Rectal Transmission of Transmitted/Founder HIV-1 Is Efficiently Prevented by Topical 1% Tenofovir in BLT Humanized Mice

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**Recommended Citation**

Chateau ML, Denton PW, Swanson MD, McGowan I, Garcia JV (2013) Rectal Transmission of Transmitted/Founder HIV-1 Is Efficiently Prevented by Topical 1% Tenofovir in BLT Humanized Mice. PLoS ONE 8(3): e60024. doi:10.1371/journal.pone.0060024

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Rectal Transmission of Transmitted/Founder HIV-1 Is Efficiently Prevented by Topical 1% Tenofovir in BLT Humanized Mice

Morgan L. Chateau¹, Paul W. Denton¹, Michael D. Swanson¹, Ian McGowan², J. Victor Garcia¹*

Introduction

Efficacious biomedical HIV prevention interventions could dramatically reduce the number of new HIV infections globally [1–8]. Microbicides (also referred to as topical pre-exposure prophylaxis [topical PrEP]) represent one of several classes (e.g. oral PrEP, treatment-as-prevention) of such interventions currently being developed [9–15]. There are multiple reasons why microbicides are attractive as tools for HIV prevention: (i) local administration of an antiretroviral gel at the site of exposure will result in higher drug levels at the intended anatomical location than can be achieved using oral PrEP [16–19] while reducing the likelihood of experiencing systemic dosing-associated toxicities [14,19]; (ii) the reduced toxicity associated with topical microbicides is expected to increase adherence [20]; (iii) microbicides are user controlled [17]; (iv) microbicides are predicted to be cost-effective [21,22]; (v) topical microbicides can be developed with combinations of viral inhibitors [23]; (vi) an ideal microbicide would be safe and effective in both rectal and vaginal compartments [24–26]; and (vii) antiviral microbicides may also protect against viruses other than HIV (e.g. herpes simplex) [27,28].

All microbicide efficacy clinical trials to date have tested the prevention of vaginal HIV transmission [5,9,20,29–36]. However, an important driver of the epidemic in both men and women is HIV transmission resulting from anal intercourse [37–44] such that rectal microbicide development is also required [20,45–49]. Proof of concept that administration of an antiretroviral gel rectally can prevent transmission of SIV/SHIV has been demonstrated for tenofovir [50] and MIV-150 [51]. Tenofovir, UC781, and nonoxynol-9 have been tested for safety and acceptability in Phase 1 rectal microbicide clinical trials and, of these three, only tenofovir is being advanced [18–20,52,53]. Therefore, our in vivo preclinical efficacy study in bone marrow–liver-thymus (BLT) humanized mice was designed to determine the efficacy of topical tenofovir for the prevention of rectal HIV-1 transmission.

BLT mice are the experimental platform of choice for this study for several reasons. For example, BLT mice harbor a de novo generated human immune system distributed throughout each animal [54–76]. In the context of this study, an important characteristic of BLT mice is their susceptibility to rectal HIV-1 transmission [60,63] due to the presence of human CD4+ T cells, macrophages and dendritic cells found throughout BLT mouse intestines, including the rectum [54,63]. Previously both topical [56] and systemic [59,60] HIV prevention interventions have been extensively tested in BLT mice for their ability to block vaginal transmission of HIV-1. The results obtained from these studies were highly predictive of the clinical trial outcomes [9,13,56,59,60,77].

An important and novel aspect of this study is the use of a MSM-derived transmitted/founder (T/F) virus [78]. Typically only one or a few virions (defined as the T/F viruses) are responsible for a mucosal transmission event in humans making
Figure 1. Experimental design and timeline. BLT mice were utilized to determine the efficacy of topically applied tenofovir to prevent rectal HIV-1 transmission. Rectal HIV-1 exposures were performed within 30 minutes following rectal application of 1% tenofovir. Plasma viral load and real-time PCR amplification of tissue associated viral DNA were used as HIV-1 detection strategies to determine whether peripheral blood samples collected at the indicated times and tissues collected at harvest contained HIV-1.

