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A novel formulation enabled transformation of 3 HIV drugs Tenofovir-Lamivudine-Dolutegravir (TLD) from Short-Acting to Long-Acting All-in-One Injectable

Running Head: **A novel long-acting 3-HIV-drugs TLD all-in-one injectable**

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ABSTRACT

***Objective*:** To develop an injectable dosage form of the daily oral HIV drugs, tenofovir (T), lamivudine (L), and dolutegravir (D), creating a single, complete, all-in-one TLD 3-drug-combination that demonstrates long-acting pharmacokinetics.

Design: Using drug-combination-nanoparticle (DcNP) technology to stabilize multiple HIV drugs, the 3 HIV drugs TLD, with disparate physical-chemical properties, are stabilized and assembled with lipid-excipients to form *TLD-in-DcNP*. *TLD-in-DcNP* is verified to be stable and suitable for subcutaneous administration. To characterize the plasma time-courses and PBMC concentrations for all 3 drugs, single subcutaneous injections of *TLD-in-DcNP* were given to nonhuman primates (NHP, *M. nemestrina*).

***Results*:** Following single-dose *TLD-in-DcNP*, all drugs exhibited long-acting profiles in NHP plasma with levels that persisted for 4 weeks above predicted viral-effective concentrations for TLD in combination. Times-to-peak were within 24 hr in all NHP for all drugs. Compared to a free-

soluble TLD, *TLD-in-DcNP* provided exposure enhancement and extended duration 7.0-, 2.1-, and 20-fold as *AUC* boost and 10-, 8.3-, and 5.9-fold as half-life extension. Additionally, DcNP may provide more drug exposure in cells than plasma with PBMC-to-plasma drug ratios exceeding one, suggesting cell-targeted drug-combination delivery.

***Conclusions*:** This study confirms that TLD with disparate properties can be made stable by DcNP to enable TLD concentrations of 4 weeks in NHP. Study results highlighted the potential of *TLD-in-DcNP* as a convenient all-in-one, complete HIV long-acting product for clinical development.

Keywords: HIV; long-acting; tenofovir; lamivudine; dolutegravir, TLD, Drug-combination-Nanoparticles.

INTRODUCTION

The 3-HIV oral drugs–tenofovir (TFV: T), lamivudine (3TC: L), and dolutegravir (DTG: D)–TLD is the WHO's recommended first-line treatment for people living with HIV. Daily oral TLD is effective for people living with HIV to achieve undetectable plasma virus levels only when taken consistently. Pill fatigue and discontinuation of daily TLD tablets increase the risk of viral rebound and progression to AIDS [1].

Cabenuva is an approved injectable containing two separate long-acting products, cabotegravir and rilpivirine. Initiation of *cabenuva* typically includes one-month daily tablet intake, with verified viral suppression before every 1-or-2-month intramuscular(IM)-assisted injection [2]. Its implementation has proven challenging [3,4]. *Cabenuva* lacks hepatitis B virus (HBV) coverage and is not recommended for people living with HIV and HBV [5].

Building on success in formulating water insoluble-and-soluble HIV drugs with biocompatible lipid-excipient to form drug-combination-nanoparticles–referred to as DcNP platform [6–8] –we investigated whether short-acting TLD can be transformed into a single long-acting formulation (with TFV providing HBV coverage). A TLD drug-combination was stabilized by two-lipid-excipients in suspension as an injectable, referred to as *TLD-in-DcNP*. This product-candidate was characterized by long-acting pharmacokinetics in NHP. We found that LA-TLD can be formulated in the DcNP dosage form, and a single subcutaneous injection in NHP yields sustained concentrations for all 3-HIV drugs for 4 weeks.

MATERIALS AND METHODS

Chemicals

Tenofovir/TFV PMPA (((1-(6-Amino-9H-purin-9-yl)propan-2-yl)oxy)methyl) phosphonic acid, Laurus, India), lamivudine/3TC and dolutegravir sodium/DTG (Cipla, India) were cGMP-grade. Lipoid GmbH (Germany) provided cGMP DSPC (1,2-Distearoyl-sn-glycero-3-phosphocholine) and mPEG$_{2000}$-DSPE (N-(carbonylmethoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt).

