Poster 1448

Forgiveness of BIC/FTC/TAF: In Vitro Simulations of Intermittent Poor Adherence Find Limited HIV-1 Breakthrough and High Barrier to Resistance

Andrew Mulato, Rima Acosta*, Stephen R. Yant, Tomas Cihlar, and Kirsten White

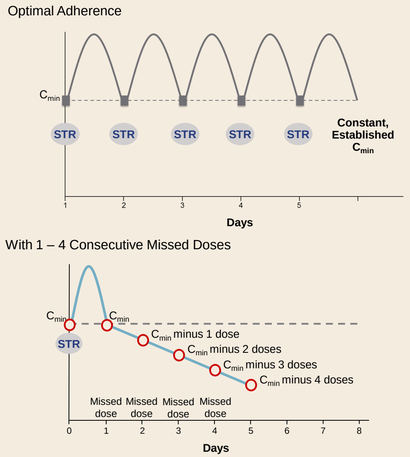
Gilead Sciences, Inc., Foster City, CA

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Tel: (650) 522-6009
Fax: (650) 522-5260

Background

- Current guidelines for first-line 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]
- The two-drug combination of the INSTI dolutegravir (DTG) and the NRTI lamivudine (3TC) (DTG+3TC) is recommended with certain restrictions based on baseline laboratory test results
- Although short lapses in adherence to antiretroviral (ARV) drugs can lead to virologic failure and emergence of drug resistance, certain drug regimens have high levels of "forgiveness" (avoiding viral rebound and resistance in the setting of suboptimal adherence)
  - Previous *in vitro* experiments have shown that when using an inoculum of wild-type HIV or HIV with low levels of the RT M184V substitution, BIC+FTC+TAF was better at preventing viral breakthrough and emergent drug resistance than DTG+3TC[3]
    - These experiments used drug concentrations at constant low levels; in the real world however, PLWH are more likely to intermittently miss one or more doses, resulting in fluctuating levels of drug
    - *In vitro* viral breakthrough experiments should be analyzed comparatively; clinical trials studying missed doses of these ARV combinations have not been conducted

Figure 1. Schematic of Antiviral Pharmacokinetics



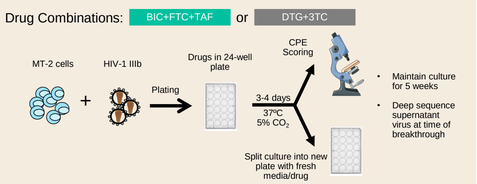
- Missing ARV doses results in a predictable decrease of systemic exposures to each drug in the regimen based on its established clinical half-life

Objective

- To investigate viral breakthrough and resistance development *in vitro* under constant low drug exposures or alternating high and low drug exposures, simulating variable adherence levels

Methods

Figure 2. *In Vitro* Viral Breakthrough Selections



- **Viral Breakthrough Selections (Figure 2):** MT-2 cells were infected with HIV-1 IIIb. Infected cells were cultured in the presence of fixed or weekly alternating 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.
- **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[4-6]. The TAF C_{min} concentration used generated the active metabolite tenofovir diphosphate (TFV-DP) at its physiological concentration in peripheral blood mononuclear cells (PBMCs) from TAF-treated individuals[7]. FTC and 3TC concentrations were set at their human plasma-free adjusted C_{min} concentrations[7-9].

