Development of a Two-Drug-Long-Acting Removable Formulation for HIV suppression

G-04

Manse Kim,[1,2,3] Sarah E. Wessel,[1,2] Rae Ann Spagnuolo,[1,2] Nurjahan Begum,[1,2] Lijun Ling,[1,2,3] Diana Battaglia,[1,2,3] Katie R. Mollan,[2,4] Amanda Schauer,[5] J. Victor Garcia,[1,2,3] Martina Kovarova*[1,2]*

Affiliations

[1]Division of Infectious Diseases, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
[2]Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
[3]Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL, USA.
[4]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
[5]UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

A single injection of a long-acting formulation with two drugs, dolutegravir and doravirine, can effectively maintain HIV suppression after discontinuation of ART in humanized mice.

Background

The long-acting formulation of antiretrovirals can increase adherence to HIV treatment, and prevent treatment failure and the development of drug resistance. Long-acting formulations have shown improved compliance compared to conventional regimens requiring frequent dosing, leading to better outcomes and treatment retention. Here, an in situ forming implant system (ISFI) was used to develop a long-acting (LA) formulation that can co-deliver two drugs with a single administration. ISFI formulations are injectable and solidified to implants after subcutaneous injection. They are fully biodegradable but can be removed to stop drug delivery during an adverse event.

Methods

First, an injectable LA formulation of non-nucleoside reverse transcriptase inhibitor doravirine (DOR-ISFI) was developed. The second drug, an integrase strand transfer inhibitor dolutegravir (DTG), was incorporated into the same formulation using co-solvent optimization. The pharmacokinetics of developed formulations were evaluated in BALB/c mice, and the pre-clinical efficacy of HIV suppression was assessed in humanized mice

Results

The DOR-ISFI delivered DOR plasma concentrations >10×IC95 for 140 days and efficiently protected against multiple vaginal HIV challenges in humanized mice. The two-drug formulation DTGDOR-ISFI co-delivered DTG and DOR in serum for 9 months above the IC90. Drugs penetrated tissues relevant for HIV infection including lymph nodes, brain, spleen, gut, and genital tracts of male and female. During preclinical evaluation of HIV maintenance therapy, this DTGDOR-ISFI effectively suppressed HIV for at least 4 weeks after discontinuation of antiretroviral therapy (ART). Removal of the implant successfully stopped the delivery of both drugs.

Conclusions

DTGDOR-ISFI is an important advance in managing HIV suppression by reducing the number of injections and simplifying LA drug delivery.

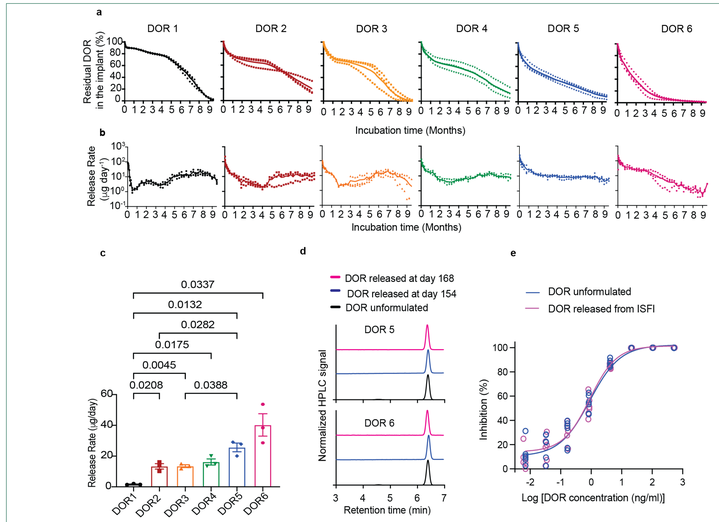


Figure 1. In vitro characteristics of drug release from DOR-ISFI formulations. DOR-ISFI formulations (30µL) were injected into a release medium (2% Kolliphor HS 15 in PBS) and incubated at 37 °C with shaking at 100 rpm (n=3 for each formulation). **a.** Percentage of remaining DOR in the implants. **b.** Daily release rates of DOR from implants. **c.** Comparison of DOR daily release rates at 1 month of incubation with a Welch's t-test. **d.** HPLC histograms of DOR release from DOR5 implants (upper panel) and DOR6 implants (lower panel) after 5 months of incubation (blue, 154 days and pink, 168 days). **e.** Non-linear regression analysis with 4-parameter curve fit of the anti-HIV activity of DOR released from DOR5 implants after 5 months of in vitro release (purple, n=6) compared to unformulated DOR (blue, n=6).

