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# PHARMACOKINETICS OF TENOFOVIR ALAFENAMIDE BY SUBCUTANEOUS IMPLANT FOR HIV PREP

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## Background

Previously, we reported on a biodegradable, retrievable, subcutaneous implant we called thin-film polymer device (TFPD) for long-term delivery of antiretroviral (ARV) drugs for pre-exposure prophylaxis (PrEP)[1, 2]. We modified fabrication and are presently developing an extruded polymer device (EXPD) that mitigates some of the deficiencies that were associated with TFPD (i.e., ARV solubility, membrane properties and device geometry). We assessed *in vitro* and *in vivo* release of tenofovir alafenamide (TAF) hemifumarate from EXPDs, to demonstrate i) sustained tenofovir diphosphate (TFV-DP) concentrations in PBMCs, ii) continuous non-fluctuating plasma concentration of TAF for extended period and iii) the safety and tolerability of EXPD implants in animals.

## Introduction

**Extruded Polymer Device (EXPD)**

- User-independent, biodegradable, subcutaneous implant capable of achieving sustained release of ARV drugs with zero-order release kinetics for HIV PrEP
- Target implant geometry of 2-2.5 mm (diameter) x 40 mm (length)
- Compatible with existing trocar applicators

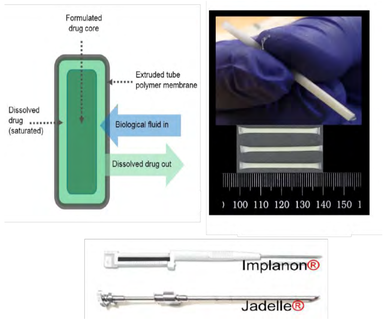



Figure 1. Systematic representation of the EXPD (upper left), EXPD prototypes (upper right) and examples of existing trocar applicators under consideration (bottom).

## Materials and Methods

- **Tenofovir alafenamide (TAF) hemifumarate** – nucleotide reverse transcriptase inhibitor (NRTI) was kindly provided by Gilead Sciences (Foster City, CA).

**Formulation of EXPD**

- Heat extruded polycaprolactone (PCL) EXPD were fabricated (2.5mm x 40mm) using 80kDa PCL (GenX Medical, Chattanooga, TN USA) to produce devices with wall thickness of 70 µm.
- Devices were loaded with a paste consisting of TAF and castor oil in a 2:1 ratio. A heat sealer was used to form continuous seams.
- Target *in vitro* release rate of <1 mg/day.
- Devices were sterilized by gamma irradiation prior to use.

***In vitro* Evaluation**

- *In vitro* release of TAF from EXPD implants was evaluated in phosphate buffered saline (PBS) pH 7.4 in a shaking incubator at 37°C and 120 rpm. Appropriate volumes (15-100 mL) and exchange rates (1-3 days) were maintained to ensure sink concentrations were not exceeded. Concentrations of TAF in buffer were quantified via UV spectroscopy.

**Pharmacokinetic Evaluation in Rabbits**

- Animal study was conducted at the Magee Womens Research Institute in accordance with IACUC guidelines using an approved protocol.
- EXPDs were surgically implanted for subcutaneous delivery of TAF into the dorsal-scapular region of adult female New Zealand White rabbits (n=6; 2.5 kg body weight). The vehicle (castor oil) EXPD implants (n=4) were used as control.
- Animals were monitored daily for signs of pain or distress.
- Blood samples were collected at 1, 3, 7, 10, and 14 days and then weekly until day 91 whereas blood samples for peripheral blood mononuclear cells (PBMCs) were collected on a weekly basis starting on day 7.
- Implants were retrieved and target tissue samples (vagina, cervix, rectum) harvested on day 49 from a subset of animals (n=2) in each group. The remaining animals were euthanized on day 91.
- Drug and metabolite concentrations were determined via liquid chromatographic-tandem mass spectrometric analysis (LC-MS/MS). Here we report plasma tenofovir (TFV) and TAF concentrations (ng/mL) and PBMC TFV diphosphate (TFV-DP) concentrations (fmol/$10^6$ cells). Below limits of qualification (BLQ) for TAF in plasma was <0.03 ng/ml), BLQ for TFV in plasma and tissue were <0.31 ng/mL and <0.05 mg/sample, respectively. The BLQ for TFV-DP in PBMC and tissue was determined to be <50 fmol/sample for both. Metabolite results were normalized to number of PBMCs or weight of tissue analyzed.
- Residual drug analysis of the devices used in the *in vitro* release and PK studies was determined by ultra performance liquid chromatographic (UPLC).
- Evaluation of tissue compatibility to the EXPDs at the site of implantation was conducted by histopathological examination and data reviewed by a pathologist .