doi:10.1371/journal.pone.0060024.g001

Table 1. BLT mice used to test the efficacy of topical tenofovir to prevent rectal HIV-1JRCSF transmission.*

| Mouse | % human CD45+ in PB at exposure | % hCD45+ hCD3+ hCD4+ in PB at exposure | Tissue Cell associated viral DNA | HIV Status |
|-------|---------------------------------|----------------------------------------|---------------------------------|------------|
| Topical Tenofovir |                       |                                        |                                  |            |
| J01   | 78                              | 87                                     | B, S, O, LN                      | Neg        |
| J02   | 69                              | 86                                     | B, S, O, LN,                    | Neg        |
| J03   | 67                              | 79                                     | B, S, O, LN                      | Neg        |
| J04   | 39                              | 83                                     | B, S, O, LN                      | Neg        |
| J05   | 78                              | 67                                     | B, S, O, LN,                    | Pos        |
| J06   | 54                              | 84                                     | B, O, LN                         | Neg        |
| J07   | 69                              | 71                                     | B, S, LN                         | Neg        |
| J08   | 73                              | 68                                     | BM, S, O, LN                    | Neg        |
| J09   | 86                              | 69                                     | ND                               | Neg        |
| J10   | 65                              | 88                                     | BM, S, O, LN                    | Neg        |
| J11   | 79                              | 86                                     | B, S, O                          | Neg        |
| J12   | 80                              | 86                                     | BM, S, O, LN                    | Neg        |
| Mean (+/− 2SD) | 70% (+/− 13) | 80% (+/− 8) | B, S, O, LN | Pos |

Vehicle |                       |                                        |                                  |            |
| J13   | 73                              | 88                                     | B, S, O, LN                      | Pos        |
| J14   | 52                              | 79                                     | ND                               | Neg        |
| J15   | 32                              | 85                                     | ND                               | Neg        |
| J16   | 84                              | 73                                     | B, S, O, LN,                    | Pos        |
| J17   | 68                              | 86                                     | ND                               | Pos        |
| J18   | 56                              | 85                                     | ND                               | Neg        |
| J19   | 31                              | 79                                     | B, S                              | Pos        |
| J20   | 83                              | 72                                     | B, O, S                          | Neg        |
| J21   | 61                              | 71                                     | S, O, LN                         | Pos        |
| J22   | 71                              | 83                                     | ND                               | Neg        |
| J23   | 79                              | 88                                     | B, S, O, LN                      | Neg        |
| J24   | 62                              | 84                                     | B, S, O, LN                      | Neg        |
| J25   | 72                              | 84                                     | ND                               | Neg        |
| J26   | 60                              | 80                                     | S, O, LN                         | Pos        |
| J27   | 75                              | 82                                     | B, S, O, LN                      | Neg        |
| J28   | 76                              | 88                                     | B, S, O, LN                      | Pos        |
| J29   | 77                              | 87                                     | ND                               | Pos        |

Mean (+/− 2SD) | 65% (+/− 16) | 82% (+/− 6) | B, S, O, LN | Pos |

*The data shown in the table includes analyses performed on both infected and uninfected mice with the text in bold used to highlight that HIV-1 was found in the indicated tissues.

Abbreviations: B – bone marrow; LN – lymph nodes; ND - not done; Neg – negative; O – thymic organoid; PB – peripheral blood; Pos – positive; and S – spleen.
doi:10.1371/journal.pone.0060024.t001
T/F viruses physiological relevant for in vivo efficacy studies of HIV prevention interventions [79,80], BLT mice were treated rectally with topical 1% tenofovir and then rectally inoculated with HIV-1JRCSF, a well characterized low passage primary isolate, or the T/F virus HIV-1THRO. We found that rectal transmission of both viruses was efficiently prevented by topical tenofovir.

**Materials and Methods**

**Preparation of BLT Mice and Characterization of Human Reconstitution**

BLT mice were prepared essentially as previously described [54–61,63,76]. Briefly, thy/liv implanted [81] and preconditioned NOD/SCID-gamma chain null (NSG) mice (Jackson Laboratories, Bar Harbor, ME) were transplanted with autologous human fetal liver CD34+ cells (Advanced Bioscience Resources, Alameda, CA) and monitored for human reconstitution in peripheral blood [59,61,63]. Mice were maintained at the University of North Carolina at Chapel Hill Division of Laboratory Animal Medicine in accordance with protocols approved by the Institutional Animal Care and Use Committee.

**Topical Application of Tenofovir and Rectal Exposure of BLT Mice to HIV-1**

Stocks of HIV-1JRCSF [82] and HIV-1THRO [78] were prepared and titered as we have previously described [57,83]. Mice were exposed rectally using 0.6 μg p24 of HIV-1JRCSF (4×10⁶ TCIU, tissue culture infectious units) and 0.7 μg p24 of HIV-1THRO (5×10⁶ TCIU). Topical tenofovir consisted of 1% tenofovir (PMPA; 9-(2-phosphonyl-methoxypropyl)-adenine) in PBS. The vehicle (placebo) control was PBS.

