Abstract 435

# Potential TDF/EVG Nanoparticle Formulation: Plasma Pharmacokinetics in Humanized Mice Model

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## *Revised* Abstract

**OBJECTIVE:** One of the highest priorities of HIV research is to have access to long-acting pharmaceutical formulations for HIV treatment and prevention. Our research aims at fabricating long lasting combination antiretroviral (cARV) drugs loaded nano-system for PrEP. Presently we are reporting on the pharmacokinetics and intracellular drug retention studies of tenofovir disoproxil fumurate (TDF) and elvitegravir (EVG) loaded nanoparticles (TDF +EVG NP) in humanized mouse model (huNSG).

**METHODS:** Formulation of cARV loaded nanoparticles (NP) by oil-in-water emulsion approach. Poly(lactic-co-glycolic acid) (PLGA), a biodegradable/ biocompatible polymer was used to formulate the TDF and EVG drugs loaded NPs (TDF+EVG NPs) using our standard methodology. For pharmacokinetic studies, female huNSG mice (Jackson Labs, Inc.) were treated with TDF+EVG NPs (50 mg/kg administered SubQ in 1 ml D5W). At specific times (1, 2, 4, 7, 10, and 14 days post-SubQ administration), mice (n=3/time point) were euthanized, blood was harvested, centrifuged, and plasma was analyzed for tenofovir (TNF) and EVG. The plasma concentrations of the drugs were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Intra-day and inter-day variability was < 4%. Plasma concentration-time was modeled using a non-compartmental modeling. Peak plasma levels and time to peak were determined by graph inspection.

**RESULTS:** TDF+EVG NP size averaged 136.7 nm and drug entrapment efficiency of TDF and EVG was 43.2% and 42.2%, respectively. Mean (± SD) peak plasma TNF and EVG levels were 362.8 ± 275.7 and 58.1 ± 28.3 ng/mL and time to peak was 7.6 and 4.3 days after SubQ administration, respectively. Using non-compartmental modeling, plasma pharmacokinetics (PK) parameters for TNF and EVG from NP formulation (TDF/EVG NP), respectively, were $AUC_{0\text{-}14}$ 35.1 ± 22.9 (TNF) and 6.8 ± 4.7 μg * h/L (EVG). Based on modeling, an interval of one SubQ dose every 7 days would provide detectable drug in plasma throughout the dosing interval above 40 ng/mL of each drug under consideration.

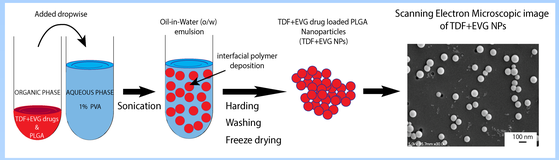
**CONCLUSION:** The simultaneous administration of TDF and EVG in single NP formulation shows prolonged PK parameters of TNF and EVG. Further tissue concentrations in female reproductive tract (FRT) and injection site are needed to determine if this will be an appropriate prevention option. This is the first report of using NRTI+ISTI in a nanoparticle formulation for PrEP.

## Introduction:

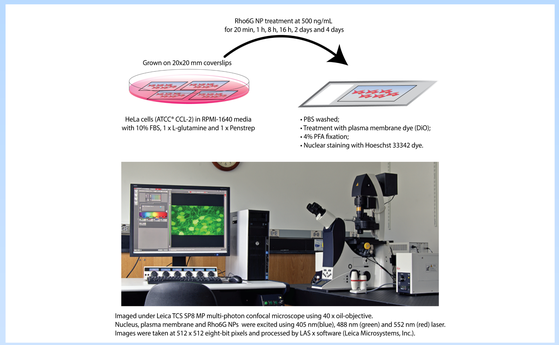
- For HIV-1 preventive campaign, additional PrEP modalities are needed to be proposed/tested.
- Currently, Truvada™ (TDF+FTC) is the only FDA-approved oral tablet for PrEP[1].
- Nanomedicines are long-acting injectables being investigated for PrEP.
- A sustained-release injectable PrEP modality could be a desired option.
- Polymeric nanomedicines containing combination ARV compounds are being investigated for PrEP.
- We report here the SubQ administration of tenofovir disoproxil fumurate and elvitegravir nanoparticles (TDF+EVG NPs) as a PrEP modality.
- The plasma PK of tenofovir (TNF) and elvitegravir (EVG) are presented.
- Finally, the TNF and EVG concentrations in FRT and injection site tissue is presented.

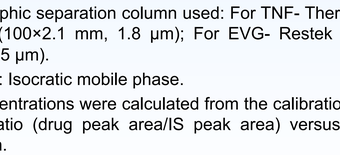
## Methods

◆ Fabrication procedure:



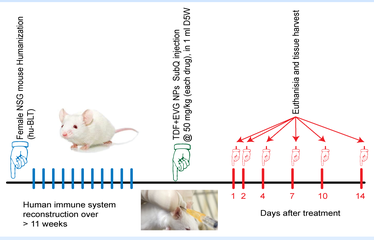

◆ Confocal analysis[2]:



Imaged under Leica TCS SP8 MP multi-photon confocal microscope using 40 x oil-objective.
Nucleus, plasma membrane and Rho6G NPs were excited using 405 nm(blue), 488 nm (green) and 552 nm (red) laser.
Images were taken at 512 x 512 eight-bit pixels and processed by LAS x software (Leica Microsystems, Inc.).