## In Vitro Release of TAF

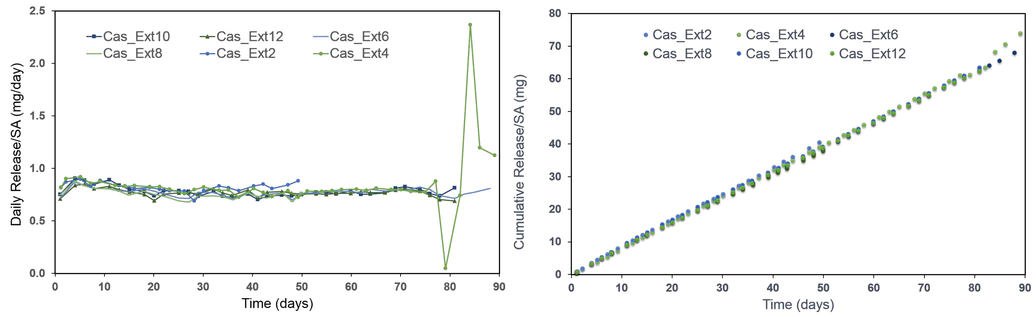



Figure 2. The daily release rate of the *in vitro* devices was determined to be 0.75±0.06 mg/d (left panel) and demonstrated sustained release and zero-order release over days 0 to 70 with a $R^2$ value of 0.98 (right panel). By day 80, some of the EXPDs exhibited erratic release profiles near the intended duration of the implant.

## Plasma and PBMC Pharmacokinetic Profile

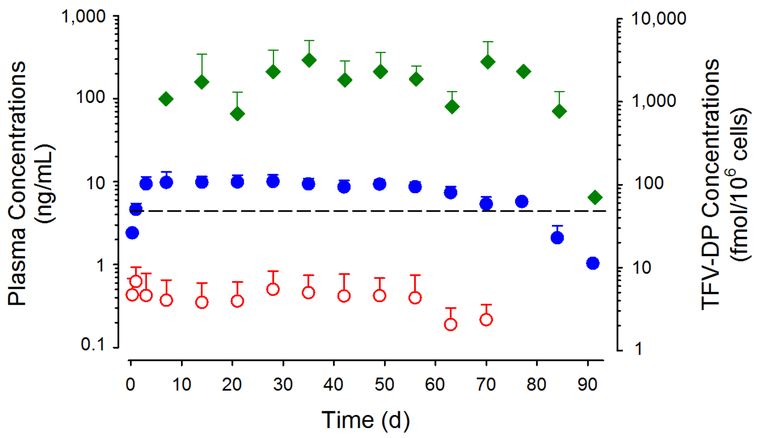



Figure 3. Overall, PBMC TFV-DP and plasma TFV were sustained throughout the 3-momth study. The pharmacokinetic results demonstrate that plasma TAF concentrations were detectable as early as 6 hours after implantation and continued to be maintained before plasma concentrations were no longer detectable after day 70. Between day 7 and day 70 where all three analytes were detectable, the median concentrations (±SD) for PBMC TFV-DP, plasma TFV and plasma TAF were 1957±846 fmol/$10^6$ cells, 9.3±1.4 ng/mL and 0.4±0.12 ng/mL, respectively. Please note that PBMC TFV-DP concentrations reported in this study are well above target for HIV PrEP[3,4].

