, we assumed that the fold effect was identical to that of 3TC.

SN2.2 Multiple NRTIs

NRTIs, which are analogs of same nucleotide (e.g, 3TC + FTC) are not recommended for medication, since they may compete for incorporation and intracellular activation (by phosphorylation). We will focus on NRTIs, which are analogs of different nucleotides, specifically tenofovir-diphosphate (a deoxyadenosine triphosphate dATP analog) combined with either emtricitabine-triphosphate, or lamivudine-triphosphate (both deoxycytosine triphosphate dCTP analogs). We will assume that the micro-kinetic parameters of the MMOA model (catalytic rate, binding affinity, excision rate) are not affected by the presence of the respective other NRTI, and nor are the concentrations of endogenous dNTPs.

Under these assumptions, the combined efficacy of two inhibitors I_1 and I_2 on viral DNA polymerization can readily be computed

$$1 - \varepsilon(I_1, I_2) = \frac{T_{0\to \mathrm{N}}(\emptyset)}{T_{0\to \mathrm{N}}(I_1, I_2)} \tag{SN2.12}$$

where the term $T_{0\to \mathrm{N}}(I_1, I_2)$ is the time required to complete viral DNA polymerization in presence of both inhibitors, which can be computed by integrating eqs. (SN2.4)–(SN2.9).

SN2.2.1 Residual cell infection

Finally, the residual cell infection in the presence of inhibitors I_1 and I_2 ($1 - \eta(I_1, I_2)$) can be computed:

$$1 - \eta(I_1, I_2) = \frac{1}{\rho_{\phi,\mathrm{RT}} + \frac{1-\rho_{\phi,\mathrm{RT}}}{1-\varepsilon(I_1,I_2)}} = \frac{1 - \varepsilon(I_1, I_2)}{1 - \rho_{\phi,\mathrm{RT}} \cdot \varepsilon(I_1, I_2)}, \tag{SN2.13}$$

where the term $\rho_{\phi,\mathrm{RT}} = 0.5$[6] is the probability to successfully complete reverse transcription before viral destruction in the absence of NRTIs.

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