Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)

National Clinical Trial (NCT) Identified Number: NCT04232540

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Protocol Number: 122319

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Principal Investigator: Angela Kashuba

<IND/IDE> Sponsor: not applicable

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All versions should have a version number and a date. Use the international date format (day month year) and write out the month (e.g., 23 June 2015).

Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
SOE, Introduction and Background	• Removed the term "real-time" throughout the document o Pertinent feasibility endpoints now use "delivery of the report to the designated research staff member within 2 hours of initiation of hair processing" rather than "…within 2 hours of hair sample collection" •	Revisions made due to Covid-19 pandemic.
	• Removed mitra sampling for the remaining 15 participants in cohort A o Now stated as "blood sample will be collected for 10 participants in Group A and a subset of participants in Group B." After Mitra is analyzed for the first 10 participants in Group B, we will reevaluate to determine if	

	additional samples are needed to validate hair samples.	
2.A.1, **6.A.1,** **6.A.2** **8.0** **10.1.1**	• V1 and V2 study activities will be conducted virtually (except hair collection and in-person patient provider clinic visits) to minimize in-person contact o Consent and screening will be conducted virtually using videoconferencing via zoom on computer, tablet or phone o All questionnaires (patient baseline, patient post-visit, patient endline, provider baseline, provider post-visit, and provider endline) will be conducted virtually through REDCap (emailed to participant or verbally administered via phone or videoconferencing) o **Hair collection will be conducted by research staff at remote locations to limit time in clinic** o **Implementation of the intervention will now be allowed in the setting of telehealth clinical visits between patient and provider in addition to in-person clinical visits** o MedViewer reports will be sent to providers via secure email o All remaining V2s will be conducted virtually. Hair and blood samples will not be collected. •	
Section 8	• Consenting will take place up to 3 days before scheduled clinic appointment with provider o Hair sample will be collected by research staff at a remote location in the 3 days between consent and the scheduled provider visit o MedViewer report will be discussed between patient and	

	provider (or patient and pharmacist, if needed) within 4 weeks of hair sample collection •	
3.0, 9.1, 9.2, 9.3, 9.4.2, 9.4.7, 9.4.8	• Seven endpoints have been revised • Statistical analysis revised	
	• Interviewer-administered portions of the baseline questionnaire have been modified o Health Literacy measure (Newest Vital Sign) removed o ART adherence Visual Analog Scale modified to allow for remote self-administration •	
	• Maximum duration on study extended by 6 months for providers (increased from 10 to 16 months) • Provider sample size range reduced (from 20-30 to 16-30)	

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

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1 PROTOCOL SUMMARY

SYNOPSIS

Title: **E**stablishing **N**ovel **A**ntiretroviral **L** **I**maging for **H**air to **E**lucidate **N**onadherence

Study Description: The proposed study is a single-arm cross-sectional study to pilot test the implementation of infra-red (IR) matrix-assisted laser desorption electrospray ionization (MALDESI) for mass spectrometry imaging (MSI) of hair among patients receiving HIV care in the University of North Carolina (UNC) Infectious Diseases (ID) Clinic. The study will investigate feasibility, acceptability, and appropriateness of utilizing the IR-MALDESI MSI assay (named MedViewer) as an investigational clinical adherence-monitoring tool. The aim of the MedViewer test is to a) longitudinally quantify, in easy to understand patient and provider reports, antiretroviral (ARV) concentrations in patient hair; and b) enhance adherence counseling conversations between people living with HIV and their medical providers through review and discussion of the MedViewer report during a clinic visit. The study will implement the MedViewer test with 50 eligible patients who are living with HIV across two viral load strata, administer brief visit-specific questionnaires to all patient and provider participants, and conduct qualitative in-depth interviews (IDIs) and quantitative endline questionnaires with a subsample of patient participants (n=30) and all provider participants. The two strata will be based on the participants' viral load within the past two years. Group A (n=25) will consist of participants with a viral load below the limit of quantification for the previous two years with documentation of at least one test in the last six months. Group B (n=25) will consist of participants who have had at least one HIV RNA test above the limit of quantification within the previous two years.

This study comprises Aim 3B of R01AI122319.

Objectives: Primary Objective:
Investigate the feasibility of delivering the MedViewer intervention as planned, the acceptability to patients of participation in the MedViewer intervention, and the appropriateness of MedViewer use for adherence counseling.

Secondary Objective:
Investigate additional dimensions of feasibility, acceptability, and appropriateness of using hair (MedViewer) to provide feedback to people living with HIV regarding longitudinal patterns of medication adherence.

Endpoints: Primary Endpoints:

1. Feasibility: Proportion of participants receiving the MedViewer report during their provider visit, as planned.

2. Acceptability: Proportion of contacted patients who are eligible for a screening visit (not including inclusion criterion 9) who agree to participate in the MedViewer Intervention pilot study.
3. Appropriateness: Perceived usefulness of the MedViewer intervention for adherence counseling (assessed through in-depth interviews).

Secondary Endpoints:

Feasibility:

1. Reasons for patient's non-receipt of the MedViewer report during a visit with provider or pharmacist within 4 weeks of hair collection (if applicable).
2. Reasons for non-discussion of the MedViewer report with provider or pharmacist (if applicable).
3. Duration of time (in minutes) from initiation of hair processing to MedViewer report delivery to designated research staff member.

Acceptability:

1. Provider-reported likelihood of recommending MedViewer to future patients.
2. Patient-reported likelihood of agreeing to future MedViewer use.
3. Patient comprehension of the MedViewer report.

Appropriateness:

1. Perceived usefulness of MedViewer to promote ART adherence.
2. Perceived impact of MedViewer use on patient-provider communication and relationship.

Study Population:

Patient participants:
We will enroll 50 patients, aged 18 or older with at least 1.0 cm natural caput hair, from the UNC ID Clinic. All participants will be people living with HIV, prescribed an ARV regimen eligible for the MedViewer test, and willing to participate. Patients will be enrolled into one of two strata: Group A will be patients with all HIV RNA results below the limit of viral quantification over the previous 2 years with documentation of at least one test in the previous six months. Group B will be patients who have had at least one HIV RNA result above the limit of viral quantification within the previous two years.

We will screen and enroll patients of all gender identities, racial identities, ethnicities, and ages (above 18 years), so the demographic distribution of screened participants reflects that of patients at the UNC ID Clinic who are living with HIV. We will enroll participants into two viral load strata as defined above for Group A (N=25) and Group B (N=25).

We will also enroll a subsample of up to 30 participants enrolled in the larger study to participate in a follow-up study visit, which will include an in-depth interview (IDI) and an endline questionnaire.

	Provider participants: We will seek to enroll all (~15-30) medical providers who see HIV-positive patients at least one half-day per week in the UNC ID Clinic, who have stated willingness and availability to comply with all study procedures for the duration of the study.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	The study will include only one main research site, UNC Medical Center, a large academic hospital-based primary and tertiary care center in Chapel Hill, North Carolina. The study procedures will be conducted in the hospital's Clinical & Translational Research Center (CTRC) and the satellite UNC Infectious Diseases (ID) Clinic, which serves approximately 1900 HIV-positive patients living with HIV from a wide catchment area.
Description of Study Intervention:	The study intervention consists of four main components: **1) Standardized training session for medical providers:** Training session (approximately 30-60 minutes) at the start of the study (and offered at alternate later times) to introduce providers to MedViewer, how the report will be obtained and shared with patients, how to interpret MedViewer report, and possible strategies to discuss results with patients using IRB-approved communication aids. **2) Informational video for patients:** Approximately eight-minute video to introduce patients to the MedViewer test, the hair collection process, and how test results can provide useful information to both patients and providers. Patients will have the opportunity to watch the video more than once if requested. **3) Hair sample, MedViewer test, and accompanying MedViewer report (patient and provider versions).** Patient participants will provide a hair sample, which will be used for a MedViewer test to generate two distinct visual reports of the results (one version for patients and one version for providers), which will be delivered to the provider to discuss with the patient during a clinic visit (telehealth or in-person) with the provider or pharmacist within 4 weeks of hair sample collection. **4) Communication aids for providers:** Aids intended to facilitate providers' discussions of MedViewer report with their patients, including a one-page reference sheet with possible strategies to counsel patients on adherence using MedViewer report and a one-page FAQ to clarify potential concerns.

The intervention will be conducted with both patient and provider participants to learn about feasibility, appropriateness, and acceptability of using MedViewer from both the patient and provider perspective.

For patients, intervention activities include watching the brief MedViewer video, providing a hair sample to be sent to the laboratory for the MedViewer test, and receiving and reviewing the MedViewer report with their provider or a pharmacist. Patients in Group B and a subset of patients in Group A will also provide a blood sample to measure antiretroviral concentrations in comparison to the hair.

For providers, intervention activities include participating in the provider MedViewer training session and integrating each patient's investigational MedViewer report into a clinic visit that occurs within 4 weeks of hair sample collection for investigational purposes. Each time the provider has an appointment scheduled with a patient participating in the study, MedViewer results will be delivered to the provider before the patient's appointment. During the appointment, the provider will review and discuss the MedViewer report with the patient as part of their routine adherence counseling discussion. The provider may refer to the communication aids, per personal preference, to help guide the MedViewer discussion. If the provider does not receive the report before the patient appointment, he or she will decide with the patient whether to postpone the provider's MedViewer discussion with the patient until the result is ready or request that the UNC Infectious Diseases Clinic HIV Pharmacist discuss the results with the patient.

To produce the MedViewer report, a trained researcher will collect a sample of hair from the participant's head and transport the sample to the laboratory per standard operating procedures. The analyst will run the MedViewer test to generate two distinct visual results reports (one version for patients and one version for providers) and send the visual MedViewer reports back to the research team member via a secure (encrypted) email. The research team member will match the patient's unique ID number back to the patient's name, print the MedViewer reports, and deliver electronic or hard-copy reports to the provider before the appointment with the participating patient.

Study Duration: The estimated time of the study, from the start of enrollment to completion of data collection, is expected to be approximately 16 months. This includes 1 month of initial provider participant enrollment, approximately 7.5 months of patient participant enrollment and data collection, and 1.5 months of final follow-up patient participant and provider participant data collection). The study duration has been extended by 6 months due to delays related to COVID-19.

Participant Duration: Patient Participants: Expected duration of patient participation in the study is one day (approximately 4 hours), with intervention activities and

quantitative data collection conducted during a single study visit that can take place over multiple days as needed.

IDI subsample of patient participants: A subsample of up to 30 patient participants will be enrolled for 2-13 additional weeks depending on how soon they complete their second study visit, including an IDI and endline questionnaire (scheduled for 28 days (-14 days/+60 days) following the MedViewer clinical visit with the provider or pharmacist). The IDI and endline questionnaires are expected to occur on a single day but may occur on separate days.

Provider Participants: Expected duration of medical provider participation in the study is approximately 10 months, corresponding to 1 month of the initial provider recruitment period (pre-patient-involvement), approximately 7.5 months conducting patient clinic visits to deliver MedViewer reports, and 6 weeks (1.5 months) after the close of patient enrollment to complete the endline questionnaire and IDI. The IDI with each medical provider will be conducted at any point after the provider has delivered MedViewer reports to at least two patients or following the close of patient enrollment, whichever comes first.

SCHEMA

1.A.1 PATIENT PARTICIPANT STUDY SCHEMA

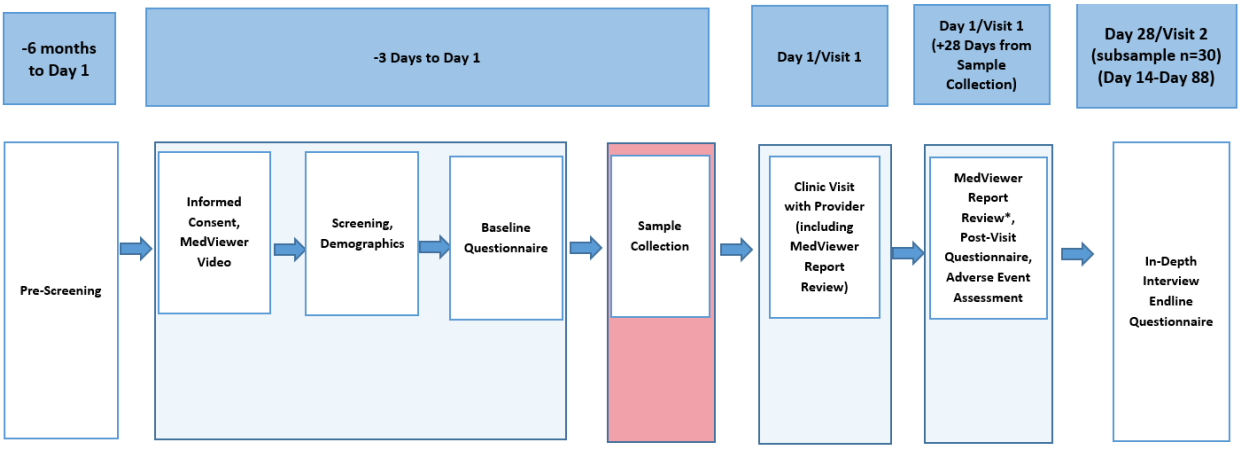


Figure 1: Patient participant study schema. Sequence of ENLIGHTEN study activities for patient participants.

*NOTE: MedViewer Report Review can be conducted up to 28 days from hair sample collection if not completed at Day 1 Clinic Visit with Providers

1.A.2 PROVIDER PARTICIPANT STUDY SCHEMA

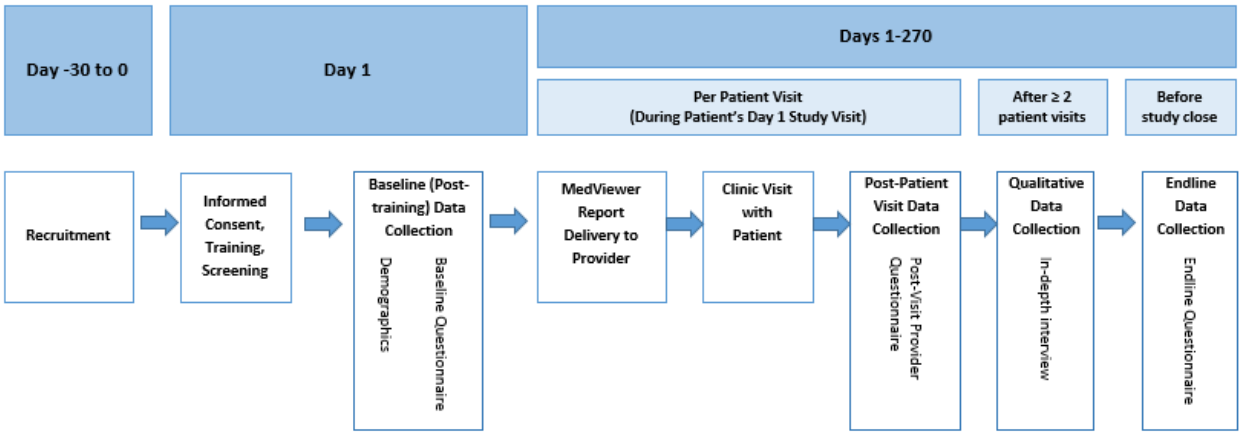


Figure 2: Provider participant study schema. Sequence of ENLIGHTEN study activities for provider participants.

SCHEDULE OF EVENTS (SOE)

There are two Schedules of Events (SOEs) shown below: one for patients and one for providers.

Patients: This table represents the SOE that will occur for <u>each</u> patient participant. Day 1 represents the day of enrollment for each patient participant.

Procedures	Pre-screening Day -6 months to Day 1	Screening and Enrollment (-3 Days to Day 1)	Study Visit 1/Day 1	Study Visit 2 Day 28 (-14/ +60 days)
Pre-screening	X			
Screening Visit		X		
Informed consent and MedViewer video		X		
Collect Study samples (Hair and Mitra*)		X		
Demographics		X		
Baseline patient questionnaire		X		
Clinic Visit with Provider			X	
MedViewer Report Discussion**			X	
Post-visit patient questionnaire			X	
In depth interviews and endline questionnaire (phone, computer-based or in-person) to subsample of ~30 patients				X
Adverse event review			X	X

*- Mitra will only be collected for participants in Group B and a subset in Group A.
**- MedViewer Report Review can be conducted up to 28 days from hair sample collection if not completed at Day 1 Clinic Visit with Providers

Providers: This table shows the SOE for provider participants. The exact duration of time for provider study visits with patients will vary based on the date of each provider's enrollment. Days -30 to 0 represent the initial provider recruitment period. Day 1 represents the first day of provider enrollment. Day 228 represents the final day of provider enrollment (corresponding with the last day of the 7.5-month period of patient enrollment and patient Study Visit 1). Day 270 (Day 228 + 42 days for endline provider data collection following the close of provider enrollment) represents study close and the end of provider involvement. Time frames represent providers' active participation in the study, not including study delays related to COVID-19.

Procedures	Initial Recruitment period Days -30 to 0	Screening/Enrollment/ Training, Baseline Data Collection, Day 1 - 228	Visits with patients Days 2 – 228	In-depth interview Days 2 – 270	Endline questionnaire Days 2 – 270
Provider recruitment	X				
Provider consent, screening, demographics		X			
Provider training		X			
Post-training questionnaire		X			
Patient Visits –Provider receipt of MedViewer reports and MedViewer discussion with patients (see patient SOE above)			X		
Post-visit provider questionnaire (1 per enrolled patient visit)			X		
In-depth interview (after seeing at least two patients or before study close, whichever comes first)				X	
Endline questionnaire					X
Adverse event review		X	X	X	X

2 INTRODUCTION

STUDY RATIONALE

This study is part of a larger research program (R01AI122319) that is generating data on a novel non-invasive and longitudinal approach to rapidly quantify antiretroviral (ARV) concentrations and provide evidence of drug ingestion. The goal is to provide the clinician/researcher and patient/study participant feedback on ARV adherence. The novel approach utilizes infra-red (IR) matrix-assisted laser desorption electrospray ionization (MALDESI) technology for mass spectrometry imaging (MSI) to visualize and quantify ARV concentrations in hair. For the ease of patient/provider communication, we have labeled this IR-MALDESI MSI approach as "MedViewer" and will refer to it as such throughout this protocol.

Adherence to ARV therapy is critical for achieving HIV RNA suppression in people living with HIV and for preventing HIV acquisition in uninfected individuals using pre-exposure prophylaxis (PrEP). Yet a high level of adherence can be challenging for people living with HIV on life-long ARVs and for HIV-negative individuals using daily PrEP who are not at daily risk for HIV acquisition. In multiple recent double-blind, placebo-controlled PrEP studies, poor adherence was primarily responsible for a lack of drug efficacy [1–4]. These studies found that counting product returns and using patient self-report significantly over predicted adherence as measured by ARV concentrations in blood plasma or cells [1]. Since the consequences of poor or intermittent adherence are significant, valid measures of adherence, particularly adherence patterns over time, are critical for optimizing the effectiveness of both HIV treatment and prevention in clinic and research settings. Blood plasma or intracellular concentration monitoring has been considered the "gold standard" for determining whether an ARV has been ingested and is a common marker for therapeutic drug monitoring or clinical trial adherence monitoring. However, this approach has its own set of limitations, including being invasive, requiring advanced processing or storage (e.g. intracellular measures), being a short-term measure of drug taking behavior (depending on the half-life of the analyte), and requiring long turn-around times or substantial sample processing prior to analysis.

We propose the use of MedViewer to quantify and visualize ARV concentrations in hair. We hypothesize that MedViewer can rapidly quantify ARV concentrations, provide non-invasive and longitudinal evidence of drug ingestion, and allow for clinician/researcher and patient/study participant feedback on adherence performance. Preliminary results from our investigation of MSI in hair suggest it has potential value in measuring longitudinal drug exposure [5]. Preliminary results from our R01 suggest that MedViewer has potential value in distinguishing between patients who don't initiate ARV medication, those who miss doses, and those who don't persist [54]. However, little is known about whether or how such an intervention tool would be accepted by patients or providers or how best to implement it in the context of a clinic setting. The aim of this pilot study is to investigate the acceptability, appropriateness, and feasibility of using MedViewer in the setting of clinical monitoring. The goal of this work is to develop a simple, noninvasive, longitudinal depiction of ARV adherence that will provide high clarity feedback for both clinicians and patients. Given that this is a feasibility pilot, no change in clinical care is anticipated from the intervention. While providers will use the investigational reports during their conversations with patients about ARV adherence, provider participants will still only utilize currently accepted measures of ARV adherence, including measuring HIV viral load, utilizing the results of HIV genotyping in the setting of viremia, and utilizing therapeutic drug monitoring of antiretrovirals in making clinical decisions.

Results from our formative work in Aim 3a suggest that a fully validated MedViewer would be an acceptable, appropriate, and feasible method for providing patients living with HIV with feedback regarding longitudinal patterns of medication adherence[6,7]. Piloting the implementation of MedViewer is critical for determining the extent to which the formative results hold true and identifying potential modifications to the MedViewer procedures for future optimal implementation at scale.

We will use 2 viral load strata for recruitment and main analyses and 3 viral load groups for some exploratory analyses. We selected and defined these strata based on several factors: **(1) clinical importance of understanding adherence and the potential effects of MedViewer on adherence among patients with different VL levels; (2) empirical data of the distribution of VL levels among patients in the UNC ID Clinic; (3) practical considerations of the need to enroll participants efficiently; (4) considerations of the statistical precision and power.**

1. **Clinical importance of understanding adherence and the potential effects of MedViewer on adherence among patients with different VL levels:** Input from the medical officer and clinic director of the study were based on clinical judgement and literature review. Based on their input, it was deemed that a 2-year viral load history will provide sufficient data to identify and distinguish patients with at least 1 detectable VL from patients with a history of ALL undetectable viral loads. ***Considered of particular importance, suboptimal adherence is most likely to occur (and hence be most important to explore) among patients with a history of at least 1 detectable viral load.*** Thus, it will be particularly useful to understand the longitudinal adherence patterns (as reflected by the concentration of medication in hair) among patients with history of having at least one detectable viral load.

   Whereas serum viral load is checked every three to six months and is challenging to relate to specific adherence patterns, ARV concentration measurements in hair provide more granular information about day-to-day adherence in the weeks or months prior to hair sample collection. Therefore, recruiting patients with a history of ALL undetectable VL levels in the past two years at this pilot stage of research, will help clinicians and researchers understand the extent to which undetectable VLs measured only periodically reflect consistently good adherence. It will also help researchers and clinicians understand the extent to which variability in concentrations of ARVs in the body/hair over time does not lead to virologic failure.

   Thus, it was deemed important to explore MedViewer among both (i) patients with histories of ALL undetectable viral loads, and (ii) patients with a history of at least 1 detectable viral load. **These same strata will be used for our main analyses.**

   Regarding recruitment and analysis of the strata of participants who had at least 1 detectable viral load, it was deemed clinically important to investigate, in an exploratory manner, longitudinal ARV concentrations of patients who are usually undetectable but experience occasional low-level "blips" in their viral load (e.g. at levels up to 1,000 or 10,000 copies/mL) compared to those with higher levels of detectable VL. The clinical etiology and full implications of such blips are not well understood, but it is increasingly evident that any time with detectable viral load confers higher risk of morbidity (including vascular inflammation, neurologic and cognitive impact, risk of malignancy, and immunologic compromise). Exploring the relationship between longitudinal patterns of ARV concentrations in hair among people having viral blips and comparing these patterns with those of undetectable and higher level detectable VL will shed light on the clinical importance of blips. Therefore, it will be important to recruit sufficient numbers of individuals who have such blips as described below in points 2-4.

2. **Empirical data of the distribution of VL levels among patients in the UNC ID Clinic.** While it will be important to recruit enough patients with blips, patients with VL between undetectable to 1,000 copies/mL (or even undetectable to 10,000 copies/mL) represent a relatively small proportion of the UNC ID Clinic population (15%), and the best viral load to use as a cut-off for determining a blip remains undefined. In descriptive analyses among over 2,000 patients living with HIV in the UNC ID Clinic with at least 1 VL measure in the last 2 years, 15% had at least 1 undetectable VL and 1

detectable VL while on ARVs. These individuals were evenly distributed among those whose highest detectable VL was <1,000 copies/mL versus those ≥1,000 copies/mL. Hence, we determined that recruitment stratification requiring balanced numbers would not be necessary to obtain sufficient numbers of patients within each of the two detectable VL groups (those below, vs. at or above, 1,000 copies/mL) in order to conduct exploratory analyses because they were evenly distributed in the clinic naturally.

3. **Practical considerations of the need to enroll participants efficiently.** Further, recruiting all eligible patients with at least 1 detectable viral load would enable recruitment to proceed faster (and more flexibly) than if stratified by the level of detectable VLs.

4. **Considerations of the statistical precision and power.** The main analyses will use these two strata (described in 9.2). However, exploratory analyses will be conducted using the 3 viral load groups: (i) all undetectable at the level of quantification of the laboratory, (ii) those with at least 1 detectable but < 1,000 copies/mL and (iii) those with at least 1 detectable VL ≥ 1,000. Given that the proportion of patients in the two upper strata are approximately equal, but combined are only 15% of the total available participants, oversampling of the detectable stratum (compared with its natural distribution) during recruitment at a 1:1 ratio (25 in detectable stratum and 25 in the undetectable stratum) will allow sufficient number of participants in each of the 3 analytic groups to enable exploratory comparisons as per the statistical power calculations.

A. BACKGROUND

Over the past 10 years, hair analysis has gained importance in forensic sciences [8], drug testing [9,10], toxicology investigations [11], and for evaluating drug adherence [12]. Hair is unique in that it has the potential to provide information about drug intake over a longer period of time compared to other biological fluids, including plasma [2], blood cells [13], and urine [14]. Advances in analytical technology have transitioned hair analysis from gas chromatography-mass spectrometry (GC-MS) methods to more efficient and sensitive liquid chromatography-triple quadrupole (LC-MS/MS) methods. However, several limitations exist to current hair analysis techniques that constrain the utility of this technology to rapidly quantify drug exposure over time and be used as a measure of adherence to inform and influence drug taking behavior:

1. LC-MS/MS QUANTIFICATION REQUIRES LARGE AMOUNTS OF HAIR. Despite the advances in sensitive LC-MS/MS instrumentation, some ARVs require collecting a thatch of up to 100 strands of hair (20). Since no one multiplex LC-MS/MS method exists to measure all ARVs in a single sample, multiple thatches are required for complete ARV evaluation. This can be a significant deterrent for individuals with short hair, for those who require hair collected between braids, or who object to the collection of large amounts for cultural reasons [15]. Conversely, mass spectrometry imaging (MSI) utilizes single hair strands for analysis. MSI is the only imaging technique that can determine the identity and distribution of drugs and their metabolites in biological matrices with one test without complicated labelling approaches. We have previously demonstrated that our IR-MALDESI MSI technique is suitable for ARV detection in biological samples [16–18], and show here that it can quantify ARVs in hair.

2. LC-MS/MS HAIR QUANTIFICATION REQUIRES TIME-CONSUMING PROCESSING. Although sensitive and specific, LC-MS/MS data requires at least 7 steps to process hair for analysis, including segmentation, decontamination, cutting or grinding, extracting, and purifying before analysis can occur [19]. In addition, these tests require processing of "blank" matrices for standard curve and quality control samples. IR-MALDESI MSI requires minimal sample processing, allowing the sample to be analyzed within two hours.

3. NO DATA EXIST ON USING REAL-TIME HAIR MONITORING IN PEOPLE LIVING WITH HIV. Because of the time-consuming nature of sample processing, real-time monitoring of adherence in hair has not yet been performed. Rather, this technique has been limited to retrospective analysis of clinical trial or clinical cohort data. Utilizing an IR-MALDESI approach can shorten the time to data generation to two hours. We will investigate the feasibility of conducting adherence monitoring in a clinic setting utilizing this novel technology.

4. LC-MS/MS PROVIDES LIMITED DATA ON ADHERENCE OVER TIME. Current homogenate methods only provide drug exposure averaged across hair strands and lengths and do not provide adherence information on a time scale. For example, for those with long hair, recent changes in adherence can confound past adherence patterns. Normalization of hair length between subjects is not routinely performed, as logistical difficulties (e.g. amount of hair curl, large number of strands needed for analysis) preclude uniformly segmenting hair. MSI combines the specificity of current LC-MS/MS methods with localization information similar to whole body autoradiography. By combining rate of hair growth (~1cm/month) with the amount of drug exposure along the length of the hair strand, drug exposure over time can be accurately determined. Furthermore, it is possible that a more granular dosing history can be constructed by mathematical modeling of the concentration data along the strand over time by incorporating partial differential equations.