## Individual PK Data

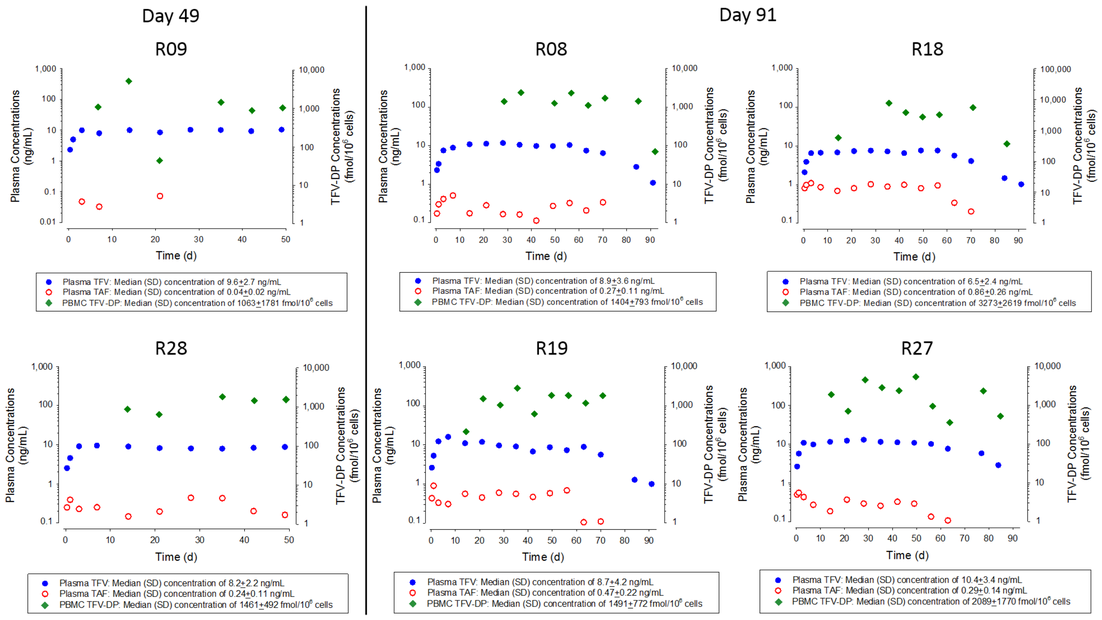



Figure 4. Data from the Day 49 (R09 and R028) and Day 91 (R08, R18, R19 and R27) animals demonstrated similar PK profiles for PBMC TFV-DP, plasma TFV, and plasma TAV concentrations indicating that the performance of the EXPD implants were nearly identical.

## TFV-DP Concentrations in Target Tissues

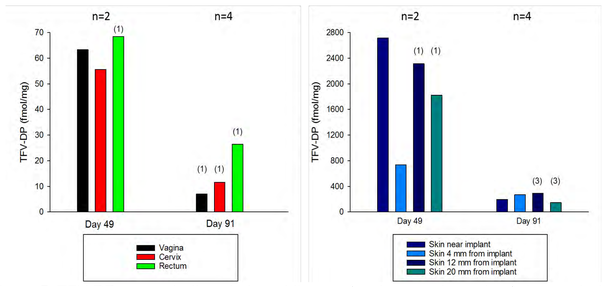



Figure 5. TFV-DP concentrations in target tissues (vagina, cervix, rectum) demonstrate that TFV-DP was detectable in most tissues at 49 days but levels decline in most tissues at day 91 (left panel). [Note: number in the parentheses indicate the number of animals with detectable levels of 2 animals on day 49 or 4 animals on day 91]. These findings indicate additional work in systemic delivery of TAF will be required to achieve higher exposure of TFV-DP in target tissues at a longer duration. The significance of high TFV-DP levels in skin tissue near the implantation site is currently unknown and warrants further investigation (right panel).

## Residual Drug Analysis of Used Implants

| Study Arm | Device ID | Initial TAF (mg) | TAF in Device (mg) | TAF Released (mg) | TAF Purity (%) |
|---|---|---|---|---|---|
| **Day 49 Devices** | | | | | |
| In Vitro | Cas_Ext_2 | 110.2 | 66.0 ± 1.7 | 44.2 | 88.6 |
| In Vitro | Cas_Ext_8 | 109.6 | 69.9 ± 1.6 | 39.7 | 90.0 |
| PK | R09 | 109.0 | 54.6 ± 0.2 | 54.4 | 83.2 |
| PK | R28 | 108.6 | 59.1 ± 1.2 | 49.5 | 83.0 |
| **Day 91 Devices** | | | | | |
| In Vitro | Cas_Ext_4 | 109.8 | 3.9 ± 0.1 | 105.9 | 10.0 |
| In Vitro | Cas_Ext_6 | 111.1 | 4.2 ± 0.1 | 106.9 | 8.7 |
| In Vitro | Cas_Ext_10 | 109.0 | 2.2 ± 0.1 | 106.8 | 5.3 |
| In Vitro | Cas_Ext_12 | 110.2 | 3.5 ± 0.1 | 106.7 | 7.6 |
| PK | R08 | 109.4 | 0.6 ± 0.03 | 108.8 | 1.5 |
| PK | R18 | 109.7 | 0.2 ± 0.0 | 109.5 | 0.4 |
| PK | R19 | 110.6 | 0.3 ± 0.02 | 110.3 | 0.5 |
| PK | R27 | 109.9 | 2.6 ± 0.5 | 107.3 | 6.5 |

The data in the table above demonstrates a good correlation for the EXPDs used in these studies in terms of *in vitro* release rate of 0.75 ± 0.06 mg/d when compared to the release rate of 0.69 ± 0.12 mg/d in the rabbit PK study. The goal of targeting *in vitro* release rate of <1 mg/day was achieved. These findings indicate that greater than 98% of TAF payload was delivered by day 91. The degradation of TAF in the devices indicate additional work (e.g., polymer thickness, different expedient, stabilizer) is required prior initiation the next *in vivo* study.

## Tissue Compatibility

While clinical observations indicated cutaneous response to the drug-containing EXPDs near the implantation site, assessment of bacterial infections were negative and histopathological changes findings were determined to be unremarkable.

## Conclusions

- TAF implants maintained sustained PBMC TFV-DP concentrations well above that predicted for HIV PrEP in humans.
- The sustained plasma TAF concentrations and the *in vivo* release rate of the active implants correlated well with the *in vitro* TAF release from similar devices.
- Taken together, these data indicate that the EXPD TAF device is suitable for continued development as a long-acting subcutaneous implant for HIV PrEP.

## References

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2. Schlesinger et al. (2016). Pharm. Res. *33(7):1649-56.*
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