Poster
TUPEB244

Forgiveness of Antiretroviral Regimens: *In Vitro* HIV-1 Viral Breakthrough with 2-Drug versus 3-Drug Regimens Simulating Variable Adherence to Treatment

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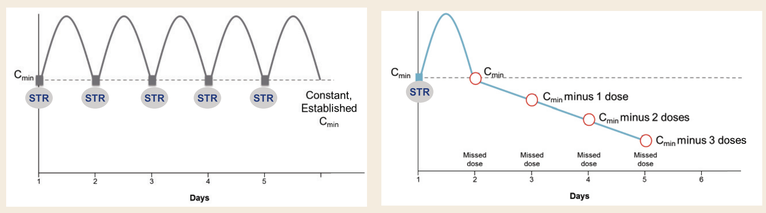
Background

- Current guidelines for the treatment of HIV-1 infection recommend triple therapy consisting of an integrase strand transfer inhibitor (INSTI) plus 2 nucleoside/tide reverse transcriptase inhibitors (NRTIs) including the single-tablet regimen (STR) of bictegravir+emtricitabine+tenofovir alafenamide (BIC/FTC/TAF) [1,2]
- BIC/FTC/TAF is approved for the treatment of HIV infection, has a high barrier to resistance [3-10], and may offer "forgiveness" (avoiding viral rebound and resistance in the setting of short-term non-adherence)
- Optimal adherence to daily oral antiretroviral therapy (ART) is important to minimize the emergence of drug resistance[4]. Whereas high adherence rates are achieved in clinical trials, treatment adherence rates in the real world are lower and not always predictable[11-13]
- A regimen's barrier to resistance is both genetic and pharmacologic. Drugs with higher resistance barriers are preferred. Variations in minimal drug concentrations (C_{min}) can occur due to natural variation between individuals, drug-drug interactions, food effects, and missed doses
  - Genetic Barrier: The number and sequence of mutations needed to generate resistance to a drug
  - Pharmacokinetic Barrier: The concentration of drug needed to suppress the virus, clinical drug concentrations, and drug half-life

Figure 1: Schematic of Antiviral Pharmacokinetics

- Missing antiretroviral doses results in a predictable decrease of systemic exposures to each drug in the regimen based on their established clinical half-life



- Recently, controlled clinical trials in treatment-naïve individuals have shown efficacy using an INSTI + 1 NRTI (dolutegravir/lamivudine; DTG+3TC) but the question remains whether the decreased number of regimen components may be less forgiving than triple therapy; virologic failure on DTG/3TC with emergent resistance to both DTG and 3TC has been reported (ACTG 5353)[28]

Objective

- To investigate BIC+FTC+TAF vs. DTG+3TC "forgiveness" by *in vitro* HIV-1 viral breakthrough selections and resistance emergence
  - In cells infected with either wild-type or low-level M184V
  - Drug exposures modeling full adherence and suboptimal adherence to ART

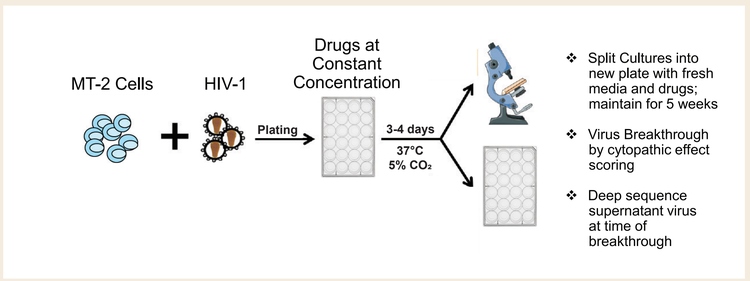
Methods

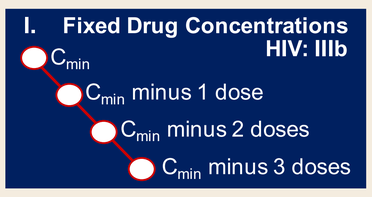
- **Viral Breakthrough Selections (Figure 2):** *In vitro* viral breakthrough selection experiments should be interpreted as a comparative *in vitro* study; clinical trials of missed doses for these ARV combinations have not been conducted. MT-2 cells were infected with HIV-1 (IIIb or clonal xxLAI strain containing varying levels of M184V, an FTC and 3TC resistance mutation). Infected cells were cultured in the presence of fixed concentrations of BIC+FTC+TAF or DTG+3TC, split every 3-4 days with fresh media containing drug, and monitored for viral breakthrough by cytopathic effect for up to 5 weeks. Supernatants containing breakthrough virus were collected and stored at the time of cytopathic effect. Statistical comparisons for experiments using the same starting virus backbone were made using Fisher's exact test.