5. LIMITED DATA EXIST ON ACCEPTABILITY OF HAIR COLLECTION FOR DRUG MONITORING. Most hair sampling to date has been conducted in the context of clinical investigations with retrospective analyses. Recently, a clinical study evaluating the use of ARV hair concentrations from Ugandan children living with HIV found collection challenging, and the investigators suggested that community education and buy-in was integral to implementing this intervention [20]. In a study that examined perspectives, beliefs, and concerns of ARV users providing hair specimens for ARV concentration, participants found this request to be unusual, and identified several cultural beliefs that could impede sample collection. However, the majority of participants were willing to give hair if provided with enough information beforehand [21]. In Aim 3a, we conducted formative work by engaging with patients and providers to understand the anticipated acceptability of a hypothetical tool to monitor ARV adherence in the clinical setting, but we lack data on acceptability from actual implementation of a longitudinal adherence monitoring tool.

6. MEDICAL PROVIDERS COULD BENEFIT FROM SUPPORT TO HELP THEM OVERCOME SUBSTANTIAL CHALLENGES THEY FACE ADDRESSING ARV ADHERENCE WITH THEIR PATIENTS. Research, including our statewide survey of NC HIV providers, has found that physicians face challenges when counseling their patients about ART adherence [22–30], and that health care professionals would benefit from additional support to effectively address adherence [30–33]. Our research, and that of others, has demonstrated that counseling with feedback can enhance medication adherence [34–40]. For example, in PACT, we tested the use of motivational interviewing with

visual feedback of MEMS adherence data in the UNC ID Clinic; the intervention group had 2.75 times higher odds of achieving >95% adherence than controls (95% CI:1.023-7.398) [41]. Through additional studies, we have documented methods for supporting HIV providers in effectively adopting new practices and tools to improve patient outcomes, and strategies to encourage patient-provider partnerships in medical decision-making [41–49]. Hair MSI adherence data will allow us to take this work a step further, using state-of-the-art technology to give providers a new tool for conducting data-driven, real time adherence counseling.

An IR-MALDESI MSI method has been validated for the quantitation of emtricitabine (FTC) and dolutegravir (DTG) in hair strands (Appendix **E.1**. As part of this validation, we have determined lower limits of quantitation for each of these two ARVs (FTC: 0.27 ng/mg; DTG: 0.04 ng/mg) based on calibration of IR-MALDESI MSI response using a series of prepared hair standards are within the range of incurred samples we have evaluated as part of Study Aim 1. These medicines are available in many combination pills, such as Truvada®, Biktarvy®, and Tivicay®, and several others. Method accuracy and precision have been evaluated from incurred samples for each of the two targeted ARVs, meeting acceptance criteria, and are also summarized in the method validation report. We have developed a quantitative workflow for rapid, sensitive analysis of hair strands that is performed in two steps. First, a calibration of IR-MALDESI MSI response to ARVs is conducted by uniformly spraying ARV standards in increasing concentration onto blank hair strands using a templated acrylic mask, resulting in a linear response to ARVs over relevant concentration ranges. Second, evaluation of all ARV response in hair strands is measured simultaneously with an internal standard (IS) and melanin biomarker.

We have investigated differences in IR-MALDESI ARV response based on hair color and cosmetic alteration (Appendix F.1). Through simultaneous assessment of ARVs and a melanin biomarker, we observed a positive correlation between IR-MALDESI ARV and melanin response in hair strands for dolutegravir, and a slightly negative correlation between emtricitabine and melanin. Normalizing the average IR-MALDESI response to daily dosing for FTC and DTG did not improve the relative standard deviation of observations. These results indicate no advantage to scaling IR-MALDESI signal abundance based on melanin content in hair. Mechanical manipulation of hair strands collected from Aim 1 were performed according to specified package instructions from commonly used products for bleaching, dyeing, and relaxer treatments. Matched treated and untreated hair strands were evaluated under the same experimental conditions to assess any changes in IR-MALDESI MSI response to ARVs resulting from the treatment process. Only dyeing resulted in decreased response for dolutegravir in hair, but all treatment regimens reduced emtricitabine below the limit of detection. Evaluation of emtricitabine in hair strands collected as part of NCT02768779 from patients reporting a variety of recent hair treatments indicated that treatments within a period of 4 weeks from sampling did reduce the IR-MALDESI signal abundance, but that treatments further in the past would not influence measurements focused on the past month of adherence. Therefore, volunteers will be excluded if they have had treatment to their hair within the past 4 weeks per self-report. For this protocol, chemical hair treatments will be defined as partial or complete bleach, dye or relaxer within the 28 days prior to the enrollment visit. Other hair treatments are not exclusionary. Volunteers enrolled in the sub-study will

also be excluded if they have self-reported chemical hair treatments within the 28 days prior to that visit.

Benchmarking of IR-MALDESI MSI longitudinal ARV profiling (Appendix D.1) has been conducted through 3-phase (single dose, daily dose, dose proportionality) directly observed therapy studies with FTC+TFV and DTG (NCT03218592). IR-MALDESI MSI longitudinal profiles from samples collected at the end of the third phase detected changes in the intensity of response to ARVs along hair strands corresponding to the prescribed phases of the directly observed therapy studies. Steady-state profile response to daily dosing across study participants was measured to establish optimal hair response and adherence thresholds analysis based on receiver operating characteristic curves of the IR-MALDESI MSI ARV profiles.

Additionally, we have conducted a formative qualitative study (Aim 3a of R01AI122319) to inform intervention development for this pilot study around feasibility, acceptability, and appropriateness of MedViewer implementation in a busy clinic environment. We collected qualitative data from 30 patients and 29 providers of the UNC ID Clinic and obtained input from two Community Advisory Boards to refine the procedures that will be used to deliver MedViewer test results to patients in this pilot study. First, we developed three prototype graphical displays of hypothetical adherence data and interviewed 30 patients living with HIV and 29 clinicians to explore their reactions to each graphical display. Patients and clinicians expressed enthusiasm about using the graphs to facilitate understanding of patient adherence. Several themes also emerged regarding enhancing usability of the graphs: Patients and clinicians questioned why graphs would show drug concentrations above the therapeutic range. For patients with lower health literacy, the graphs did not provide enough understanding of the link between medication-taking and drug concentrations in hair - these patients also preferred pictographs over bar or line graphs. Clinicians preferred daily drug concentration data in bar graphs accompanied by information about the measure's variability. These findings indicated a need for separate visual results displays for patients and providers, with provider displays offering more complex information. [6]

We also sought patient and provider opinions about applying real-time feedback into patient-provider conversations about adherence. Both patients and providers suggested that identifying patterns of non-adherence with real-time feedback could facilitate problem-solving, provide motivation to improve adherence, and reinforce the importance of optimal adherence to ART by comparing adherence and viral load results. Some providers worried that discussing real-time monitoring results could harm the patient-provider relationship by implying mistrust of patient-reported adherence. A few providers were also concerned that adherence monitoring could discourage patients' retention in care if they feel they are being "policed." Similarly, several patients felt that receiving suboptimal results could make participants feel defeated and reduce their adherence motivation. To address these concerns, we found that communication guidance to providers about discussing real-time MedViewer Report with patients should focus on both optimizing adherence and mitigating negative perceptions of adherence monitoring to preserve trust and engagement in care. While our formative work indicates that patients and providers anticipated finding MedViewer acceptable and appropriate, no data exist regarding actual acceptability, appropriateness, or feasibility of MedViewer implementation in a busy clinic, a gap that we will address with this study. [7]

Our proposed study is highly significant in that it not only addresses the limitations of current biologic approaches to quantifying adherence but also provides solutions to many of the current analytical

limitations of hair analysis. Our novel comprehensive engagement of stakeholders for determining optimal approaches to providing hair adherence data will allow a complete understanding of strengths and limitations of implementing this approach in a clinical setting. Achieving the goals set forth in this application will significantly enhance our understanding of drug pharmacology in hair and place hair data generated by MedViewer in context for clinical studies and clinical practice. Although these data may not, by themselves, solve all issues related to measuring adherence, our current success in mass spectrometry evaluation of ARVs in complicated matrices, track-record of measuring ARVs by IR-MALDESI MSI, and expertise in social and behavioral research highly justifies further pharmacologic investigations into the use of hair imaging to quantify adherence. The approach we discuss here has widespread implications in clinical and research settings.

RISK/BENEFIT ASSESSMENT

2.A.1 KNOWN POTENTIAL RISKS

Risks from participation in this study and from exposure to the intervention are minimal.

Risks to patient participants

- **Video.** Although the MedViewer intervention video is designed to introduce patients to MedViewer in an approachable manner, it is possible that patients will experience discomfort or anxiety watching a video about providing a hair sample for conducting the MedViewer test and about antiretroviral medication circulating in their body depositing in their hair. To minimize this risk, in addition to having designed the video with extensive input from patients, we will also inform patient participants prior to watching the video that it is currently for investigational use only and that they may stop it at any time if they feel uncomfortable.

- **Baseline and post-visit questionnaires**: Immediate known potential risks to participating in the questionnaires include the potential for patients to feel uncomfortable or experience anxiety when being asked questions about their medication adherence and about their interaction with their provider. To minimize this risk, questions are designed to be asked in a neutral, non-judgmental manner and patients will be provided assurance that their information will remain private, will not be known to their provider nor affect their medical care. They will also be informed that they may stop or skip any questions that they do not wish to answer.

- **Hair collection**: There is no foreseeable risk with hair collection. No hair related adverse events have been reported on prior hair collection protocols, and we will collect hair samples on this protocol using the same operating procedures.

- **Blood collection**: Blood will be collected via phlebotomy or fingerstick. Risks associated with blood draws include bleeding, discomfort, feelings of dizziness or faintness, and/or bruising, swelling and/or infection. A finger stick is a bit uncomfortable and may cause some soreness and bruising. Very rarely, the site can become infected. The research team will attempt to minimize these risks by having all samples collected by trained professionals using aseptic technique.

- **MedViewer test and report:** It is possible that discussing MedViewer report may alter patient-provider communication during the clinic visit, such that the patient and/or provider feels uncomfortable. Patient participants may feel anxious or embarrassed reviewing their MedViewer Report with the provider or pharmacist or uncomfortable if the Med-Viewer results contradict self-reported adherence. Additionally, misinterpretation of MedViewer report may lead to misunderstanding of or confusion about adherence patterns and potentially misinformed conversations about patient medication-taking. To minimize these risks the study team will take every measure to answer patient questions about the report and to normalize nonadherence nonjudgmentally during the MedViewer video. The study team will also train providers to clearly understand and explain MedViewer results and inform both patient and provider participants that MedViewer reports are currently for investigational use only and that no clinical decisions should be made based on MedViewer reports. The study team will also ask patients, in post-visit questionnaires and (for the subsample) IDIs, to report the extent to which they perceive that the report and their beliefs about their medication-taking align.

- **Confidentiality:** Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions. Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others and that social harms may result (i.e., because participants could become known as HIV-infected). For example, participants could be treated unfairly or discriminated against or could encounter lack of acceptance by their families and/or communities. Several systems are in place to maintain participant confidentiality. All hard copies of study records, including all documents with personally identifiable information, will be kept in a locked drawer in a locked office to which only the study investigators will have access. Samples, study data, and study report forms will not contain patient names; rather they will be labeled with a unique study identification (ID) number with a combination of letters and numbers. The file that links participant identification numbers to their names and other identifiable information will be password-protected and kept by one study investigator on a secure internal computer network, separate from the study data. All electronic data for this study will be stored on a dedicated University server which contains extensive protections and securities. The server is housed and administered at the server farm at the Manning data center located at 211 Manning Drive Chapel Hill, NC 27599-3420. Though every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required; UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. Additionally, all patient recruitment and screening phone calls will be conducted in a private room with the door closed to prevent inadvertent disclosure of participant information. All recruitment emails will use a standard IRB-approved script including only general information that the patient may be eligible for a research study at the UNC Medical Center and requesting that they contact the study staff by phone, if interested, to learn more.

- **Long-range risks:** There are no known long-range risks of the study.

- **Unknown risks:** There may be risks unknown to the study researchers, so all patients are encouraged to report all problems.

Additional risks to a subsample of patients participating in in-depth interviews (IDI)

- **Semi-structured IDIs**: Risks to participation in the interviews includes the potential for patient participants to feel uncomfortable or experience anxiety when being asked in-depth questions about their medication adherence and about their experiences during adherence counseling with MedViewer. To minimize these risks the study team will conduct the interviews in a neutral and non-judgmental manner and patients will be reminded that they can stop at any time or skip any questions that make them feel uncomfortable. In a case in which a patient does experience anxiety that is particularly uncomfortable, they will be offered the opportunity for a referral to a mental health counselor in the clinic.

- **Confidentiality**: In addition to the steps mentioned above to maintain participant confidentiality, all interviews and brief questionnaires will take place in a private room with a closed door or on a secure IRB approved video or phone call in a private room with a closed door to prevent inadvertent disclosure of participant information. All interview data, including audiotapes, will be stored either in a locked drawer in a locked office to which only the study investigators will have access or electronically in a password-protected folder on the dedicated university server.

- **Long range risks:** There are no known long-range risks of the study.

- **Unknown risks:** There may be risks unknown to the study researchers, so all patients are encouraged to report all problems.

Risks to provider/pharmacist participants

- **Questionnaires**: Immediate known risks to participating in the questionnaires include the potential for providers to feel uncomfortable or experience anxiety when being asked questions about their patients' medication adherence and about their adherence counseling practices. To minimize these risks the study team will conduct the interviews in a neutral and non-judgmental manner and providers will be reminded that they can stop at any time or skip any questions that make them feel uncomfortable.

- **MedViewer test and report:** It is possible that discussing MedViewer report may alter patient-provider communication during the clinic visit, such that the patient and/or provider feels uncomfortable. To minimize this risk, will inform provider participants that the MedViewer Report is currently for investigational use only. Providers may spend extra time with patients to discuss MedViewer report, which may prolong the appointment and delay the providers' schedule for the day. As a primary goal of this study is to assess whether the use of the report will prolong patient visits or be a feasible, useful and acceptable tool, we may not be able to fully prevent this risk. To minimize it, however, we will obtain feedback after each patient visit about both patient and provider experiences with the tool.

- **Semi-structured IDIs**: Risks to participation in the interviews includes the potential for providers to feel uncomfortable or experience anxiety when being asked about their patients' medication adherence and about their adherence counseling practices. To minimize these risks the study team will conduct the interviews in a neutral and non-judgmental manner and providers will be

reminded that they can stop at any time or skip any questions that make them feel uncomfortable.

- **Confidentiality:** Participation in research includes the risks of loss of confidentiality and discomfort with personal nature of questions. Although the study site makes every effort to protect participant privacy and confidentiality, provider participants' involvement in the study could become known to others, and it is possible, although not likely, that social harms may result. The same steps listed above to maintain patient confidentiality will be used to maintain provider confidentiality, and all in-depth interviews will take place in a private room or through a secure IRB approved video or phone call.

- **Long-range risks:** There are no known long-range risks of the study.

- **Unknown risks:** There may be risks unknown to the study researchers, so all participants are encouraged to report all problems.

2.A.2 KNOWN POTENTIAL BENEFITS

Participants may not receive a direct benefit from being in this study, as this research is designed to generate information to benefit others in the future. While there are no yet known benefits of the intervention, one possible immediate benefit is that the viewing investigational longitudinal adherence information in the investigational MedViewer report may facilitate provider-patient communication and help patients develop strategies for improving their adherence. Additionally, patients may become more knowledgeable about ARV medication and their medication-taking patterns. Through the MedViewer intervention video, patients will learn how medication is distributed in their body and how the MedViewer test works. It is possible that increased information and knowledge will help patients become more adherent to their medication leading to longer-term health benefits.

Additionally, medical providers may find MedViewer useful in facilitating adherence counseling with their patients, prompting patients to offer the provider more information that the provider can use to more appropriately tailor their counseling to patients' circumstances. Knowledge to be gained from this study has the potential to inform further development of the MedViewer test, a novel method to improve medication adherence of people living with HIV and other chronically medicated patients. Such a method has the potential to, in turn, improve patients' health outcomes by facilitating their medication adherence patterns and helping them reach optimal levels of the indicated medication.

2.A.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The proposed research presents very minimal risks to the participants and has the potential to benefit the participants by assisting in the development of a new tool to help patients improve their medication-taking behaviors. The information to be gained from this study cannot be obtained without exposure of participants to the intervention.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Investigate the feasibility of delivering the MedViewer intervention as planned, the acceptability to patients of participation in the MedViewer intervention, and the appropriateness of MedViewer use for adherence counseling	1. Feasibility: proportion of participants receiving the MedViewer report during their provider visit, as planned (i.e. the results are delivered to designated research staff member within 2 hours of initiation of hair processing and the results are discussed with provider/pharmacist within 4 weeks of hair collection). 2. Acceptability: proportion of contacted patients who are eligible for a screening visit (not including inclusion criterion 9) who agree to participate in the MedViewer intervention pilot study. 3. Appropriateness: perceived usefulness of the MedViewer intervention for adherence counseling.	Evaluation of these implementation domains will indicate the potential for future application of MedViewer in routine care and will help to identify needed modifications to the MedViewer intervention to improve delivery. Proctor et al.'s (2011) framework for outcomes in implementation research indicates the importance of assessing feasibility, acceptability, and appropriateness [50].
Secondary		
Investigate additional dimensions of feasibility, acceptability, and appropriateness of using hair IR-MALDESI MSI (MedViewer) to provide patients living with HIV with feedback regarding longitudinal patterns of medication adherence	Feasibility: 1. Reasons for patient's non-receipt of the MedViewer report during a visit with provider or pharmacist within 4 weeks of hair collection (if applicable). 2. Reasons for non-discussion of the MedViewer report with provider or pharmacist (if applicable). 3. Duration of time (in minutes) from initiation of hair processing to MedViewer report delivery to designated research staff member. Acceptability: 1. Provider-reported likelihood of recommending MedViewer to future patients. 2. Patient-reported likelihood of agreeing to future MedViewer use. 3. Patient comprehension of the MedViewer report. Appropriateness: 1. Perceived usefulness of MedViewer to promote ART adherence. 2. Perceived impact of MedViewer use on patient-provider communication and relationship.	Evaluation of these implementation domains will indicate the potential for future application of MedViewer in routine care and will help to identify needed modifications to the MedViewer procedure to improve delivery. Proctor et al.'s (2011) framework for outcomes in implementation research indicates the importance of assessing feasibility, acceptability, and appropriateness [50].
Exploratory		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Assess exploratory aspects of MedViewer feasibility and acceptability, and patient and provider views of and experiences with the MedViewer test. Explore the impact of adherence counseling using MedViewer on ART adherence and hypothesized mechanisms of change (adherence information, motivation, behavioral skills).	<u>Feasibility:</u> 1. Patient-reported maximum out of pocket cost willing to pay for MedViewer. 2. Cost of MedViewer delivery per person. 3. Compatibility of MedViewer with current clinic practices. <u>Acceptability:</u> 1.Patient-reported reasons for declining participation in the MedViewer intervention pilot study. 2. Reasons would/would not agree to (patient-reported) or recommend (provider-reported) future MedViewer use. 3. Acceptability of specific components of MedViewer procedure (a-d): a. Sufficiency of provider training & materials. b. Sufficiency of patient education (video). c. Provider satisfaction with results delivery (discussion format and content). d. Patient satisfaction with results delivery (person, discussion format, and content) <u>Adherence-Related:</u> 1. ART adherence measured by self-reported assessment of 3-day, 7-day, and 30-day adherence. 2. Adherence information (qualitative assessment of information/ understanding gained of patient adherence resulting from use of MedViewer) 3. Adherence motivation (quantitative and qualitative assessment of adherence motivation resulting from use of MedViewer) 4. Adherence behavioral skills (quantitative and qualitative assessment of adherence behavioral skills and self-efficacy resulting from use of MedViewer)	Preliminary assessment of MedViewer's impact on the intended behavioral outcome (ART adherence) and hypothesized mechanisms of influence (based on Information, Motivation, and Behavioral Skills model) will be used inform the design of a future randomized trial to evaluate the effect of MedViewer on these endpoints [51].
Further explore the accuracy of the MedViewer test as an adherence measure	1. ARV concentrations in blood collected for first patient study visit and follow-up patient study visit, when applicable. 2. Patient viral load assessed with a clinical care visit linked to a patient-provider MedViewer visit and abstracted from the medical record by study staff. 3. $\log_{10}$ antiretroviral drug concentrations in hair collected for first patient study visit and follow-up patient study visit, when applicable.	These endpoints will allow for comparison of reported concentrations of medication in hair with recent ART adherence, as measured via Mitra and current viral load. These comparisons will provide further evidence to assess MedViewer accuracy (in addition to that gathered in Aims 1,2) among a larger group of patients.

4 STUDY DESIGN

OVERALL DESIGN

This is a pilot study to assess the feasibility, acceptability and appropriateness of delivery of the investigational MedViewer intervention in an academic Infectious Diseases clinic with adult patients living with HIV who have been prescribed ARV medication by a UNC provider in the last three months. We hypothesize that patients and medical providers will find IR-MALDESI MSI (MedViewer) to be an acceptable, appropriate, and feasible method for providing patients living with HIV with feedback regarding longitudinal patterns of medication adherence. As this does not entail a medication, trial phase is not applicable.

The proposed study is a single-site, single-arm cross-sectional pilot study that will implement the MedViewer intervention to 50 eligible patients living with HIV and administer brief, post-visit patient questionnaires (Appendix C.2) and provider questionnaires (Appendix C.6). A subset of up to 30 patient participants and all provider participants will also participate in semi-structured in-depth interviews (Appendix C.3 and Appendix C.7, respectively) (IDIs) and endline questionnaires (Appendix C.4, Appendices C.8-9).

SCIENTIFIC RATIONALE FOR STUDY DESIGN

To address the proposed study objective to pilot test the MedViewer intervention for feasibility, acceptability, and appropriateness, a single arm study is appropriate. This study will provide data to inform a subsequent larger randomized controlled trial to assess efficacy of the intervention to improve patient outcomes, including medication adherence and viral load. Data will be collected from both patient and provider participants as well as clinic tracking records. The combination of quantitative and qualitative data collection from various sources will provide information about various aspects of feasibility, acceptability, and appropriateness of the MedViewer intervention from the patient, provider, and clinic perspective.

JUSTIFICATION FOR DOSE

There are no study medication products in this pilot study. Patient participants will be on clinically acceptable ARV regimens as prescribed by their regular HIV care providers. No alterations in patients' medication lists will be undertaken as part of this study.

Patient exposure to the study intervention will be measured as time, in minutes. Time spent by patients to undergo the intervention will include 1) approximately eight minutes of exposure to the MedViewer video (with the opportunity to watch the video multiple times, as needed); 2) approximately 2-5 minutes to collect the hair sample; and 3) the duration of time spent reviewing the MedViewer report with the provider/pharmacist during the provider or pharmacist visit (estimated at 2-10 minutes). The video observation, hair sample collection, and review and discussion of the MedViewer report may each occur on different days (with up to 3 days between the video and hair sample collection and up to 4 weeks between hair sample collection and MedViewer report review and discussion). The study intervention has been designed to introduce the minimum amount of time necessary to run the MedViewer test and report the results back to the patient while maintaining accuracy and

confidentiality. Use of daily adherence monitoring feedback during adherence counseling has shown promise as an effective strategy to promote medication adherence [35,39]. As this is a cross-sectional pilot study, there will be one single exposure to the MedViewer intervention.

Provider exposure to the study intervention will also be measured as time in minutes, including approximately 30-60 minutes for the MedViewer provider training as well as the duration of time spent reviewing the MedViewer report with the patient during the provider or pharmacist visit (estimated 2-10 minutes). Unlike patient participants, providers will be exposed to the intervention over multiple time points. Providers will be exposed to the intervention each time they have an appointment with a patient who is participating in the study throughout the 7.5 months of patient enrollment. Providers will have varying levels of exposure depending on the number of enrolled patients they see as part of the study.

END OF STUDY DEFINITION

A patient participant is considered to have completed the study if he/she/ze/they has completed all aspects of the study, including the last scheduled procedure (i.e. the post-visit patient questionnaire, and/or the blood sample, when applicable, whichever comes last) shown in the Schedule of Events (SOE) in Section 1.3. Further, participants in the IDI subsample (approximately 30 patients) are considered to have completed the study when they have completed the aforementioned study procedures, as well as the IDI and endline questionnaire, within 28 days (-14/+ 60 days) of the MedViewer visit with the provider or pharmacist, as shown in the SOE in Section 1.3. A provider participant is considered to have completed the study after the last patient participant has completed the study and the provider has completed both the IDI and the endline provider questionnaire.

Based on our prior experience with other similar studies and the rate of potentially eligible patients seen in the clinic per week, we anticipate recruiting patients for 33 weeks (7.5 months). After IRB approval, we will recruit providers for a period of approximately 4 weeks (1 month) before beginning patient recruitment. We will also include up to six weeks (1.5 months) of follow-up data collection for provider participants after the last day of patient participant enrollment. Thus, we anticipate that the study activities will last up to 44 weeks (10 months). The end of the study is defined as completion of the last visit or procedure shown in the SOE in the study globally for the last study participant.

5 STUDY POPULATION

INCLUSION CRITERIA

Patient participants: To be eligible to participate in this study as a patient participant, an individual must meet all the following criteria:

1. Documentation of HIV-1 infection by means of any one of the following:
    a. Documentation of HIV diagnosis in the medical record by a licensed health care provider;
    b. OR HIV-1 RNA detection by a licensed HIV-1 RNA test demonstrating >1000 RNA copies/mL;

c. OR any licensed HIV screening antibody and/or HIV antibody/antigen combination test confirmed by a second licensed HIV test such as a HIV-1 Western blot confirmation or HIV rapid Multispot antibody differentiation test.

NOTE: A "licensed" test refers to a US FDA-approved test, which is required for all IND studies. Non-US sites are encouraged to use FDA-approved methods; if not available, then each non-US site must use a test that has been certified or licensed by an oversight body within that country and validated internally. WHO (World Health Organization) and CDC (Centers for Disease Control and Prevention) guidelines mandate that confirmation of the initial test result must use a test that is different from the one used for the initial assessment.

2. At least 18 years of age on the day of consent.
3. Documentation of HIV viral loads over 2-year period prior to screening.
4. Has been a patient at the UNC ID Clinic for at least 90 consecutive days prior to the date of enrollment in the study (i.e. attended first appointment with the UNC ID Clinic at least 90 days prior to the date of enrollment in the study).
5. Has attended at least one HIV appointment with the UNC ID Clinic within the 365 days prior to the date of enrollment in the study.
6. Has been prescribed one of the ARV medications eligible in this study (Dolutegravir, Emtricitabine) by a UNC provider for at least 90 days prior to the date of enrollment in the study.
7. Has an HIV appointment scheduled with the UNC ID Clinic during the enrollment period of the study with a medical provider enrolled in the study.
8. Documentation that the individual has provided consent to participate in the study and has been informed of all pertinent aspects of the study.
9. Has stated willingness and availability to comply with all study procedures for the duration of the study.
10. Literate in English.
11. Has at least 1.0 cm of natural caput hair.
12. As enrollment will occur in a stratified manner based on 2 viral load strata, (n = 25 for patients with viral loads below the limit of quantification, n = 25 for those with viral loads above the limit of detection), once a stratum quota is reached, patients whose viral loads fall within that stratum will not be eligible to participate in the study.

Provider participants: To be eligible to participate in this study as a provider participant, an individual must meet all the following criteria:

1. Medical provider (including, but not limited to: attending physician, ID fellow, nurse practitioner, physician assistant, or a designated HIV Care pharmacist) for UNC ID Clinic patients living with HIV.
2. Provides medical care for patients at the UNC ID Clinic at least one half-day per week (i.e. 4 hours per week).
3. Evidence of a personally signed and dated informed consent form indicating that the participant has been informed of all pertinent aspects of the study.
4. Has stated willingness and availability to comply with all study procedures for the duration of the study.

A. EXCLUSION CRITERIA

We will target patients of diverse ages, gender identities, racial identities, and ethnicities to test MedViewer with different hair types and capture a range of patient perspectives (reflective of the diversity of patients seen in the clinic who are people living with HIV) on acceptability, appropriateness, and feasibility of MedViewer test procedures.

Patient participants: Pregnant patients will not be excluded from the study as participation does not pose any increased risk during pregnancy to the mother or fetus.

An individual who meets any of the following criteria will be excluded from participation as a patient participant in this study:

1. Previous participation in the study IGHID 11530- Formative Sub-Study for Novel Mass Spectrometry Imaging Methods to Quantify Antiretroviral Adherence.
2. Deemed, by medical provider in UNC ID Clinic, too ill, or other relevant reason, to participate in the study.
3. Prior history of clinically significant alteration of the gastrointestinal system or drug absorption capability, including but not limited to: gastrectomy, total colectomy
4. Any chemical hair treatment with dye, bleach, or relaxers within the 4 weeks prior to sampling.