The exposure timeline (Figure 1) consisted of rectal application of vehicle or of 1% tenofovir less than 30 minutes prior to rectal application of virus. Rectal exposures with HIV-1JRCSF and HIV-1THRO were performed essentially as previously described [60,63] except that all the mucosal exposures were carried out atraumatically and without simulated rectal intercourse [84]. All rectal applications of vehicle or inhibitor as well as virus were performed while mice were anesthetized [60,63]. After viral exposure, mice were returned to their housing to recover and were then monitored longitudinally for evidence of HIV-1 infection as indicated below.

**Analysis of HIV-1 Infection of BLT Mice**

Infection of BLT mice with HIV-1 was monitored at the indicated time intervals in peripheral blood by determining plasma levels of viral RNA using real time PCR (limit of detection 750 copies/ml) [55,56] and by monitoring CD4+ T cell percentages by flow cytometry [59,60]. At necropsy, tissues were harvested and mononuclear cells isolated as previously described [54,56,59,61,63]. Mononuclear cells were washed, enumerated and tested using real time PCR for the presence of HIV-1 DNA (limit of detection 10 copies) [56,57,59,60].

Sequence analysis was performed on plasma RNA samples in the sole case of breakthrough infection of a tenofovir-treated, HIV-1JRCSF-exposed BLT mouse. The entire reverse transcriptase gene from plasma HIV-1 RNA amplification products was sequenced. No resistance mutations in reverse transcriptase were present [85–88].

**Statistics**

All statistical analyses (alpha level: 0.05) were performed using Prism v. 5 (Graph Pad Software). Kaplan-Meier plots indicate the percentage of animals that are HIV-1 positive in the peripheral blood at each time point analyzed. Power analysis calculation for experimental group sample sizes were determined as previously determined [54,61].

| Table 2. BLT mice used to test the efficacy of topical tenofovir to prevent rectal HIV-1THRO transmission.* |
|---------------------------------------------------------------|
| **Mouse** | % human CD45+ in PB at exposure | % hCD45+ hCD3+ hCD4+ in PB at exposure | **Tissue Cell associated viral DNA** | **HIV Status** |
| Topical Tenofovir | | | | |
| T01 | 56 | 83 | B, S, O, LN | Neg |
| T02 | 81 | 81 | B, S, O, LN | Neg |
| T03 | 82 | 77 | B, S, O, LN | Neg |
| T04 | 87 | 82 | B, S, O, LN | Neg |
| T05 | 24 | 80 | B, S, O, LN | Neg |
| T06 | 29 | 80 | B, S, O, LN | Neg |
| Mean (+/- 2SD) | 60% (+/-28) | 81% (+/-2) | | |
| Vehicle | | | | |
| T07 | 61 | 82 | B, S, O, | Pos |
| T08 | 85 | 81 | B, S, O, LN | Neg |
| T09 | 70 | 76 | B, S, O | Pos |
| T10 | 86 | 78 | B, S, O | Pos |
| T11 | 42 | 83 | B, S, O | Pos |
| T12 | 56 | 77 | ND | Neg |
| T13 | 83 | 75 | B, S, OLN | Neg |
| T14 | 73 | 78 | ND | Pos |
| Mean (+/- 2SD) | 70% (+/-16) | 79% (+/-3) | | |

*The data shown in the table includes analyses performed on both infected and uninfected mice with the text in bold used to highlight that HIV-1 was found in the indicated tissues. 

Abbreviations: B – bone marrow; LN – lymph nodes; ND - not done; Neg – negative; O – thymic organoid; PB – peripheral blood; Pos – positive; and S – spleen.

doi:10.1371/journal.pone.0060024.t002
Figure 2. Analysis of peripheral blood and tissues for the presence of HIV-1JRCSF after rectal exposure in the presence or absence of topical tenofovir. (A–B) Longitudinal analyses of peripheral blood plasma viral RNA (A) and the percentage of peripheral blood CD3\(^{+}\) T cells also expressing CD4 (B) are presented for vehicle (left) and topical tenofovir (right) -treated BLT mice exposed rectally to HIV-1JRCSF. (C) Real-time PCR analysis of tissues for presence or absence of HIV-1 DNA. Thin dashed lines represent the limit of detection for the respective assays. Error bars indicate standard error of the mean. Open symbols are used to depict data from HIV negative mice and closed symbols are used to depict data from HIV positive mice.