Methods

TLD-in-DcNP injectable suspension was prepared following a process previously detailed [6,9], which was adjusted here. The adjustments were that 5.66 mmol DTG; 5.97mmol HCL; 40.27mmol DSPC and 4.49mmol $mPEG_{2000}$-DSPE were dissolved in 472mL ethanol at 70°C; 28mL of 200mM $NaHCO_3$ buffer containing 5.85mmol TFV and 5.85mmol 3TC was added; solution was spray-dried (4M8Trix Unit; ProCepT, Belgium) under controlled-solvent-removal process to get *TLD-in-DcNP* powder. *TLD-in-DcNP* powder in 0.45%w/v NaCl-20mM $NaHCO_3$ buffer suspension was held at 75°C; homogenized (Emulsiflex-C5; Avestin, Canada) to achieve stable particles (50-70nm). The suspension was cooled to 25°C and stored at 4°C. The TLD % associated with DcNP, measured by equilibrium dialysis under 200-time-excess-volume, was 21.5%-T, 15%-L, and 95%-D. Finally, *TLD-in-DcNP* (52±5nm) sterile suspension was NHP-ready.

Pharmacokinetic Study

Seven NHPs *(Macaca nemestrina*, 7-12kg) were enrolled under approved Institutional Animal Care and Use Committee protocol. 5 NHP were dosed 6.2, 5.1, 10 mg/kg with *TLD-in-DcNP*, while 2 NHPs were dosed with the free-mixture TLD, both by subcutaneous route in the back mid-scapular region. Blood samples for the *TLD-in-DcNP* were collected at 0, 0.25, 0.5, 1, 3, 5, 8, 24, 48, 120, 168, 192, 336, 50, 672 hr (4 weeks) for drug analysis–the free TLD samples were collected with same schedule, but until 120 hr. Furthermore, in 4 NHP dosed with *TLD-in-DcNP*, in the 48 and 168 hr blood samples, peripheral blood mononuclear cells (PBMC) were isolated, frozen immediately to avoid drug loss, and analyzed for drug content. For TFV and 3TC, we could only measure the total content (parent+metabolites), without discerning triphosphates due to in-column conversion to parent TFV or 3TC. All 3-drugs in cell and plasma were analyzed using a published-validated LC-MS/MS assay [10], adapted for TLD with specific mass (m/z). Based on 5µL injection, LOD/LOQ were recorded at 0.25/0.05ng/mL for DTG, 0.59/0.2ng/mL for TFV, and 0.49/0.16ng/mL for 3TC.

Noncompartmental pharmacokinetic analysis was carried out using MATLAB2023a. Times-to-peak, peak concentrations (C_{max}), concentrations at 4 weeks (C_{4w}), apparent terminal half-lives, and areas under the curves until 4 weeks (*AUC*) were calculated (directly from integrating time-concentration curve based on trapezoidal rules) for each animal and reported as mean ± SD. Based on sample quality, DTG assay for NHP#5's PBMC and free DTG in NHP#7's plasma did not meet quality standard, and therefore they were not included in the final analysis.

For antiviral activity, an *in-vitro* HIV-1 inhibition assay was performed to estimate the 90% inhibitory drug concentration (IC_{90}), as previously described [6], in presence of a serum protein. The IC_{90} for *TLD-in-DcNP* and free mixture TLD (fixed-dose-ratio) were established as 0.3, 0.2, and 0.25 ng/mL for TFV, 3TC and DTG, respectively, and similar between free and DcNP combinations.

RESULTS

After systematic studies with DSPC and $mPEG_{2000}$-DSPE lipid excipients, TLD 3-HIV drugs with disparate water solubilities (TFV, 3TC, DTG: Log-P -3.4, -1.3, 2.4) were successfully stabilized

in a DcNP nanosuspension. The final *TLD-in-DcNP* suspension contained 6.2, 5.1, 10 mg/kg TLD, and 115.1, 45.8 DSPC and $mPEG_{2000}$-DSPE (mg/mL). No NHP safety issues, including injection site reactions, were observed with both *TLD-in-DcNP* and free. Drug concentrations in plasma and PBMC were determined following a subcutaneous dose: [TFV], [3TC] and [DTG] were detectable in plasma throughout a 4-week NHP study (Figure 1A). In comparison, plasma drug levels in the NHP receiving TLD-free dosage fell below detectable levels within 3 days.