Provider participants: An individual who meets any of the following criteria will be excluded from participation as a provider participant in this study:

1. Not willing or able to participate in any of the provider training sessions for this study or any form of make-up training session with research team.

B. LIFESTYLE CONSIDERATIONS

<Not Applicable>

C. SCREEN FAILURES

Screen failures are defined as participants who are pre-screened via the IRB-approved questionnaire and are found to not currently be eligible for any reason. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demographic characteristics, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Rescreening: Participants may be rescreened at any point during open recruitment if their eligibility requirements align with the protocol. Rescreened participants should be assigned the same participant number they were assigned for the initial screening (if they were assigned one).

D. STRATEGIES FOR RECRUITMENT AND RETENTION

Recruitment and retention strategies for patient participants:

We plan to enroll 50 patient participants who are patients at a single U.S. site, the UNC ID Clinic, a hospital-based outpatient clinic in Chapel Hill, North Carolina. Data collection will occur in the UNC ID Clinic, the Clinical & Translational Research Center (CTRC), and possibly other areas of the UNC-Chapel Hill campus. We will enroll participants into 2 viral load strata, with a target of 25 participants in Group A –the lower viral load stratum (those with viral load below the limit of viral quantification over the previous 2 years with documentation of at least one test in the previous six months) – and 25 participants in Group B –the upper viral load stratum (those who have had at least one HIV RNA result above the limit of viral quantification within the previous two years). We anticipate enrolling an average of 6.67 patients per month (1.52 per week), over the course of 7.5 months (33 weeks), to reach our target of 50 total participants. We will screen and enroll patients of all gender identities, racial identities, ethnicities, and ages (above 18 years), such that the demographic distribution of screened participants reflects that of patients at the UNC ID Clinic who are people living with HIV.

Of all the people living with HIV who attend the UNC ID Clinic, 95% have consented to having their patient information available in a secure clinic database where it can be viewed to identify potential eligibility for open research studies and to being notified of studies for which they are potentially eligible (as per IRB form 99-MED-408). The ID Clinic has a full-time research screener to assess patient eligibility for open research projects. We will use the IRB-approved screening and recruitment process that has been used in the ID Clinic for over ten years, whereby the clinic screener pre-screens patients in the clinic database and alerts research staff to potentially eligible patients. Specific recruitment methods used (phone, email, in person) for any individual patient participant will depend upon the methods for which that patient has previously provided permission (on IRB form 99-MED-408).

We will use the following recruitment strategies and steps:

- Posting IRB-approved culturally appropriate flyers in designated areas of the clinic for the duration of study enrollment with information about the name and overarching purpose of the study.

- Contacting prospective, pre-screened patients, who have given permission to be contacted by phone, before their next scheduled HIV appointment, using an IRB approved script to notify them about the study and verify their eligibility using a brief standardized IRB-approved script/screening form. Phone calls will be conducted in a private room to prevent inadvertent disclosure of participant information.

- Contacting potentially eligible patients, who have given permission to be contacted by secure email, before their next scheduled HIV appointment, using an IRB-approved template to notify them about the study, provide them with a contact phone number, and ask them, if interested, to contact the study staff by phone to undergo a brief IRB-approved initial screening process. Emails will be sent from an encrypted email server. Follow-up screening phone calls will be conducted in a private room to prevent inadvertent disclosure of participant information.

- For patients who cannot be contacted by phone and/or email after multiple (a maximum of three) attempts and who have an in person clinical visit schedule with their provider, research staff will approach them in the clinic waiting room (if the patient has given permission to do so) on the day of their visit to notify them that they may be eligible for a study. Those who are interested will be asked to move to a private room in the UNC ID Clinic or research clinic to undergo further screening and, if eligible and interested, informed consent. (Appendix B.1) The private room will have a closed door to prevent inadvertent disclosure of subject information.

- If one or more of the basic inclusion criteria are not met, the individual will be informed that they are not eligible.

Research staff will obtain informed consent from each potential participant before starting any study procedures according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs.

The UNC ID Clinic saw 1900 patients living with HIV in 2018 (approximately 150 patient visits/month). Based on the number of patients seen in the ID Clinic, the high percentage of participants prescribed eligible ARV medications for this study, the ID Clinic's commitment to public health research, and past experience with recruitment, we do not expect difficulty enrolling an adequate number of patient participants. Since the onset of the COVID 19 epidemic, the UNC ID Clinic has shifted to conducting the option of telehealth clinical visits with providers. In person visits are also still currently permitted. Based on reports from the UNC ID Clinic Medical Director, with the shift to including the option for telehealth visits, the volume of patient visits scheduled and attended has increased, therefore enhancing our capacity to identify eligible patients based on the number of patients who are scheduled for clinical visits.

Recruitment and retention procedures for in-depth interview (IDI) subsample of up to 30 patient participants:

We will also enroll a subsample of up to 30 patient participants (from those enrolled in the larger study) to participate in a follow-up study visit, including an IDI and brief endline questionnaire. We will invite every enrolled patient to participate.

To recruit participants into the IDI subsample, we will use the following strategies:

- During the initial study visit for the main study, research staff will inform all participants about the follow-up visit activities and invite them to participate. However, to ensure that the subsample is representative of the full study sample, accounting for time of enrollment in the study (i.e. to obtain approximately equal numbers of patients at various points during the enrollment period for the larger study), we will monitor the rate of subsample enrollment every month and adjust our recruitment methods, as needed, to increase the likelihood of obtaining a representative sample of participants throughout the duration of the study.

- During the study visit, all communication regarding the IDI subsample activities and informed consent will take place in a private room with a closed door or via secure IRB-approved video conferencing or phone call conducted in a private room with a closed door to prevent inadvertent disclosure of participant information (Appendix B.2).

Research staff will obtain informed consent from each prospective subsample participant before starting any study procedures according to the standards set for the in the ICH Good Clinical Practice guidelines and per unit SOPs.

Recruitment and Retention Procedures for Provider Participants:

We will also aim to recruit and enroll all (~15 but up to 30) medical providers who provide care to patients in the ID Clinic (for example, attending physicians, fellows, nurse practitioners, physician assistants, nurses, and pharmacists).

Providers will be recruited through the following strategies/steps:

- Posting an IRB-approved informational flyer in designated areas of the UNC ID Clinic, including the physician work room and in providers' mailboxes at the ID Clinic.

- Contacting providers through secure email (using an existing list of current providers in the UNC ID Clinic provided to research staff by Co-Investigator and Clinic Medical Director, Claire Farel, MD) to notify them about the study and invite them to provide their availability to undergo screening with research staff and provide written informed consent, if interested and eligible. The emails will also include information about the provider training session required for all enrolled providers prior to seeing patients for their MedViewer study visits. Invitation emails will use an IRB-approved template. Invitation emails will be sent from an encrypted email server.

- Interested providers will be sent the IRB-approved informed consent form prior to meeting with study staff for informed consent so that they can review it ahead of time. (Appendix B.3)

- Presenting IRB-approved verbal and PowerPoint announcements about the study during weekly Infections Disease conferences and other relevant events typically attended by ID Clinic providers. The announcements will include information about the provider training sessions

- Holding multiple provider training sessions on different dates. Attendance of a training is required for enrolled providers to see patients for MedViewer study visits. For providers who cannot participate in any of the group training sessions, study staff will provide the opportunity to complete a one-on-one training with a research staff member.

As practitioners at a state medical facility, the clinic providers are a highly dedicated group of clinicians with a clinic culture that embodies and reflects their strong commitment to public health and to clinical research to improve health outcomes in North Carolina. Based on this commitment to public health and the research team's past experience with recruitment, we do not anticipate difficulty enrolling ID Clinic providers to participate in this study.

Participating providers will be compensated for completing study activities over the entire study period, including study activities related to each of their individual patients who participates in the study over the 7.5 month patient recruitment period. This amount reflects the assumption that each participating provider's activities will involve multiple patient visits. Since providers will participate in the study for up to 10 months, they will be compensated for completing the IDI and endline questionnaire to encourage retention.

6 STUDY INTERVENTION

STUDY INTERVENTION(S) ADMINISTRATION

6.A.1 STUDY INTERVENTION DESCRIPTION

The study intervention includes four components:

1. **Standardized training session for medical providers:** This 30-60 minute training session, designed to orient providers to the MedViewer test and prepare them to participate in this study, will be held at the start of the study before enrolling patient participants and at various points throughout the study. The training will introduce the providers to the MedViewer patient video (see component 2), the MedViewer test and report (see component 3), and provider communication aids (see component 4). The training will also prepare providers to incorporate the delivery of the investigational MedViewer test results into discussions with patients during routine HIV care appointments by providing an opportunity to practice interpreting and discussing MedViewer results with patients using an example MedViewer report. The training session will be required for all enrolled providers participating in the study before providers can be scheduled to see enrolled patients for visits to review their MedViewer report. Before the training session, in the provider's private office, another private space on campus, or via IRB-approved videoconferencing or phone call, via an IRB-approved e-consent process, study staff will obtain written informed consent from providers who are both eligible and interested in enrolling in the study. Training sessions may be in-person or virtual. Multiple training dates will be offered to providers. For providers who are interested in participating in the study but unable to attend a scheduled group training session, study staff will offer one-on-one training (Appendix A.1). Supplemental training sessions with the same training materials will be offered, as needed.

2. **Informational video for patients:** This approximately eight-minute video will introduce patients to the MedViewer test, the hair collection process, and how test results can be useful for conversations between patients and providers about adherence. More specifically, the video will explain: 1) how ARVs are processed in the body and end up in hair; 2) what the hair sample collection process will be like; 3) how the test results are produced in the lab; 4) how the hair sample will be disposed of; 5) how the provider may review the results with the patient; and 6) how the test results can inform conversations about the patient's adherence. The video will also address potential patient concerns about the test (as identified in a formative study conducted prior to intervention development). Potential concerns include, but are not limited to, whether it is painful to provide a hair sample and how patient privacy will be protected. This video will be part of the informed consent process for patient participants to facilitate their understanding of the MedViewer aspect of the study. Eligible and interested patients will watch the video during the informed consent process either in a private room located in the UNC CTRC or ID Clinic or via an IRB-approved videoconferencing call. Patients will have the opportunity to watch the video multiple times, as needed. (Appendix A.2)

3. **Hair sample, MedViewer test, and accompanying MedViewer report (patient and provider versions).** After providing informed consent, patient participants will provide a hair sample for

MedViewer testing. Hair sample collection will take place in the ID Clinic or CTRC or in a private remote location to minimize clinic exposure, where a trained clinical research staff member will pluck five strands of hair from the back of the patient's head. The research team member will then place the hair on a foil package and affix the distal end of the hair with a label. The folded hair foil will be placed into a resealable biohazard bag in a closed container and then transported promptly to the lab for MedViewer testing in accordance with EHS policy. At the lab, the imaging scientist will place the sample in the MedViewer machine to run the test and generate two distinct visual reports of the results (one version for patients and one version for providers). The scientist will send the report via secure email to the research team, who will print the report and deliver a hard-copy or electronic version to the appropriate provider participant in the ID Clinic. (Appendix A.3)

The patient version of the investigational report will show a monthly calendar display of color-coded results indicating whether daily ARV drug concentrations in the patient's body were at optimal or sub-optimal concentrations each day (Appendix D.1). Each report will have a key of relevant information about the test. The investigational provider report will have bar graph of daily drug concentrations as well as information reflecting the variability of the lab test. The investigational patient report will be formatted as a calendar with dichotomous color assignment to each day on the report indicating an optimal or sub-optimal medication concentration. All reports will be clearly labeled that this information is for investigational research use only. (Appendix A.3)

During the patient's regularly scheduled appointment at the ID Clinic, the goal is for the provider and patient to view the investigational reports together and use them to have a conversation about the patient's ART medication adherence. If the MedViewer report is not available during the patient's regularly scheduled ID Clinic appointment, the provider and patient participants will discuss them during a separately scheduled MedViewer appointment within four weeks of hair sample collection. The provider will not follow a standardized script to discuss the results with the patient; rather, the provider will conduct the appointment based on their clinical judgement and discretion, drawing on information from the provider training session (see Component 1) and communication aids (see Component 4), medical expertise, and the individual needs and circumstances of the patient participant. If the provider chooses to follow suggested communication strategies listed in the reference sheet (see Component 4), he or she may discuss MedViewer reports with patients by: explaining the summary statistics printed with the results or pointing out any patterns of insufficient drug concentrations and adherence successes; asking patients about event-level and/or chronic and psychosocial causes of missed doses; asking patients about successful adherence strategies; working with patients to identify personalized strategies that could help patients overcome causes of missed doses in the future or to replicate successes; offering encouragement for good adherence; and working with patients to set goals for improving future adherence.

The investigational MedViewer reports will NOT be entered into the patient's Electronic Health Record (in EPIC) or become a formal part of the clinical patient record or be used for clinical decision making. After the provider and patient have reviewed the MedViewer reports, both versions of the report will be destroyed in accordance with standard medical documents with a certified copy of each stored in the research record. The research team will document receipt of the MedViewer report by the provider on the study forms.

If the provider does not receive the MedViewer reports on time for the scheduled appointment, the research team will notify the provider, who will decide to either 1) delay seeing the patient for the scheduled appointment until the report arrives; or 2) meet with the patient at the scheduled appointment time and schedule a separate appointment to review the MedViewer report with the patient within 4 weeks of hair sample collection; or 3) request that the clinic HIV care pharmacist discuss the MedViewer report with the patient within 4 weeks of hair sample collection. This decision will also be based on whether the patient is willing or able to attend a separate appointment to receive the results and whether they are comfortable receiving the results from the pharmacist. Any of the aforementioned appointments or discussions may occur in-person or virtually, as appropriate for the patient and provider and/or pharmacist. The research team will work with the patient, initial provider, and clinic pharmacist using procedures as specified in a study SOP.

4. **Communication aids for providers:** Communication aids for providers, intended to facilitate providers' discussions of the investigational MedViewer reports with their patients, will include a one-page reference sheet with information and tips to interpret the MedViewer results reports, understand the accuracy and certainty of different aspects of the test, and discuss the results with patients. The document will include possible strategies to counsel patients on adherence using the investigational MedViewer report and a one-page FAQ to clarify potential concerns. The counseling reference sheet will indicate the potential utility of the investigational MedViewer test as an objective, real-time measure of longitudinal adherence that provides concrete adherence feedback for patients and lists suggested steps, covered during the provider training, to counsel patients on adherence using MedViewer report. These include:

    - reviewing the MedViewer report with patients
    - understanding potential implications of patterns of missed or successful doses
    - strategizing to overcome challenges and replicate successes
    - motivating patients to improve or maintain adherence

    The communication aid and reference sheet will be delivered to providers along with MedViewer reports. The IRB-approved FAQ sheet will include logistical information about the MedViewer test, information about test sensitivity and specificity to detect missed doses, as well as questions and concerns raised by providers during the formative study prior to intervention development. Research staff will update the FAQ sheet, as necessary, to include additional questions and concerns that arise throughout study implementation. The FAQ sheet will be placed in the provider workroom and exam rooms within the ID Clinic. (Appendix A.3)

6.A.2 DOSING AND ADMINISTRATION

Patient participants: For patients, the MedViewer intervention is a single intervention, with a single intervention exposure (over one, and in rare cases multiple, in-person or virtual clinic visits). Each exposure to the intervention will include 1) watching an informational video about the MedViewer test 2) providing a hair sample that will be sent to a laboratory for MedViewer testing, and 3) receiving the MedViewer visual results report from the provider or pharmacist during the regularly scheduled clinic visit or a separate MedViewer appointment within 4 weeks of hair collection. The MedViewer intervention will be administered similarly for each patient participant, with slight variations in timing

and level of exposure to individual components, based on the patient-provider interactions as well as clinic flow on the day of the study visit.

Exposure to the MedViewer tool will be measured as time in minutes. Each participant will first view the same informational video (approximately 8 minutes long) on a tablet in private room or over a secure IRB-approved videoconferencing call during the consent process. Within three days following informed consent, the patient will have a hair sample collected, which should take approximately 2-5 minutes. The research team collects the hair in the exact same way with each patient according to standard SOPs. For patients in Group B and a subset in Group A, a matched blood sample will be collected for research purposes only. The patient's blood will be wicked onto a Mitra microsampling device that is based on Volumetric Absorptive Microsampling (VAMS) technology. The Mitra device has advantages over DBS cards and filter paper in the areas of collection, processing, and extraction. The Mitra device provides the key benefits of working with dried blood, but with a volumetrically accurate, stable dried blood sample that reduces the incidence of reworks.

While we will aim to schedule hair sample collection within 4 days prior to the scheduled clinical HIV appointment with the provider, we will allow the clinical visit to take place up to 4 weeks following hair sample collection. At the next clinical visit that occurs after and within 4 weeks of hair sample collection, the patient participant will meet with their provider and the provider will incorporate review and discussion of MedViewer report into usual care. Since the provider will not use a standardized script to discuss the results with the patient, the degree of exposure to the MedViewer report will vary among participants. Because this is a feasibility study, mode of exposure to the results report (e.g. over videoconferencing versus in-person and provider-delivery versus pharmacist) may vary across patient participants, as appropriate, based on clinic flow, availability of the MedViewer report, and whether the visit with the provider was scheduled in person or by videoconferencing. For example, if, for any reason, a provider does not receive the MedViewer report for a patient participant prior to the patient's scheduled clinical visit, the visit may be postponed until the report is delivered to the provider or a separate follow-up- appointment may be scheduled with the same provider or the designated clinic pharmacist for review and discussion of the MedViewer report. If the patient does not receive the report from their provider or pharmacist and does not complete a post-visit questionnaire, they will be eligible to return to participate in another baseline visit in the future.

Provider participants: All enrolled providers will have a similar time of study exposure, with the opportunity to be enrolled from study initiation, or their individual date of enrollment, through the estimated 16-month duration of the study, including 10 months of active participation in the study, comprised of 7.5 months of patient enrollment. Providers will have a standard, single exposure to the in-person or virtual provider training with the option to participate in supplemental training sessions, as needed. Providers will also have a single initial exposure to the communication guides, which they can use during visits with patients, as desired. However, providers will have varying levels of exposure to receiving patients' MedViewer results and reviewing results with patients, depending on their patient population and number of their patients participating in the study. In the case of clinic pharmacists, exposure will depend on the number of patients referred to them for the MedViewer report discussion. Quality of exposure to patients' MedViewer report will also depend on clinic flow and whether providers receive the results on time for patients' scheduled HIV appointments.

PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.A.3 ACQUISITION AND ACCOUNTABILITY

To create and distribute the MedViewer report of results, hair sample processing by IR-MALDESI-IMS methods will be performed in the lab located in the Genetic Medicine Building in the Eshelman School of Pharmacy. All samples will be processed according to SOPs. This laboratory has validated analytical methods for all currently marketed antiretrovirals by LC-MS/MS techniques, and is CLIA- (#34D1022136) and CAP- (LAP#7521077; AU-ID#1589458) accredited. However, the MedViewer technology is considered an experimental approach indicated for investigational use only. The validation reports for emtricitabine and dolutegravir can be found in Appendix E.

6.A.4 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

There are no study drug products on this study, nor will we be utilizing the services of Investigational Drug Services.

As the intervention centers around delivery and discussion of the MedViewer report of results, we describe its appearance, packaging, and labeling. The study intervention has been designed and will be manufactured by the study team. Each patient participant's MedViewer report will be individualized to reflect the measured concentration of ARV in their hair over time. (Appendix A.3)

6.A.5 PRODUCT STORAGE AND STABILITY

There are no study products that are part of the intervention for which we would need to assess stability or provide storage.

6.A.6 PREPARATION

There are no study products on this protocol that need preparation in the pharmacy.

MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

As this is a single arm pilot feasibility study in which all participants will receive the same intervention, there will be no randomization of intervention assignment. Similarly, as all patient participants will receive the intervention and data are only being collected on patients participating in the study, blinding of data collectors or other researchers to participants' intervention assignment will not be feasible, necessary, or practical. In addition, as the design of the intervention requires providers to deliver the MedViewer report to participating patients, it is not feasible for the providers to be blinded to patients' intervention assignment/study participation.

To obtain a representative subsample of patients to participate in the IDIs, we will initially invite all patients to participate in the follow-up study. We will monitor the rate of subsample enrollment every month, adjusting our recruitment methods, as needed, to increase the likelihood of obtaining a representative subsample of participants throughout the duration of the study.

STUDY INTERVENTION COMPLIANCE

This study will be conducted in full compliance with the protocol and all study procedures will be tracked for compliance as outlined in the CQMP. The protocol will not be amended without prior written approval by the protocol chair and the NIAID Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRB prior to implementing the amendment.

Provider Training Attendance. At the provider training, attendance will be taken, and the research team will note all participating providers and whether all slides and intended activities were covered. During any supplemental trainings that take place, attendance and training content will be documented in study records.

CONCOMITANT THERAPY

There are no concomitant therapy exclusions in this protocol.

6.A.7 RESCUE MEDICINE

Not Applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/ WITHDRAWAL

A. DISCONTINUATION OF STUDY INTERVENTION

If one participant experiences a Serious Adverse Event (SAE) deemed related to the study, enrollment will be held while the protocol undergoes review.

B. PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Enrolled participants are free to withdraw from participation in the study at any time and for any reason upon request. Participants may also choose to withdraw from the intervention for any reason but still complete other study activities.

An investigator may withdraw a patient participant from the study for the following reasons:

- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation – related or unrelated to the study – develops after enrollment such that continued participation in the study would not be in the best interest of the participant.

- If a patient presents a safety risk to the research staff.

- If their participation in the study is disruptive to the study or the clinic.

- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

- At the discretion of the provider that pursuing on study is not in the best interest of the patient.

An investigator may withdraw a provider participant from the study for the following reasons:

- If their participation is disruptive to the study or the clinic.
- If participation is no longer of interest to the provider participant

The reason for any participant's discontinuation or withdrawal from the study will be recorded in the research record. While the study is intended to be completed in one day and thus discontinuation or withdrawal are unlikely, as data are collected at several time points during the study visit, it is possible that some participants will not be fully retained. Participants who sign the informed consent form but do not receive the study intervention will be replaced. Participants who sign the informed consent form and receive the full study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study will not be replaced. For example, participants who do the intervention but leave or are withdrawn (e.g. due to being too sick) before completing the post-visit questionnaire would not be replaced. And participants who do *some* but not all of the intervention (e.g. get hair plucked but don't wait for result), will be replaced.

C. LOST TO FOLLOW-UP

Because most study procedures for patients are designed to take place over several time points within several weeks of enrollment,(based on a combination of participant availability, provider schedule, MedViewer test timeline), there are several points at which patient participants may be lost to follow-up.

Patient participants: Patient participants will be considered lost to follow-up if, after enrollment, they do not present for any scheduled component of the intervention within the allowable timeframe and are not reachable to reschedule (including hair sample collection or their visit with their provider/pharmacist at which MedViewer results are to be discussed) or on the days of their study visit activities, they leave the study site or become unreachable at any point before completing the intervention or study activities and they do not explicitly inform research staff that they would like to withdraw from the study.

Patient participants who are part of the IDI subsample will be considered lost to follow-up if they do not attend their scheduled follow-up interview, are unreachable for their interview, or do not complete their interview and endline questionnaire and they both: 1) do not explicitly inform research staff they

would like to withdraw from the study; and 2) they are unreachable by phone, email, or at the study site within 60 days of their missed IDI).

The following actions will be taken if a patient participant fails to present for a scheduled study visit or interview:

- The research team will attempt to contact the participant, reschedule the missed visit within 60 days of the initially scheduled date, and ascertain if the participant wishes to and/or should continue in the study.

- The research team will allow some study activities to be completed by phone, secure video conferencing, email, or secure online completion of study questionnaires, if needed to facilitate completion of all study activities. Until we have verified that the person we are speaking to is the intended patient participant, we will not provide any information that we are calling from a UNC clinic. To ensure that the person we are speaking to on the phone is the intended patient, in addition to obtaining at enrollment, detailed information about whether and where it is okay to leave messages, we will use a standardized script that asks the individual to confirm their identity.
- Before a participant is deemed lost to follow-up following failure to attend a scheduled activity, a research team member will make every effort to regain contact with the participant (maximum of 3 attempts) within 14 days of the missed Visit 1 activity or 60 days for Visit 2 activity. These contact attempts will be documented in the chart.

- Should the participant continue to be unreachable, they will be considered lost to follow-up.

Provider participants: Provider participants will be considered lost to follow-up if, after enrollment, they do not complete any intervention or study activities and they both 1) do not explicitly inform research staff that they would like to withdraw from the study; and 2) are unreachable by phone, email, or in-person before study close.

The following actions will be taken if a provider participant fails to complete intervention or study activities (i.e. post-visit questionnaires, endline questionnaire, IDI) before study close:

- For missed study activities, the research team will attempt to contact the provider, request that the provider complete the activity, and ascertain if the provider participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, a research team member will make every effort to regain contact with the provider (with up to 3 phone calls or emails prior to study close). These contact attempts will be documented in a Study Participation Case Report Form.

- Should the participant continue to be unreachable before study close, he or she will be considered lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

A. EFFICACY ASSESSMENTS

Administration of questionnaires or other instruments:
All patient questionnaires will be administered through computer-assisted self-interviewing (CASI), unless otherwise noted. In some cases, questionnaire items may be administered verbally by a trained research team member via computer-assisted personal interviewing (CAPI), if needed. There will also be a paper copy of the questionnaires available as backup, if needed. All provider questionnaires will be administered through CASI when possible or a paper copy of the questionnaire, unless otherwise noted. All in-depth interviews will be conducted by trained research team members using semi-structured interview guides and will be digitally recorded with participant consent.

PATIENT ASSESSMENTS

Patient chart and medication review: For screening purposes, UNC ID Clinic screeners or a trained research team member will review medical records of patients who have consented to having their patient information available in a secure clinic database, where it can be viewed to identify potential eligibility for open research studies (as per IRB form 99-MED-408).

Patient screening questions: Using the list of potentially eligible study participants, the research team will attempt to contact prospective participants by phone, or – if needed secure (encrypted) email -- to conduct or arrange for a pre-screening phone call before the scheduled clinic visit (as outlined in Section 5.3). This will be done with a standardized IRB-approved questionnaire. If contact with the patient cannot be established before a scheduled in-person clinic visit, the research team will approach the patient in the clinic for pre-screening. During the phone call or in-person screening, the research team will assess patient eligibility using a standard IRB-approved screening form with questions pertaining to patient age, amount of time as a patient at the UNC ID Clinic, HIV appointments in the last year, current and past prescribed ARV medications and length of caput hair (which needs to be at least 1 cm in length).

Provider screening and demographics questions: Following informed consent, the research team will assess provider eligibility using a standard screening form with questions pertaining to number of clinic hours per week and type of provider. Providers will also be asked to report demographic characteristics including gender, age, race/ethnicity, and length of time working as a provider at UNC.

Reasons for patient participation decline: If an eligible prospective participant declines participation in the study, and does not provide informed consent, the research team will ask them to indicate the primary reason(s) for this refusal from a list of common reasons for declining participation; an open-ended option will also be available and the patient may refuse to answer. This will be documented in the database.

Patient withdrawal/discontinuation reasons: If an enrolled participant withdraws from the intervention or from the study at any point after providing informed consent, the research team member will ask them to indicate the primary reason(s) for withdrawing. Similarly, if an investigator discontinues or withdraws a participant from the intervention or study, the investigator will record the reason for participant discontinuation or withdrawal in the chart.

Hair Sample and MedViewer laboratory test: During the first study visit, the research team will collect 5 plucked strands of hair from the patient to be delivered to the laboratory for MedViewer testing per local SOPs. The patient will receive the MedViewer report from the provider during the clinic visit.

Pharmacokinetic Blood Sample: During the first study visit, either before or after hair sample collection, the research team member or another trained clinician will collect a blood sample via fingerstick or phlebotomy for a subset of patient participants in Group A and patient participants in Group B. The blood sample will be used for measuring concentrations of antiretrovirals for comparison with the MedViewer results. Our lab has validated antiretroviral tests for drug monitoring. Patient participants who came in advance of their visit to have blood drawn for this clinical visit or those who will not have blood drawn on the day of the provider visit will have their blood collected during the first day of their study visit for study purposes. This is necessary because drug concentrations collected on days after receiving the MedViewer report may be affected by receipt of the MedViewer report. ARV concentrations in this blood sample will be compared with the MedViewer report findings to indicate the extent to which it reflects adherence behavior before the clinic visit. These samples were obtained in the directly observed therapy study in Aim 2 of the grant for benchmarking purposes, along with hair. Blood sample storage, processing and analysis will be conducted in the CTRC and UNC School of Pharmacy Clinical Pharmacology and Analytical Chemistry (CPAC) Lab located on the 1st floor of Genetic Medicine Building on UNC campus.

