Effects of the anti-HIV pill Truvada on gene transcription in the gastrointestinal tract of HIV-uninfected individuals

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PI: Florian Hladik, MD PhD
Subcontract PI: Joanne Stekler, MD MPH

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1. Subcontract Study Sites and Key Personnel

Administration:
University of Washington Center for AIDS Research
Box 359931, Harborview Medical Center
325 Ninth Avenue
Seattle, WA 98104

Performance Site:
UW AIDS Clinical Trials Unit
Box 359929, HMC

Principal Investigator:
Florian Hladik, MD, PhD
Department of Obstetrics and Gynecology
1959 NE Pacific Street, Box 356460
Seattle, WA 98195-6460
(206) 667-6848
(206) 667-7767
E-mail: fhladik@fhcrc.org

Subcontract Principal Investigator:
Joanne Stekler, MD MPH
(206) 744-8312
jstekler@uw.edu

Collaborator:
Ann Collier, MD
(206) 744-3293
acollier@uw.edu

2. Study Schema

Purpose: To determine the effects of the anti-HIV pill Truvada on gene transcription in the upper and lower gastrointestinal tract

Design: Open-label, cohort study

Population: HIV-negative men initiating HIV pre-exposure prophylaxis (PrEP)

Study size: Up to 20 evaluable subjects

Regimen: Truvada (tenofovir plus emtricitabine) prescribed for daily use by participants' primary care providers

Duration: Each subject will have an evaluation including blood draw, upper endoscopy and anoscopy, with biopsies prior to initiating PrEP and approximately 2 months after initiating PrEP. We will ask for confirmation of negative HIV test results 1-2 months after study completion.

3. Background and Rationale

Current antiretroviral treatment (**ART**) is extremely effective in controlling HIV replication and in many patients suppresses the number of virions measurable in peripheral blood to undetectable levels. Nevertheless, whenever ART is stopped, HIV levels rebound due to the reactivation of integrated HIV proviruses in resting $CD4^+$ T lymphocytes [1]. During effective ART, it would theoretically require 60 years of treatment to eliminate the latent reservoir in T cells [2].

Maintenance of the latent viral reservoir depends on viral silence and cellular proliferation. Some latently-infected cells do not express viral proteins and because of this silence escape immune system detection. Proliferation of virally-silent cells then increases the host pool of viral DNA [3-5]. It is typically assumed that the maintenance of infection is entirely determined by characteristics of HIV itself and its effects on immune activation, so little attention has been given to the possibility that ART drugs could contribute to viral latency.

Tenofovir, a phosphonated nucleoside reverse transcriptase inhibitor (**NRTI**), is being considered for topical pre-exposure prophylaxis (**PrEP**), so we conducted a comprehensive systems biology assessment of its impact on the mucosa in MTN-007, a rectal microbicide trial [6-8]. Surprisingly, tenofovir's effects bear potential relevance to HIV cure. It strongly inhibited the transcription of many nuclear proteins, suggesting that it could contribute to silencing integrated HIV provirus. The drug also inhibited the anti-inflammatory function of mucosal epithelial cells and stimulated signatures of increased cell growth, suggesting that it could contribute to proliferation of latently-infected cells.

These findings prompted our hypothesis that tenofovir, and perhaps NRTIs in general, have unappreciated effects on HIV latency, and may in fact prevent HIV cure by promoting the survival of cells with integrated provirus. Thus, effectively suppressing HIV with an NRTI-free regimen could have curative potential.

An important step for investigating this hypothesis is the determination whether tenofovir's inhibition of anti-inflammatory immunological function observed after topical use extends to oral administration. Drug levels in the rectum are lower after oral dosing than topical application [9, 10], but the cumulative effect of longer-term oral treatment likely leads to similar functional consequences. To investigate the effect of oral NRTI treatment on gut immune homeostasis, we plan to perform global transcriptome analysis using similar mRNA expression microarrays as in MTN-007, as well as digital droplet PCR analyses for specific anti-inflammatory genes such as IL-10, in a cohort of Seattle MSM who give rectal biopsies before and after three months of oral PrEP with Truvada. Samples will also be tested for active NRTI drug levels.