The plasma concentration peaks for *TLD-in-DcNP* were all recorded within the first day (Figure 1B); comparable to the equivalent free-mixture. *TLD-in-DcNP's AUC* were 7.0-, 2.1-, and 20-fold higher, for TFV, 3TC, and DTG, respectively (Figure 1B). Effects of DcNP on TLD half-life extension were recorded as 10-, 8.3-, and 5.9-fold over the free-soluble drug-combination equivalent. All drug concentrations were above the estimated IC_{90} value for the 4-week study.

Effects of DcNP on intracellular TLD enhancement were notable as the average cell-to-plasma ratio were greater than one, for all 3 drugs (Table 1). Intracellular TFV concentration in PBMC was 2.2- and 3.1-fold higher than plasma, at 48 and 168 hr, respectively. Similarly, [total] 3TC in PBMC was 3- and 15-fold higher than in plasma. Finally, DTG concentration in PBMC was 29- and 4.1-fold higher than in plasma (Table 1).

Collectively, these data indicate that water-soluble TFV, 3TC, and water-insoluble DTG combined into the single *TLD-in-DcNP* injectable provided with 4 weeks plasma exposure in NHP above detectable, measurable, and IC_{90}-predicted levels.

DISCUSSION

A stable-scalable 3-in-1 HIV drug combination, based on a novel injectable drug combination DcNP platform was successfully made. This novel *TLD-in-DcNP* formulation in NHP sustained plasma levels for 4 weeks despite having disparate physiochemical properties of TFV, 3TC, and DTG. The higher intracellular PBMC drug levels relative to plasma for all three drugs suggest that DcNP deliver these antivirals into cells with efficiency. Given oral TLD is a current WHO-recommended "one-pill-a-day" HIV treatment, having a long-acting TLD based on DcNP dosage form may overcome daily pill fatigue. The subcutaneous LA-TLD dosage-forms may provide a self-administration option as opposed to assisted IM-injection. While the 4-week duration of the 3 drugs in *TLD-in-DcNP* may be shorter than the eventual every-two-month dosing with LA-CAB and LA-RPV [11–13], the LA *TLD-in-DcNP* reaches C_{max} without delay and within 1 day (Figure 1B). As sustained/extended-release dosage forms, LA-CAB and LA-RPV deposited in the muscular space exhibit delayed peak plasma concentrations which are typically reached by 7-10 days [12]. To ensure tolerability and address delays in reaching therapeutic levels, an oral lead-in phase is advisable for *cabenuva* treatment. However, for *TLD-in-DcNP*, an oral lead-in may not be necessary due to shorter times-to-peak, and because of the well-documented safety profile of TLD in humans. This could facilitate clinical implementation.

Previously, the DcNP technology was reported to transform short-acting drug combinations into long-acting combinations [7,8,14–16]. We have leveraged the physical and chemical interactions

of water-soluble and insoluble drug substances with amphipathic lipid-excipients at varying temperatures. As a result, DcNP technology is able to stabilize physically disparate water-soluble and -insoluble HIV and cancer drugs, such as lopinavir, ritonavir, efavirenz, dolutegravir or paclitaxel with water-soluble lamivudine, tenofovir, or gemcitabine [7,8,14–16]. The capacity of formulating multiple agents as one injectable suspension is notable in the HIV long-acting landscape as DcNP can transform water-soluble drugs tenofovir and lamivudine into a long-acting product along with water-insoluble DTG [17].

The 3-HIV drugs containing a potent integrase inhibitor in one complete HIV-treatment may also provide sustained viral suppression due to higher and more consistent intracellular drug levels. By week 1 in NHP, cell-to-plasma ratios for all 3-TLD drugs exhibited 3 to 15-fold higher than what found in plasma with potential synchronized TLD enhancement. As TFV is the current active drug substance of interest (formulated as prodrug TAF or TDF) to treat people with chronic HBV, *TLD-in-DcNP* could provide antiviral coverage for people with HBV and HIV-HBV.

To inhibit viral replication, TFV and 3TC are phosphorylated intracellularly to the active metabolites (TFV-dP and 3TC-tP). Previously, we reported with DcNP, TFV and 3TC, PBMCs isolated from NHP exhibited 60-70% of intracellular total-TFV concentrations as TFV-dP [14], and 40% of 3TC as 3TC-tP [8]. These data were similar to that reported for oral TDF and 3TC [18–20]. While remaining to be directly measured, it is likely that DcNP formulated 3TC and TFV in the PBMCs are biologically active with similar fractions of active metabolites to that with oral dosages.