Patient baseline questionnaire: After informed consent and screening and prior to receiving and discussing the MedViewer report, the patient will be administered a baseline questionnaire by a trained research team member using the CASI system. Questionnaires may also be administered verbally by a trained research team member (via CAPI), if needed. Questionnaire items will pertain to: self-rated health, patient sociodemographic information (time to travel from home to ID Clinic; age; sex assignment at birth and gender identity; sexual orientation; race/ethnicity; current marital status; education level; income in past year; current employment status; health insurance status and type; method of paying for ART), comprehension and sufficiency of video content, self-reported ART adherence over the past 3 days, past 7 days, and past 30 days, and adherence motivation and self-efficacy. (Appendix C.1)

Patient post-visit questionnaire: As soon as possible following the clinic appointment during which MedViewer report receipt is intended to occur, patients will be asked to complete a 13-item questionnaire comprising questions regarding their experience receiving and discussing the MedViewer report with their provider. Questionnaire items will pertain to: affirmation of MedViewer report receipt, person report delivered/discussed by, future likelihood of using MedViewer and recommending to others, perceived comprehension of MedViewer report, satisfaction with the adherence counseling discussion, acceptability of wait time, acceptability of hair sample collection, satisfaction with the MedViewer report, maximum acceptable future out-of-pocket cost of MedViewer test, perceived usefulness of MedViewer to promote ART adherence, perceived impact of MedViewer use on patient-provider communication and relationship, and adherence motivation and self-efficacy. (Appendix C.2)

Patient in-depth interview (IDI): A subsample of up to 30 patients will complete semi-structured IDIs (either in-person or via IRB-approved videoconferencing) during the second study visit, approximately four weeks (28 days (-14/+ 60 days) following the MedViewer visit with the provider or clinic pharmacist. If the visit to discuss MedViewer is scheduled but does not occur, the second study visit will take place 28 days (-14/+60 days) following the date on which the MedViewer visit was scheduled to occur. Trained research assistants will conduct the interviews using semi-structured interview guides. Interview topics

will include: perceived usefulness of MedViewer for adherence counseling, reasons would/would not agree to/recommend future MedViewer use, satisfaction with patient education video, comprehension of MedViewer report (cognitive interview), satisfaction with adherence counseling discussion using MedViewer report, attitude toward wait time and hair sample collection, anticipated effect of regular MedViewer use on patient-provider communication and relationships, and perceived effect of MedViewer on comprehension of own adherence behavior, adherence motivation, and adherence behavioral skills. (Appendix C.3)

Patient endline questionnaire: The subsample of patients completing IDIs will also complete a brief structured endline questionnaire. Questionnaire items will pertain to: self-reported percentage of missed doses over past 3 days, past 7 days, and past 30 days, perceived usefulness of MedViewer to promote ART adherence, maximum out-of-pocket cost willing to pay for MedViewer, adherence self-efficacy, and adherence motivation. (Appendix C.4)

PROVIDER ASSESSMENTS

Provider participation refusal: If an eligible prospective provider participant declines participation and does not provide informed consent, the research team will ask them to indicate the primary reason(s) for this refusal from a list of common reasons for declining participation with an open-ended option. The research assistant will record this information in the screening and enrollment database.

Provider withdrawal/discontinuation reasons: If an enrolled provider withdraws participation at any point, the research assistant will ask them to indicate the primary reason(s) for withdrawing their participation. Similarly, if an investigator discontinues or withdraws a provider participant from the study, the investigator will record the reason for participant discontinuation or withdrawal from the study on the Study Participation Case Report Form.

Provider post-training baseline questionnaire: All clinicians participating in the provider training will complete a self-administered post-training evaluation questionnaire, which will include an assessment of satisfaction with the training quality, content, perceived knowledge gained of the MedViewer test and study procedures, and self-efficacy to deliver MedViewer report to patients. (Appendix C.5)

Provider post-visit questionnaire: After each patient study visit, providers will complete a brief questionnaire pertaining to the content of the MedViewer counseling session (one questionnaire per patient). Providers will be asked to complete the questionnaire pertinent to a particular patient by the end of day of the patient participant visit, ideally immediately after the relevant patient appointment, whenever possible. Providers who do not complete the questionnaire on the day of the relevant patient appointment will be contacted by the study team the next day and asked to complete it as soon as possible (within seven days after the visit). On the questionnaire, providers will indicate if the MedViewer report was discussed with the patient. If results were not discussed, they will indicate reasons for non-discussion. If results were discussed, the provider will indicate the estimated time spent discussing the MedViewer results patient, their perceptions of patient comprehension of the results, perceived usefulness of the report in promoting and motivating patient adherence, and perceived usefulness of the MedViewer Report in improving their understanding of patient adherence and in promoting quality of the adherence discussion with the patient. (Appendix C.6)

Provider in-depth interview: All providers will participate in an IDI within 180 days of seeing at least 2 patient participants or before study close, whichever comes first. Trained research team members will conduct the interviews using semi-structured interview guides. Interview topics will include: usefulness

of MedViewer for adherence counseling, perceived usefulness of MedViewer in encouraging patients to sustain or improve adherence, reasons would/would not recommend future MedViewer use, satisfaction with the MedViewer report and adherence counseling using the report, perceived ease of delivering and discussing MedViewer results during typical appointment, perceived level of disruption to clinic flow by MedViewer procedures, and the anticipated effect of regular MedViewer use on patient-provider communication and relationships. (Appendix C.7)

Provider endline questionnaire: Providers will complete an endline questionnaire within 6 weeks of closure of the patient participant enrollment period or before closing their participation in the study, whichever comes first. Questionnaire items will pertain to: likelihood of recommending MedViewer to other patients, satisfaction with adherence counseling discussions using MedViewer report, perceptions of the most appropriate clinician for future MedViewer counseling, perceived usefulness of MedViewer in encouraging patients to sustain or improve adherence, and influence of MedViewer use on patient-provider communication and relationships. (Appendix C.9)

ADMINISTRATIVE DATA COLLECTION

Detailed Patient Contact Information Form: For each enrolled participant we will collect detailed contact information in the event that we have to reach them regarding study procedures.

Participation database: The research team will maintain a database of participants approached or contacted for participation, screened for eligibility, enrolled, and withdrawn from the study. These data will be used to assess participation acceptance rates and to assess differences between participants and non-participants.

Sample Tracking and Processing: All samples will be collected, processed and transported in accordance with local standard operating procedures. All necessary sample data will be collected, recorded and tracked on study forms with detailed chain of custody and documentation of compliance with all processing procedures that will include but is not limited to time of sample collection and time of MedViewer report delivery to provider or pharmacist.

B. SAFETY AND OTHER ASSESSMENTS

Safety Monitoring

Close cooperation between the Protocol Chair(s), study site Investigators, NIAID Medical/Program Officer, and other relevant parties will be necessary in order to monitor patient safety and to respond to occurrences of toxicity in a timely manner. The team will have regular calls and/or meetings during the period of study implementation, and additional ad hoc calls will be convened if required.

The study site investigators are responsible for continuous close monitoring of all AEs that occur among study patients after enrollment, and for alerting the team if unexpected concerns arise.

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are the first layer of this tiered system and are responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the Protocol Safety Review Team (PSRT) if unexpected concerns arise. The PSRT will consist of the following study site investigators: Angela Kashuba, PharmD (Principle Investigator), Cynthia Gay, MD (Study Physician), Carol Golin, MD

(Co-Investigator), Claire Farel, MD (Co-Investigator), Amanda Poliseno (Project Manager) and Alexandra Munson, MPH (Behavioral Studies Research Coordinator) along with the Biostatistician.

During the trial, the PSRT will review safety reports (all AEs included, independent of determination of relatedness to study products/intervention) and conduct calls to review the data as appropriate. If necessary, experts external to the protocol team, representing expertise in the fields of microbicides, biostatistics, medical ethics or other specialty may be invited to join the PSRT safety review.

Procedures in place as a part of safety monitoring include the assessment and documentation at each study contact after consent with a patient participant of any adverse events (as defined and described below in 8.3). In addition, all study procedures will be carried out by qualified personnel as described in their respective sections in the protocol. Finally, to reduce variability in the quality of study procedures, all hair and blood samples will be processed in one central laboratory, all participants will view the same video to receive information about MedViewer and the study questionnaires will be administered using a standardized CASI system, incorporating the CAPI system and verbal administration of questionnaires by trained research team members, if needed.

Screening, Eligibility and Enrollment of Patient Participants
Several steps are in place to ensure safety during screening and enrollment procedures:

- **Study Visibility.** To enhance patient awareness of and hence comfort with the study, we will place IRB-approved informational flyers in the clinic.

- **Pre-consented use of database**. Of all the patients in the UNC ID Clinic who are living with HIV, approximately 95% have consented to having their patient information available in a secure clinic database, where it can be viewed to identify potential eligibility for open research studies, and to being notified of studies for which they are potentially eligible (as per IRB form 99-MED-408). Patients in the database have indicated all the methods of contact to which they have and have not agreed for contact by researchers for potential participation.

- **Pre-screening**. In addition, the ID Clinic has a full-time research screener to assess patient eligibility for open research projects. We will use the IRB-approved screening process that has been used in the ID Clinic for over ten years, whereby the clinic screener or a trained research team member will pre-screen patients scheduled for an appointment in the upcoming week in the clinic database. To pre-screen patients, using the clinic database, the screener or trained research team member will assess for eligibility criteria outlined in sections 5.1 and 5.2 under an IRB-approved limited waiver of HIPAA. At regular intervals, the clinic screener will share the list of scheduled patients who meet eligibility in pre-screening to the research team.

- **Recruitment**. Using the list of potentially eligible study participants the research team will contact potentially eligible patients who have given permission by phone before their next scheduled HIV appointment using an IRB-approved phone screening questionnaire to notify them about the study and assess their interest. Phone calls will be conducted in a private room to prevent inadvertent disclosure of participant information. Patients may also contact study staff in response to IRB-approved study recruitment flyers.

- **Screening**. For participants who express interest on the phone, the researchers will continue with the phone contact to verify their eligibility using a brief standardized IRB-approved

script/screening form (with questions pertaining to patient age, amount of time as a patient at the UNC ID Clinic, HIV appointments in the last year, current and past prescribed ARV medications and length of caput hair), and if confirmed to be eligible for a screening visit, schedule them for the first study visit.

- **Alternate Recruitment and Screening.** If contact with the patient cannot be established by phone despite multiple attempts (as outlined in section 5.5) before the patient visit, the RA may use several alternate approaches to recruit, screen and schedule the patient. First for patients who have given permission, the RA may contact them by email using an IRB-approved script to invite them to call for further screening by phone. For patients who call for further screening, the RA would follow phone-screening procedures outlined above. Second, for patients who cannot be contacted by phone or email after multiple attempts, and who are scheduled for an in-person clinical visit with their provider, the research staff will approach them in the ID Clinic waiting room (if the patient has given permission to do so) on the day of their visit to notify them that they may be eligible for a study. Those who are interested will be asked to move to a private room in the ID Clinic or CTRC to undergo further screening and, if eligible and interested, informed consent. The private room will have a closed door to prevent inadvertent disclosure of subject information.

- **Exclusion.** If one or more of the basic inclusion criteria are not met, or an exclusion criterion is met, the subject will be informed that they are not eligible.

- **Consent.** Research staff will obtain written informed consent from each potential participant before starting any study procedures according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs.

Screening and Enrollment of Patient IDI Subsample. We will also enroll a subsample of patients to participate in follow-up IDIs and endline questionnaires. We will use the following process to recruit and enroll a target of up to 30 patients for this portion of the study:

- **Screening and Recruitment.** During the initial study visit for the main study, research staff will inform eligible participants about the IDI subsample activities and invite them to participate. Every enrolled participant in the main pilot study will be invited to participate in the IDI. We will monitor the rate of subsample enrollment every month and adjust our recruitment methods, as needed. This approach will be used to ensure that the subsample is representative of the larger study sample, including regarding time of enrollment in the study (so that we obtain equal numbers of patients enrolling early and late in the larger study).

- **Consent.** Research staff will obtain a second written informed consent from each potential participant before starting study procedures for the IDI according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs.

- **Monitoring of Enrollment Rate.** We will monitor the rate of subsample enrollment every month and adjust our recruitment methods as needed to increase the likelihood of obtaining a representative sample of participants throughout the duration of the study. Specifically, if after two months of enrollment, more than 15 participants have enrolled, we will invite every other participant to enroll.

- All communication regarding the IDI subsample activities and informed consent will take place in-person or by IRB-approved phone or videoconferencing call in a private room with a closed door to prevent inadvertent disclosure of participant information.

Screening and Recruitment Procedures for Provider Participants: We will also aim to recruit and enroll all (~15 and up to 30) medical providers who provide medical care to patients in the ID Clinic (attending physicians, fellows, nurse practitioners, physician assistants, nurses, and pharmacists).

- **Information Provision.** We will use an IRB-approved verbal and written announcements about the study and training during weekly Infectious Disease conferences typically attended by ID Clinic providers.

- **Recruitment.** A recruitment email that uses an IRB-approved script will be sent to providers by the research team to invite them to attend a training session about the MedViewer test. Providers will be asked to RSVP to the email to sign up for or decline attendance of the training. Attendance of a training is required for their study participation, however, participation in the training does not require their participation in the study. Invitation emails will be sent from an encrypted email server.

- **Consent.** Research staff will obtain written informed consent from each potential provider participant before starting any study procedures according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs.

Alternate training. For providers who cannot participate in the original training, we will provide the opportunity to attend an alternate training session or to complete a one-on-one training with a research staff member. Supplemental training will be offered, as needed.
Assessment of study intervention adherence: See Section 6.4

Assessment of Adverse events: Adverse events, including serious adverse events will be documented throughout the duration of the study. See Section 8.3.

C. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.C.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).] As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time during the study period.

In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to seek evaluation where the study

clinician is based, and to request that the clinician be contacted upon their arrival. Sites will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an adverse event. All participants reporting an untoward medical occurrence will be followed clinically, until the occurrence resolves (returns to baseline) or stabilizes.

The site clinicians will determine AE resolution or stabilization in their best clinical judgment but may seek PSRT medical consultation regarding follow-up or additional evaluations of an AE. Study site staff will document in source documents all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. Study staff also will record all AEs on study logs.

8.C.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

No ARVs or HIV-related conditions will be considered for expedited reported as protocol-related adverse events.

Study staff will report all AEs that meet serious adverse event (SAE) reporting requirements according to the DAIDS-defined “standard” reporting requirements. Information on all AEs will be included in reports to any applicable government and regulatory authorities. Study staff will report information on all AEs and SAEs to the IRB and the DAIDs Medical Officer in accordance with all applicable regulations and requirements.

8.C.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

All AEs will be categorized by severity, by the study physician, using the following guidelines to describe severity.

- **Grade 1 Mild** – Events require minimal or no treatment and do not interfere with the participant’s daily activities.
- **Grade 2 Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- **Grade 3 Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. [Of note, the term "severe" does not necessarily equate to "serious".]

- **Grade 4 Life-threatening** – A life-threatening event is one where the patient is in immediate danger of death unless intervention is done. It does not mean that the patient may die at some time in the future from the event or may have died if the event had been more serious or specific.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) will have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect. Determination of relationship to study intervention will be based on what is known about the natural history of the underlying disease, a subject(s) concurrent illness(es), concomitant therapy, study-related procedures, accidents, temporality and other external factors.

Instead of SAE reporting category, the SUSAR (Suspected, Unexpected Serious Adverse Events) reporting category will be utilized.

The terms used to assess the relationship of an event to the study intervention are:

Related—There is a reasonable possibility that the AE may be related to the study intervention

Not Related—There is not a reasonable possibility that the AE is related to the study intervention

We will also document the outcome of the AE, categorizing the outcome as unknown, ongoing, death, resolved, or resolved with sequelae.

8.C.3.3 EXPECTEDNESS

Expected adverse reactions are AEs that are known to occur for the study intervention being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction. There are no known AEs that are expected to occur as a result of patient exposure to the MedViewer intervention. However, the study physician will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.C.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The research team will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days after the last day of study participation. At each study contact occurring after consent, the researchers will inquire about the occurrence of ***unsolicited*** AE/SAEs since the last visit using a standardized questionnaire. Events will be followed for outcome information until resolution or stabilization. In addition, the study staff will inquire in a ***solicited*** manner about whether the participant has experienced: 1) any negative changes in their relationship with their provider; 2) any unplanned disclosure of their HIV status.

8.3.5 ADVERSE EVENT REPORTING

Information regarding all AEs regardless of seriousness or severity will be recorded in the participant's source files. Grade 1 clinical symptoms (non-laboratory, e.g., headache, nausea) that lead to a temporary or permanent hold of study intervention, and all Grade 2 and higher AEs, will be collected on standard case report forms (CRFs) for entry into the study database and future reporting to appropriate agencies at stated intervals consistent with all local policies.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

Expedited Adverse Event Reporting

No ARVs or HIV-related conditions will be reported as expedited reporting as protocol-related adverse events.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Any new safety information learned during the course of study conduct that might affect participant willingness to participate will be shared.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

Not Applicable.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Protocol Safety Review Team (PSRT), which includes the study PI. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;

- A detailed description of the event, incident, experience, or outcome;

- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;

- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the PSRT and study sponsor in accordance with the DAIDS-defined "standard" reporting requirements, which is as

soon as possible, but in no event later than within 7days of the investigator becoming aware of the event.

- Any other UP will be reported to the IRB and to the PSRT/study sponsor in accordance with the DAIDS-defined "standard" reporting requirements, which is as soon as possible, but in no event later than within 7 days of the investigator becoming aware of the problem.

- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) in accordance with the DAIDS-defined "standard" reporting requirements, which is within 7 days of the IRB's receipt of the report of the problem from the investigator.]

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Any new safety information or problems that occur during the conduct of the study that might affect a participant's willingness to participate will be shared.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL ENDPOINTS AND MEASURES

Our study objectives are to:

1. Primary Objective: Investigate the feasibility of delivering the MedViewer intervention as planned, the acceptability to patients of participation in the MedViewer intervention, and the appropriateness of MedViewer use for adherence counseling.

2. Secondary Objective: Investigate additional dimensions of feasibility, acceptability, and appropriateness of using hair (MedViewer) to provide patients living with HIV feedback regarding longitudinal patterns of medication adherence.

3. Exploratory Objective 1: Assess exploratory aspects of MedViewer feasibility and acceptability, and patient and provider views of and experiences with the MedViewer test.

4. Exploratory Objective 2: Explore the impact of adherence counseling using MedViewer on ART adherence and hypothesized mechanisms of change (adherence information, motivation, behavioral skills).

5. Exploratory Objective 2: Further explore the accuracy of the MedViewer test.

As this is a feasibility study, the objective is to accurately assess and describe outcomes among the study sample. Our analytic approach for quantitative endpoints, therefore, focuses on calculating precise estimates of the outcomes rather than testing hypotheses. Our analytic approach for qualitative endpoints focuses on describing participant perceptions, attitudes, and recommendations in depth.

- Primary Endpoint(s):

1. *Feasibility*: proportion of participants receiving the MedViewer report during their provider visit, as planned (i.e. the result is delivered to the designated research staff member within 2 hours of initiation of hair processing and the results are discussed with provider/pharmacist within 4 weeks of hair collection).

   We anticipate 80% or more of participant visits will meet criteria for this primary endpoint.

   Time period: This is a cross-sectional study; here and elsewhere, except where specified otherwise, the time period is a single study visit. Activities in Study Visit 1 may occur over multiple days.

2. *Acceptability*: proportion of contacted patients who are eligible for a screening visit (not including inclusion criterion 9) who agree to participate in the MedViewer intervention pilot study.

   We anticipate 80% or more of contacted, screening eligible, patients (not including inclusion criterion 9) will agree to participate in the MedViewer intervention pilot study.

3. *Appropriateness:* perceived usefulness of the MedViewer intervention for adherence counseling; assessment will occur via in-depth interviews.

   Hypothesis testing and precision estimates are not applicable. A description of planned qualitative analyses of in-depth interviews (IDIs) is provided below in section 9.5.

- Secondary Endpoint(s):

Feasibility:

1. Reasons for patient's non-receipt of the MedViewer report during a visit with provider or pharmacist within 4 weeks of hair collection (if applicable). Descriptive analysis.

2. Reasons for non-discussion of the MedViewer report with provider or pharmacist (if applicable). Descriptive analysis.

3. Duration of time (in minutes) from initiation of hair processing to MedViewer report delivery to the designated research staff member. We anticipate the duration will be 120 minutes or less in approximately 80% or more of the study participants.

Acceptability:

1. Provider-reported likelihood of recommending MedViewer to future patients. We anticipate 80% or more of providers will score 3 or greater on the 5-point likelihood of future MedViewer use item.

2. Patient-reported likelihood of agreeing to future MedViewer use. We anticipate 80% or more of participants will score 4 or greater on the likelihood of future MedViewer use item.

3. Patient comprehension of MedViewer report. We anticipate 80% or more of patients will score 3 or greater (out of 4) on the comprehension measure. We further anticipate that most patients completing cognitive interviews will display good comprehension of the report content.

Appropriateness:

1. Perceived usefulness of MedViewer to promote ART adherence. Rating of patients' and providers' perceived usefulness of MedViewer to promote ART adherence measured by multi-5-item scales.
    i. Patients: We anticipate a mean score of 4.0 or higher
    ii. Providers: We anticipate a mean score of 4.0 or higher

    We further anticipate that a majority of patients and providers completing in-depth interviewers will express that they feel MedViewer is useful to promote ART adherence.

2. Perceived impact of MedViewer use on patient-provider communication and relationship: Rating of comparative overall satisfaction with patient-provider interaction during the MedViewer visit as compared to a typical visit rated on a 5-point comparative satisfaction scale.

    Patients: We anticipate a mean score of 4.0 or higher.

    Providers: We anticipate a mean score of 4.0 or higher.

    We further anticipate that a majority of patients and providers competing in-depth interviews will express that they feel MedViewer is useful to promote ART adherence.

- Exploratory Endpoints:

Exploratory Feasibility Endpoints:

1. Patient-reported maximum out-of-pocket cost willing to pay for MedViewer. We will estimate the proportion of participants who would be willing to pay a range of potential out-of-pocket costs of MedViewer (range: $0 to $250). Descriptive analysis.

2. Cost of MedViewer delivery per person. Descriptive analysis and cost estimation.

3. Compatibility of MedViewer with current clinic practices. Qualitative assessment of provider perceptions.

Exploratory Acceptability Endpoints:

1. Patient-reported reasons for declining participation in the MedViewer intervention pilot study. Categorical variable. Descriptive analysis.

2. Reported reasons would/would not agree to (patient-reported) or recommend (provider-reported) future MedViewer use. Qualitative analysis.

3. Acceptability of specific components of MedViewer procedure (a-d):
    a. Sufficiency of provider training & materials: Assessed by provider-reported self-efficacy, knowledge and perceived quality rated on a 5-point scale). We anticipate 80% or more of providers will have a mean score of 4 or greater. Additional qualitative assessment and description.

    b. Sufficiency of patient education (video): Assessed by four knowledge questions – we anticipate that 90% of patients will correctly answer each question; 2 self-efficacy questions rated on a 5-point response scale - we anticipate 80% or more of patients will have a mean score of 4 or greater. Additional qualitative assessment and description.

    c. Provider satisfaction with results delivery (person, discussion format, and content), with specific items to be assessed by provider on 5-point scale. We expect 80% or more of providers will score 4 or greater on each item. Additional qualitative assessment and description.

    d. Patient satisfaction with results delivery (person, discussion format and content), wait time, hair sample collection and MedViewer report, with specific items to be assessed by patients on a 5-point scale. We expect 80% or more of patients will have a mean score of 4 or greater. Additional qualitative assessment and description.

Exploratory Adherence-Related Endpoints:

1. ART adherence (self-reported assessment of 3-day, 7-day, and 30-day adherence) for IDI subsample. Assessed during patient study visit 1 and follow-up patient study visit for IDI subsample.

2. Adherence information (qualitative assessment of information/understanding gained of patient adherence resulting from use of MedViewer). Assessed during follow-up patient study visit for IDI subsample.

3. Adherence motivation (quantitative and qualitative assessment of adherence motivation resulting from use of MedViewer). Assessed during follow-up patient study visit for IDI subsample.

4. Adherence behavioral skills (quantitative and qualitative assessment of adherence behavioral skills and self-efficacy resulting from use of MedViewer). Assessed during follow-up patient study visit for IDI subsample.

Exploratory Endpoints for Assessment of Accuracy of Hair as an Adherence Measure:

1. ARV concentrations in blood collected for first patient study visit and a follow-up patient study visit, when applicable.

2. Patient viral load assessed with a clinical care visit linked to a patient-provider MedViewer visit (within the last 6 months) and abstracted from the medical record by study staff.

3. Log_{10} antiretroviral drug concentrations in hair collected for first patient study visit and follow-up patient study visit, when applicable.

   We will estimate log_{10} ARV hair concentrations in 3 viral load (VL) groups (undetectable, detectable but $\leq$ 1,000, and > 1,000 copies/mL) and plan to conduct hypothesis testing. Alternative Hypothesis: There will be a difference in log_{10} ARV hair concentrations among the 3 viral load strata such that log_{10} ARV hair concentrations will be highest in those with VL below the limit of quantification for the lab and lowest in those with VL > 1,000.

9.2 SAMPLE SIZE DETERMINATION

Primary sample size consideration: We will estimate the probability (p) that participants have their hair sample-based MedViewer report delivered as planned (for primary feasibility) within each VL strata with a corresponding 95% Wilson-Score binomial confidence interval (CI). The same approach will be used for acceptability, appropriateness, and additional binary endpoints.

A sample size of 50 would enable sufficient precision for a pilot study to estimate main and secondary outcomes as well as sufficient power for exploratory data analyses. Specifically, the table below shows the anticipated CI half-widths under the statistically conservative assumption that p=0.50 (50%, the point of highest variance) and p=0.80 (80%, as anticipated) assuming n=25 individuals with undetectable VL and n=25 with detectable VL. When the sample is split evenly between the low VL and high VL strata (n=25 per stratum), each stratum has a high probability of achieving a CI half-width of 0.19 (19%) or narrower if p=0.50 and 0.18 (18%) or narrower if p=0.80.

Table: Precision Calculations for full sample (N = 50)

Total Sample Size	**Low VL (undetectable) n=**	**High VL (detectable) n=**	**Assumed true probability**	**Precision: 95% CI Half-Width**	**P (achieving this precision)**
50	25	25	p=0.5	Low VL 0.19 High VL 0.19	95% 95%

			p=0.8	Low VL 0.18 High VL 0.18	95% 95%

CI = confidence interval (two-sided); VL = viral load; P(·) = probability

For primary outcomes with homogeneous results by VL strata, we will use a weighted analysis to estimate the overall proportion for feasibility, acceptability and appropriateness at the UNC clinic, with weights corresponding to the VL distribution in the UNC CFAR HIV Clinical Cohort (UCHCC).

For exploratory analysis, we will further divide the 2 VL groups to all for consideration of 3 VL groups (below level of viral quantification (which is <40 at UNC), detectable to <1000, and 1000+ copies/mL). Based on analyses of preliminary clinical cohort viral load data, we anticipate the participants with detectable VL to be approximately evenly split between the 25 to <1000 and 1000+ copies/mL VL groups, respectively. Association between VL group and each primary outcome for acceptability and appropriateness will be explored using a 3x2 χ^2 test for trend (i.e., Cochran–Armitage test for trend) although as an exploratory analysis, the study is not powered to identify statistically significant differences.

Association between VL group and each primary outcome for acceptability and additional exploratory analyses evaluating VL as continuous with left-censored observations <40 copies/mL can compare the VL distributions between participants who find the hair evaluation appropriate vs. those who do not (anticipated outcome group sizes of n=25 and 10 assuming 80% of participants report the hair evaluation is appropriate).

For additional exploratory analysis, we will also conduct descriptive analyses of the primary outcomes with homogeneous results by VL strata for the 40 patient participants who participated in the study following modifications made in response to the COVID 19 pandemic to explore estimation of the overall proportion of this subsample for feasibility, acceptability and appropriateness, using weighted analysis with weights corresponding to the VL distribution in the UCHCC. The table below shows the anticipated CI half-widths under the statistically conservative assumption that p=0.50 (50%, the point of highest variance) and p=0.80 (80%, as anticipated) assuming n=20 individuals with undetectable VL and n=20 with detectable VL. When the sample is split evenly between the low VL and high VL strata (n=20 per stratum), each stratum has a high probability of achieving a CI half-width of 0.21 (21%) or narrower if p=0.50 and 0.20 (20%) or narrower if p=0.80.