We plan to compare findings in the rectal mucosa to findings in duodenal biopsies, because drug concentrations after oral medication are expected to be higher in the upper GI tract than in the rectum. The small bowel has been implicated as an important site for harboring residual HIV provirus during suppressive ART [11, 12], which is somewhat paradoxical, since high ART concentrations should in theory restrict the size of the viral reservoir. Determining NRTI drug concentrations and the drugs' transcriptional effects in the duodenum – both has never been done before - may help to resolve this paradox.

For comparative purposes, we plan to perform similar studies with peripheral blood cells.

Lastly, NRTIs are mutagenic to E. coli [13, 14]. In conjunction with their broad transcriptional effects, as uncovered by our studies (see above), this implies that NRTI drugs may also change

the composition of the gut microbiome. By disrupting the gut microbiome and causing pro-inflammatory gene expression changes [15], tenofovir/emtricitabine may further perturb gut immune homeostasis. Altered immune homeostasis could then feed back to alter the gut microbiome, because a disruption of the mucosa's tolerogenic response to GI commensal bacteria could compromise the protective immunological niche that commensals need to thrive [16]. For these reasons we will also obtain cytobrushes from the rectal and duodenal mucosa to analyze the gut microbiome by 16S rRNA gene sequencing on the Illumina Miseq platform.

4. Inclusion Criteria

- HIV-negative
- Male gender at birth
- Age ≥18 years old
- Intent to initiate PrEP in the next 1-2 months.
- Willingness and ability to provide informed consent for study participation
- Willingness to undergo all required study procedures

5. Exclusion Criteria

- Creatinine clearance < 60mL/min
- Platelet count below the normal reference
- Coagulation (PT/PTT) tests above the normal reference
- Any prior use of PrEP
- Use of PEP within 30 days prior to study entry
- Receipt of
    - Anti-coagulant medications (e.g. warfarin). Aspirin is allowable.
    - Systemic corticosteroid medications
    - NSAID use >2 days/week
- Signs or symptoms of acute HIV infection within 14 days of study entry
- Plan to leave the Seattle area in the subsequent 2.5 months
- Any condition or substance use that, in the opinion of the study investigator, would interfere with study participation.

6. Study Procedures and Visits

Study visits:
Visits (See Schedule of Evaluations, Appendix 1) will occur at the UW AIDS Clinical Trials Unit and the GI endoscopy suite at Harborview Medical Center.

Screening
The following will occur at the Screening visit

- Informed consent for screening and study participation
- Release of Information signed to obtained follow-up HIV test 30-60d after study completion
- Targeted medical history
- Targeted physical exam
- Laboratory testing, if not documented within the prior month:
  Venipuncture for:
  - HIV testing (4th generation antibody/antigen testing)
  - Complete blood count
  - PT/PTT
  - Renal function panel
- Scheduling for entry procedures

Entry (must occur within 30 days of the Screening Visit and before inititiation of PrEP)
The following will occur at the Entry visit

- Targeted history and physical exam
- Venipuncture
  - 4 PAXgene tubes with 2.5 mL each (RNA expression studies)
  - 3 ACD tubes with 10 mL each (PBMC isolation and storage; plasma storage)
- Anoscopy with 5 rectal biopsies and one cytobrush
- EGD with 5 duodenal biopsies and one cytobrush
- Compensation for study visit
  Anoscopy $100
  EGD $250
- Scheduling for 2 month procedures

Follow-up (must occur within 50-80 days and ideally 50-66 days of beginning PrEP)
The following will occur at the follow-up visit

- Targeted history and physical exam
- Venipuncture
  - 4 PAXgene tubes with 2.5 mL each (RNA expression studies)
  - 3 ACD tubes with 10 mL each (PBMC isolation and storage; plasma storage)
- Anoscopy with 5 rectal biopsies and one cytobrush
- EGD with 5 duodenal biopsies and one cytobrush
- Compensation for study visit
  Anoscopy $100
  EGD $250

HIV testing follow-up (30-60 days after follow-up EGD and anoscopy)
Request to obtain HIV test from PCP. If not available, visit for venipuncture for HIV testing (4th generation antibody/antigen testing)

7. **Study Regimens**

Truvada (tenofovir 300mg + emtricitabine 200mg) for daily use will be prescribed by the participant's primary care physician. All prescription refills and monitoring will occur through that mechanism.

8. Concomitant Studies and Medications

Subjects will be allowed to participate in any other HIV research study as long as all protocol-specified evaluations can be performed and the other study does not prohibit participation or provide medications that interact with Truvada.