The predicted target concentrations of the 90% antiviral activity as TLD-combination in serum, IC_{90} were noted to be similar between DcNP vs free-soluble formulation. The IC_{90} estimated with serum protein, were noted to be 0.2 to 0.3 ng/mL (Figure 1A). The IC_{90} values for TLD are based on the fixed-dose combination, which may be significantly lower than the reported protein-adjusted IC_{90} for each drug. For instance, the IC_{90} for the single-agent DTG is 64 ng/mL [19], whereas in the TLD formulation, we found it as 0.25 ng/mL. Moreover, comparing the oral dose-normalized exposure of DTG (AUC/dose) between NHPs and humans [21,22], the elevated UGT metabolic clearance of DTG in NHPs could potentially result in a 24-fold increase in DTG exposure in humans [23]. Thus, given a 10mg/kg dose in NHP, the levels observed could be upscaled to therapeutic ranges for humans.

Previously, we reported that drugs in DcNP formulations do not form a local depot [24]. Instead, DcNP are rapidly and completely absorbed by the lymphatic, as opposed to blood capillary system. The DcNP enable preferential drug penetration into lymphatic, instead of less permeable blood, vessels as a first-passage after subcutaneous dosing; there was no significant retention at the injection site [25]. It is likely that all-drugs in *TLD-in-DcNP* remain associated with the DcNP particles, contributing to *in-vivo* stability leading to long-acting pharmacokinetics for all drugs. Earlier studies with *TFV-in-DcNP* containing lopinavir/ritonavir introduced into systemic circulation by IV dosing indicates that greater than 90% of TFV remained associated with DcNP in blood circulation [24]. Therefore, the observed peaks followed by sustained levels in the time-course are likely due to a combination of lymphatic "first pass" absorption, cellular uptake and retention, and *in-vivo* formulation stability [24,26,27].

Collectively, the successful transformation of short-acting to long-acting *TLD-in-DcNP* may provide an all-in-one long-acting first-line, complete HIV treatment. This innovative approach utilizes lipid-stabilized drug-combination in suspensions, resulting in prolonged pharmacokinetics, enhanced *AUC,* and increased concentrations in PBMC. With dose adjustments, this novel all-in-one 3 HIV drug formulation opens new possibilities for long-acting HIV therapy, potentially transforming the lives of people living with HIV and HIV-HBV.

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REFERENCES

1 Dybul M, Fauci AS, Bartlett JG, Kaplan JE, Pau AK. **Guidelines for Using Antiretroviral Agents among HIV-Infected Adults and Adolescents: The Panel on Clinical Practices for Treatment of HIV***. *Ann Intern Med* 2002; **137**:381–433.

2 ViiV (GSK). VOCABRIA (cabotegravir) tablets and CABENUVA (cabotegravir extended-release injectable suspension and rilpivirine extended-release injectable suspension) - CENTER FOR DRUG EVALUATION AND RESEARCH APPLICATION NUMBER: 212887Orig1s000 212888Orig1s000 INTEGRATED REVIEW. 2022.https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/212887Orig1s000,212888Orig1s000IntegratedR.pdf (accessed 22 May 2023).

3 Cooper SE, Rosenblatt J, Gulick RM. **Barriers to Uptake of Long-Acting Antiretroviral Products for Treatment and Prevention of Human Immunodeficiency Virus (HIV) in High-Income Countries**. *Clinical Infectious Diseases* 2022; **75**:S541–S548.

4 Kityo C, Cortes CP, Phanuphak N, Grinsztejn B, Venter F. **Barriers to Uptake of Long-Acting Antiretroviral Products for Treatment and Prevention of HIV in Low- and Middle-Income Countries (LMICs)**. *Clinical Infectious Diseases* 2022; **75**:S549–S556.

5 Corcorran MA, Kim HN. **Chronic Hepatitis B and HIV Coinfection**. *Top Antivir Med* 2023; **31**:14–22.

6 Freeling JP, Koehn J, Shu C, Sun J, Ho RJY. **Anti-HIV Drug-Combination Nanoparticles Enhance Plasma Drug Exposure Duration as Well as Triple-Drug Combination Levels**

in Cells Within Lymph Nodes and Blood in Primates. *AIDS Res Hum Retroviruses* 2015; **31**:107–114.