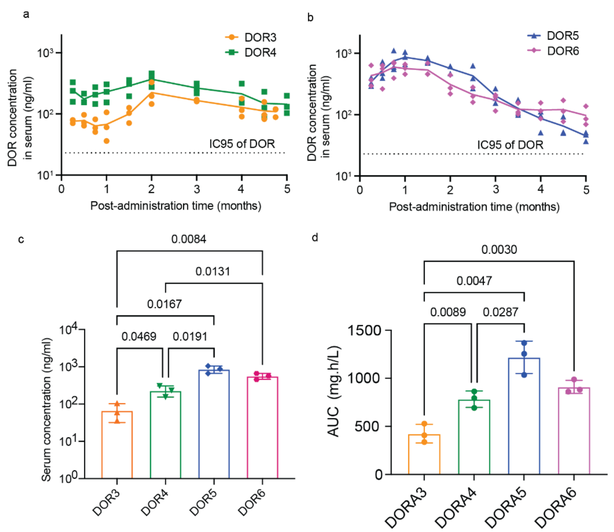


Figure 2. Levels of DOR in serum after DOR-ISFI administration. BALB/c mice received a single subcutaneous injection (50 µL) of DOR-ISFI formulations. **a**. Serum DOR concentrations in mice treated with DOR3 (n=3) or DOR4 (n=3). **b**. Serum DOR concentrations in mice treated with DOR5 (n=3) or DOR6 (n=3). **c**. Comparison of serum DOR concentrations in mice injected with DOR3, DOR4, DOR5, or DOR6 at 28 days post ISFI administration (Welch's t-test). **d**. Comparison of DOR serum area under the concentration-time curve (AUC) at 20 weeks post-administration (Welch's t-test)

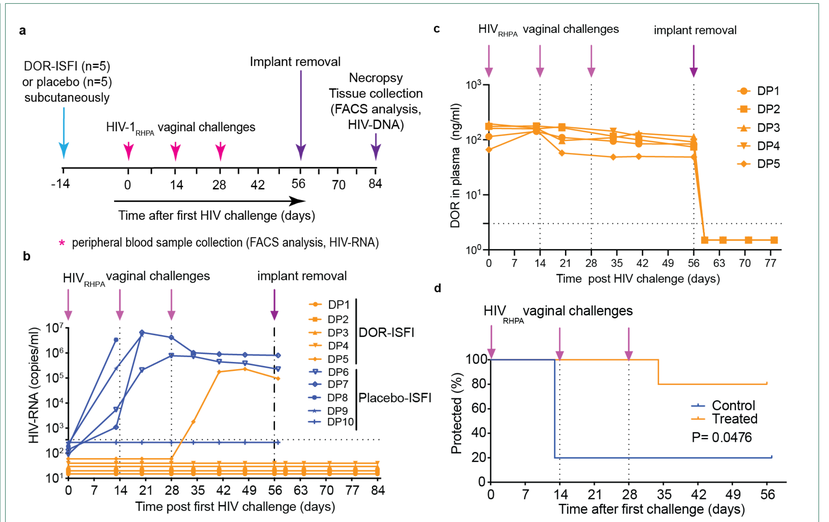


Figure 3. A single dose of DOR-ISFI prevents multiple high-dose HIV-1 vaginal infection. a. Humanized mice were injected with 50 µl of placebo (n = 5) or DOR5 (n = 5) and vaginally challenged to HIV-1_{RHPA} (3.0×10^5 TCIU). The removal of implants from four DOR5-treated mice was performed 4 weeks after the last HIV challenge. Cell-associated HIV-DNA levels in multiple organs were measured at the end of the experiment. **b**. HIV-RNA levels in the plasma of control (blue) and DOR5-treated (yellow) mice. **c**. DOR plasma concentrations. **d**. Time-to-event Kaplan-Meier plot illustrating the estimated probability of protection through day 56 (P = 0.0476, exact log-rank test).