doi:10.1371/journal.pone.0060024.g002
mission in BLT mice. Kaplan-Meier plot indicates the time to CD4 infected vehicle control mice maintained similar peripheral blood peripheral blood. The breakthrough infection mouse and the HIV-1JRCSF transmission between the vehicle and topical tenofovir arms. experiment, we also monitored the levels of CD4 transcriptase gene was sequenced. Over the course of this breakthrough virus were identified when the entire reverse have a 'breakthrough' infection with readily detectable plasma viral RNA (Figure 2A). One tenofovir treated mouse was found to have a 'breakthrough' infection with readily detectable plasma viral RNA (Figure 2A). No tenofovir resistant mutations from this breakthrough virus were identified when the entire reverse transcriptase gene was sequenced. Over the course of this experiment, we also monitored the levels of CD4 T cells in peripheral blood. The breakthrough infection mouse and the infected vehicle control mice maintained similar peripheral blood CD4 T cell levels to the HIV-1 negative mice (Figure 2B), as we have previously observed with this CCR5-tropic HIV-1 isolate in BLT mice [59,60].

Prior to defining topical tenofovir treated BLT mice as protected from rectal HIV-1 transmission, we tested tissues harvested from these mice for the presence of cell-associated HIV-1 DNA. All mice without plasma viral RNA were also found to be negative for viral DNA in all tissues evaluated (e.g. bone marrow, spleen, human thymic organoid and lymph nodes) confirming the lack of HIV-1 transmission in these animals (Figure 2C; Table 1). The HIV status and time to plasma viremia were then combined to generate a Kaplan-Meier plot of the protection from rectal HIV transmission provided by either the vehicle or topical tenofovir (Figure 3). Log rank analysis (p = 0.03) confirmed that topical tenofovir prevents rectal HIV-1JRCSF transmission in BLT mice.

Rectal Transmission of Transmitted/Founder HIV-1THRO is Prevented by Topical Tenofovir

HIV-1THRO is a CCR5-topic, MSM-derived T/F virus [78]. A total of 14 BLT mice were exposed rectally to HIV-1THRO (Figure 4). Eight mice received vehicle and six mice received tenofovir. Five of the mice receiving vehicle were infected as determined by the presence of plasma virus RNA (Figure 4A). In contrast, none of the tenofovir treated BLT mice (0/6) exposed rectally to HIV-1THRO exhibited plasma viremia (Figure 4A). In addition to plasma viremia, we also monitored the levels of human CD4 T cells in the peripheral blood of all the HIV-1THRO exposed mice. The levels of human CD4 T cells in the infected mice did not change throughout the course of infection (Figure 4B).

To confirm the lack of HIV-1 infection of the tenofovir treated mice we used real time PCR to determine the presence of cell-associated HIV-1 DNA in tissues obtained from these mice. None of the mice treated with tenofovir had detectable levels of viral DNA in any of the tissues examined (Figure 4C; Table 2). In contrast, the presence of viral DNA in tissues from infected animals was readily confirmed (Figure 4C; Table 2). Log rank analysis of these results presented in a Kaplan-Meier plot (Figure 5) revealed that topical tenofovir administered prior to exposure to BLT mice prevents rectal transmission of the physiologically relevant T/F virus, HIV-1THRO (p = 0.02).

Discussion

Mucosal infection after sexual intercourse is the most common route of HIV-1 transmission worldwide which makes the cervicovaginal and rectal mucosa the two most important anatomical sites for viral exposure [91]. Receptive anal intercourse has the highest risk of HIV-1 infection and accounts for most new infections in the US [92,93]. Nevertheless, the vast majority of past and ongoing clinical trials for HIV prevention using topical microbicides have focused on preventing vaginal HIV-1 acquisition [5,9,20,29–36]. The formulation of tenofovir 1% gel used in the RMP-02/MTN-006 Phase 1 rectal safety study was the same formulation used vaginally in the CAPRISA 004 trial [9,19]. Unfortunately, there was a significant increase in gastrointestinal adverse events seen in the RMP-02/MTN-006 study, possibly due to the hyperosmolar nature of the gel [19,20]. We therefore elected to evaluate the efficacy of tenofovir directly, in the absence of any type of gel, to make a clear determination of the potential efficacy of tenofovir for the prevention of rectal HIV transmission. Our study supports the choice of tenofovir as an appropriate active pharmaceutical ingredient around which a specifically engineered microbicide can be designed for rectal [18–20] or dual compartment use [23,26].
Figure 4. Analysis of peripheral blood and tissues for the presence of HIV-1_{THRO} after rectal exposure in the presence or absence of topical tenofovir. (A–B) Longitudinal analyses of peripheral blood plasma viral RNA (A) and the percentage of peripheral blood CD3+ T cells also expressing CD4 (B) are presented for vehicle (left) and topical tenofovir (right) -treated BLT mice exposed rectally to HIV-1_{THRO}. (C) Real-time PCR analysis of tissues for presence or absence of HIV-1 DNA. Thin dashed lines represent the limit of detection for the respective assays. Error bars indicate standard error of the mean. Open symbols are used to depict data from HIV negative mice and closed symbols are used to depict data from HIV positive mice.