7 Perazzolo S, Shireman LM, Koehn J, McConnachie LA, Kraft JC, Shen DD, *et al.* **Three HIV Drugs, Atazanavir, Ritonavir, and Tenofovir, Coformulated in Drug-Combination Nanoparticles Exhibit Long-Acting and Lymphocyte-Targeting Properties in Nonhuman Primates**. *JPharmSci* 2018; **107**:3153–3162.

8 McConnachie LA, Kinman LM, Koehn J, Kraft JC, Lane S, Lee W, *et al.* **Long-Acting Profile of 4 Drugs in 1 Anti-HIV Nanosuspension in Nonhuman Primates for 5 Weeks After a Single Subcutaneous Injection**. *JPharmSci* 2018; **107**:1787–1790.

9 Kinman L, Brodie SJ, Tsai CC, Bui T, Larsen K, Schmidt A, *et al.* **Lipid–Drug Association Enhanced HIV-1 Protease Inhibitor Indinavir Localization in Lymphoid Tissues and Viral Load Reduction: A Proof of Concept Study in HIV-2287-Infected Macaques**. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2003; **34**:387–397.

10 Koehn J, Ding Y, Freeling J, Duan J, Ho RJY. **A simple, efficient, and sensitive method for simultaneous detection of anti-HIV drugs atazanavir, ritonavir, and tenofovir by use of liquid chromatography-tandem mass spectrometry**. *Antimicrob Agents Chemother* 2015; **59**:6682–6688.

11 Andrews CD, Spreen WR, Mohri H, Moss L, Ford S, Gettie A, *et al.* **Long-Acting Integrase Inhibitor Protects Macaques from Intrarectal Simian/Human Immunodeficiency Virus**. *Science* 2014; **343**:1151–1154.

12 Spreen W, Ford SL, Chen S, Wilfret D, Margolis D, Gould E, *et al.* **GSK1265744 Pharmacokinetics in Plasma and Tissue After Single-Dose Long-Acting Injectable Administration in Healthy Subjects**. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2014; **67**:481–486.

13 Margolis DA, Boffito M. **Long-acting antiviral agents for HIV treatment**. *Curr Opin HIV AIDS* 2015; **10**:246–252.

14 Kraft JC, McConnachie LA, Koehn J, Kinman L, Collins C, Shen DD, *et al.* **Long-acting combination anti-HIV drug suspension enhances and sustains higher drug levels in lymph node cells than in blood cells and plasma**. *AIDS* 2017; **31**:765–770.

15 Yu J, Mu Q, Perazzolo S, Griffin JI, Zhu L, McConnachie LA, *et al.* **Novel Long-Acting Drug Combination Nanoparticles Composed of Gemcitabine and Paclitaxel Enhance Localization of Both Drugs in Metastatic Breast Cancer Nodules**. *Pharmaceutical Research* 2020; **37**:197.

16 Perazzolo S, Zhu L, Lin W, Nguyen A, Ho RJY. **Systems and Clinical Pharmacology of COVID-19 Therapeutic Candidates: A Clinical and Translational Medicine Perspective**. *Journal of Pharmaceutical Sciences* 2021; **110**:1002–1017.

17 Romano JW, Baum MM, Demkovich ZR, Diana F, Dobard C, Feldman PL, *et al.* **Tenofovir Alafenamide for HIV Prevention: Review of the Proceedings from the Gates Foundation Long-Acting TAF Product Development Meeting**. *AIDS Research and Human Retroviruses* 2021; **37**:409–420.

18 Durand-Gasselin L, Van Rompay KKA, Vela JE, Henne IN, Lee WA, Rhodes GR, *et al.* **Nucleotide Analog Prodrug, Tenofovir Disoproxil, Enhances Lymphoid Cell Loading Following Oral Administration in Monkeys**. *Mol Pharm* 2009; **6**:1145–1151.

19 Bazzoli C, Bénech H, Rey E, Retout S, Salmon D, Duval X, *et al.* **Joint Population Pharmacokinetic Analysis of Zidovudine, Lamivudine, and Their Active Intracellular Metabolites in HIV Patients ▾**. *Antimicrob Agents Chemother* 2011; **55**:3423–3431.