PEP

Participants seeking PEP will be referred to their PCP or to the Madison Clinic at Harborview. If subjects receive PEP, they will not be eligible for the follow-up procedures.

9. Data Collection and Analyses

All blood (except for routine blood work and HIV testing) and tissue specimens will be transported to the Hladik Laboratory. Venipuncture specimens can be transported at room temperature. Biopsy specimens should be collected in RNAlater (n=2), formalin (n=1), and in empty tubes (n=2) and transported on ice.

RNA expression studies will be performed in the Hladik Lab using Illumina 24-plex microarray hybridization chips. Drug levels in blood and tissue cells will be measured by mass spectrometry in the laboratory of Dr. Craig Hendrix at Johns Hopkins University in Baltimore. Microbiome sturdies will be done in the laboratory of Dr. David Fredricks. Samples for these analyses will be distributed and shipped to the Hendrix and Fredricks Labs from the Hladik Lab. All specimens sent from the clinic to the Hladik Lab, and then on to the Hendrix and Fredricks Labs, will be de-identified, i.e., specimen labels and accompanying information sheets will not allow the PI or any laboratory members to identify the study subjects. Communication with study subjects will only be done by clinic personnel.

All tests and data analyses will be performed for research purposes only. All experimental data will be analyzed by the Hladik Group, with help from collaborator Dr. Paul Edlefsen, a bio-mathematician with experience in high dimensional data analysis.

Microarray data will be analyzed by standard statistical tools available from the Bioconductor Suite in R as described in detail previously [15]. Microarray findings may be confirmed by immunohistochemical staining of tissue sections, also performed and analyzed in the Hladik Lab.

Drug levels will be measured by mass spectrometry and analyzed as described in detail in previous publications [17-19].

Microbiome data will be identified to species and genus levels by high-throughput, multiplexed 16S targeted amplicon sequencing on a Miseq platform by 16S rRNA targets to normalized and pooled libraries (performed by GeneWiz).

To confirm gene expression data, we may perform a number of follow-up tests with the samples, including: (1) immunohistological staining of tissues; (2) flow cytometric analysis of blood and tissue cells; (3) proteomics assays with lysed tissue cells, cytobrush supernatants, lysed blood cells, and blood plasma. We will not perform genetic DNA testing and we will not establish permanent cell lines from the samples.

13. Human Subjects Considerations

Human subjects application
This project and all materials will be reviewed and approved by the UW Human Subjects Division prior to conduct of the study and in annual progress reports if the project continues beyond the one year study period. Written informed consent will be obtained from all study participants.

Procedures
Endoscopy: The specific procedural risks of EGD with biopsies are bleeding, infection, perforation, and adverse reaction to the sedating medications. We quote risk of any of these happening to be less than 0.01%.

Anoscopy: Risks of anoscopy with biopsies are highly uncommon but could include mild to moderate discomfort, bleeding, or infection. Participating in receptive anal intercourse after anoscopy might lead to an increased risk for transmission of HIV. Participants will be advised to avoid receptive anal intercourse for the 24 hours prior to anoscopy and to use condoms for receptive anal intercourse for 7 days after anoscopy or until no blood is found.

Confidentiality
All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to study staff.

As part of the study, subjects will be given a study-specific code (e.g. Initials-####). All study-specific laboratory specimens, reports, study data collection, and administrative forms will be identified by this study code.

All records that contain names or other personal identifiers (e.g. consent forms, contact information) will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems.

Communicable disease reporting
HIV is exempt from reporting requirements in the state of Washington as long as persons diagnosed with HIV are referred to One on One or their primary care providers for care. Participants will be made aware of all reporting requirements during the study informed consent process.

Appendix 1: Schedule of evaluations

	Screening	Entry[1]	2 month	30-60d after study
Clinic visit	X	X	X	X [3]
Screening consent	X			
Study consent	X			
HIV testing	X		X	
CBC/Coags/renal function[2]	X			
EGD/anoscopy		X	X	
Blood volume (mL)	20	36	42	?
Length of visit (min)	60	180	180	15

[1]The Entry Study visit must occur within 30 days of the screening visit and before initiation of PrEP

[2]If not documented within the prior month.

[3]If results are not available from the PCP/ROI signed at study screening.

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