Table 1. Drug Concentrations for Cell Culture Equivalents

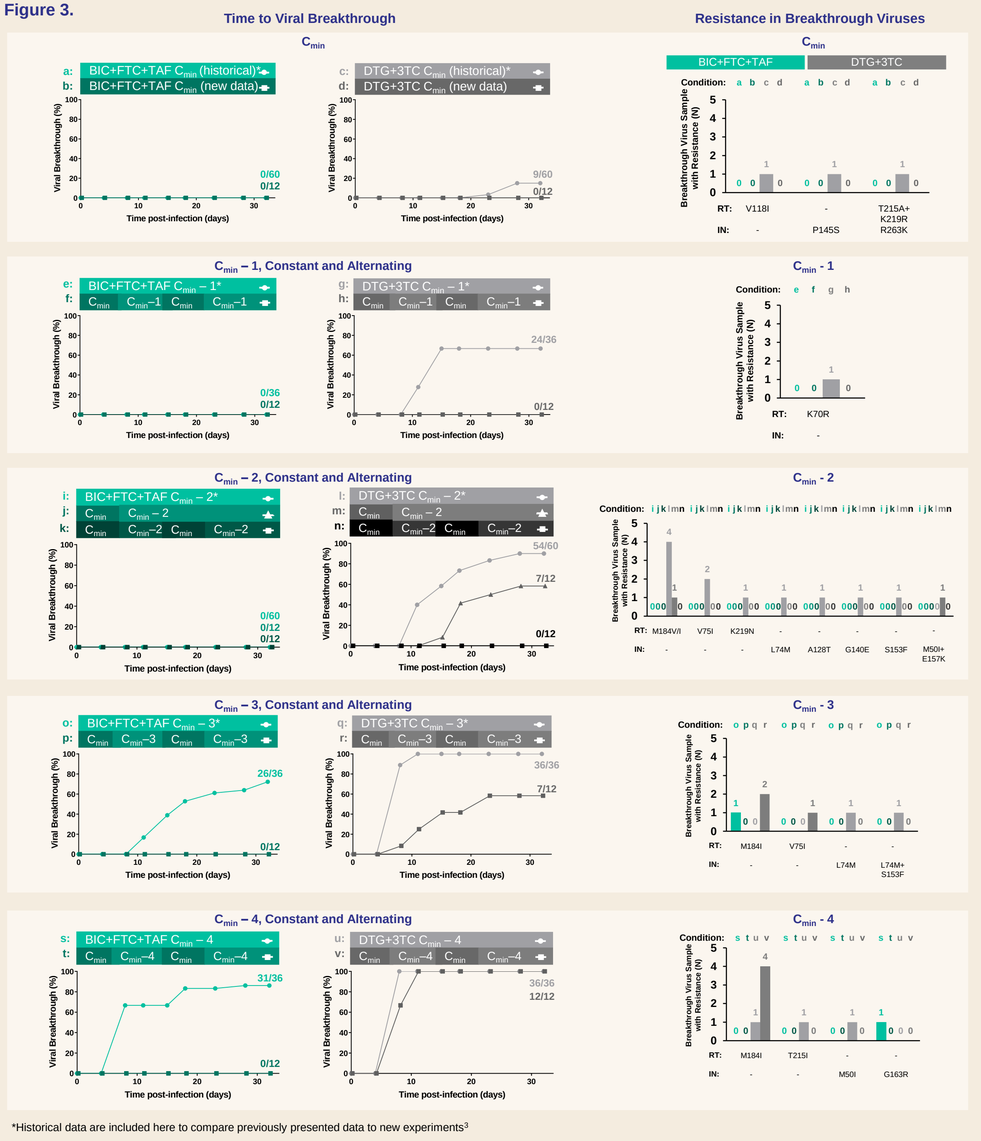
	BIC+FTC+TAF			DTG+3TC	
	BIC	FTC	TAF	DTG	3TC
Clinical Dose (mg)	50	200	25	50	300
Molecular Weight (g/mol)	449.4	247.2	534.5	419.4	229.3
Clinical C_{min} (µg/mL)[a]	2.61	0.096	0.008	1.11	0.042
Clinical C_{min} (nM)	5808	388	15	2515	265
Human Serum Shift[b]	43.6	1.0	1.0	27.5	1.0
Cell Culture Equivalent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

a. Median C_{min} values obtained 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[4]
c. C_{min} / Human Serum Shift values
d. $t_{1/2}$ for FTC, TAF, and 3TC represent intracellular half-life of the active di- or tri-phosphate metabolite[7-9]; $t_{1/2}$ values for BIC and DTG from USPI

- **Simulation of Missed Doses:** To simulate 1, 2, 3, or 4 missed doses (C_{min} minus 1 dose, C_{min} minus 2 doses, C_{min} minus 3 doses, C_{min} minus 4 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 was determined as C_{min}*(0.5^{24*X/$t_{1/2}$}). Drug concentrations alternated between C_{min} and C_{min} – X in each selection.
- **Genotypic Analyses:** Each viral breakthrough supernatant was sequenced by next generation sequencing (Seq-IT, Germany) and mutations were reported if present at ≥2%. Mutations were observed between 2.1% and 44.1% per well.