20 Else LJ, Jackson A, Puls R, Hill A, Fahey P, Lin E, *et al.* **Pharmacokinetics of Lamivudine and Lamivudine-Triphosphate after Administration of 300 Milligrams and 150 Milligrams Once Daily to Healthy Volunteers: Results of the ENCORE 2 Study**. *Antimicrob Agents Chemother* 2012; **56**:1427–1433.

21 Moss L, Wagner D, Kanaoka E, Olson K, Yueh YL, Bowers GD. **The comparative disposition and metabolism of dolutegravir, a potent HIV-1 integrase inhibitor, in mice, rats, and monkeys**. *Xenobiotica* 2015; **45**:60–70.

22 Cottrell ML, Hadzic T, Kashuba ADM. **Clinical Pharmacokinetic, Pharmacodynamic and Drug-Interaction Profile of the Integrase Inhibitor Dolutegravir**. *Clin Pharmacokinet* 2013; **52**:981–994.

23 Akazawa T, Uchida Y, Miyauchi E, Tachikawa M, Ohtsuki S, Terasaki T. **High Expression of UGT1A1/1A6 in Monkey Small Intestine: Comparison of Protein Expression Levels of Cytochromes P450, UDP-Glucuronosyltransferases, and Transporters in Small Intestine of Cynomolgus Monkey and Human**. *Mol Pharmaceutics* 2018; **15**:127–140.

24 Perazzolo S, Shireman LM, McConnachie LA, Koehn J, Kinman L, Lee W, *et al.* **Integration of Computational and Experimental Approaches to Elucidate Mechanisms of First-Pass Lymphatic Drug Sequestration and Long-Acting Pharmacokinetics of the Injectable Triple-HIV Drug Combination TLC-ART 101**. *JPharmSci* 2020; **109**:1789–1801.

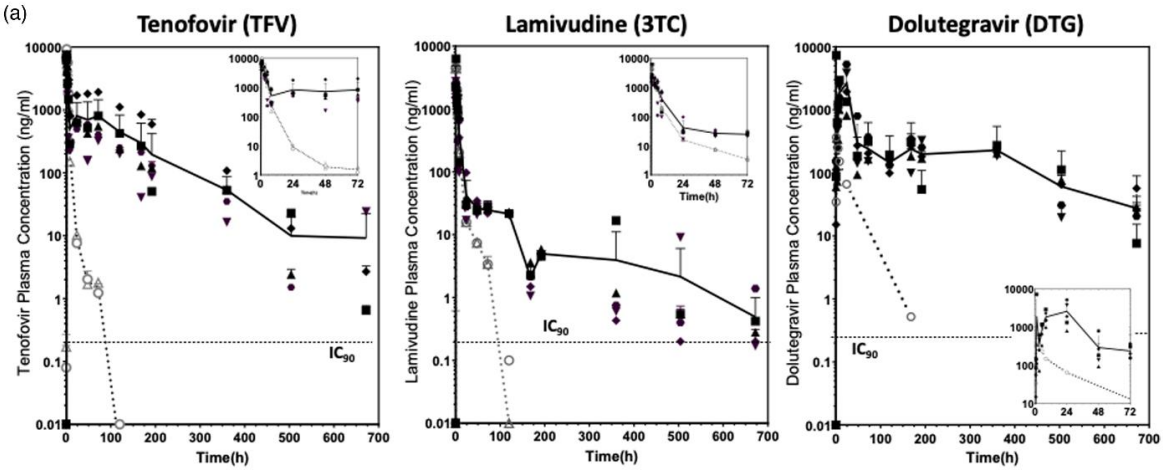
25 Kraft JC, Treuting PM, Ho RJY. **Indocyanine green nanoparticles undergo selective lymphatic uptake, distribution and retention and enable detailed mapping of lymph vessels, nodes and abnormalities**. *Journal of Drug Targeting* 2018; **26**:494–504.

26 Perazzolo S, Shireman LM, Shen DD, Ho RJY. **Physiologically Based Pharmacokinetic Modeling of 3 HIV Drugs in Combination and the Role of Lymphatic System after Subcutaneous Dosing. Part 1: Model for the Free-Drug Mixture**. *JPharmSci* 2022; **111**:529–541.