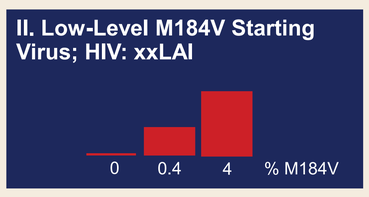
Figure 2. *In Vitro* Breakthrough Selection Methodology

Drugs: BIC + FTC + TAF or DTG + 3TC

- Viral Breakthrough Methodology







- **Test Drug Concentrations (Table 1):** BIC and DTG concentrations were calculated using their human plasma clinical trough concentrations (C_{min}) according to their prescribing information and adjusted for human plasma protein binding[15-17]. The TAF C_{min} concentration generated tenofovir-diphosphate (TFV-DP) at its physiological concentration in PBMCs from TAF-treated individuals[18]. FTC and 3TC concentrations were set at their human plasma-free adjusted C_{min} concentrations[18-20].
- **Missed Doses:** To simulate 1, 2, or 3 consecutive missed doses (C_{min} minus 1 dose, C_{min} minus 2 doses, and C_{min} minus 3 doses), drug concentrations were adjusted by their plasma half-lives for BIC and DTG and active metabolite half-lives for the NRTIs (TAF, FTC, and 3TC). C_{min} – X doses determined as C_{min}*(0.5^{24*X/ $t_{1/2}$}). Drug concentrations were kept constant in each selection.

Table 1. Drug Concentrations for Cell Culture Equivalents

	BIC + FTC + TAF			DTG + 3TC	
	BIC	FTC	TAF	DTG	3TC
Clinical Dose (mg)	50	200	25	50	300
Molecular Weight (g/mol)	449.4	247.2	534.5	419.4	229.3
Clinical C_{min} (µg/mL)[a]	2.61	0.096	0.008	1.11	0.042
Clinical C_{min} (nM)	5808	388	15	2515	265
Human Serum Shift[b]	43.6	1.0	1.0	27.5	1.0
Cell Culture Equivalent C_{min} (nM)[c]	133	388	15	91	265
$t_{½}$ (hr)[d]	17	37	116	14	17.5

a. C_{min} values are median values from United States prescribing information (USPI)
b. BIC and DTG data generated in-house by standard equilibrium dialysis shift in human serum versus cell culture media[15]
c. C_{min} / Human Serum Shift
d. $t_{1/2}$ for FTC, TAF, and 3TC represent intracellular half-life of the active di- or tri- phosphate metabolite[18-20]; $t_{1/2}$ values for BIC and DTG from USPI

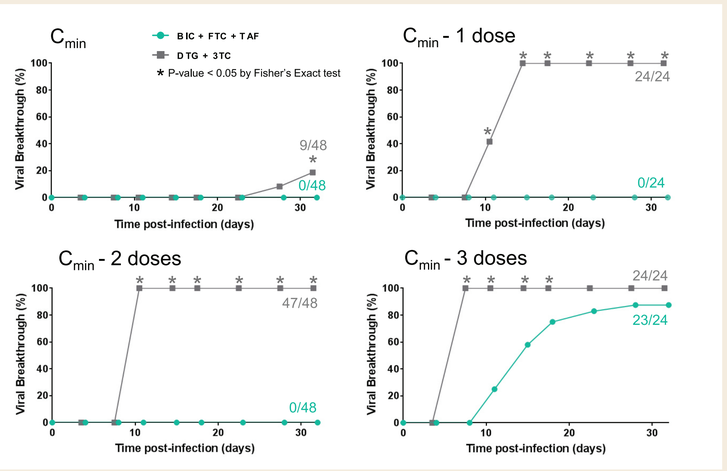
- **Genotypic Analyses:** Each well with viral breakthrough was sequenced by next generation sequencing (Seq-IT) and mutations were reported if present at >2%. Mutations were observed between 2.1% and 99% frequency per well.

