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Primary CD4 Subsets are Similarly Loaded by Tenofovir Alafenamide

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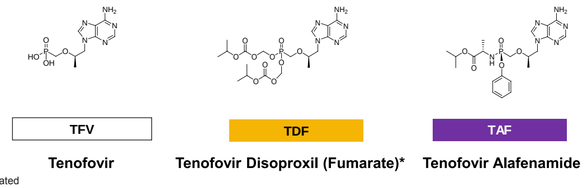
Introduction

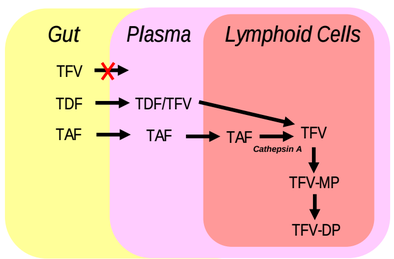
- Tenofovir alafenamide (TAF) is a new oral prodrug of the HIV-1 NRTI tenofovir (TFV) (Figure 1)
- TAF is more stable in human plasma and delivers more intracellular TFV diphosphate (TFV-DP) in PBMCs compared to tenofovir disoproxil fumarate (TDF) (Figure 2)[1,2]
- In a 10 day monotherapy study, 25 mg TAF resulted in a 5-fold increase in intracellular TFV-diphosphate (TFV-DP) and a 90% reduction in plasma TFV (compared to 300 mg TDF)[3]
- In vitro analysis showed that CD4+ T-cells and Monocyte-Derived-Macrophages (MDM) from diverse populations were equivalently loaded when treated with TAF[4]
- TFV-DP levels among subsets of primary human CD4+ T-cells following TAF treatment has not been established. Intracellular TFV-DP levels were evaluated and compared in multiple CD4+ T-cell subsets at clinically relevant TAF concentrations

Figure 1. Structure of Tenofovir and its Prodrugs



*: fumarate salt not indicated

Figure 2. TAF Improves the Delivery of Intracellular TFV-DP and Reduces Circulating TFV

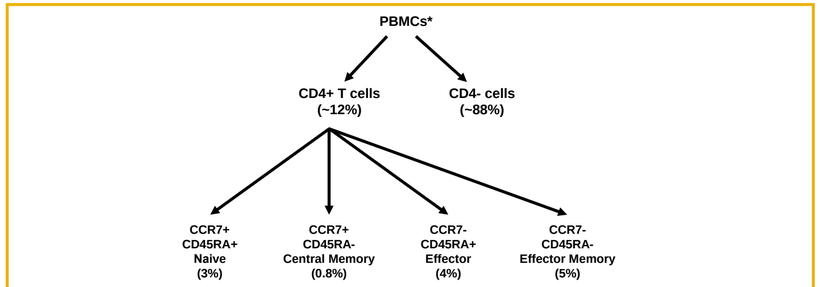


Methods

- TAF loading studies were conducted in total CD4+ T cells, as well as in naive, effector, central memory, and effector memory CD4+ T cell subsets from healthy human donors
- Naïve, memory or total CD4+ T cells were enriched using magnetic beads and CD4 subsets were isolated by fluorescence-analysis cell sorting based on CD3, CD4, CD45RA, and CCR7 expression (Figure 3)
  - Each CD4 subset comprised ≤ 5% of the total PBMC population; LeukoPaks were used to achieve the desired cell number for these analyses
- TAF loading was evaluated using a 2 hour pulse and washout method (Figure 4)
  - Method mimicked in vivo exposure, where >95% TAF is eliminated from plasma within 2 hours of dosing[5]
  - Cells were harvested at 2 and 24 hours post-treatment initiation
- Two concentrations of TAF were evaluated
  - Previous studies demonstrated that treating PBMCs with 200-400 nM TAF resulted in intracellular TFV-DP levels comparable to those observed *in vivo* with 25 mg TAF dose (0.677 µM)[5] (Figure 5)
- Harvested cells were spun through an oil layer (Nyosil M25). Cell extracts (containing TFV-DP) were generated from the purified cell pellets with 70% MeOH and TFV-DP was measured by LC/MS/MS

