Long-Acting Emtricitabine Prodrugs Provide Protection From HIV Infection *In Vivo*

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Background

- Antiretroviral drugs are predominantly administered orally for both therapy and pre-exposure prophylaxis (PrEP).
- Despite ease of administration, oral delivery is prone to patient non-adherence exacerbated for some drugs by pill fatigue and gastrointestinal intolerance **(1)**.
- By decreasing frequency of administration, long-acting injectables (LAIs) may offer an effective strategy to circumvent these issues **(2)**.
- We report here a preclinical assessment of LAI semi-solid prodrug nanoparticle (SSPN) formulations of novel emtricitabine (FTC) prodrugs to prevent HIV infection.

- SSPNs of FTC carbamate/carbonate prodrugs were generated using a proprietary emulsion-templated freeze-drying technology.
- 2 lead formulations were tested for their ability to prevent HIV infection in NSG-cmah$^{-/-}$ mice humanised by CD34+ cell transplantation.
- Animals received 140 mg/kg FTC equivalent (SSPN 9 or 10) via 2 intramuscular injections vs an untreated control (n= 7-6 per group).
- At days 7 and 14 mice were challenged intraperitoneally with a 10^3 $TCID_{50}$ dose of HIV_{ADA}.
- Animals were sacrificed at 28 days post infection.
- Plasma samples were taken for determination of viral load (VL). Tissue samples were collected for viral RNA and proteins detection via RT-PCR and immunohistology.

Methods

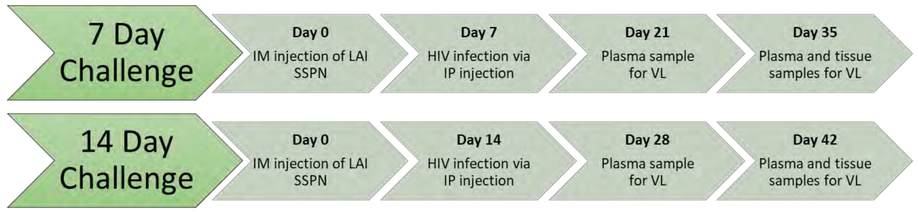

Figure 1 shows the experimental design used to investigate the potential for LAI SSPN formulations to prevent HIV infection

Results

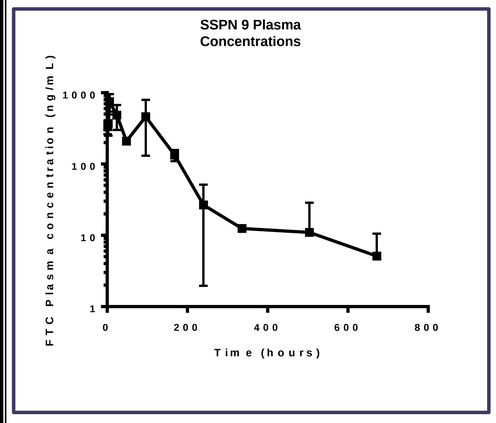

Figure 2 PK profile of the SSPN 9 over 28 days following 2 intra-muscular injections (1 injection in each leg, 70 mg/kg mg/kg based on FTC content). PK was examined in BALB/c mice in order to inform PD studies.

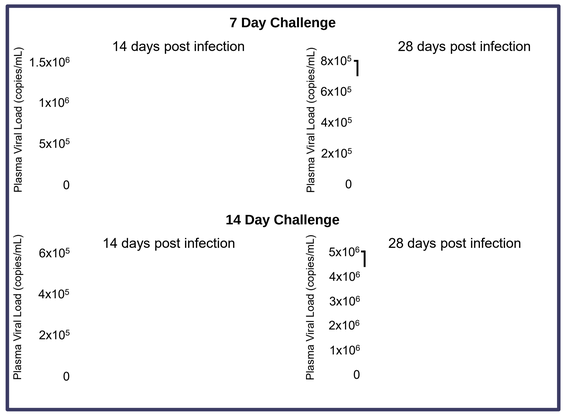

Figure 3 plasma viral load at 7 and 14 days post IM injection. Plasma samples were taken 14 days and 28 days post HIV infection.