Results

I. Regimen Forgiveness by *In Vitro* Breakthrough Selections: Constant Drugs levels at C_{min} and After Missing 1-3 Consecutive Doses (HIV-1 IIIb virus)

BIC + FTC + TAF	DTG + 3TC
C_{min} (no missed doses)	C_{min} (no missed doses)
C_{min} after missing 1 dose	C_{min} after missing 1 dose
C_{min} after missing 2 doses	C_{min} after missing 2 doses
C_{min} after missing 3 doses	C_{min} after missing 3 doses

I. Time to Viral Breakthrough: C_{min} and After Missing 1-3 Consecutive Doses (N=144)



I. Resistance in Breakthrough Viruses at C_{min} and After Missing 1-3 Consecutive Doses

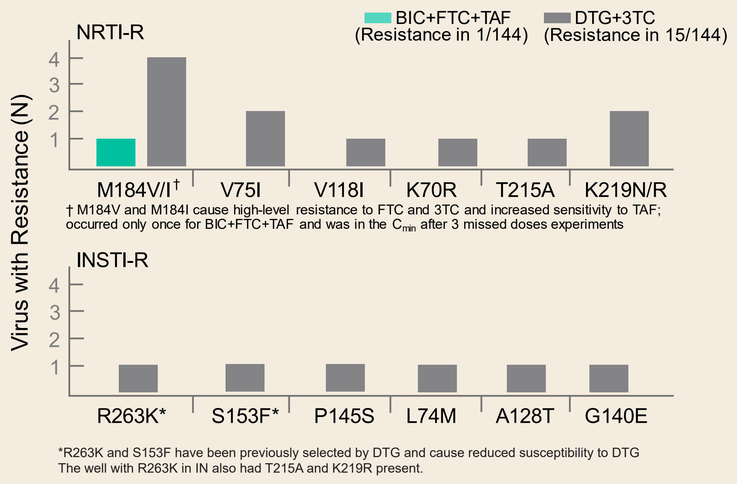
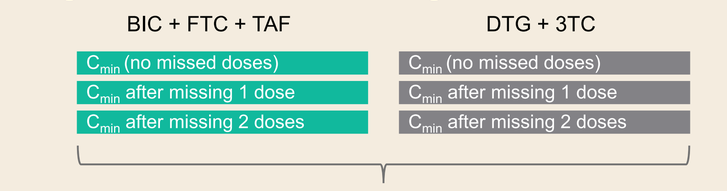


Table 2. Mechanisms of Forgiveness and Barrier to Resistance

	Mechanisms	BIC+FTC+TAF	DTG+3TC
Drug Levels	**Long plasma or intracellular $t_{1/2}$**		
	BIC or DTG[16-17]	17 hr	14 hr
	FTC-TP or 3TC-TP[18-20]	37 hr	17.5 hr
	TFV-DP[18]	116 hr	na
	Long IN/DNA dissociation $t_{1/2}$ for BIC or DTG[21-22]	132 hr	71-78 hr
Synergy and Mechanisms of Synergy	**Combination antiviral activity**		
	BIC + FTC or DTG + 3TC[15,23-25]	Synergy	Synergy
	BIC + TAF[15]	Synergy	na
	TFV + FTC[23-24]	Synergy	na
	TFV-chain-termination stabilized by Dead-End Complex with FTC-TP[25]	Increased TFV Activity	na
Resistance Profile	**Phenotype of M184V**		
	BIC or DTG[16]	Sensitive	Sensitive
	FTC or 3TC[17-18]	Resistant	Resistant
	TFV (TAF)[26]	Hypersensitive	na

II. Regimen Forgiveness by *In Vitro* Breakthrough Selections: Using Low-Level M184V xxLAI Starting Virus Mixtures



Note: Clonal xxLAI may have slower breakthrough kinetics than passaged IIIb virus