Table: Precision Calculations for subsample of participants enrolled after COVID 19 modifications (N = 40)

Total Sample Size	Low VL (undetectable) n=	High VL (detectable) n=	Assumed true probability	Precision: 95% CI Half-Width	P (achieving this precision)
40	20	20	p=0.5	Low VL 0.21 High VL 0.21	>95% >95%
			p=0.8	Low VL 0.20 High VL 0.20	>95% >95%

9.3 POPULATIONS FOR ANALYSES

We plan to enroll a total of 50 patient participants, which will be split into 25 patients in Group A and 25 patients in Group B. We plan to enroll ~15 and up to 30 provider participants.

Screening eligible patient cohort: This cohort will include all potential patient participants who were found to be eligible to participate in a screening visit (not including inclusion criterion 9), regardless of whether they enrolled in the study or not.

Enrolled patient cohort: The enrolled patient cohort will include all patient participants who screened eligible and signed an informed consent form.

Per-protocol delivery patient cohort: This cohort will include all patient participants enrolled in the intervention who had their hair sample collected and had a MedViewer report delivered to designated research staff member.

Viral load patient subgroups: Patients are enrolled based on their being in one of two viral load strata defined as those patients with two consecutive years of undetectable (below the level of viral quantification) viral loads and those patients with at least one detectable (at or above the level of viral quantification) viral load within the previous two consecutive years.

In-depth interview patient subcohort: (Statistical analyses are not applicable to in-depth interviews.)

Post-COVID modification enrolled patient subcohort: This will include members of the enrolled patient cohort who enrolled in the study after the COVID modification to study procedures. Analysis conducted on this cohort will be done solely for exploratory descriptive purposes.

Enrolled provider cohort: This will include all providers who screened eligible for and signed an informed consent form to participate in the study.

For this study, analysis of the primary feasibility outcome (proportion of participants receiving MedViewer report during their provider visit as planned) will be conducted on the enrolled patient cohort.

Analysis of the primary acceptability outcome (proportion of contacted patients eligible for a screening visit who agree to participate will be conducted on the screening eligible patient participant cohort.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

As this is a pilot feasibility study, our analytic approach focuses on calculating precise estimates of the outcomes, rather than on making comparisons or testing hypotheses. Descriptive statistics of categorical variables will be presented as counts and percentages, and descriptive statistics of continuous variables

will be presented as means with standard deviations or median, 25th to 75th percentiles and ranges. We will plot and visually inspect the distribution of continuous variables. Generally, we do not anticipate variable transformations other than those pre-specified in the endpoints / measures section (e.g., $\log_{10}$ viral load).

With respect to estimation, a maximum likelihood estimate (MLE) will be presented together with a 95% confidence interval (CI) for the proportion, or arithmetic mean of a continuous variable. Estimated proportions will be presented with a corresponding Wilson-Score 95% CI and estimated means will be presented with a corresponding t-distribution 95% CI. If an estimated proportion is unexpectedly near the 0 or 1 boundary (e.g., <0.1 or >0.9), an exact Clopper-Pearson 95% CI will be used as a sensitivity analysis.

Main protocol analyses will be conducted within the 2 enrollment viral load strata separately, as the strata sample sizes were selected for stratum-specific estimation and precision. We anticipate that patients in Group B will be over-sampled for study participation compared to the general clinic population. At the time of final analysis, data from the UNC CFAR HIV Clinical Cohort (UCHCC) will be used to define weights for each viral load stratum, such that a combined, weighted analysis can be used to generalize back to the clinic population. Details of a combined analyses will be pre-specified in a separate analysis plan, with consideration to additional patient characteristics such as gender, race/ethnicity, and age. Some exploratory analyses will be conducted using the 3 VL groups as described in Section 9.2.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINTS

Primary Feasibility Endpoint: This primary endpoint (proportion of patient participants receiving the MedViewer report during their clinic visit as planned), is the proportion of participants who both: (i) had their MedViewer report delivered to the designated research staff member within 2 hours of initiation of hair processing and (ii) discussed the results with a provider (i.e. their medical provider or the HIV Care Pharmacist) within 4 weeks of hair collection.

- **Measurement/observation:** To assess the primary feasibility endpoint, the numerator will be calculated using two separate data sources. First, to assess whether the designated research staff member received the patient's MedViewer report within 2 hours of initiation of hair processing a study tracking log will be maintained by the study team on which the following times will be recorded: hair collection, hair transport, hair processing, and delivery of the report to the designated research staff member. The study team will also note which clinician reviewed the report with the patient, and in their self-report post-visit questionnaires patients will be asked to indicate whether the MedViewer report was discussed following clinic visits with their medical provider and/or the HIV Care Pharmacist. As a backup data source, providers will also be asked in their post-visit questionnaire if they discussed the report with the patient.

- **Scale:** This will be a single dichotomous (yes/no) measure. Patients will be counted in the numerator of the primary outcome if:
    - (i) the study team member indicated on the tracking log that, yes, study staff received the patient' results within 2-hours of initiation of hair processing in the lab. This will be determined by subtracting Time 1 (when hair processing was initiated) from Time 2 (when results were delivered to the designated research staff member, and

- (ii) the patient self-reported on the post-visit questionnaire that, yes, they discussed results with the provider. As a backup data source, providers will also be asked in their post-visit questionnaire if they discussed the report with the patient.

- **Statistical analysis:** The proportion of enrolled participants who achieved the primary feasibility endpoint will be estimated. An estimated proportion and corresponding 95% Wilson-Score CI will be used to analyze this endpoint and all similar dichotomous (i.e., binary) endpoints.

- **Statistical procedures:**
  - **Covariates and factors:** Not applicable.
  - **Rationale for covariates:** Not applicable.
  - **Details to check assumptions:** If an estimated proportion is unexpectedly near the 0 or 1 boundary (e.g., above 0.9), an exact Clopper-Pearson 95% CI can be used as a sensitivity analysis.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage.

- **Populations for Analysis:** Enrolled patient cohort.

- **Missing data**: All enrolled patients will contribute to the analysis denominator; those failing to achieve the feasibility success definition (for any reason) will be counted as a failure for the feasibility outcome. If the patient has missing data on the pertinent item of the patient post-visit questionnaire, we will substitute the missing value with the provider's response to the parallel item in the provider post-visit questionnaire.

Primary Acceptability Endpoint: This primary endpoint is assessed based on the proportion of contacted patients who are eligible, who agree to participate I the MedViewer intervention pilot study.

- **Measurement/observation:** Data for this measure that will be obtained from the study participation log.

- **Scale:** This will be a single dichotomous (yes/no) measure for each individual participant. To create this measure, the denominator will include all potential patient participants who were contacted and found to be eligible for the study as documented on their pre-screening questionnaire. The numerator will be those who agreed to participate in the study as documented by their informed consent form. The primary endpoint is descriptive.

- **Statistical analysis:** The proportion of potential patient participants eligible for a screening visit (not including inclusion criterion 9) who achieved the primary acceptability endpoint (accepted enrollment) will be estimated. An estimated proportion and corresponding 95% Wilson-Score CI will be used to analyze this endpoint.

- **Statistical procedures:**
  - **Covariates and factors:** Not applicable.
  - **Rationale for covariates:** Not applicable.

- **Details to check assumptions:** Exact Clopper-Pearson 95% CI can be used as a sensitivity analysis.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage.

- **Populations for Analysis:** Screened Eligible patient cohort.

- **Missing data:** Not applicable; all patients found eligible for a screening visit (not including inclusion criterion 9) will be counted in the analysis.

Primary Appropriateness Endpoint: This primary endpoint (perceived usefulness of MedViewer for adherence counseling) will be assessed via IDIs and thus statistical considerations do not apply for this endpoint. Qualitative data collection and analytic methods used for IDIs is described below in section 9.5.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINTS

Secondary Feasibility Endpoint: Reasons for patient's non-receipt of the MedViewer report during a visit with provider or pharmacist within 4 weeks of hair collection (if applicable).

- **Note: analysis is not dependent on findings of primary endpoint although it does use a data point that is part of the primary endpoint.*

- **Measurement/observation:** Reasons for patient's non-receipt of MedViewer report during a visit with provider or pharmacist within 4 weeks of hair collection (if applicable) will only be assessed for the subsample of patient participants for whom non-receipt of the MedViewer report is reported (either by a provider in the provider post-visit questionnaire, or by a research team member in a study activities tracking form). Reasons for non-receipt will be assessed using a one-item multiple-choice question with a "check all that apply" approach in provider post-visit questionnaires and an open-ended response on study tracking forms completed by the research team. The multiple responses of common reasons for non-receipt of results will also include an open-ended (other) option.

- **Scale:** Each response option will be a dichotomous (yes/no) item.

- **Statistical procedures:** Statistical analyses will report, for each response option:
  - The proportion of patients in the subsample (patient participants for whom non-receipt of the MedViewer report is reported) for whom each response option is endorsed.
  - Covariates and factors, rationale for covariates: Not applicable.
  - Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage.

- **Populations for Analysis:** MedViewer non-receipt patient cohort.

- **Missing data:** A complete-case analysis will be used; participants with missing reasons will be listed as "Reason Missing" and not counted in the denominator for percentages.

Secondary Feasibility Endpoint: Reasons for patient's non-discussion of the MedViewer report with provider or pharmacist (if applicable).

- **Note: analysis is not dependent on findings of primary endpoint although it does use a data point that is part of the primary endpoint.*

- **Measurement/observation:** Reasons for patient's non-discussion of the MedViewer report during visit (if applicable) will only be assessed for the subsample of patient participants for whom non-discussion of the MedViewer report is reported (either by the patient in the post-visit patient questionnaire or by a provider in the post-visit provider questionnaire). Reasons for non-discussion will be assessed using a one-item multiple-choice question with a "check all that apply" approach in the provider post-visit questionnaires. The multiple responses of common reasons for non-discussion of results will also include an open-ended (other) option.

- **Scale:** Each response option will be a dichotomous (yes/no) item.

- **Statistical procedures:** Statistical analyses will report, for each response option:
    - The proportion of patients in the subsample (patient participants for whom non-receipt of the MedViewer report is reported) for whom each response option is endorsed by the patient or provider.
    - Covariates and factors, rationale for covariates: Not applicable.
    - Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage.

- **Populations for Analysis:** MedViewer non-discuss patient cohort.

- **Missing data:** A complete-case analysis will be used; participants with missing reasons will be listed as "Reason Missing" and not counted in the denominator for percentages.

Secondary Feasibility Endpoint: Duration of time (in minutes) from initiation of hair processing to MedViewer report delivery to designated research staff member. This variable is defined as the amount of time elapsed (in minutes) from the time of initiation of hair processing in the lab to time of results delivery to designated research staff member. This variable will be determined by subtracting Time 1 (when hair processing is initiated) from Time 2 (when results were delivered to the designated research staff member.)

- **Note: This analysis is not dependent on findings of primary endpoint*

- **Measurement/observation:** This variable will be measured using the study visit hair sample tracking log. The log will be maintained by the study team who will report the time at which each study activity occurs.

- **Scale:** Duration of time will be determined based upon subtracting the time of initiation of hair processing from the time of report delivery.

- **Statistical procedures:** We will conduct descriptive statistics for a continuous outcome of time (in minutes) indicating the mean, standard deviation, range and distribution of the time elapsed.
    - Covariates and factors: Not applicable.
    - Rationale for covariates: Not applicable.
    - Details to check assumptions: The distribution of time elapsed will be plotted.

- **Planned presentation of results:** Results will be presented in a table as a mean and SD. Results may also be presented as a box plot or histogram.

- **Populations for Analysis:** Per-Protocol delivery patient cohort.

- **Missing data:** A complete-case analysis will be used; those with missing data will be excluded.

Secondary Acceptability Endpoint: Provider-reported likelihood of recommending MedViewer to future patients.

- **Measurement/observation:** This measure will be assessed using a single Likert-type item among providers in a self-reported questionnaire administered to providers by study team member in the endline questionnaire. Providers will be asked to rate their likelihood of recommending MedViewer, if available for regular use, to other patients in the future.

- **Scale:** This item will be rated on a 4-point scale (definitely would not recommend to definitely would recommend).

- **Statistical analysis:** We will calculate the proportion of providers reporting each response category (count/denominator and percent), respectively.

- **Statistical procedures:**
    - Covariates and factors: Not applicable.
    - Rationale for covariates: Not applicable.
    - Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage for each of the four response categories.

- **Populations for Analysis:** Per-Protocol delivery provider cohort.

- **Missing data:** A complete-case analysis will be used; those with missing data will be excluded.

Secondary Acceptability Endpoint Patient-reported likelihood of agreeing to future MedViewer use.

- **Measurement/observation:** This measure will be assessed among patients in the self-reported post-visit questionnaire administered by the study team member after the participant has received and discussed their MedViewer report with their provider, (or completed their visit

without a discussion). This will be measured using a Likert-type item. Participants will be asked to rate their future likelihood of agreeing to MedViewer use if recommended by a provider in the future.

- **Scale:** These items will be rated on a 4-point scale (definitely would not use to definitely would use).

- **Statistical analysis:** For each item, we will calculate the proportion of patients reporting each response category (count/denominator and percent), respectively.

- **Statistical procedures:**
  - Covariates and factors: Not applicable.
  - Rationale for covariates: Not applicable.
  - Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage.

- **Populations for Analysis:** Per-Protocol delivery patient cohort.

- **Missing data:** A complete-case analysis will be used; those with missing data will be excluded.

Secondary Acceptability Endpoint. Patient comprehension of MedViewer report as perceived by patients and providers, respectively.

- **Measurement/observation**: In the post-visit questionnaire following the MedViewer report discussion, patients will be asked to rate their comprehension of the information presented in report. Providers will complete a parallel item in their post-visit questionnaire to assess their view of how well patients understood the information in the MedViewer report. Patient comprehension of the report will also be assessed qualitatively: during the patient IDIs, we will conduct a cognitive interview to assess patient comprehension of adherence information presented in MedViewer report.

- **Scale:** The patient item will be rated on a 4-point scale (very difficult to understand to very easy to understand). The provider item will be rated on a 5-point scale (understood poorly to understood excellently). Qualitative responses will have no scaling.

- **Statistical analysis:** Questionnaire items: for each item, we will calculate the proportion of patients and reporting each response category (count/denominator and percent), respectively. We will calculate similar proportions for provider responses regarding their perception of each patient's comprehension, as a proportion of patients. In depth interview: we will summarize the extent to which patients understood the content of the reports and describe the primary content and formatting features of the report which were poorly understood by participants, if applicable.

- **Statistical procedures:**
  - Covariates and factors: Not applicable.

- Rationale for covariates: Not applicable.
- Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage. Cognitive interview results will be summarized narratively.

- **Populations for Analysis:** Questionnaire items: Per-protocol delivery patient and provider cohorts; IDI questions: IDI patient sub-sample.

- **Missing data:** A complete-case analysis will be used; those with missing data will be excluded.

Secondary Appropriateness Endpoint: Perceived usefulness of MedViewer to promote ART adherence. We anticipate a mean score across three items each rated on a 5-point usefulness scale of 4.0 or higher.

- **Measurement/observation:** Perceived usefulness will be measured in the patient post-visit questionnaire administered by the study team member following the MedViewer discussion; provider post-visit questionnaire following each visit with an enrolled patient; patient and provider endline questionnaires; and in the patient and provider IDIs. Participants will be asked to rate their agreement with statements regarding MedViewer's usefulness in promoting: 1) adherence over the next 30 days; 2) adherence motivation; 3) skills and strategies to avoid missed doses.

- **Scale:** These items will be rated on a Rated on a 5-point scale (not at all useful to extremely useful).

- **Statistical analysis:** For each item, we will calculate the proportion of patients and providers reporting each response category (count/denominator and percent), respectively.

- **Statistical procedures:**
  - Covariates and factors: Not applicable.
  - Rationale for covariates: Not applicable.
  - Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage.

- **Populations for Analysis:** Per-protocol delivery patient and provider cohorts.

- **Missing data:** A complete-case analysis will be used; those with missing data will be excluded.

Secondary Appropriateness Endpoint: Perceived impact of MedViewer use on patient-provider communication and relationship.

Patients: We anticipate a mean score of 4.0 or higher on one item rated on a 5-point comparative satisfaction scale (much less satisfied than usual to much more satisfied than usual) of 4.0 or higher.

Providers: We anticipate a mean score of 4.0 or higher on one item rated on a 5-point scale (very negatively affected relationships to very positively affected relationships).

- **Measurement/observation**: In the post-visit questionnaires administered to patients following the MedViewer I, patients will be asked to rate the comparative satisfaction with the patient-provider interaction during the MedViewer visit as compared to a typical visit. In the questionnaires administered to providers at endline, providers will be asked to rate the effect of using MedViewer on their relationships with their patients. During the patient and provider IDIs, participants will be asked to discuss the anticipated effect of regular MedViewer use on patient-provider communication and relationships.

- **Scale:** The patient questionnaire item will be rated on a 5-point scale (much less satisfied than usual to much more satisfied than usual). The provider questionnaire item will be rated on 5-point scale (very negatively affected relationships to very positively affected relationships). IDI questions will have no scaling.

- **Statistical analysis:** Questionnaire items: for each item, we will calculate the proportion of patients and providers reporting each response category (count/denominator and percent), respectively. IDI questions: N/A; for analytic methods, see section 9.5.

- **Statistical procedures:**
  - Covariates and factors: Not applicable.
  - Rationale for covariates: Not applicable.
  - Details to check assumptions: Not applicable.

- **Planned presentation of results:** Results will be presented as a count/denominator and percentage; Qualitative results will be summarized and presented thematically.

- **Populations for Analysis:** Per-protocol patient and enrolled provider cohorts.

- **Missing data:** Questionnaire items: Per-Protocol delivery patient and provider cohorts; IDI questions: IDI patient sub-sample.

9.4.4 SAFETY ANALYSES

Adverse Events Definitions and Reporting Requirements

An adverse event is defined as any untoward medical occurrence in a clinical research participant administered an investigational product and which does not necessarily have a causal relationship with the investigational product. As such, an AE can be an unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to the product. This definition is applied to all the study groups and is applied to all groups beginning from the time of sequential assignment. The term “investigational product” for this study refers to all study products listed in this protocol. Study participants will be instructed to contact the study site staff to report any AEs they may experience at any time between the study interval. In the case of a life-threatening event, they will be instructed to seek immediate emergency care. Where feasible and medically appropriate, participants will be encouraged to

seek evaluation where the study clinician is based, and to request that the clinician be contacted upon their arrival. Sites will obtain written permission from the participant to obtain and use records from non-study medical providers to complete any missing data element on a CRF related to an adverse event. All participants reporting an untoward medical occurrence will be followed clinically, until the occurrence resolves (returns to baseline) or stabilizes.

The site clinicians will determine AE resolution or stabilization in their best clinical judgment but may seek PSRT medical consultation regarding follow-up or additional evaluations of an AE. Study site staff will document in source documents all AEs reported by or observed in enrolled study participants regardless of severity and presumed relationship to study product. Study staff also will record all AEs on case report forms.

Serious Adverse Events

Serious adverse events (SAEs) will be defined by the Manual for Expedited Reporting of Adverse Events to DAIDS (Version 2.1, dated March 2017) as AEs occurring at any dose/intervention that:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires inpatient hospitalization or prolongation of existing hospitalization

The following are examples of hospitalization that will not be considered to be AEs:

- Protocol-specified admission (e.g. for procedure required by study protocol)
- Admission for treatment of target disease of the study, or for pre-existing condition (unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator)
- Diagnostic admission (e.g. for a work-up of an existing condition such as persistent pretreatment lab abnormality)
- Administrative admission (e.g. for annual physical)
- Social admission (e.g. placement for lack of place to sleep)
- Elective admission (e.g. for elective surgery)

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Study staff will report all AEs that meet serious adverse event (SAE) reporting requirements according to the DAIDS-defined “standard” reporting requirements. Information on all AEs will be included in reports to the US FDA, and other applicable government and regulatory authorities. Study staff will report information on all AEs and SAEs to the IRB in accordance with all applicable regulations and requirements.

Social Harms Reporting

Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms may result. For example, participants could be treated unfairly or discriminated against, or could have problems being accepted by their families and/or communities. Social harms that are judged by the Investigator of Record to be serious or unexpected will be reported to the responsible site IRB at least annually, or according to their individual requirements. In the event that a participant reports social harm, every effort will be made by study staff to provide appropriate care and counseling to the participant, and/or referral to appropriate resources for the safety of the participant as needed. While maintaining participant confidentiality, study sites may engage their CABs in exploring the social context surrounding instances of social harm.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

We will conduct descriptive statistics to characterize the samples of patient and provider participants.

First, we will describe the sample characteristics of patient participants according to the following demographic characteristics: self-rated health; hair coloring and other treatments, extensions; number of visits in the ID Clinic in the past year; years/months since became a patient in the ID Clinic; time to travel from home to clinic; age; sex assignment at birth and gender identity; sexual orientation; race/ethnicity; current marital status; education level; income in past year; current employment status; health insurance status and type; and method of paying for ART.

Second, we will compare, among those who are eligible for a screening visit, those agreeing and not agreeing to participate with regard to their age (continuous), gender (including but not limited to the following genders: male/female/trans male to female/trans female to male), viral load strata, and race/ethnicity (assessing all racial options as indicated in the baseline questionnaire) as well as recording reasons for non-participation, using a χ^2 test (categorical) or a Wilcoxon rank-sum test (continuous). If expected cell counts are below 5 a Fisher's exact test will be used rather than a χ^2 test.

Third, we will characterize the enrolled patient IDI subsample with regard to their self-reported age, sex, gender identity, race, ethnicity, marital status, educational level, income, employment status, insurance status, self-reported last 30-day adherence, duration of diagnosis, involvement in medical care, length of time it takes for them to get to the clinic, hair treatments, as well as, from chart review, their last viral load and CD4 count.

9.4.6 PLANNED INTERIM ANALYSES

No interim analyses are planned.

9.4.7 SUB-GROUP ANALYSES

For sub-group analyses, the primary endpoints (feasibility, acceptability, and appropriateness) will be analyzed by viral load strata, and by current gender and self-reported race/ethnicity, and for the post-COVID modification subsample. Key, emergent qualitative themes for subgroups will be described, as well as drug exposure in blood and hair samples during the intervention.

We will present listings of individual participant-level data for the primary endpoints.

9.4.8 EXPLORATORY ANALYSES

Exploratory analyses will be conducted to provide supplemental information regarding MedViewer intervention feasibility, acceptability and appropriateness to further inform modifications to the testing of the intervention in a larger trial. A separate full analysis plan will be developed for exploratory endpoints but a general description of our approach is provided below.

Exploratory Feasibility Endpoints:

- Patient-reported maximum out-of-pocket cost willing to pay for MedViewer. The amount that patients would be willing to pay out of pocket for MedViewer in the future will be measured in two ways. First, using the post-visit and endline questionnaires, patients will report the maximum dollar amount that the they would be willing to pay out-of-pocket for one use of the MedViewer test (adapted from Jonas et al., 2010 and Grutters et al., 2009 willingness to pay measures) [52,53]. We will report summary statistics, including the mean (with 95% CI), standard deviation, median, range and 25^{th} to 75^{th} percentile of the amount of money patients reported being willing to pay. The distribution of maximum willing to pay out-of-pocket costs will be displayed using boxplots by viral load strata.

- Cost of MedViewer delivery per patient. This will be measured as a continuous variable using the study tracking form which will keep record of staff and study activity time. Specifically, this will be the total cost of MedViewer program delivery (based upon administrative cost data that will include the cost of sample collection kits, cost of lab analytes, processing and equipment, costs for utilizing CTRC and clinic exam rooms, records of staff time spent on intervention procedure), and for patients undergoing remote sample collection, the cost of staff travel (e.g. mileage, fuel, etc.,) to collect the samples from participants. Staff time will be estimated based on time log assessments made during 4 randomly selected weeks per year. The total costs will be divided by the number of patients to whom the MedViewer intervention was delivered. Providers will indicate the estimated additional/reduced time in minutes spent with each patient due to the use of the MedViewer report; a sum of the total administrative cost data and total costs of staff time spent on intervention procedure as assessed using recorded (i.e., logged) staff time, reports from providers indicating the estimated additional/reduced time in minutes they spent with each patient due to the use of the MedViewer report and salary data. The total cost divided by the number of patients to whom the MedViewer was delivered will be the estimated cost of delivery per person.

- Compatibility of MedViewer with current clinic practices. Qualitative assessment of provider perceptions. IDI questions will ask about perceived ease of delivering and discussing MedViewer results during typical appointment and perceived level of disruption to clinic flow by MedViewer procedures.

Exploratory Acceptability Endpoints:

- Patient-reported reasons for declining participation in the MedViewer intervention receipt of MedViewer (declining or withdrawing participation). This information will be collected using two

data sources: the study pre-screening form (reasons for declining participation) and the study termination form (reasons for withdrawing participation). A list of reasons will be available for the study team member to select why the participant declined or withdrew participation, and an option to write in a reason will also be provided. We will describe the number of people nominating each reason for declining/withdrawing participation.

- Reasons would/would not agree to (patient-reported) or recommend (provider-reported) future MedViewer use. Through in-depth interviews, we will elicit explanations of the reported likelihood of agreeing to (patients) or recommending (providers) future MedViewer use. Participants will be further asked to discuss the primary reasons for this attitude toward future MedViewer use. For data collection and analytic procedures, see section 9.5.

- Patient comprehension of report. Will also explore patient comprehension of the MedViewer report qualitatively during individual IDIs with the subset of patients. As described in 9.5, these interviews will be administered by a trained team member who will use a semi-structured interview guide.

- Acceptability of specific components of MedViewer procedure (a-d):
    a. Sufficiency of provider training & materials. Assessed by provider-reported self-efficacy, knowledge and perceived quality rated on a 5-point scale). We anticipate 80% or more of providers will have a mean score of 4 or greater. Additional qualitative assessment and description.

    b. Sufficiency of patient education (video): Assessed by 4 knowledge questions – we anticipate that 90% of patients will correctly answer each question; 2 self-efficacy questions rated on a 5-point response scale - we anticipate 80% or more of patients will have a mean score of 4 or greater. Additional qualitative assessment and description.

    c. Provider satisfaction with results delivery (person, discussion format, and content), with specific items to be assessed by provider on 5-point scale. We expect 80% or more of providers will score 4 or greater on each item. Additional qualitative assessment and description.

    d. Patient satisfaction with results delivery (person, discussion format and content), wait time, hair sample collection and MedViewer report, with specific items to be assessed by patients on a 5-point scale. We expect 80% or more of patients will have a mean score of 4 or greater. Additional qualitative assessment and description.

Exploratory Adherence-Related Endpoints:

- ART adherence measure by self-report (open-ended self-report of missed doses over the past 3 days, 7 days, and 30 days).

- Adherence information (qualitative assessment of information/ understanding gained of patient adherence resulting from use of MedViewer). Assessed during follow-up patient study visit for IDI subsample.

- Adherence motivation (quantitative and qualitative assessment of adherence motivation resulting from use of MedViewer). Assessed during follow-up patient study visit for IDI subsample.

- Adherence behavioral skills (quantitative and qualitative assessment of adherence behavioral skills and self-efficacy resulting from use of MedViewer). Assessed during follow-up patient visit for IDI subsample.

Exploratory Endpoints for Assessment of Accuracy of Hair as an Adherence Measure:

- ARV concentrations in blood collected for first patient study visit and follow up patient study visit, when applicable (blood sample analysis).

- Patient viral load assessed with a clinical care visit linked to a patient-provider MedViewer visit (within the last 6 months) and abstracted from the medical record by study staff.

- $\log_{10}$ antiretroviral drug concentrations in hair collected for first study visit and follow-up patient study visit, when applicable. We will explore the relationship between $\log_{10}$ ARV hair concentrations and viral load strata. In analyses of the association between VL group and $\log_{10}$ ARV hair concentrations using one-way ANOVA we will pre-specify that the impact of race-ethnicity (as well as additional potential measured confounders) will be explored with a general linear model.

9.5 QUALITATIVE DATA COLLECTION AND ANALYSES

IDI participants will complete an interview approximately one hour in length to discuss the interview topics pertaining to the relevant endpoints noted above. IDIs will be conducted by trained research staff experienced in qualitative research. Interviews will be guided by the use of a semi-structured interview guide which will include open-ended questions corresponding to each qualitative endpoint. Interviewers will be allowed the flexibility to probe patient responses and pursue discussion diverging from the initial interview questions if it is relevant to the endpoints of interest. Each interview will be digitally recorded and transcribed verbatim to text for analysis.