doi:10.1371/journal.pone.0060024.g004
Our goal was to evaluate the in vivo efficacy of a rectal microbicide candidate for inclusion into a rectal microbicide to prevent HIV-1 acquisition. We focused on rectal HIV transmission because this route of virus spread continues to be a major contributor to the number of men and women becoming infected with HIV [37–43]. We chose a topical intervention because of the many potential benefits associated with this drug delivery route [14,17,19–28]. BLT mice were chosen as the experimental platform for this evaluation because previous studies have shown that FDA approved drugs prevent mucosal HIV transmission of the human primary virus isolate HIV-1JRC SF in this model [56,59,60]. Here when BLT mice were pretreated with topical tenofovir (or vehicle) and then rectally exposed to HIV-1JRCSF, we found that topical tenofovir efficiently prevents rectal transmission of HIV-1JRCSF (Figures 2 and 3; Table 1).

To extend and expand on this observation we also evaluated the protective effect of tenofovir using a second virus, HIV-1THRO. HIV-1THRO is a MSM-derived T/F virus and therefore its evaluation in the context of rectal transmission is of significant relevance [78]. T/F viruses represent the one or few founder viruses that undergo amplification in local T cells and subsequent systemic dissemination after mucosal exposure [78–80,94]. These T/F viruses use CCR5 as a coreceptor for entry and replicate poorly in monocyte/macrophages relative to T cells [78]. Despite their intrinsic relevance, T/F viruses have not been previously used for in vivo transmission studies in animal models. We found that HIV-1THRO transmits rectally in BLT mice and that its transmission can be efficiently prevented by pretreatment with rectally applied tenofovir (Figures 4 and 5; Table 2).

Analysis of the data from two HIV-1 isolates indicates that 1 of 18 BLT mice became infected despite treatment with topical 1% tenofovir prior to rectal HIV-1 exposure, while 13 of 25 vehicle treated BLT mice became infected (p = 0.002 Fisher’s exact test) (Tables 1 and 2). In an in vivo study using non-human primates (NHP), 2 of 6 macaques became infected despite treatment with topical 1% tenofovir 15 minutes prior to rectal SIV exposure, while 3 of 4 vehicle treated macaques became infected (30). The conclusion reached by the authors of the macaque study and our conclusion of the study presented here are the same – topical tenofovir can inhibit rectal transmission of SIV [50], primary HIV-1 (Figure 3) and T/F HIV-1 (Figure 5).

Topical microbicides are of significant interest in HIV prevention because they achieve high local drug concentrations capable of preventing HIV transmission with reduced risk for toxicity [14,17,19]. The in vivo preclinical efficacy data presented here together with previous data from NHP [50] show that topical tenofovir can efficiently block rectal transmission. The incorporation of a physiologically relevant T/F HIV-1 into this study of rectal HIV prevention increases its translational value. The results presented here show the importance of animal models for the evaluation of HIV-1 prevention strategies and demonstrate the potential for efficacy of tenofovir-based rectal microbicides in humans. Future studies will leverage the results from this work and the BLT model to perform dose-ranging tenofovir studies, evaluate rectal-specific gel formulations containing tenofovir and evaluate other topical rectal microbicide agents for efficacy.

**Acknowledgments**

We thank P. Anton and C. Dezzutti for their critical comments regarding this manuscript. We thank Drs. I. Chen and John Kappes for providing pJRCSF and pTHRO.o/2626, respectively, via the AIDS Research and Reagent Program. We would like to thank former and current lab members and veterinary technicians at UNC Division of Laboratory Animal Medicine for their assistance with various technical aspects of this work.

**Author Contributions**

Conceived and designed the experiments: MLC PWD MDS IM JVG. Performed the experiments: MLC PWD MDS. Analyzed the data: MLC PWD MDS JM JVG. Wrote the paper: MLC PWD JVG.

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