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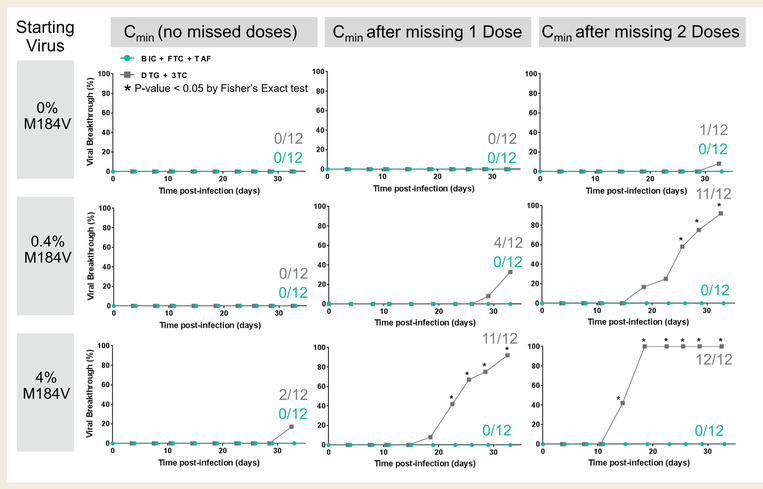

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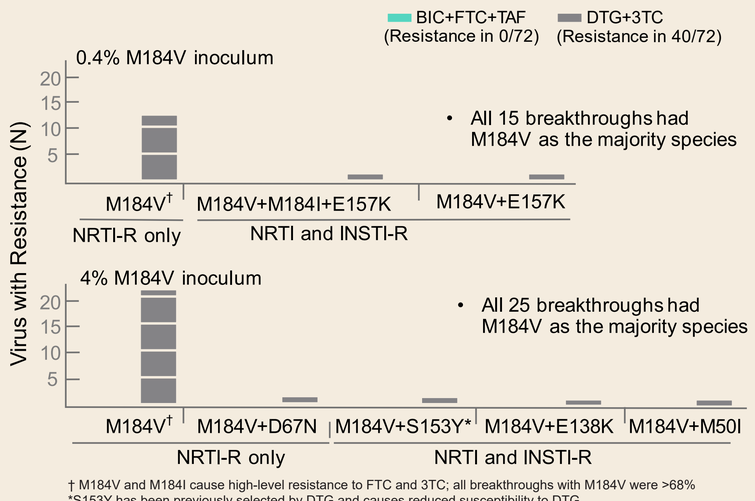

Conclusions

- *In vitro* viral breakthrough selection experiments help define the resistance barrier of ARV drug combinations and the resistance mutations that emerge. Here, two series of breakthrough experiments were conducted starting with wild-type or low-level M184V HIV and antiretroviral regimens set at drug *in vivo* concentrations or concentrations mimicking short lapses in adherence (1 to 3 consecutive missed doses)
- Using wild-type HIV, BIC+FTC+TAF was better at preventing *in vitro* viral breakthrough and emergent drug resistance compared to the 2-drug combination of DTG+3TC
  - BIC+FTC+TAF had viral breakthrough only in the wild-type experiments under the lowest drug concentrations tested (C_{min} after missing 3 doses). Only one viral breakthrough had emergent resistance with M184I in RT. In total, 23/252 wells had breakthrough and 1 had emergent resistance
  - In the wild-type experiments, DTG+3TC had 104/144 wells breakthrough and 15 of these had emergent resistance consisting of M184V/I and other mutations in RT, and other mutations in IN, including R263K and S153F, and one with resistance mutations in both RT and IN mutations. M184I has also been reported in DTG+3TC two-drug *in vitro* breakthrough experiments by another group[27]
- M184V causes resistance to FTC and 3TC and is one of the most frequent mutations to occur during virologic failure, is archived, and can be transmitted[29-31]. Pre-existing M184V at low levels in the inoculum had viral breakthroughs with DTG+3TC and high-level M184V with or without additional substitutions compared to BIC+FTC+TAF where no breakthrough occurred
- These *in vitro* studies suggest that the favorable pharmacology, antiviral synergy, and resistance profile provided by BIC+FTC+TAF may be more protective against virologic breakthrough and emergent resistance than DTG+3TC in the setting of non-adherence
- *In vitro* studies of the 'forgiveness' of ART regimens are important as they may help to predict regimen durability and risk of resistance in the real world, where sub-optimal adherence is more common

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