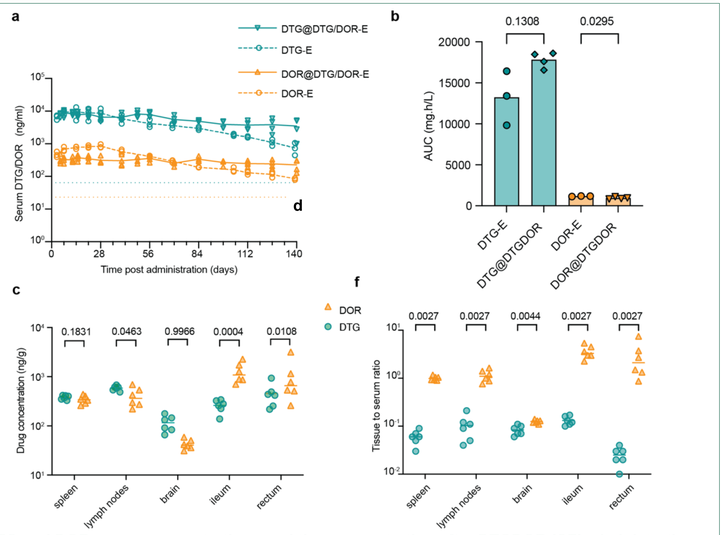


Figure 4. DTG and DOR serum concentrations and tissue penetration after DTGDOR-ISFI administration. BALB/c mice received a single subcutaneous injection (50 µL) of the DTGDOR-E two-drug formulation or one of the single-drug formulations DTG-E or DOR-E. **a**. Serum DTG and DOR concentrations in mice administered DTGDOR-E (n=4), DTG-E (n=3) or DOR-E (n=3). **b**. Comparison of DTG and DOR serum concentrations AUC in mice administered DTGDOR-E, DTG-E or DOR-E formulations 5 months post-administration (Welch's t-test). DTG and DOR levels (**c**) and tissue-to-serum ratio (**d**) in tissues

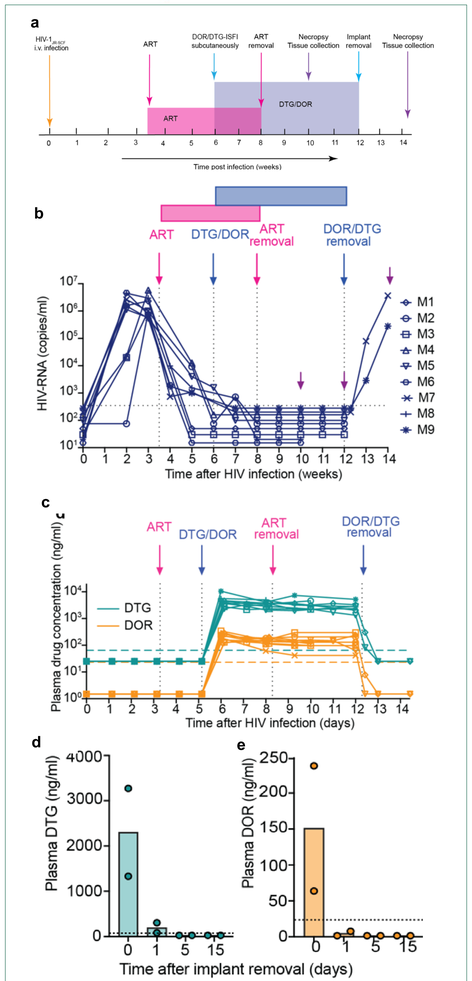


Figure 5. DTGDOR-E formulation maintains HIV suppression for 6 weeks. a. Experimental design for HIV maintenance therapy. Humanized mice were infected with HIV and the established infection was treated with ART chow (RAL, TDF, FTC) for 4.5 weeks. **b**. Plasma HIV-RNA levels in DTGDOR-E treated mice. Dashed line, HIV-RNA limit of detection (346 copies/ml) **c.** Plasma concentrations of DTG and DOR during the course of the experiment. **d, e**. After implant removal, plasma concentrations of DTG (d) and DOR (e). Dashed lines, DTG IC90=64 ng/ml (d, e, teal) and DOR IC95=23 ng/ml (d, f, yellow).

Acknowledgments

This work was funded from National Institutes of Health grants R01AI40799 (JVG) and R01HD100584P (MK).