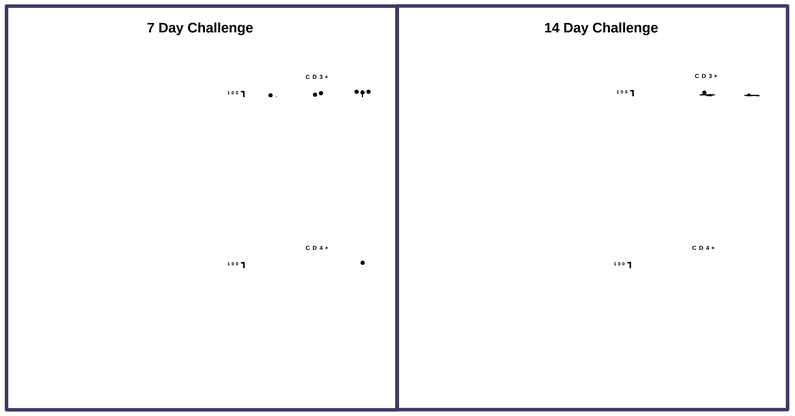

Figure 4 Characterisation of human PBMCs by flow cytometry. Blood samples were collected at point of termination and stained for expression of CD45 mononuclear cells and CD3 T Cells. T cells were also stained for CD4 and CD8 expression assessed.

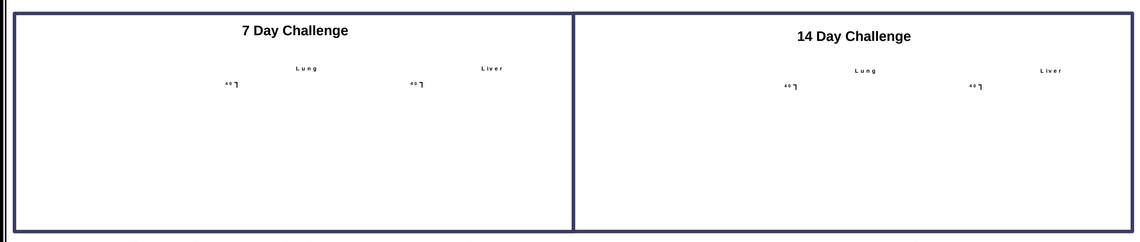

Figure 5 shows detection of HIV-1 Gag-p24 rna via PCR in humanised mice following HIV infection 7 and 14 days post IM injection. Tissue samples from spleen, lung and liver were taken 28 days post HIV infection.

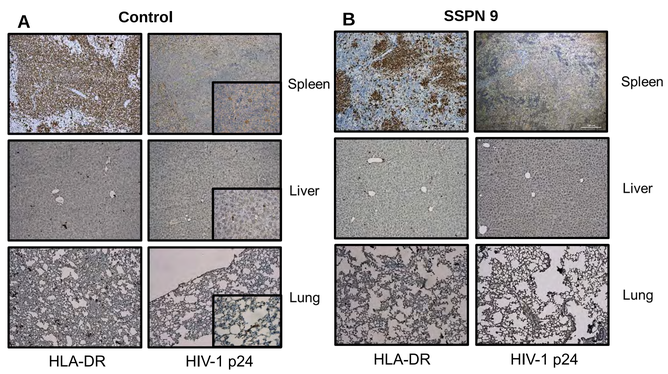

Figure 6 Representative histopathology samples taken from spleen, liver and lung tissues 7 days post IM injection from the **A)** control group and **B)** SSPN 9 treated group. Tissue samples were collected 28 days post HIV infection. Samples were stained for HLA- DR and Gag-p24 **6A** highlight Gag-p24 positive staining in the control group (brown). No Gag-p24 was detected in the SSPN 9 treated group.

- Mice treated with SSPN 9 and 10 demonstrated undetectable VL (700 copies/mL detection limit), and HIV RNA remained undetectable 28 days post infection in plasma, spleen, lung and liver in all animals for the 7 day challenge.
- Following 14-day challenge, mice treated with SSPN 9 demonstrated undetectable HIV in plasma and all tissues.
- Mice treated with SSPN 10 demonstrated 2 mice had detectable plasma VL (4.77 x 10^3 copies/mL) and 3 mice showed presence of HIV RNA in plasma and proteins in spleen, lung and liver in day 28.
- HIV was detectable in all untreated animals.

Conclusions

- The data presented here demonstrate both formulations were 100% effective at preventing HIV infection 7 days post LAI administration.
- Following 14 days, SSPN 9 prevented HIV infection in 100% of mice while SSPN 10 prevented infection in 50% of mice.
- These data indicate great potential for delivering FTC via LAI and the approach may support LAI development for PrEP.
- Further studies will aim to optimise formulations to produce exposure beyond 14 days and to assess applications in therapy as part of a combination.

References

(1) Curley *et al*, Advances in nanomedicine drug delivery applications for HIV therapy, Future Science OA, 2017

(2) Owen A & Rannard S, Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy, Advanced Drug Delivery Reviews, 2016