Results

Figure 3.



*Historical data are included here to compare previously presented data to new experiments[3]

Table 2. Summary of Viral Breakthrough and Resistance Development

In Vitro Dosing (By Week)					Breakthrough Frequency (Resistance Development)			
					BIC+FTC+TAF		DTG+3TC	
Week 1	Week 2	Week 3	Week 4	Week 5	Break-through (n/N; %)	With Resistance (n)[b]	Break-through (n/N; %)	With Resistance (n)[b]
C_{min} (constant)[a]					0/60; 0	0	9/60; 15	Other (3)
C_{min} (constant)					0/12; 0	0	0/12; 0	0
C_{min}-1 (constant)[a]					0/36; 0	0	24/36; 67	Other (1)
C_{min}	C_{min}-1	C_{min}	C_{min}-1		0/12; 0	0	0/12; 0	0
C_{min}-2 (constant)[a]					0/60; 0	0	54/60; 90	M184V/I (4); Other (7)
C_{min}	C_{min}-2				0/12; 0	0	7/12; 58	M184I (1); Other (1)
C_{min}	C_{min}-2	C_{min}	C_{min}-2		0/12; 0	0	0/12; 0	0
C_{min}-3 (constant)[a]					26/36; 72	M184I (1)	36/36; 100	Other (2)
C_{min}	C_{min}-3	C_{min}	C_{min}-3		0/12; 0	0	7/12; 58	M184I (2); Other (1)
C_{min}-4 (constant)					31/36; 86	Other (1)	36/36; 100	M184I (1); Other (2)
C_{min}	C_{min}-4	C_{min}	C_{min}-4		0/12; 0	0	12/12; 100	M184I (4)

a. Previously reported data. b. Other mutations are listed in Figure 3.

Summary of Results

- Alternating drug exposures between low concentrations and short exposures to higher concentrations lowered the occurrences of viral breakthrough
  - Viral breakthrough with BIC+FTC+TAF occurred in 2/11 conditions
    - Breakthrough was seen at the lowest two constant drug exposures (C_{min}-3 and C_{min}-4 simulating missing 3 and 4 consecutive doses)
    - Breakthrough was prevented with short C_{min} drug exposures
  - Viral breakthrough with DTG+3TC occurred in 9/11 conditions
    - Breakthrough occurred in drug exposures as high as constant C_{min}; lower constant drug exposures led to faster and more complete breakthrough
    - Intermittent C_{min} exposures helped delay or prevent breakthrough
- Resistance-associated mutations were observed in some cultures with viral breakthrough, in both the alternating drug exposure and constant drug exposure experiments
  - 2 of 57 (3.5%) BIC+FTC+TAF breakthroughs and 29 of 185 (16%) DTG+3TC breakthroughs had emergent resistance mutations

Conclusions

- BIC+FTC+TAF has high *in vitro* forgiveness and consistent protection against the emergence of drug resistant HIV during simulations of short lapses in adherence
- Higher DTG+3TC exposure, whether constant or intermittent, was better at preventing or delaying viral breakthrough than lower DTG+3TC exposures, but DTG+3TC was consistently less forgiving than BIC+FTC+TAF
- Prevention of viral replication and resistance development is necessary to maintain lifelong viral suppression, particularly in the real world where drug adherence is often imperfect

References

1. DHHS. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. December 18, 2019; **2**. EACS. Guidelines v10.0. November 2019; 3. Mulato A, et al. IAS (2019) Presentation TUPEA103; **4**. Tsiang M, et al. AAC (2016) 60(12): 7086-7097; **5**. BIKTARVY® United States Prescribing Information; **6**. TIVICAY® United States Prescribing Information; **7**. Dickinson L, et al. AAC (2015) 59(10): 6080-6086; **8**. Else LJ, et al. AAC (2011) 56(3): 1427-1433; **9**. Yuen GJ, et al. AAC (2004) 48(1): 176-182.

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