Analysis

All interviews will be digitally recorded and transcribed. Data will consist of four key steps: 1) Reading for Content: We will begin with data reading until content becomes intimately familiar. As data are reviewed, emergent themes will be noted. 2) Coding: A list of structural codes related to the interview questions will be developed. Code definitions will be documented in a codebook. Qualitative research assistants will be trained to apply the codes using software for qualitative analysis. The codebook will be piloted with 5 interview transcripts: each transcript will be double coded to reconcile code application, and codes and rules for their application were modified as needed. To ensure inter-coder reliability, 10%

of data will be double-coded. Independent coders will review areas of discrepancy until complete agreement is achieved on coded text; 3) Data reduction: We will summarize participant responses pertaining to each interview topic and describe variation in responses between individuals or among subgroups. We will work with the data related to each code to identify principal sub-themes that reflect finer distinctions in the data; 4) Data display: Matrices and tables that categorize and display data will be used to help facilitate comparisons (e.g. across viral load strata).

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

The investigators will obtain informed consent from each patient before starting any study procedures according to the standards set forth in the ICH Good Clinical Practice guidelines and per unit SOPs. The process will include reviewing consent forms with potential patients in a confidential setting and explaining all risks and benefits associated with participation of the study. This involves reading over the IRB-approved consent form with the patient in a private space, soliciting questions from the patient, allowing the patient ample time alone to review the form, soliciting questions again, and then offering the patient the opportunity to sign the consent form or provide verbal consent for follow-up IDI study visits using an IRB-approved verbal consent process. To ensure understanding, study staff will ask questions of the patients regarding study procedures. The consent forms will use language that is sufficiently simple for lay persons to comprehend. Patients will not be coerced into participating. Children under the age of 18 years, decisionally impaired adults and non-English speakers will not be enrolled in this study. Each patient will be provided with a photocopy of all documents that they sign. The informed consent process will cover all elements of informed consent required by research regulations. In addition, the process specifically will address the following topics of importance to this study:

- The unknown safety and unproven efficacy of the study interventions
- The importance of patients in both study groups to the success of the study
- The importance of adherence to the study visit and procedures schedule
- The potential medical risks of study participation (and what to do if such risks are experienced)
- The potential social harms associated with study participation (and what to do if such harms are experienced)
- The limited benefits of study participation
- The distinction between research and clinical care
- The right to withdraw from the study at any time

The informed consent process will include an assessment, through a series of questions, of each potential patient's understanding prior to enrollment and sequential assignment of concepts identified by the protocol team as essential to the informed consent decision. Patients who are not able to demonstrate adequate understanding of key concepts after exhaustive educational efforts will not be enrolled in the study.

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

One IRB-approved informed consent document will be reviewed with all patients on study. All patients will also review and sign a HIPAA form approved by the IRB. During the consent process, the patient participants will watch the short, IRB-approved video, as noted above, describing the MedViewer and what to expect.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Our informed consent process begins with the initial patient participant contact and provider participant contact and continues until study completion.

Most of our initial patient contact will be through phone contact, during which an IRB-approved phone screening questionnaire will be administered. If the patient agrees and passes the screening, they will provide basic demographic data needed to schedule their appointment in the clinical research management system (CRMS) and this is done under an IRB-approved limited HIPAA waiver. Full HIPAA and informed consent occur during the first patient visit.

Most of our initial provider contact will be through email, during which an IRB-approved email invitation to attend the provider training and participate in the study will be sent. If the provider is willing to participate, they will schedule their training with the research team and complete informed consent process prior to the training. Full informed consent and screening will occur prior to the provider training.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

The study may be discontinued at any time by the NIAID, site investigators, site IRBs, or other government or regulatory authorities. For any study that is prematurely terminated or temporarily suspended, the PI will promptly inform study patients, the IRB, and the sponsor providing the reason(s) for the termination or temporary hold.

When a study is prematurely terminated, refer to Section **7, Study Intervention Discontinuation and Participant Discontinuation/Withdrawal**, for handling of enrolled study participants.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients or to provider participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Confidentiality will be maintained by storing all specimens for current and future use with a unique identifying number, which will be linked to the subject's name, social security number, address, telephone number, and hospital medical record (MR) number. The principal investigators and study staff will be the only people with access to the identifying information. Any information provided to other people working on this study will be given with the study ID number, not other identifying information. The records will be secured in a locked file cabinet in a locked room in a badge access only office suite of the principal investigator.

All electronic data for this study will be stored on a dedicated University server which contains extensive protections and securities.

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed. Any blood sample specimens will be stored in the secure lab of the Principal Investigator at the address noted previously in this protocol. No data will be transmitted outside of study staff. All samples will be destroyed in according with Environmental Health and Safety policies and local SOPs one year after the results have been published. No samples will be stored long term.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Provide the name and contact information of the Principal Investigator and the Medical Monitor.

Principal Investigator	***Medical Officer***
Angela Kashuba, BScPhm, PharmD, DABCP, FCP, Professor	*Cynthia Gay, MPH, MD, Associate Professor*
Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy	*Division of Infectious Diseases, Department of Medicine UNC School of Medicine*
Address: CB# 7569, 3318 Kerr Hall, 310 Pharmacy Lane Chapel Hill NC, 27599-7569, USA	*Address: 130 Mason Farm Rd. (Bioinformatics), CB# 7030 Chapel Hill, NC 27599-7030, USA*
Phone Number: 919-966-9998	*Phone Number: 919-843-2726*
Email: akashuba@unc.edu	*Email: cynthia_gay@med.unc.edu*

10.1.6 SAFETY OVERSIGHT

A multi-tiered safety review process will be followed for the duration of this study. The study site investigators are the first layer of this tiered system and are responsible for the initial evaluation and reporting of safety information at the participant level, and for alerting the Protocol Safety Review Team (PSRT) if unexpected concerns arise. The PSRT will consist of the following study site investigators: Angela Kashuba, PharmD (Principle Investigator), Cindy Gay, MD (Medical Officer), Amanda Poliseno (Project Manager) along with the Biostatistician.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s):

- Onsite monitoring will occur at regular intervals. Comprehensive source monitoring will occur. Database monitoring will be targeted at 10% of data entry.

- Monitoring follow up clarifications will be completed within 14 business days as noted in our local SOPs.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Quality Control and Quality Assurance

The study site will conduct quality control and quality assurance procedures in accordance with Requirements for Clinical Quality Management Plans at DAIDS Funded and/or Supported Clinical Research Sites available at:
(http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/QMPPolicy.pdf).

Data Review
Upon completion of a study or study interval, all study related documentation should be incorporated in the Study Folder (Data Packet or Ancillary Records). The Study Folder may consist of folders, 3 ring binders, accordion files, or other filing system, as appropriate.
All analysts are responsible for ensuring the lab notebooks are completely filled according to SOP-0318 "Using CPAC Lab Notebooks".

The data packet will be reviewed and approved by the QC officer and Lab Director or Assistant Director to verify accuracy and completeness of the lab notebooks and data packets. Checklists have been generated that define specific items that require review by the QC officer as well as the Lab Director or Assistant Lab Director. Separate checklists are used for Tier 1 (Research), Tier 2 (Qualified Methods), and Tier 3 (Validated Methods/Proficiency Testing/Therapeutic Drug Monitoring) studies. Checklists are also included for the review of Validated Method SOPs (200 Series), Analytical Validation Reports (400 Series), and Qualified Method SOPs (700 Series).

Data Reporting

Manifest: Once the analysis is complete for a study, the results should be entered into the study Manifest. At a minimum, the Manifest should contain the following:

- Run ID
- Sample ID
- CPAC ID
- Species
- Matrix
- Time point
- Analyte
- Concentration
- Comments

Security: After the data packet has been reviewed and approved by the QC officer and Lab Director or Assistant Director, the manifest should be locked to prevent any data changes, and then may be released to the Sponsor.

Quality Assurance:

- All analyses must be reviewed and approved by the QC officer and Lab Director or Assistant Director prior to reporting results.
- All analysts responsible for performing any bioanalytical tests have been trained in all applicable procedures.
- Hardcopies of test results will be kept in CPAC for 2 years. Hardcopies will then be transferred to an archive facility and kept up to 7 years.
- Following completion of every run, the instrument data and result tables are copied to the secure network (M Drive) for storage. Backups are also performed nightly by the Information Technology department for the Department of Pharmacotherapy and Experimental Therapeutics.
- Training records are kept in the Annual Training and Continuing Education binder.

Archiving Procedures

Upon completion of a study or study interval, all study related documentation should be included in the study folder. A study folder should be created for each study and contain the following: Sample receipt records, sample processing forms, sample disposal records, analytical run records, and sample analysis report.

Conditions for archiving:

- A dedicated archive facility
- An individual responsible
- An index of the archive contents
- Records of entry and data examined
- Data should be logged into the archive within a reasonable period of time
- Appropriate data protection measures

Record Retention Policy: The following table lists the documents that should be retained at CPAC for the retention period of 7 years after the close-out date. Arrangements may be made with the Sponsor for storage or disposal after 7 years.

- Study specific data, chromatograms, reports
- Contracts and agreements
- Patient chart records

The following table lists the documents that should be retained at CPAC for up to 20 years.

- Bioanalytical Methods
- Standard Operating Procedures
- Analytical Validation Reports
- Lab notebooks
- Equipment calibration and maintenance records
- Proficiency testing records
- Audit inspection reports
- Employee training records

Study Coordination: All procedures, documentation, subject contact, data management, clinical monitoring, personnel training and regulatory requirements will be performed in accordance to policies governed by the University of North Carolina Clinical Pharmacology and Analytical Chemistry Laboratory. These policies are located in department and study specific SOPs.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

The study site will maintain source data/documents in accordance with Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (http://www3.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/PDF/Source DocPolicy.pdf).

The investigator will maintain, and store securely, complete, accurate and current study records throughout the study. The investigator will retain all study records for at least three years following the completion of the study, unless directed otherwise by the National Institutes of Health (NIH). Study records must be maintained on site for the entire period of study implementation.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 14 working days of identification of the protocol deviation, or within 14 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported annually to our local IRB and NIH. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers 1 year after the completion of the primary endpoint by contacting the lead contacts notated through ClinicalTrials.gov .

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.]

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. This is required by our local research offices at the onset of IRB submission and no less than annually. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

10.2 ADDITIONAL CONSIDERATIONS

N/A

10.3 ABBREVIATIONS

AE	Adverse Event
ACTG	AIDS Clinical Trials Group
ANOVA	Analysis of Variance
ART	Antiretroviral therapy
ARV	Antiretroviral
CAPI	Computer Assisted Personal Interviewing
CASI	Computer Assisted Self Interviewing
CD4	Cluster of Differentiation 4
CDC	Centers for Disease Control and Prevention
CFAR	Center for AIDS Research
CFR	Code of Federal Regulations
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CPAC	Clinical Pharmacology and Analytical Chemistry
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
CTRC	Clinical and Translational Research Center
DAIDS	Division of Acquired Immunodeficiency Syndrome i
EAE	Expedited Adverse Event
EDTA	Ethylene diamine tetraacetic acid
ENLIGHTEN	Establishing Novel AntiretroviraL Imaging for Hair to Elucidate Nonadherence
FAQ	Frequently Asked Questions
FDA	Food and Drug Administration
GC-MS	Gas Chromatography-Mass Spectrometry
GCP	Good Clinical Practice
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
ID	Infectious Disease
ID Number	Identification Number
IDI	In-Depth Interview
IGHID	Institute for Global health and Infectious Diseases
IR	Infra-red
IRB	Institutional Review Board
LC-MS/MS	Liquid Chromatography-Triple Quadruple
MALDESI	Matrix-Assisted Laser Desorption Electrospray Ionization

MO	Medical Officer
MOP	Manual of Procedures
MSI	Mass Spectrometry Imaging
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PBPK	Physiologically Based Pharmacokinetic
PI	Principal Investigator
PO	Program Officer
PrEP	Pre-Exposure Prophylaxis
PSRT	Protocol Safety Review Team
QA	Quality Assurance
QC	Quality Control
RA	Research Assistant
RNA	Ribonucleic Acid
RSC	Regulatory Support Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SNP	Single Nucleotide Polymorphisms
SOE	Schedule of Events
SOP	Standard Operating Procedure
TDM	Therapeutic Drug Monitoring
UNC	University of North Carolina at Chapel Hill
UP	Unanticipated Problem
US	United States
VL	Viral Load
WHO	World Health Organization

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
1.1	18Feb2020	Protocol language update	Improved clarity
2.0	20Aug2020	Incorporates LOA #1, changes to study procedures	Study procedures changed to remote operations due to COVID-19

11 REFERENCES

[1] Agot K, Taylor D, Corneli AL, Wang M, Ambia J, Kashuba ADM, et al. Accuracy of Self-Report and Pill-Count Measures of Adherence in the FEM-PrEP Clinical Trial: Implications for Future HIV-Prevention Trials. AIDS Behav 2015;19:743–51. doi:10.1007/s10461-014-0859-z.

[2] Baxi SM, Liu A, Bacchetti P, Mutua G, Sanders EJ, Kibengo FM, et al. Comparing the novel method of assessing PrEP adherence/exposure using hair samples to other pharmacologic and traditional measures. J Acquir Immune Defic Syndr 2015;68:13–20. doi:10.1097/QAI.0000000000000386.

[3] Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, Nair G, et al. Tenofovir-based preexposure prophylaxis for HIV infection among African women. N Engl J Med 2015;372:509–18. doi:10.1056/NEJMoa1402269.

[4] Haberer JE, Baeten JM, Campbell J, Wangisi J, Katabira E, Ronald A, et al. Adherence to antiretroviral prophylaxis for HIV prevention: a substudy cohort within a clinical trial of serodiscordant couples in East Africa. PLoS Med 2013;10:e1001511. doi:10.1371/journal.pmed.1001511.

[5] Rosen EP, Thompson CG, Bokhart MT, Prince HMA, Sykes C, Muddiman DC, et al. Analysis of Antiretrovirals in Single Hair Strands for Evaluation of Drug Adherence with Infrared-Matrix-Assisted Laser Desorption Electrospray Ionization Mass Spectrometry Imaging. Anal Chem 2016;88:1336–44. doi:10.1021/acs.analchem.5b03794.

[6] Pack AP, Golin CE, Hill LM, Carda-Auten J, Wallace DD, Cherkur S, et al. Patient and clinician perspectives on optimizing graphical displays of longitudinal medication adherence data. Patient Educ Couns 2019. doi:10.1016/j.pec.2018.12.029.

[7] Hill LM, Golin CE, Pack AP, Carda-Auten J, Wallace DD, Cherkur S, et al. Using real-time adherence feedback toenhance communication about adherence to antiretroviral therapy: Patient and clinicianperspectives. JANAC 2019.

[8] Springfield AC, Cartmell LW, Aufderheide AC, Buikstra J, Ho J. Cocaine and metabolites in the hair of ancient Peruvian coca leaf chewers. Forensic Sci Int 1993;63:269–75.

[9] Agius R, Kintz P, European Workplace Drug Testing Society. Guidelines for European workplace drug and alcohol testing in hair. Drug Test Anal 2010;2:367–76. doi:10.1002/dta.147.

[10] Jurado C, Sachs H. Proficiency test for the analysis of hair for drugs of abuse, organized by the Society of Hair Testing. Forensic Sci Int 2003;133:175–8.

[11] Appenzeller BMR, Tsatsakis AM. Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: state of the art, critical review and future needs. Toxicol Lett 2012;210:119–40. doi:10.1016/j.toxlet.2011.10.021.

[12] Hickey MD, Salmen CR, Tessler RA, Omollo D, Bacchetti P, Magerenge R, et al. Antiretroviral concentrations in small hair samples as a feasible marker of adherence in rural Kenya. J Acquir Immune Defic Syndr 2014;66:311–5. doi:10.1097/QAI.0000000000000154.

[13] Adams JL, Sykes C, Menezes P, Prince HMA, Patterson KB, Fransen K, et al. Tenofovir diphosphate and emtricitabine triphosphate concentrations in blood cells compared with isolated peripheral blood mononuclear cells: a new measure of antiretroviral adherence? J Acquir Immune Defic Syndr 2013;62:260–6. doi:10.1097/QAI.0b013e3182794723.

[14] Tests for drugs of abuse. Med Lett Drugs Ther 2002;44:71–3.

[15] Pergament D. It's Not Just Hair: Historical and Cultural Considerations for an Emerging Technology. Chicago-Kent Law Review 1999;75.

[16] Barry JA, Robichaud G, Bokhart MT, Thompson C, Sykes C, Kashuba ADM, et al. Mapping antiretroviral drugs in tissue by IR-MALDESI MSI coupled to the Q Exactive and comparison with LC-MS/MS SRM assay. J Am Soc Mass Spectrom 2014;25:2038–47. doi:10.1007/s13361-014-0884-1.

[17] Bokhart MT, Rosen E, Thompson C, Sykes C, Kashuba ADM, Muddiman DC. Quantitative mass spectrometry imaging of emtricitabine in cervical tissue model using infrared matrix-assisted laser desorption electrospray ionization. Anal Bioanal Chem 2015;407:2073–84. doi:10.1007/s00216-014-8220-y.

[18] Thompson CG, Bokhart MT, Sykes C, Adamson L, Fedoriw Y, Luciw PA, et al. Mass spectrometry imaging reveals heterogeneous efavirenz distribution within putative HIV reservoirs. Antimicrob Agents Chemother 2015;59:2944–8. doi:10.1128/AAC.04952-14.

[19] Vogliardi S, Tucci M, Stocchero G, Ferrara SD, Favretto D. Sample preparation methods for determination of drugs of abuse in hair samples: A review. Anal Chim Acta 2015;857:1–27. doi:10.1016/j.aca.2014.06.053.

[20] Olds PK, Kiwanuka JP, Nansera D, Huang Y, Bacchetti P, Jin C, et al. Assessment of HIV antiretroviral therapy adherence by measuring drug concentrations in hair among children in rural Uganda. AIDS Care 2015;27:327–32. doi:10.1080/09540121.2014.983452.

[21] Coetzee B, Kagee A, Tomlinson M, Warnich L, Ikediobi O. Reactions, beliefs and concerns associated with providing hair specimens for medical research among a South African sample: a qualitative approach. Future Virol 2012;7:1135–42. doi:10.2217/fvl.12.100.

[22] Barfod TS, Hecht FM, Rubow C, Gerstoft J. Physicians' communication with patients about adherence to HIV medication in San Francisco and Copenhagen: a qualitative study using Grounded Theory. BMC Health Serv Res 2006;6:154. doi:10.1186/1472-6963-6-154.

[23] Fehringer J, Bastos FI, Massard E, Maia L, Pilotto JH, Kerrigan D. Supporting adherence to highly

active antiretroviral therapy and protected sex among people living with HIV/AIDS: the role of patient-provider communication in Rio de Janeiro, Brazil. AIDS Patient Care STDS 2006;20:637–48. doi:10.1089/apc.2006.20.637.

[24] Golin CE, Smith SR, Reif S. Adherence counseling practices of generalist and specialist physicians caring for people living with HIV/AIDS in North Carolina. J Gen Intern Med 2004;19:16–27.

[25] Krummenacher I, Cavassini M, Bugnon O, Schneider MP. An interdisciplinary HIV-adherence program combining motivational interviewing and electronic antiretroviral drug monitoring. AIDS Care 2011;23:550–61. doi:10.1080/09540121.2010.525613.

[26] Nachega JB, Morroni C, Zuniga JM, Schechter M, Rockstroh J, Solomon S, et al. HIV treatment adherence, patient health literacy, and health care provider-patient communication: results from the 2010 AIDS Treatment for Life International Survey. J Int Assoc Physicians AIDS Care (Chic) 2012;11:128–33. doi:10.1177/1545109712437244.

[27] Reif S, Smith SR, Golin CE. Medication adherence practices of HIV/AIDS case managers: a statewide survey in North Carolina. AIDS Patient Care STDS 2003;17:471–81. doi:10.1089/108729103322395500.

[28] Smith SR, Golin CE, Reif S. Influence of time stress and other variables on counseling by pharmacists about antiretroviral medications. Am J Health Syst Pharm 2004;61:1120–9.

[29] Valverde E, Beer L, Johnson C, Blair JM, Mattson CL, Sanders C, et al. Prevention counseling practices of HIV care providers with patients new to HIV medical care: medical monitoring project provider survey, 2009. J Int Assoc Provid AIDS Care 2014;13:127–34. doi:10.1177/2325957413516496.

[30] Wilson IB, Laws MB, Safren SA, Lee Y, Lu M, Coady W, et al. Provider-focused intervention increases adherence-related dialogue but does not improve antiretroviral therapy adherence in persons with HIV. J Acquir Immune Defic Syndr 2010;53:338–47. doi:10.1097/QAI.0b013e3181c7a245.

[31] Amico KR. Standard of Care for Antiretroviral Therapy Adherence and Retention in Care from the Perspective of Care Providers Attending the 5th International Conference on HIV Treatment Adherence. J Int Assoc Physicians AIDS Care (Chic) 2011;10:291–6. doi:10.1177/1545109711406734.

[32] Amico KR, Mugavero M, Smith L, Crane H, Quinlivan E, Roytburd K, et al. A brief provider survey to characterize retention and adherence standard of care support for patients in HIV care 2014.

[33] Thompson MA, Mugavero MJ, Amico KR, Cargill VA, Chang LW, Gross R, et al. Guidelines for improving entry into and retention in care and antiretroviral adherence for persons with HIV: evidence-based recommendations from an International Association of Physicians in AIDS Care panel. Ann Intern Med 2012;156:817–33, W. doi:10.7326/0003-4819-156-11-201206050-00419.

[34] Bachman Desilva M, Gifford AL, Keyi X, Li Z, Feng C, Brooks M, et al. Feasibility and Acceptability of a Real-Time Adherence Device among HIV-Positive IDU Patients in China. AIDS Res Treat 2013;2013:957862. doi:10.1155/2013/957862.

[35] Finocchario-Kessler S, Catley D, Thomson D, Bradley-Ewing A, Berkley-Patton J, Goggin K. Patient communication tools to enhance ART adherence counseling in low and high resource settings. Patient Educ Couns 2012;89:163–70. doi:10.1016/j.pec.2012.03.020.

[36] Golin CE, Earp J, Tien H-C, Stewart P, Porter C, Howie L. A 2-arm, randomized, controlled trial of a motivational interviewing-based intervention to improve adherence to antiretroviral therapy (ART) among patients failing or initiating ART. J Acquir Immune Defic Syndr 2006;42:42–51. doi:10.1097/01.qai.0000219771.97303.0a.

[37] Kohnert K-D, Heinke P, Vogt L, Salzsieder E. Utility of different glycemic control metrics for optimizing management of diabetes. World J Diabetes 2015;6:17–29. doi:10.4239/wjd.v6.i1.17.

[38] Mistry N, Keepanasseril A, Wilczynski NL, Nieuwlaat R, Ravall M, Haynes RB, et al. Technology-mediated interventions for enhancing medication adherence. J Am Med Inform Assoc 2015;22:e177-93. doi:10.1093/jamia/ocu047.

[39] Reich WA. Medication adherence feedback intervention predicts improved human immunodeficiency virus clinical markers. Int J Nurs Pract 2013;19:577–83. doi:10.1111/ijn.12100.

[40] Tanenbaum ML, Leventhal H, Breland JY, Yu J, Walker EA, Gonzalez JS. Successful self-management among non-insulin-treated adults with type 2 diabetes: A self-regulation perspective. Diabet Med 2015;32:1504–12. doi:10.1111/dme.12745.

[41] Golin CE, Groves J, Carda-Auten J, Gould M, White B, Pence B, et al. Accessing and Adhering to Medical Care after Prison Release: IndividualsMotivating to Participate in Adherence, Care and Treatment (imPACT) Trial 2013.

[42] Golin CE, Davis RA, Przybyla SM, Fowler B, Parker S, Earp JA, et al. SafeTalk, a multicomponent, motivational interviewing-based, safer sex counseling program for people living with HIV/AIDS: a qualitative assessment of patients' views. AIDS Patient Care STDS 2010;24:237–45. doi:10.1089/apc.2009.0252.

[43] Golin CE, Earp JA, Grodensky CA, Patel SN, Suchindran C, Parikh M, et al. Longitudinal effects of SafeTalk, a motivational interviewing-based program to improve safer sex practices among people living with HIV/AIDS. AIDS Behav 2012;16:1182–91. doi:10.1007/s10461-011-0025-9.

[44] Golin CE, Patel S, Tiller K, Quinlivan EB, Grodensky CA, Boland M. Start Talking About Risks: development of a Motivational Interviewing-based safer sex program for people living with HIV. AIDS Behav 2007;11:S72-83. doi:10.1007/s10461-007-9256-1.

[45] Gardner LI, Marks G, O'Daniels CM, Wilson TE, Golin C, Wright J, et al. Implementation and

evaluation of a clinic-based behavioral intervention: positive steps for patients with HIV. AIDS Patient Care STDS 2008;22:627–35. doi:10.1089/apc.2007.0210.

[46] Lewis CL, Golin CE, DeLeon C, Griffith JM, Ivey J, Trevena L, et al. A targeted decision aid for the elderly to decide whether to undergo colorectal cancer screening: development and results of an uncontrolled trial. BMC Med Inform Decis Mak 2010;10:54. doi:10.1186/1472-6947-10-54.

[47] Patel SN, Golin CE, Marks G, Grodensky CA, Earp JA, Zeveloff A, et al. Delivery of an HIV prevention counseling program in an infectious diseases clinic: implementation process and lessons learned. AIDS Patient Care STDS 2009;23:433–41. doi:10.1089/apc.2008.0189.

[48] Sheridan SL, Golin C, Bunton A, Lykes JB, Schwartz B, McCormack L, et al. Shared decision making for prostate cancer screening: the results of a combined analysis of two practice-based randomized controlled trials. BMC Med Inform Decis Mak 2012;12:130. doi:10.1186/1472-6947-12-130.

[49] Thrun M, Cook PF, Bradley-Springer LA, Gardner L, Marks G, Wright J, et al. Improved prevention counseling by HIV care providers in a multisite, clinic-based intervention: Positive STEPs. AIDS Educ Prev 2009;21:55–66. doi:10.1521/aeap.2009.21.1.55.

[50] Proctor E, Silmere H, Raghavan R, Hovmand P, Aarons G, Bunger A, et al. Outcomes for implementation research: conceptual distinctions, measurement challenges, and research agenda. Adm Policy Ment Health 2011;38:65–76. doi:10.1007/s10488-010-0319-7.

[51] Fisher JD, Fisher WA, Misovich SJ, Kimble DL, Malloy TE. Changing AIDS risk behavior: effects of an intervention emphasizing AIDS risk reduction information, motivation, and behavioral skills in a college student population. Health Psychol 1996;15:114–23. doi:10.1037/0278-6133.15.2.114.

[52] Jonas DE, Russell LB, Chou J, Pignone M. Willingness-to-pay to avoid the time spent and discomfort associated with screening colonoscopy. Health Econ 2010;19:1193–211. doi:10.1002/hec.1545.

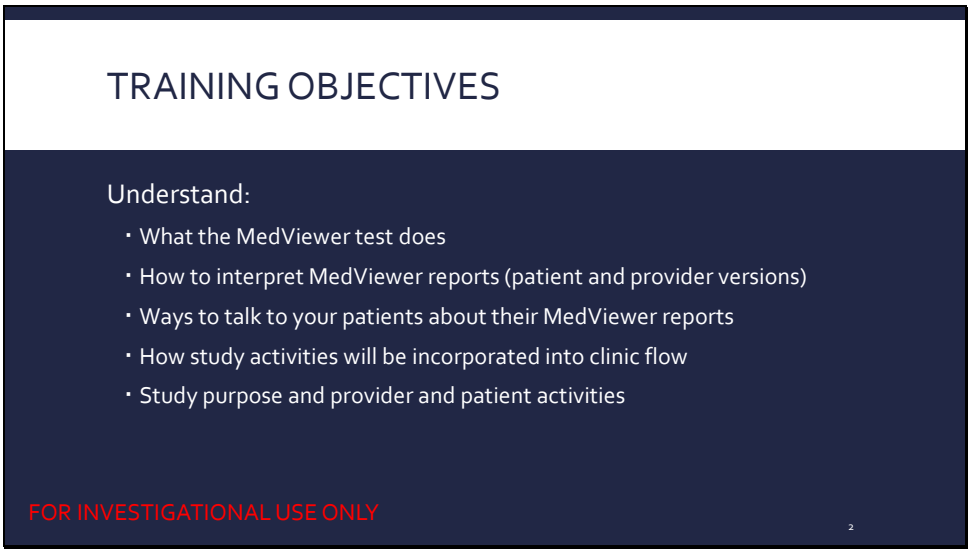
[53] Grutters JPC, Anteunis LJC, Chenault MN, Joore MA. Willingness to pay for a hearing aid: comparing the payment scale and open-ended question. J Eval Clin Pract 2009;15:91–6. doi:10.1111/j.1365-2753.2008.00959.x.

