Urine Assay for Tenofovir to Monitor Adherence to Tenofovir/Emtricitabine as PrEP

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### Background
- Tenofovir/Emtricitabine (TDF/FTC) reduces HIV transmission and is approved for pre-exposure prophylaxis (PrEP).
- PrEP for HIV prevention is at least 92% effective when taken daily.¹-⁴
- Adherence is critical for the success of PrEP.
- Current adherence measurements, self-report, and plasma tenofovir (TDF) levels are inadequate tools for monitoring adherence in real-time.⁵

### Objective
- Our goal was to develop and validate a urine assay to measure TDF levels to objectively monitor adherence to PrEP.

### Methods
- We conducted 3 cohort studies to validate the assay:
  1. Qualitative evaluation of the relationship of urine TDF to plasma TDF in 10 HIV+ subjects with undetectable HIV viral loads on a TDF-based regimen
  2. Quantitative evaluation of TDF clearance in plasma and urine over 7 days in 10 HIV-negative subjects who received a single dose of TDF/FTC
  3. Concordance between plasma and urine TDF levels over time was assessed in a 16 week study of 10 HIV-negative individuals on daily TDF/FTC for PrEP

### Results

#### The assay
- We developed a semi-quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) urine assay with high sensitivity/specificity for TDF.
- This assay allowed us to determine TDF concentrations in log categories between <10 ng/mL to >10,000 ng/mL.

#### Cohort 1 (Relationship between urine and plasma concentration in well-controlled individuals)

- 100% concordance btw presence of TDF in plasma & urine (PPV 100%, 95 CI, 0.63-1; NPV 100%, 95 CI, 0.05-1)
- TDF concentration 3-4 logs higher in urine than plasma

#### Cohort 2 (Clearance of TDF in plasma & urine)
- TDF detected for >7 days in urine and 2-4 days in plasma after a single dose of Truvada
- Urine TDF is detected for >7 days in urine and 2-4 days in plasma
- TDF is detected for >10 ng/mL to >100 ng/mL
- TDF concentration range: >10 ng/mL to >10,000 ng/mL
- Tenofovir (TDF) calibration curve in human urine over a concentration range of 10 to 10000 ng/mL. A known concentration of TDF was injected into a human urine sample (a solid) and measured using LC-MS/MS (y axis). [TDF] = tenofovir concentration.

#### Cohort 3 (Urine assay in patients on PrEP)
- TDF detected in 93% of urine samples (concentration range: >10 ng/mL to >10,000 ng/mL)
- TDF detected in 74% of plasma samples (concentration range: >10 ng/mL to >100 ng/mL)
- Urine TDF concentration > 1000 ng/mL highly predictive of presence of TDF in plasma (>10 ng/mL) (PPV 0.88, 95%CI, 0.69-0.97; NPV 0.88, 95%CI, 0.47-0.99)

### Conclusions
- The urine assay correlates highly with plasma TDF levels and is more sensitive for TDF over 7 days.
- Levels of urine TDF could be used to distinguish between recent adherence with a dose of TDF in last 48 hours (>1000ng/mL), low adherence (>100ng/mL), and non-adherence with >1 week since the last dose (<10 ng/mL).

### Future Steps
- Will real-time adherence monitoring with the urine assay lead to clinically significant and sustained increase in adherence to PrEP?
- Can the urine assay be further developed into a point of care test?

### References