◆ Mouse study design:




◆ **LC-MS/MS procedure:**

- Stock solutions: EVG in methanol; Tenofovir (TNF) in water:methanol (80:20, v/v).
- Working standard solutions was prepared in water:methanol (50:50, v/v).
- Labeled internal standards spiking solution: Serial diluting of stocks in methanol.
- Plasma calibration curve: EVG- from 5 to 2000 ng/mL; TNF- 25 to 2000 ng/mL.
- Sample processing:

  *Plasma samples*- Protein precipitation in methanol.

  *Tissue samples*- Homogenized in water (1:2, w/v) followed by protein precipitation in methanol
- LC-MS/MS Instrument: An Agilent 1200 HPLC system (Agilent Technologies, CA, USA) coupled with AB Sciex API 3200 Q Trap with an electrospray ionization (ESI) source (Applied Biosystems, Foster City, CA).
- Chromatographic separation column used: For TNF- Thermo Accucore RP-MS column (100×2.1 mm, 1.8 μm); For EVG- Restek BD BiPh column (2.1×50 mm, 5 μm).
- Method used: Isocratic mobile phase.
- Sample concentrations were calculated from the calibration curve based on peak area ratio (drug peak area/IS peak area) versus nominal analyte concentration.

## Results

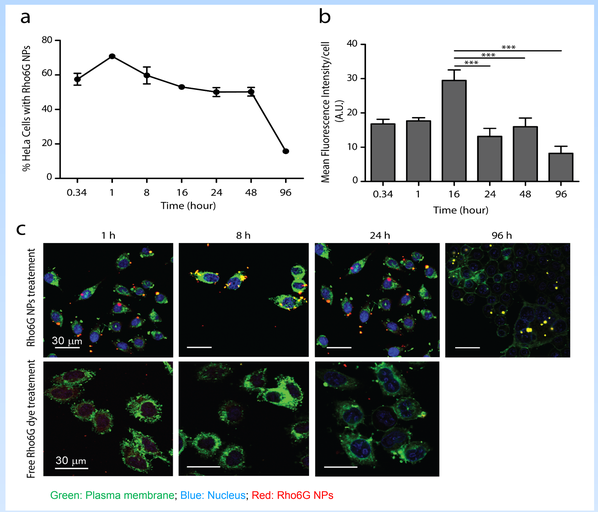
❖ **Physical-chemical properties of TDF+EVG NP**

| | Size (nm) | PDI | Zeta Potential (mV) |
|---|---|---|---|
| TDF+EVG NP | 141.9 ± 2.1 | 0.13 ± 0.01 | -35.65 ± 2.85 |

PDI = polydispersity (measure of size distribution)

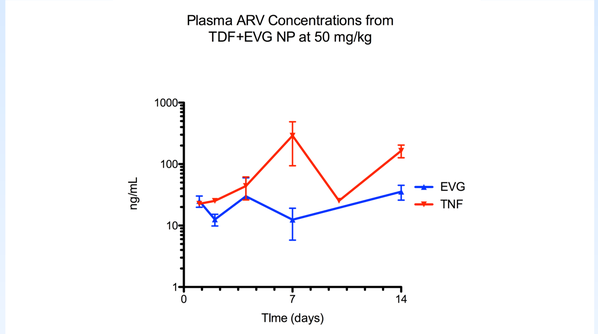
Drug entrapment efficiency TDF 43.2% EVG 42.2%

❖ **Confocal image:**




Green: Plasma membrane; Blue: Nucleus; Red: Rho6G NPs

❖ **Plasma pharmacokinetic results:**



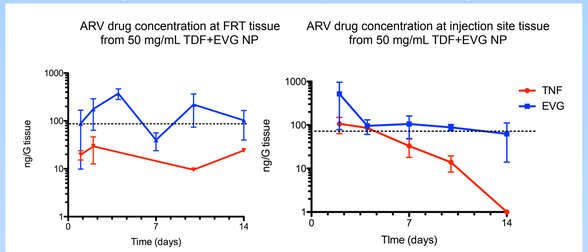

❖ Plasma concentration-time data was entered into WinNonlin to calculate AUC and Cl/F for EVG and TNF

- Mean (SD) EVG $AUC_{0\text{-}14}$ = 6892.6 (4699.5) ng * h/mL
- Mean (SD) TNF $AUC_{0\text{-}14}$ = 35,067 (22,911.8) ng * h/mL
- Mean (SD) EVG Cl/F = 0.29 (0.25) L/h
- Mean (SD) TNF Cl/F = 0.046 (0.023) L/h

## Results

❖ **Tissue ARV drug concentration results:**

A. FRT ARV drugs concentrations: B. Injection site ARV drug concentration:




## Conclusion

- ❖ The TDF+EVG NP were < 200 nm in size and uniform in size distribution, thus can penetrate in various organs through epithelial fenestrations.
- ❖ Enhanced TDF+EVG encapsulation efficiency.
- ❖ The NPs show extended retention within the cytoplasm, therefore potentially can be a long-acting injectable modality for PrEP.
- ❖ Plasma AUC demonstrate extended release over time, thus has sustained-release properties for PrEP.
- ❖ High cARV drug levels in FRT and injection site may be protective against HIV-1

## Acknowledgement

❖ We thank the Integrated Biomedical Imaging Facility core center at Creighton University School of Medicine for the ability to use the confocal microscope to capture images.

## References

1. http://www.prepwatch.org/
2. Mandal S, et al. *AIP Advances* 2015;5:084803.

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