[54] Gilliland WM, et al. MDeveloping IR-MALDESI Mass Spectrometry Imaging of HIV Medications in Hair as a Clinical Tool for Measuring Patient Adherence. Abstract 294793, TP279. Annual Meeting of the American Society for Mass Spectrometry, San Diego, CA, 2018.

APPENDICES

APPENDIX A. INTERVENTION MATERIALS

A.1 PROVIDER TRAINING MATERIALS



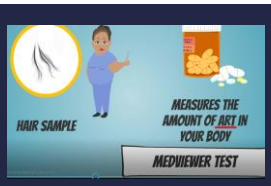
WHAT IS MEDVIEWER?

NOVEL APPROACH to quantify daily, longitudinal ART drug concentrations in hair

- Infra-red (IR) matrix-assisted laser desorption electrospray ionization (MALDESI) technology for mass spectrometry imaging (MSI)
- 5 strands scalp hair ≥ 1cm in length

CONCRETE adherence feedback

- Results show days of likely missed ART doses over past several weeks
- Can be used by patients and providers *together* to identify possible patterns of missed doses of ART



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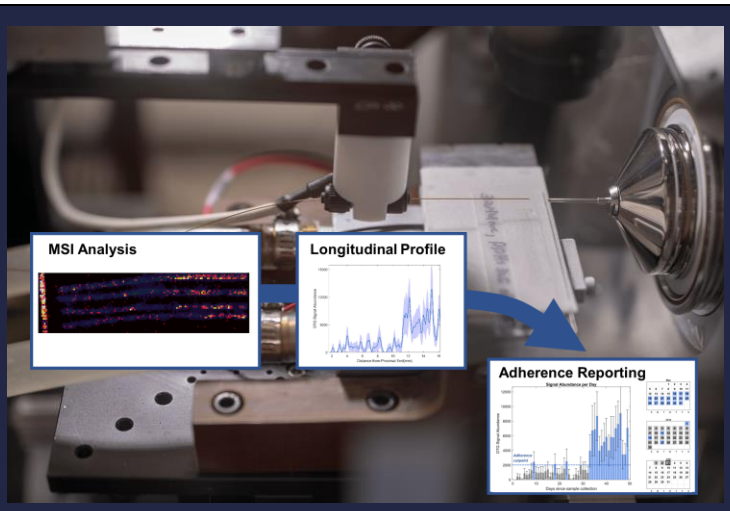

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ENLIGHTEN STUDY AIMS

1. Develop mass spectrometry technology (MedViewer) for ART drugs
2. Develop and validate mathematical benchmarks for IR-MALDESI MSI adherence monitoring
3. a. Formative study (2016-2018) informed MedViewer video and report development

3. **b. Feasibility Pilot: In the setting of real-time clinical monitoring, investigate:**
   - **Feasibility**
   - **Acceptability**
   - **Appropriateness**

   … of using MedViewer to provide feedback on longitudinal medication adherence patterns

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PILOT STUDY DURATION

January 2020 – [Month] 2021

- **January 2020:** initiate provider recruitment
- **January 2020- [Month] 2021:** visits with enrolled patients (~2-3 per provider)
- **[Month] 2021 – [Month] 2021:** ~5-minute endline questionnaire (online) and in-depth interview (if not already completed)

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PATIENT PARTICIPANTS

Patient participants (n=50)

- Age ≥ 18 and HIV-positive
- Has a scheduled appointment with a provider enrolled in study
- Has documentation of HIV viral loads over the last 2 years
- Has been a UNC ID patient for ≥ 90 days
- Attended ≥ 1 clinic visit in last year
- **On Dolutegravir or Emtricitabine ≥ 90 days**
- **≥ 1 cm hair not treated in last 4 weeks**

Low VL (n=25) (undetectable*)	High VL (n=25) (detectable**)	
< 40 c/mL	40 to <1000 c/mL	≥1000 c/mL

*all HIV RNA results below the limit of viral quantification over the previous 2 years
**at least one HIV RNA above the limit of viral quantification within previous 2 years

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PATIENT INFORMATIONAL VIDEO

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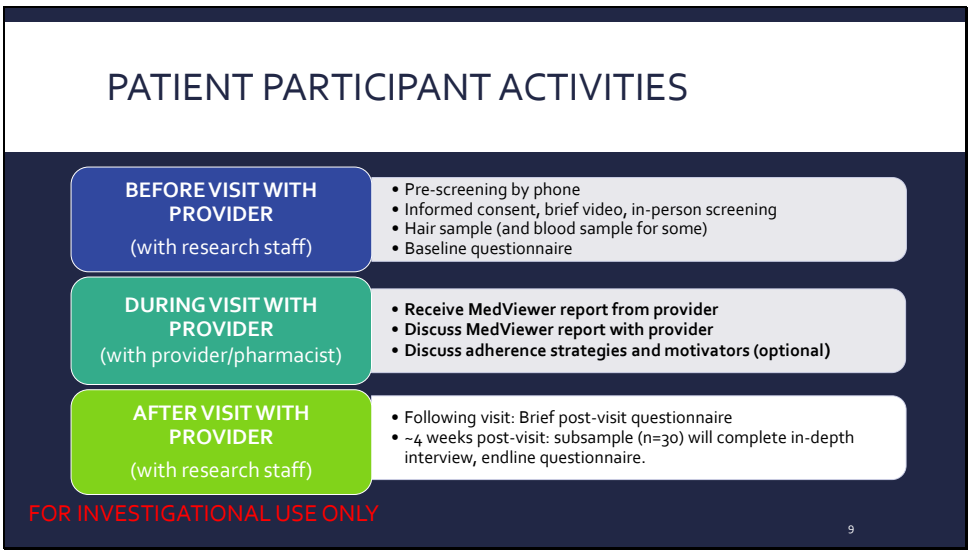
PATIENT PARTICIPANT ACTIVITIES
BEFORE VISIT WITH PROVIDER
(with research staff)
• Pre-screening by phone
• Informed consent, brief video, in-person screening
• Hair sample (and blood sample for some)
• Baseline questionnaire
DURING VISIT WITH PROVIDER
(with provider/pharmacist)
• Receive MedViewer report from provider
• Discuss MedViewer report with provider
• Discuss adherence strategies and motivators (optional)
AFTER VISIT WITH PROVIDER
(with research staff)
• Following visit: Brief post-visit questionnaire
• ~4 weeks post-visit: subsample (n=30) will complete in-depth interview, endline questionnaire.
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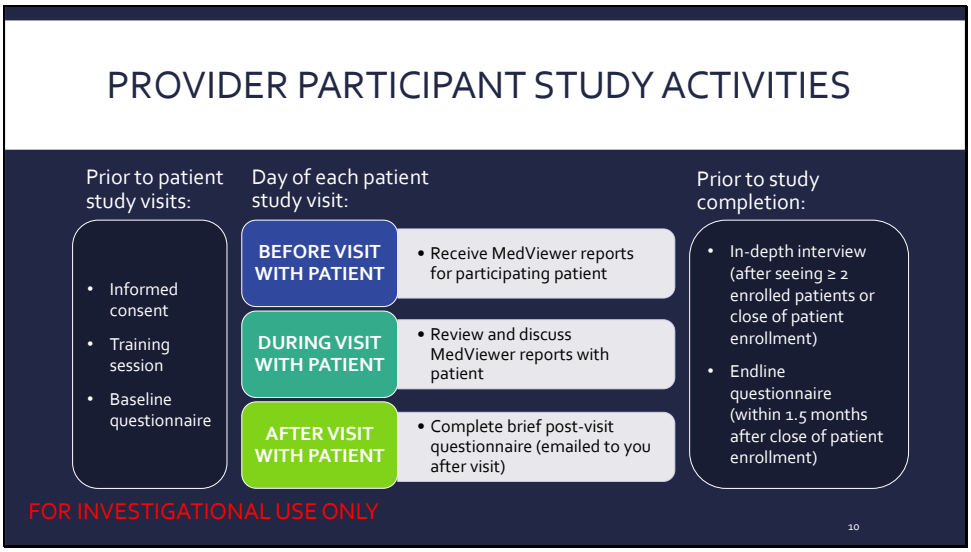
PROVIDER PARTICIPANT STUDY ACTIVITIES
Prior to patient study visits:
• Informed consent
• Training session
• Baseline questionnaire
Day of each patient study visit:
BEFORE VISIT WITH PATIENT
• Receive MedViewer reports for participating patient
DURING VISIT WITH PATIENT
• Review and discuss MedViewer reports with patient
AFTER VISIT WITH PATIENT
• Complete brief post-visit questionnaire (emailed to you after visit)
Prior to study completion:
• In-depth interview (after seeing ≥ 2 enrolled patients or close of patient enrollment)
• Endline questionnaire (within 1.5 months after close of patient enrollment)
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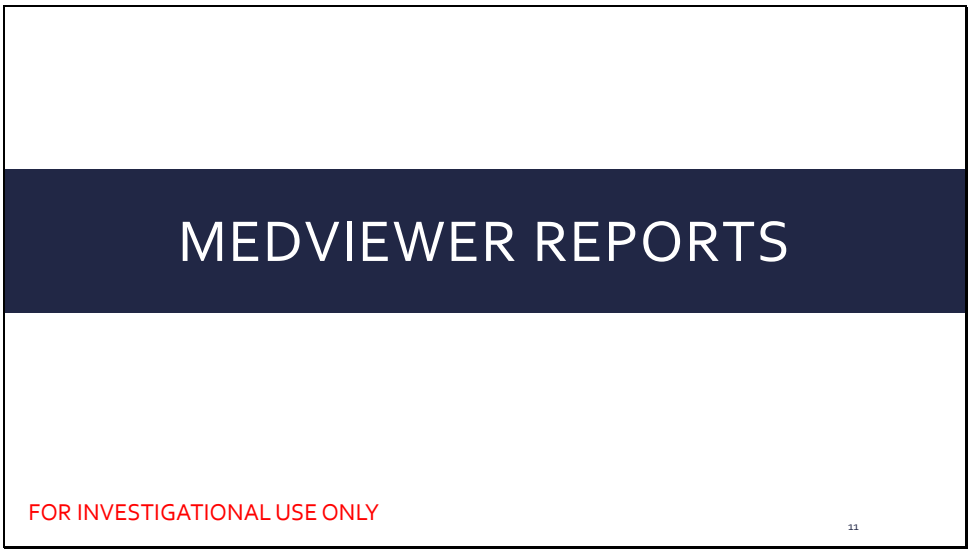
MEDVIEWER REPORTS
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11

Calendar report:

- Recommended for use by patients
- Days with likely missed doses indicated

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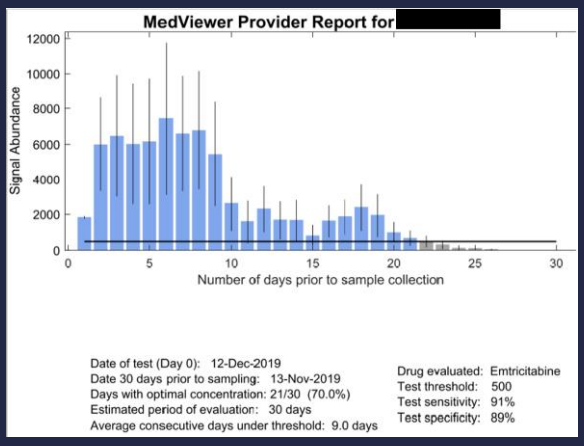

Bar-chart report:

- Recommended for use by providers
- Shows daily ART drug levels
- Contains information about specificity and sensitivity of test to detect missed doses of ART

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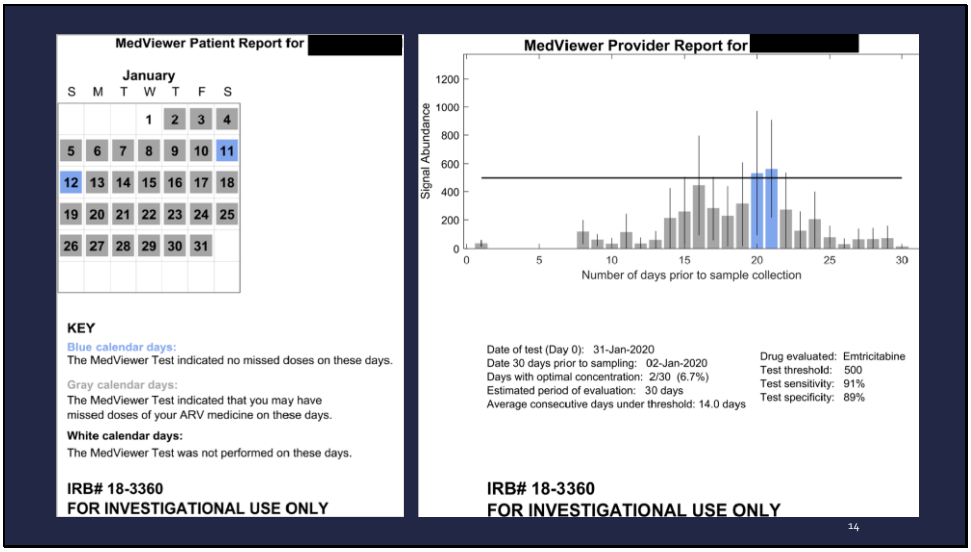

COMMUNICATION AID AND FAQ

- One-page reference sheet with possible strategies to discuss MedViewer results and ART adherence with patients (see attachment)
- One-page FAQ sheet to clarify potential questions or concerns about MedViewer and the ENLIGHTEN study (see attachment)

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COMMUNICATION AID: INTERPRETING MEDVIEWER REPORTS

- Assay still in development, so no clinical decisions should be made on the basis of the report
- Interpret reports in collaboration with your patient
- Consider the limitations of the report:
  - Designed to under- rather than over-report likely missed doses
  - Precision bars represent variability across a patient's 5 hair strands
  - Days in the reports might not represent exact dates of successful and missed doses. Rather, the dates indicated can provide an approximation to help identify patterns of missed doses

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FAQ: ACCURACY OF MEDVIEWER REPORTS

Test sensitivity and specificity

- MedViewer ROC curves based on evaluation of DTG and FTC in hair strands collected after DOT
- Adherence cut-off values of MedViewer signal abundance:
  - Maximize likelihood that a daily concentration is not misclassified as a missed dose (specificity)
  - Still achieves reasonable likelihood of identifying missed doses (sensitivity)
- Sensitivity and specificity values included on provider report

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IF DELAYS OCCUR WITH REPORT

If MedViewer reports are not ready before the patient's scheduled appointment with their provider:

- A research staff member will notify the provider before the appointment
- The provider will determine, with patient input, whether to:
  - Delay the scheduled visit until later that day when the MedViewer report is ready OR
  - Schedule a separate visit to review the report with the patient within 4 weeks OR
  - Request that the UNC ID Clinic HIV pharmacist discuss the reports with the patient within 4 weeks

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POST-VISIT PROVIDER SURVEY

5 Questions:

1. Did you show your patient their MedViewer report during the visit? (If not→ Reasons)
2. How much time did you spend discussing the report with your patient? (If none→ Reasons)
3. How useful would you say that the MedViewer report was for helping you understand this patient's ART adherence?
4. How useful would you say it was for helping you have a productive conversation with this patient about their ART adherence?
5. How well would you say your patient understood the information in their MedViewer report?

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WHAT'S NEXT FOR YOU?

Study activities	Research team will…
Baseline questionnaire	send you a link via email following this training
Review MedViewer report with patient(s) during routine clinic visit	deliver the MedViewer report to you before each patient's scheduled visit (either in-person or via secure email)
Post-visit questionnaire(s)	send you a link via email after each patient MedViewer visit
In-depth interview	contact you to schedule the interview
Endline questionnaire	send you a link via email before study close

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QUESTIONS?

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BASELINE QUESTIONNAIRE

Please complete your questionnaire as soon as possible.

You will receive an email with the link.

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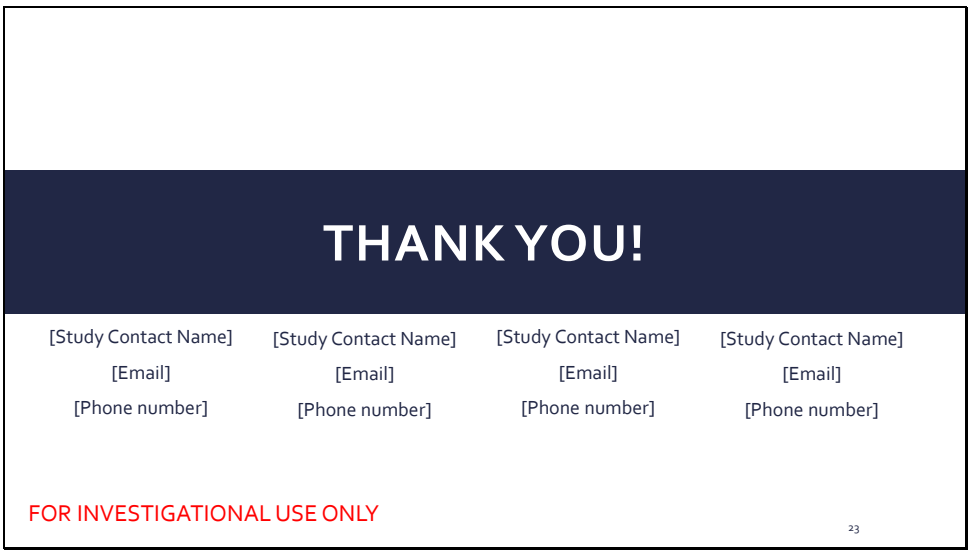
THANK YOU!
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FOR INVESTIGATIONAL USE ONLY
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A.2 PATIENT VIDEO STORYBOARD

Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)
MedViewer Patient Education Video Storyboard

Notes about video:

- The video will be approximately 8 minutes in length and include a diverse group of real and animated individuals in terms of race/ ethnicity and age.
- Closed captioning will be available for participants who feel they would benefit from that.
- The video will open with the UNC Center for AIDS Research logo, followed by a title.
- The video will include the following disclaimer information:
    - **Text to appear on screen after the title screen and before Frame 1:** "The MedViewer test described in this video is for investigational use only. No clinical decisions will be made using MedViewer results."
    - **Voiceover to accompany text on screen**: "The MedViewer test described in this video is for investigational use only. By this we mean that the MedViewer test is still being developed and studied. Therefore, medical providers will not make any clinical decisions based on MedViewer results."
    - **Text to be added to the top of MedViewer report shown in the video and used in the study:** "For investigational use only"
    - **Text to be written on final screen after Frame 8:** "The MedViewer test described in this video is for investigational use only. No clinical decisions will be made using MedViewer results."
    - **Small watermark to go in bottom left corner of the screen throughout the entire video:** "For investigational use only"
- Participants will watch the video on tablets with headphones in a private room of the CTRC before signing the informed consent form to participate in the study.
- The consent form will provide instructions to show the video during the section titled: "What will happen to me if I participate in this research?"

FRAME 1: Purpose of video

VIDEO: FILMED IN THE ID CLINIC	AUDIO SCRIPT	CAST
• Show both Provider #1 and Patient #1 standing together inside the ID Clinic. • Then, Provider #1 and Patient #1 will alternate speaking directly to the camera. • The tone should be natural and welcoming.	• Hi! I'm <Patient #1> and I come to the infectious disease clinic for care and management of my HIV. (Patient #1) • And I'm <Provider #1>. I work here in the infectious disease clinic. We have some exciting information for you. (Provider #1) • You and I already know that to stay healthy while living with HIV, we need to take antiretroviral medicine (or ART) every day. Now, researchers at the University of North Carolina have developed a test called MedViewer that can measure the amount of ART in our bodies over time. The test can help us, and our health care providers, know if we are getting enough ART to keep staying healthy. (Patient #1) • We'd like to tell you a little bit more about this. We hope that by the end of this video, you'll understand the MedViewer test and how it can improve how you and your provider manage your HIV. (Provider #1)	• Provider #1 • Patient #1

FRAME 2: How ARVs are processed in the body and end up in the hair

VIDEO **MOSTLY ANIMATION WITH NARRATION AND SOME FILMING IN THE ID CLINIC**	**AUDIO SCRIPT**	**CAST**
• Show a short shot of Patient #1 saying the first line. Patient #1 will then narrate the rest of the frame – which will be animated. • A text slide will indicate this frame is covering: "How ART is processed in the body and ends up in the hair". Music will accompany it. • Animation will show a patient ingesting ART pills and those pills being absorbed in body, ending in hair. Accompanying text will point out key points.	• To begin, let's look at what happens when you take your medicine. • When you swallow a pill, like ART, it travels to your stomach, where it dissolves into tiny particles. • These particles continue into your intestines, and then cross into your bloodstream. Your blood carries the medicine all over your body, helping to fight the HIV virus. • Small amounts of medicine even travel into your hair. • The new MedViewer test will measure the amount of ART in your hair and this represents how much ART is in your body.	• Patient #1

FRAME 3: Introduction to test, purpose of test, and what test is measuring

VIDEO **MIX OF FILM AND ANIMATION: FILMING IN THE ID CLINIC**	**AUDIO SCRIPT**	**CAST**

• Begin with Provider #1 speaking directly to the camera for the first bullet.
• Patient #1 will then speak directly to the camera for the second bullet.
• Patient #1 will then narrate while animated images pop up to show comparisons between the VL and MedViewer tests (the sub-bullets).
  ○ Comparison 1: virus vs. ART
  ▪ Show a vile of blood on the left hand side of the screen. Next to it, show an animated image for a virus. Use text to highlight the fact that the VL test measures the amount of VIRUS in the body.
  ▪ Next, show a sample of 4 hairs and a provider with tweezers on the left of the screen. Next to it (on the right) show a bottle of medication/ pills. Use text to highlight the fact that the MedViewer test measures the amount of ART in the body.
  ○ Comparison 2: single vs. longitudinal measure
  ▪ On the left show someone getting their blood drawn and then show a calendar with Viral Load on ONE day. Viral Load should be written on the bottom right of the frame.
  ▪ Then, show someone on the left and an image of a full calendar representing the fact that MedViewer can detect many time points. Show a person with short hair and a couple of weeks highlighted on the calendar. Then, show a person with long hair and a whole month highlighted. MedViewer should be written on the bottom right of these frames.
  ○ Length of hair: Show a video image of Patient #1 stating that you must have 1cm of hair. Show a ruler and a pea to provide a visual of the length.
	• Health care providers, like me, want to make sure your body is getting enough ART to fight HIV. The MedViewer test, along with your HIV viral load, will help you and your doctor manage your HIV better than ever before. (Provider #1)
	• Because you may be familiar with the viral load test already, I'll tell you how MedViewer is different. (Patient #1)
	  ○ First, the viral load test uses a BLOOD sample to measure the amount of <u>VIRUS</u> in your body. MedViewer uses a HAIR sample to measure the amount of <u>ART</u> in your body.
	  ○ Second, the viral load test only shows the amount of virus at ONE point in time – when you provided the sample. But, MedViewer shows the amount of ART in your body at MANY points in time.
	  ▪ If you have very short hair, MedViewer will show the past couple of WEEKS; if you have long hair it will show a MONTH.
	• But, to be able to use MedViewer, you have to have scalp hair that's at least 1cm long. That's about the width of a pea. (Patient #1)
		• Provider #1
		• Patient #1

FRAME 4: Hair sample collection process

VIDEO **ALL FILMED: IN THE ID CLINIC**	**AUDIO SCRIPT**	**CAST**
• Patient #1 will continue to do all the narration here. The first shot should show him talking to the camera in front of a closed clinic room door. • Next, show a health provider (Provider #2) talking to a patient (Patient #2) while Patient #1 narrates. Then, show Provider #2 picking up tweezers. ○ The camera should see Patient #2 appearing calm and interested – and agreeing to having her hair plucked. • The camera will then show Provider #2 selecting 4 strands of hair to pluck from the back of Patient #2's head. The camera will remain zoomed in until the provider plucks the hair – all five strands at one time. • The next shot will be a close-up of the provider securely attaching the plucked hair to foil with an ID label that has a unique ID# printed on it. The label will also indicate the distal and scalp ends of the hair for the lab. • The last shot will be a close-up of Provider #2 folding the foil and placing the foil-wrapped hair into a secure container.	• Let's see a close-up of how this new test works! • A trained staff member will select hair from the back of your head and use clean tweezers to gently pluck it. He or she will pluck about five strands. • Once the hair is collected, the staff member will attach the hair to a foil sheet using a sticky label with a unique ID number. Your name or any other identifying information about you, will *NOT* be on the label. • The staff member will fold the foil and place it in a secured container.	• Patient #1 • Provider #2 • Patient #2

FRAME 5: Lifespan of hair sample collection, test, sample disposal, and results

VIDEO **ALL ANIMATION WITH NARRATION**	**AUDIO SCRIPT**	**CAST**

• To introduce this frame, a slide will ask "What happens next?" and music will accompany it.
• Patient #1 will continue to do the narration for this frame.
• For each bullet point, animation will show:
  ○ Sample carried/transported into the UNC hospital
  ▪ Image of researcher walking to lab with a sealed container and sample being received by the analyst
  ○ Sample being examined in the lab
  ▪ Image of analyst (in a lab coat) putting hair into a representation of the MedViewer machine
  ○ Sample being destroyed
  ▪ Image of hair sample being incinerated
  ○ 2 hours
  ▪ An animated view of the reception/ waiting room and a clock counting 2 hours
  ○ Results sent by secure email to the ID Clinic
  ▪ Image of analyst and results, and then a secure email being sent and received (we see research staff member in the clinic delivering a confidential folder to a provider)
  ○ Provider discussing results with patient
  ▪ Image of patient and provider talking across from each other
  ○ Provider and patient talking amicably together in a clinic room
	• Then, the trained staff member will carry it to the UNC testing lab, and deliver it to the analyst.
	• The analyst will place the hair in the equipment to run the test.
	• Once the test is done, the analyst will permanently dispose of the hair.
	• Your provider will then review your results with you.
		• Patient #1

FRAME 6A [BAR GRAPH RESULTS OPTION]: How provider will review results with the patient and how results will be used

VIDEO **MIX OF FILM AND ANIMATION: FILM IN THE ID CLINIC**	**AUDIO SCRIPT**	**CAST**
• The first shot should be a quick image of Patient #2 and Provider #3 sitting down talking together in a clinic room, talking about the MedViewer results. They should be referring to and looking at a copy of the results so it's clear what they are discussing. • Then, show Patient #2 speaking directly into the camera for the first bullet. • The second bullet point, should show the results with animation highlighting specific features of the report that correspond to what the patient is discussing. • The last bullet should show Patient #2 and Provider #3 discussing the results in a clinic room.	• Hi! I'm <name> and I'm a patient as well. My provider and I just went over my MedViewer results, and I would like to share them with you. (Show patient) • The results look like this. These are all the days that the MedViewer detected and measured the amount of medicine in my body. Over here is a key that explains what we're looking at. The blue areas show the days when I had enough ART in my body. But there are also some areas that are white and blank. These are days I did not have enough medicine in my body. (Show bar graph version of results)	• Patient #2 • Provider #3

FRAME 6B [CALENDAR RESULTS OPTION]: How provider will review results with the patient and how results will be used

VIDEO **MIX OF FILM AND ANIMATION: FILM IN THE ID CLINIC**	**AUDIO SCRIPT**	**CAST**
• The first shot should be a quick image of Patient #2 and Provider #3 sitting down talking together in a clinic room, talking about the MedViewer results. They should be referring to and looking at a copy of the results so it's clear what they are discussing. • Then, show Patient #2 speaking directly into the camera for the first bullet. • The second bullet point, should show the results with animation highlighting specific features of the report that correspond to what the patient is discussing. • The last bullet should show Patient #2 and Provider #3 discussing the results in a clinic room.	• Hi! I'm <name> and I'm a patient as well. My provider and I just went over my MedViewer results, and I would like to share them with you. (Show patient) • The results look like this. These are all the days that the MedViewer detected and measured the amount of medicine in my body. Over here is a key that explains what we're looking at. The blue areas show the days when I had enough ART in my body. But, there are also some areas that are white and blank. These are days I did not have enough medicine in my body. (Show calendar graph version of results)	• Patient #2 • Provider #3

FRAME 7: Addressing potential concerns

VIDEO **MIX OF FILM AND ANIMATION: FILM IN THE ID CLINIC OR ELSEWHERE**	**AUDIO SCRIPT**	**CAST**

• First shot should be of Patient #1 in the reception area of the ID Clinic.
• Patient #1 will do the narration for the full frame, except the questions.
• The questions will be asked by diverse, animated, individuals. For each question, show an animated individual on the screen.
• For each answer, show images previously shown in the video:
  • 1) Hair being plucked while a patient smiles/ seemingly not bothered
  • 2) The bar or calendar results graphs previously used in the video – which animated highlights showing days HIV medicine was detected.
  • 4) The research member receiving a secure email, printing results, putting them into a folder labelled "Confidential", and delivering them to a provider
  • The last shot will show the ID number from the results.
	• So now that you know what MedViewer is and what it does, we want to address some questions you may have. (Patient #1)
	1) Does it hurt? (Animated individual)
	  • No! It doesn't. A clinically trained staff member will pluck several strands of hair. You may feel a brief pinch from the plucking, but it's not painful. (Patient #1)
	2) What information about me will this test collect? (Animated individual)
	  • MedViewer will only measure and report information on HIV medicines in your body. (Patient #1)
	3) Should I be concerned about confidentiality and privacy? (Animated individual)
	  • The research team will do everything possible to protect your confidentiality and privacy. The staff member and your provider are the only people who will see both your results and your name – everyone else will only see your unique ID number. (Patient #1)
		• Patient #1

FRAME 8: Summary of information covered

VIDEO **MIX OF FILM AND ANIMATION: FILM IN ID CLINIC OR ELSEWHERE**	**AUDIO SCRIPT**	**CAST**

• The first shot will be of Patient #1 in the clinic – just for the first bullet.
• Patient #1 will then narrate the rest of the bullets.
  • Subsequent shots should reuse images previously seen in the video.
  ▪ Image of hair strands and tweezers with text indicating MedViewer measures ART in the body – with ART underlined
  ▪ Image of Provider #2 plucking hair from Patient #2 who is smiling/ relaxed
  ▪ Image of hair being taken to the lab for testing
  ▪ Image of hair being incinerated
  ▪ Image of results being securely delivered to the provider
  ▪ Image of two hours on a clock
  ▪ Image of patient with short hair and a calendar showing ART detection for a couple of weeks
  ▪ Image of a patient with longer hair and a calendar showing ART detection for a month
  ▪ Image of Patient #2 and Provider #2 discussing results in a clinic room
  • The last frame should be a images of all the people who were filmed as part of this video: Provider #1, Provider #2, Provider #3, Patient #1 and Patient #2
	• Now, let's review! (Show Patient #1 speaking; narrate sub-bullets)
	  • MedViewer is a test that measures the amount of ART in your body using hair samples.
	  • A trained staff member will pluck a small number of strands of hair from your head and take them to the lab for testing.
	  • After testing, all hair samples will be permanently disposed of, and your results will be sent securely to the clinic.
	• Remember, the results will show amounts of ART in your body over the past couple of weeks or a month, depending on the length of your hair.
	  • With this information, you and your provider can see how you are taking your medication.
	• We hope you've enjoyed hearing about MedViewer. If you have any questions, please feel free to ask your health care providers. Thanks for listening!
		• Patient #1

A.3 PROVIDER COMMUNICATION AIDS

MedViewer Communication Aid

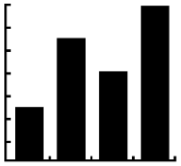
MEDVIEWER CURRENTLY FOR INVESTIGATIONAL USE ONLY

MEDVIEWER REPORTS

- Reports show days with likely missed ART dose(s) during the weeks prior to hair sample collection.
- Two versions:

Calendar version

- Days with likely missed doses indicated.
- Recommended for patients.