Results

Figure 3. CD4 Subsets & Isolation Strategy



*% of cell population within PBMCs are indicated

Adapted from Maecker *et al. (6)*

Figure 4. TAF Loading Procedure

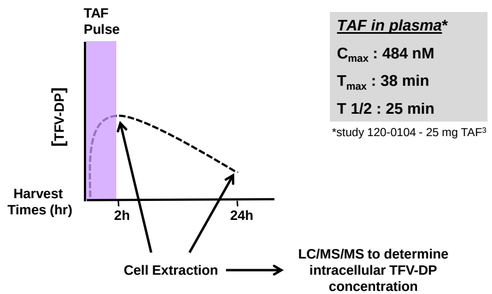
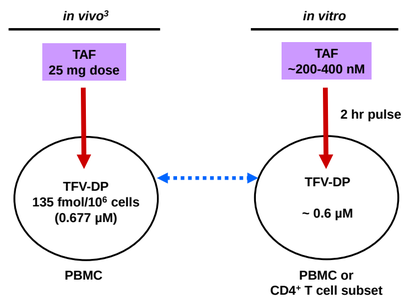


Figure 5. Experimental Design



- TAF loading studies in PBMCs demonstrated that a 2h pulse followed by a 22h washout of 200-400 nM TAF achieved TFV-DP levels comparable to those observed in vivo following 10 days of 25 mg QD TAF[5] (Figure 5)
- Comparable TFV-DP levels were achieved in total CD4+ T cells as well as all CD4+ T cell subsets after a 2h pulse with either 200 or 400 nM TAF (Figure 6)
- Minimal variation in intracellular TFV-DP was observed across donors
- There was no significant decrease in TFV-DP levels across all subsets evaluated after a 22 hour washout (Table 1); TFV and TFV-MP provided intracellular reserve for TFV-DP (not shown)
- The long intracellular half-life of TFV-DP was observed with all subsets (Table 1), consistent with clinical study results demonstrating sustained TFV-DP levels in PBMCs following TAF dosing[3]
- A trend for higher TFV-DP levels in the effector and central memory subsets compared to the other subsets was observed

Figure 6. Intracellular TFV-DP Levels are Stable over 24h in All CD4+ T Cell Subsets

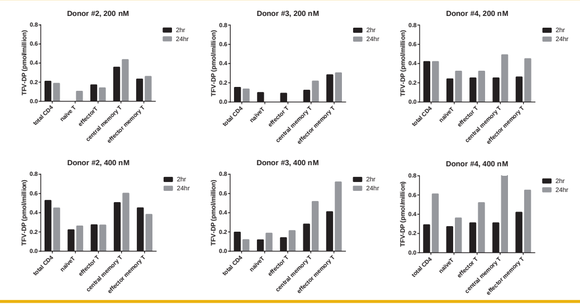


Table 1. Average TFV-DP Levels and TFV-DP Ratio in CD4+ T Cell Subsets

TAF pulse	CD4+ T-cell categories	TFV-DP levels; µM (2h)*	TFV-DP levels; µM (24h)	TFV-DP Ratio (24h/2h)
200 nM	Total CD4+	1.30	1.23	0.9
	Naïve	0.83	1.05	1.2
	Effector Memory	1.28	1.68	1.3
	Effector	0.85	1.15	1.3
	Central Memory	1.20	1.90	1.5
400 nM	Total CD4+	1.68	1.96	1.1
	Naïve	1.01	1.35	1.3
	Effector Memory	2.12	2.92	1.3
	Effector	1.21	1.66	1.3
	Central Memory	1.82	3.25	1.7

*:average values; calculated with a cell volume 0.2 µL/10^6 cells

Conclusions

- **CD4+ T cell subsets were similarly loaded by TAF *in vitro*, with all subsets achieving high and persistent levels of TFV-DP, similar to the TAF exposure observed in PBMCs *in vivo*[3] and *in vitro*[5]**
- **Maintenance of TFV-DP levels 24h post-treatment initiation suggest that high levels of TFV-DP will be maintained across most relevant CD4+ T cell subsets in patients receiving TAF QD**
- **The observed TAF exposures *in vitro* were also similar to those observed in clinical studies with E/C/F/TAF[7] and other TAF fixed dose combinations[8]**
- **Sustained TFV-DP levels observed in this study are in line with previous findings that TFV-DP has the longest intracellular half-life for the NRTI class[9] and may be beneficial for patients that miss a dose**
- **The higher TFV-DP levels in the effector and central memory subsets may have implications for maintaining viral suppression in latent viral reservoirs and for future HIV cure efforts**

References

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