27 Perazzolo S, Shen DD, Ho RJY. **Physiologically Based Pharmacokinetic Modeling of 3 HIV Drugs in Combination and the Role of Lymphatic System after Subcutaneous Dosing. Part 2: Model for the Drug-combination Nanoparticles**. *Journal of Pharmaceutical Sciences* 2022; **111**:825–837.

Figure 1. Effects of DcNP dosage form on the time-course of plasma tenofovir (TFV or T), lamivudine (3TC or L), and dolutegravir (DTG or D) in non-human primates (NHPs). *Panel A*: plasma drug concentrations over time are presented after a single subcutaneous dose of the *TLD-in-DcNP* or free-soluble mixture given to NHP. *Panel B:* noncompartmental pharmacokinetic (PK) parameters results. *Panel A.* All drugs in NHP receiving *TLD-in-DcNP* show extended time as prolonged TFV, 3TC, and DTG presence in the plasma as opposed to free-soluble drugs control at equivalent subcutaneous dose of 6.2 mg/kg TFV, 5.1 mg/kg 3TC, and 10 mg/kg DTG. Filled symbols represent plasma drug concentrations of TLD drugs in DcNP for 5 NHP, as mean ± SD. Empty symbols represent TLD given as free-soluble TLD combination in 2 NHP as mean (only one NHP with free DTG was available). Horizontal dashes lines denote the *in vitro* IC_{90} values in serum (protein) as a fixed-ratio presented in the *TLD-in-DcNP* or fee-soluble drug-in-combination. In each graph, there is an inset representing a zoomed-in view of the first 72 hours after injection. ***Panel B.*** Noncompartmental PK parameter estimates are based on the concentration-time course data presented in *Panel A*. Each PK parameter is presented as mean ± SD. *Time-to-peak* is the time needed for the drug to rise to the first identifiable concentration peak collected at concentration C_{max}. C_{4w} is the drug concentration at 4 weeks; *AUC* is the area under the curve based on integration of plasma time-courses using classical trapezoidal rules for the 4-weeks study; *AUC* ratio as the fold enhancement of TLD formulated in DcNP after a single dose; $t_{1/2,z}$ as the apparent terminal elimination half-life.

(a)



(b)

Parameters	Tenofovir (TFV) DcNP	Tenofovir (TFV) Free	Lamivudine (3TC) DcNP	Lamivudine (3TC) Free	Dolutegravir (DTG) DcNP	Dolutegravir (DTG) Free
Time-to-peak (hr) [a]	**1.0**	1.0	**1.0**	1.0	**16** ± 4.0	1.0
C_{max} (µg/mL)	**6.1** ± 0.7	6.7	**3.0** ± 0.8	2.1	**2.0** ± 1.2	0.37
$AUC_{0\text{-}4w}$ (hr•µg/mL)	**146** ± 41	21	**20** ± 2.0	9.5	**173** ± 19	8.5
AUC ratio (DcNP/Free)	**7.0**		**2.1**		**20**	
$t_{1/2,z}$ (hr)	**88** ± 35	8.6	**74** ± 16	8.9	**112** ± 34	19

Table 1. Effects of DcNP formulated 3-HIV drugs, TLD on enhanced cellular concentrations as measured in the Peripheral Blood Mononuclear Cells from NHP.*

Sample Type	**NHPs dose with TLD in DcNP dosage form**					
	Tenofovir (T)		**Lamivudine (L, 3TC)**		**Dolutegravir (D)**	
	48 h	**168 h**	**48 h**	**168 h**	**48 h**	**168 h**
[Cell]	1,631.8 ± 114.7	989.0± 136.9	50.6 ± 4.5	13.4 ± 10.5	1303.8 ± 107.6	278.0 ± 74.9
[Plasma]	731.3 ± 190.8	320.5 ± 89.5	16.8 ± 5.8	0.9 ± 0.3	45.1 ± 18.0	68.6 ± 22.6
Cell/Plasma	**2.2**	**3.1**	**3.0**	**15**	**29**	**4.1**

*NHP, non-human primates *M. Nemestrina* were given a single subcutaneous injection of *TLD-in-DcNP* dosage form as described in Materials and Methods. The peripheral blood mononuclear cells [PBMC or Cell] were immediately isolated from blood samples and analyzed for drug concentrations in the PBMCs. Data as mean±SD. PBMC-to-plasma noted as cell/plasma drug ratio are based on the mean values.