Bar chart version

- Daily ART levels detected in hair and adherence threshold indicated.
- Recommended for providers.

- Look over both the calendar and bar chart versions before discussing results with your patient.

INTERPRETING MEDVIEWER REPORTS

- This assay is still in development, so **no clinical decisions should be made on the basis of the report.**
- Interpret MedViewer reports in collaboration with your patient. Use both the reports and your patient's memory to understand your patient's medication-taking.
- Consider the limitations and levels of measurement certainty represented in the report:
  - The reports have been designed to under- rather than over-report likely missed doses.
  - Precision bars for each day in bar chart represent variability across each patient's 5 hair strands.
  - Days in the reports may not represent exact dates of successful and missed doses (see FAQ).

USING MEDVIEWER REPORTS WITH YOUR PATIENTS

First step to use with each patient:

REVIEW & CHECK
MedViewer results with patient
	• Review and explain MedViewer report results to your patient.
	• Check in with patient to see how well patterns of missed doses generally align with patient's recollection.
	  ○ If patient corroborates, consider optional steps below.
	  ○ If patient *does not* corroborate, you may choose to conclude the conversation about the report.

Optional additional steps:

UNDERSTAND
reasons for missed doses/successes
	• Discuss **causes** of successful and missed doses. Ask about:
	  ○ Event-level causes of missed doses.
	  ○ Chronic and psychosocial causes of missed doses.
	  ○ Strategies that yielded success.

STRATEGIZE **to overcome challenges/ replicate successes**	• Develop strategies with patient to overcome causes of missed doses. • Discuss ways to replicate adherence success.
MOTIVATE **to improve or maintain adherence**	• Provide praise for good adherence. • Work with patient to set goals for adherence.

MedViewer Frequently Asked Questions (FAQs)

Q: How will confidentiality of hair samples and MedViewer reports be ensured?

A: All hair samples and MedViewer reports will be labeled with a unique participant ID that contains no identifying information about the patient. Once the MedViewer test is complete, all hair samples will be incinerated. The research team will store a copy of the MedViewer report in the secure research record after your appointment with the patient. Please securely dispose of the MedViewer report and/or delete the email containing the report following your MedViewer discussion with the patient.

Q: How will I receive the MedViewer report during the pilot study?

A: A study team member will deliver the MedViewer report either a) to your workstation at the ID Clinic or b) via secure email before your scheduled appointment with the patient participant. In the case that the report is delayed and not available before the appointment, there will be several possible options (see next question.)

Q: What happens if the MedViewer report is not ready by the time I am scheduled to see the patient?

A: If you do not receive the MedViewer report on time for the scheduled appointment with your patient, the research team will notify you before the appointment. You can then decide, with patient input, whether to a) delay seeing the patient until later that day when the report arrives; b) meet with the patient at the scheduled appointment time and schedule a separate visit to review the MedViewer report within 4 weeks; or c) request that the clinic HIV care pharmacist discuss the MedViewer report with the patient within 4 weeks.

Q: How does MedViewer compare to a viral load assay?

A: While viral load results allow you to assess the impact of your patient's ART regimen on viral suppression at a given point in time, viremia may or may not be attributable to sub-optimal adherence to ART and may only reflect recent adherence behavior. Similarly, an undetectable viral load may not reflect perfect adherence. The MedViewer report shows the level of drug absorbed by the body on a daily basis over several weeks, providing a longitudinal indicator of daily adherence. Once available for routine use, MedViewer can be used alongside viral load tests to guide patient counseling and treatment and offer another tool that can be used in conversations about adherence.

Q: How accurate are MedViewer results?

A: Test sensitivity and specificity: Receiver operating characteristic (ROC) curves have been determined for MedViewer based on evaluation of dolutegravir (DTG) and emtricitabine (FTC) in hair strands collected following directly observed therapy. Based on these results, adherence cut-off values of MedViewer signal abundance have been selected to maximize the likelihood that a daily

concentration is not misclassified as a missed dose (specificity) while still achieving reasonable likelihood of identifying missed doses (sensitivity). Sensitivity and specificity values will be included on each provider report.

Dates in report: The dates of missed doses shown in the report might not correspond to exact dates of adherence behavior. Rather, the dates indicated can provide an approximation to help identify patterns of missed doses.

Q: How do different hair conditions affect the test results?

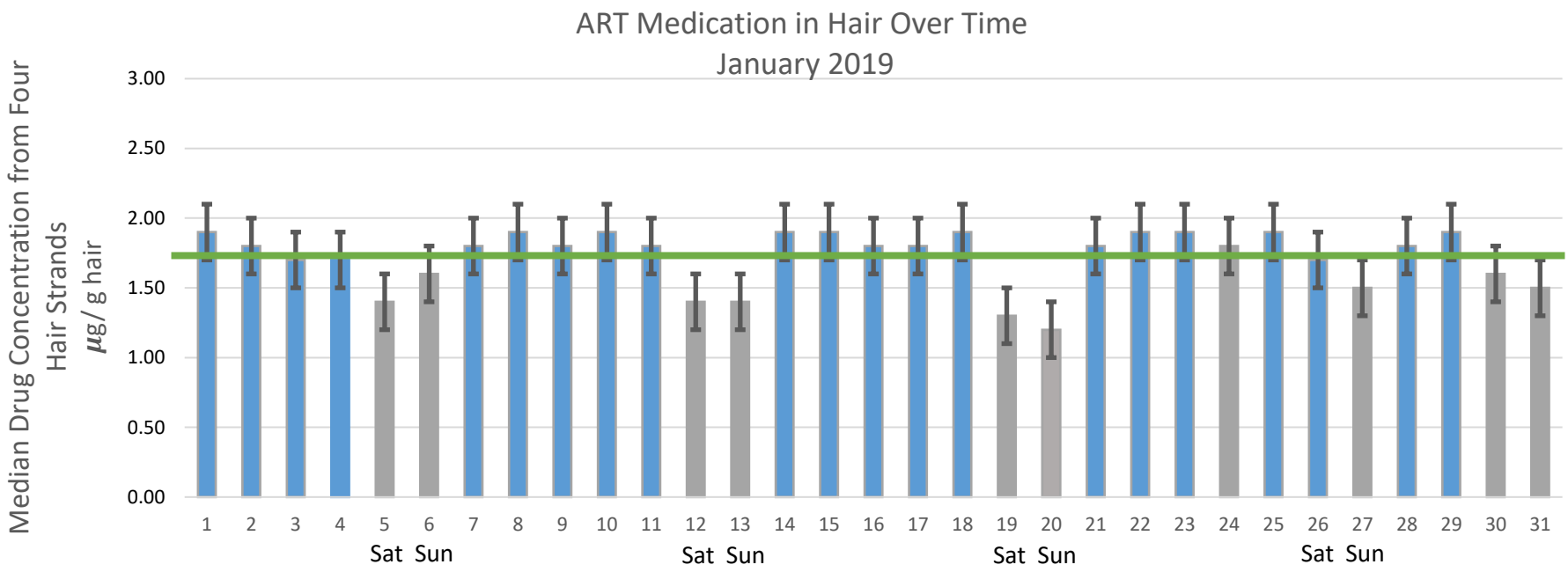
A: MedViewer results are accurate and valid for hair treated more than 4 weeks prior to hair collection. Thus, patients are eligible to participate if their most recent chemical hair treatment was more than 4 weeks prior to their study enrollment date. However, patients who have received chemical hair treatment with bleach, dye or relaxers during the 4 weeks prior to their screening visit will not be eligible to participate in the study. Other treatments such as hair straightening and moisturizing do not affect the detectable level of drug in hair measured by the MedViewer test.

MEDVIEWER RESULTS REPORTS:

FOR INVESTIGATIONAL USE ONLY

Example provider report with hypothetical patient data:

(Note: The provider report displayed below is an example of what the MedViewer provider report may look like during the clinical trial. Final report displays to be used in the trial are being developed as additional pharmacokinetic data become available.)



Green Line: Daily dosing target
Black Lines: Min/max values from four hair strands
Blue Bars: Days at or above daily dosing target
Gray Bars: Days below daily dosing target

Summary Statistics:

- Date of test: February 3, 2019
- Test sensitivity: 100%. Test specificity: 73%
- Drug tested: Dolutegravir
- Drug concentration detected: Last 3-4 weeks
- Average concentration: 1.72 µg/g hair
- Days with optimal concentration: 13/24 (54%)
- Average number of contiguous days below threshold: 2 (9%)

FOR INVESTIGATIONAL USE ONLY

Example patient report with hypothetical patient data:

(Note: The patient report displayed below is an example of what the MedViewer provider report may look like during the clinical trial. Final report displays to be used in the trial are being developed as additional pharmacokinetic data become available.)

January 2019						
Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**	**Sunday**
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

Key:

Blue: Your body had ENOUGH ART medicine. Excellent! When your body has enough medicine, it can help make the virus undetectable. It can also lower your risk of transmitting the virus to other people.

White: Your body did NOT have enough medicine. Talk with your provider about how to make sure you get the right amount.

Gray: MedViewer not conducted for these days.

Results Summary:

- Your MedViewer test was conducted on February 3, 2019.
- MedViewer detected ART medicine in your hair from the last 4 weeks.

APPENDIX B. INFORMED CONSENT FORMS

B.1 PATIENT STUDY VISIT 1 INFORMED CONSENT FORM

IRB TEMPLATE
Version 2.0-12/5/2018

DO NOT CHANGE THIS FIELD-IRB USE ONLY

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants (Verbal Consent)

Consent Form Version Date: v2.0 dated 15 October 2020
IRB Study # 18-3360
Title of Study: Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)
Principal Investigator: Angela Kashuba
Principal Investigator Department: UNC Eshelman School of Pharmacy-Division of Pharmacotherapy and Experimental Therapeutics
Principal Investigator Phone number: (919) 966-9998
Principal Investigator Email Address: akashuba@unc.edu
Funding Source and/or Sponsor: NIH National Institute of Allergy and Infectious Diseases (NIAID)

CONCISE SUMMARY
MedViewer is the name of a new technology being developed at UNC to measure the amount of antiretroviral therapy (ART) medicine in the body using hair. The purpose of this study is to determine if MedViewer is an acceptable, appropriate, and convenient way to measure drug concentrations in hair on the same day as a routine medical visit. Most participants will be enrolled in this study for one day; however, study activities may occur over multiple days if needed. Your clinic provider will also be a participant in this research study. During the study visit, which will last several hours, participants will first provide a hair sample and complete a baseline survey. A small portion of patients may also be asked to provide a blood sample as well. The hair sample will be sent to the laboratory for MedViewer testing and a report of the results will be sent to the medical provider. Then, the patient and provider will look at the MedViewer report and discuss the results, although since the MedViewer is still being developed, the results are for investigational research use only. Participation in this research is completely voluntary. You do not have to participate in this study in order to receive your regular health care, and there may be no direct benefit to you. Risks of participation could include social harms or loss of

confidentiality. After the appointment with the provider, participants will complete a short survey to share how they felt about using MedViewer and talking about their results with their provider. Study participants will receive a $70.00 Visa® gift card, as well as parking tokens and food, as needed. A small subsample of participants will be asked to participate in a second virtual visit to complete an interview. This follow-up study visit is discussed in more detail in a separate consent form.

What are some general things you should know about research studies

You are being asked to take part in a research study. To join the study is voluntary. You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a participant with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

Over the last few decades, many new medicines called antiretroviral therapy (ART) have been developed to treat Human Immunodeficiency Virus (HIV). The development of these medicines has greatly increased the quality of health care that can be offered to people living with HIV; however, these medicines only work if patients take them continuously. Whether a patient is taking their ART medicine continuously as prescribed is called adherence. Currently, to measure adherence we count pills and measure the amount, or concentration, of the ART medicine in blood. However, using blood to measure adherence only tells us about the few days before the blood draw. There is a need to find a way to measure adherence that could give us this information for a longer period of time between clinic visits.

We are developing a new technology called MedViewer, which, once finalized, will allow us to measure ART medicine in hair using a laser attached to a detector in the laboratory. Using hair, we can potentially measure adherence for a longer period of time than we can with blood, and it is less invasive than taking a blood sample. In previous research studies, we developed an initial

version of MedViewer and tested it in hair from people across a variety of races, ethnicities, and genders as well as those that do and do not use chemicals to treat their hair. The purpose of this current study is to test if results from the MedViewer can be produced in real-time during a clinical setting, and if using MedViewer is acceptable and appropriate for patients and providers. Real-time is the term we use to describe the amount of time a patient spends in the clinic for a regularly scheduled appointment. Ideally, real-time would be two hours or less from the time the hair sample is collected. In this study, we will be comparing the concentrations of ART medicine in hair with the concentrations of the same ART medicine in blood. We will enroll both patients and providers into this study. Providers will be trained on how to read the reports and how to use them as a discussion tool during patient visits.

If you decide to participate in this study, we will collect a hair sample. We will also collect a blood sample from a small subset of patients. You will also complete a baseline survey. We will update your medical history and document any chemical treatments you use on your hair. At your regularly scheduled appointment, whether in person or virtual, you and your provider will go over the results of the hair analysis and discuss findings potentially related to your medication adherence. Because MedViewer is still being developed and studied, your provider will not make any clinical decisions based on the MedViewer results. At the end of this visit, you will be asked to complete a short survey about your opinions and experience with MedViewer, this will be done electronically either by videoconferencing or email. The information you share with us will help us improve MedViewer for potential use in the future.

We will also ask providers to complete surveys before and after reviewing the reports with their patients to learn about their experience using MedViewer and how useful they thought it was.

We will be asking patients living with HIV and taking ART medicines to participate in this study. The ART medicines that we will be studying are emtricitabine and dolutegravir. These medicines are available in many combination pills, such as Truvada®, Biktarvy®, and Tivicay®, and several others.

You are being asked to participate in this study because you are at least 18 years of age and meet our eligibility criteria.

Are there any reasons you should not be in this study?

You should not be in this study if you:

- Are younger than 18 years old
- Are bald or have hair less than 1.0cm in length on the back of your head
- Are not HIV-infected
- Have any condition which, in the opinion of the investigators, is likely to interfere with study procedures or ability to affect drug absorption.

- Are not currently taking one of the ART medications under investigation
- Have chemically treated your hair using dye, bleach, or relaxers in the last 28 days.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately 50 people in this research study. Any subject unable to complete sample collection will be withdrawn, and a new participant will be enrolled as a replacement.

How long will your part in this study last?

Your participation in this study will last approximately one day. A small subset of participants will be enrolled into a cohort that completes a follow-up interview and an endline survey. If you participate in that cohort, there will be a separate consent that will review the details of that portion.

What will happen if you take part in the study?

Screening Visit

The screening visit is designed to ensure that you should be in this study. This visit will be conducted remotely via videoconferencing if possible.

Procedures that will take place during the screening visit include:

- A review of the Informed Consent document that will cover all aspects of the research study, including risks and benefits. We will give you a chance to have all of your questions answered, review the forms in private, and sign if you choose.
- As part of the consent, a short video will be shown describing the hair collection process and explaining what the results will look like and how they will be shared with you. The MedViewer test is still being studied by researchers, and therefore is for investigational research use only. The video will show you how, once the MedViewer test is finalized, the results could be used to better understand your adherence behavior and potentially to come up with strategies to help you improve or maintain adherence.
- You will have a chance to have all your questions answered and sign the Informed Consent if you choose.

If you choose to participate, and you continue to meet eligibility requirements, the sampling portion of your visit will be scheduled.

Sampling Visit (to be performed on the same day as the Screening Visit if possible)

After informed consent, we will collect a sample of hair for analysis. This may be done at a remote location to minimize clinic exposure. We will pluck 5 strands of hair from the back of your head with tweezers, so that we can look at medication in the hair root. It will be chosen from a discrete location and be hidden as much as possible. On average, a person can lose about 100 strands of hair per day.

After we collect the hair needed, we will have you complete a short baseline survey to obtain some basic information and learn about how you take the ART medicine you have been prescribed. For a small subset of participants, we will also collect a sample of your blood through either a needlestick or a fingerstick to compare the amount of medication found in the blood with the amount of medication found in hair. If you are having normal clinic labs drawn, we can have an additional tube drawn, about a half teaspoon (3mL), at the same time. Alternatively, we may be able to collect the blood through a fingerstick only (0.5mL). A finger stick is the term we use to describe pricking the tip of the finger to get a small amount of blood. You may have heard this term to describe how diabetic patients check their blood sugar. Your samples will only be used for research purposes and will not include any whole genome sequencing.

During your regularly scheduled clinic appointment, your provider will go over the results of the MedViewer hair analysis. This may be done as part of an in person clinic visit or a virtual telehealth visit. If you and your provider are not able to discuss the MedViewer reports, you and your provider will decide whether to discuss the results at a later time or whether you would like to discuss your MedViewer results with the HIV clinic pharmacist. Remember, MedViewer is still being developed and researched, and it is currently for investigational use only. If you do choose to participate your provider will not make any clinical decisions based on your MedViewer test results at this time.

Follow-up

At the completion of your clinic visit, you will complete a short computer-based survey about your experience in the study.

Additional Study Activities

A small subset of participants (about 30 of the total 50 participants) will be invited to complete an interview and brief survey about their experience with MedViewer at a later date. You are not required to participate in these additional study activities in order to participate in the main study, and there is a separate consent form that will discuss the additional study activities in greater detail.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

What are the possible risks or discomforts involved from being in this study?

Blood Draws

Risks with blood draws include pain, bleeding, bruising, infection, or swelling at the site of the needle sticks**,** as well as the possibility of dizziness or fainting during needle sticks. A finger stick is a bit uncomfortable and may cause some soreness and bruising. Very rarely, the site can

become infected. These risks will be minimized by using an aseptic (clean) technique as well as having experienced nursing and lab staff perform blood draws.

Medication Adverse Events

Although HIV medications do cause some side effects, you are already receiving the antiretrovirals, and this study does not change your risk of having a drug-related side effect. You will not be given any new medications as part of this study.

Hair

There is no foreseeable risk with the donation of hair.

Video

Although the short MedViewer intervention video is designed to introduce participants to the investigational MedViewer test in an approachable manner, it is possible that participants will experience discomfort or anxiety watching a video about providing a hair sample for conducting the MedViewer test and about antiretroviral medication circulating in their body depositing in their hair.

Baseline and post-visit surveys

Immediate known potential risks to participating in the surveys include the potential for participants to feel uncomfortable or experience anxiety when being asked questions about their medication adherence and about their interactions with their provider.

MedViewer assay and report

It is possible that discussing the MedViewer report may alter participant-provider communication during the clinic visit, such that the participant and/or the provider feels uncomfortable. Participants may feel anxious or embarrassed reviewing their MedViewer report with the provider or pharmacist, or uncomfortable if the MedViewer results contradict self-reported adherence. Additionally, the MedViewer report may lead to misunderstanding of or confusion about how you have been taking your medications and potentially misinform conversations with your provider about your medication taking patterns. To minimize these concerns, your provider will also continue to monitor your medication adherence through the normal means with your standard clinic lab values. Your provider will not make any clinical decisions based on MedViewer results because the MedViewer is currently for investigational use only. The results of this research will help to determine how, once fully developed, MedViewer could be used in a clinical setting.

Social Harms

There may be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some the questions you will be asked, some of the procedures that will be

done, or some of the test results that you will receive. You may also experience stigma as a result of being involved in a clinical research study.

Confidentiality

Participation in clinical research includes the risks of loss of confidentiality and discomfort with personal nature of questions. Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others and that social harms may result as a result of a loss of confidentiality.

There may be uncommon or previously unknown risks. You should report any problems to the researcher.

If you choose not to be in the study, what other treatment options do you have?

You do not have to be in this research study in order to continue to receive treatment.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will information about you be protected?

The investigators will protect your privacy in the following ways:

- All study records are kept in a locked drawer in a locked office under badge access or stored electronically on a secure internal computer network or server, accessed using a password-protected computer that can only be accessed by members of the study team.
- Only the study team will have access to any personally identifiable information about you.
- No samples or study report forms will contain your name. You will be assigned a study identification number that only contains your initials and a set of numbers. One study investigator will keep the file that connects this number to you on a secure internal computer network.
- All interviews will take place in a private examination room.
- Identifiable phone messages will not be left on your voicemail, and you will only be contacted by study staff in a manner that you agree upon.
- All samples will be destroyed within 12 months of the study being published.
- None of your samples will ever be used for commercial profit.

No participants will be identified in any report or publication resulting from this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if a disclosure is ever required, UNC-Chapel Hill will take any steps allowable by law to protect

the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the NIH) for purposes such as quality control or safety.

Under North Carolina law, confidentiality does not extend to certain communicable diseases, such as syphilis, gonorrhea, and chlamydia or other illnesses that put others at risk. If the researchers become aware that participants have such an illness, they are required to report it to state authorities.

The study coordinator will keep the signed original consent form and a copy will be given to you. A copy of this informed consent will also be placed in your medical record.

What is a Certificate of Confidentiality?

To help us protect your privacy, the study team has obtained a Certificate of Confidentiality from the National Institutes of Health (NIH). With this certificate, the researchers cannot be forced to disclose any information that can be used to identify you, even by court subpoena, in any federal, state, local or civil, criminal, administrative, legislative or other proceedings. The researchers would use the certificate to resist any demands for information that would identify you, as described.

The Certificate cannot be used to resist a demand for information from personnel of the United States government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an employer, insurer, or other person receives your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate does not limit the researchers from disclosing information that may include details about child abuse, homicidal or suicidal intent, or other information deemed appropriate. The researchers will not voluntarily release any information about you under this Certificate.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. If you are hurt, become sick, or develop a reaction from something that was done as part of this study, the researcher will help you get medical care, but the University of North Carolina at Chapel Hill nor the National Institute of Health (NIH) has not set aside funds to pay you for any such injuries, illnesses or reactions, or for the related medical care. Any costs for medical expenses will be billed to you or your insurance company. You may be responsible for any co-payments and your insurance may not cover the costs of study related injuries. If you think you have been injured from taking part in this study, call Dr. Cindy Gay at 919-216-2156. She will let you know what you should do. By signing this form, you do not give up your right to seek payment or other rights if you are harmed as a result of being in this study.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be receiving a $70.00 Visa® gift card for taking part in this study. You will also receive parking tokens and snacks/meals as needed for the duration of your participation in this study. You will only be paid for the portions of the study that you complete. If you do not complete the study, your payment will be adjusted:

- You will receive $5.00 for the hair sample.
- You will receive $15.00 for the blood sample.
- You will receive $20.00 for the baseline survey.
- You will receive $10.00 for the post-visit survey.

You will also receive $20.00 on your Visa® gift card to cover any telephone minutes or internet data you may have purchased to complete this study visit.

We do not reimburse for childcare.

We do our best to have your payment available on the day of your visit, but there may be times when this is not possible. We will get you payment as soon as possible after the visit, in the manner in which you prefer.

Will it cost you anything to be in this study?

It will not cost you anything to be in this study. If by chance you ever receive a bill for study activities, please notify the study team right away so this can be fixed.

Who is sponsoring this study?

This research is funded by NIH National Institute of Allergy and Infectious Diseases (NIAID). This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What if you have questions about your rights as a research participant?

All research on human participants is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

IRB Study # 18-3360

Title of Study: Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)

Participant's Agreement:
I have asked all the questions I have at this time. By giving my verbal consent, I voluntarily agree to participate in this research study. I understand that my participation is voluntary and I can withdraw my consent to participate at any time.

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

B.2 PATIENT STUDY VISIT 2 INFORMED CONSENT

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Verbal Consent - Adult Participants - Patient participants (follow-up subsample)

Consent Form Version Date: Version 2.0, 15 October 2020
IRB Study # 18-3360
Title of Study: Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)

Principal Investigator: Angela Kashuba
Principal Investigator Department: UNC Eshelman School of Pharmacy-Division of Pharmacotherapy and Experimental Therapeutics
Principal Investigator Phone number: (919) 966-9998
Principal Investigator Email Address: akashuba@unc.edu
Funding Source and/or Sponsor: NIH National Institute of Allergy and Infectious Diseases (NIAID)

CONCISE SUMMARY
The study activities described in this consent are part of a larger research study at the UNC Infectious Diseases (ID) Clinic to learn about MedViewer, a test being developed to show how people take their antiretroviral therapy (ART) medicine over time. The MedViewer test uses a patient's hair sample to create a report that shows whether ART doses were missed over the past several weeks. MedViewer is currently for investigational use only, so medical providers will not make any clinical decisions based on MedViewer results. Your participation is completely voluntary. You are already enrolled as a participant in the larger ENLIGHTEN study. This consent form describes a second part of the study in which you may choose to participate. The purpose of this part of the study is to learn more about your experience with MedViewer. If you choose to participate, the additional study activities will be: doing an interview and a survey about your experience with MedViewer. These study activities should take about 1.5 to 2 hours, in total, to complete. If you choose to participate you will be one of about 30 patient participants in this part of the study. The information you share with us will be used to improve the MedViewer test and learn how it can be used in the medical clinics by patients and medical providers in the future, once the test is finalized. You do not have to participate in this study in order to receive your health care, and there may be no direct benefit to you. Risks of participation could include social harms or loss of confidentiality. For your participation in this study, you will receive up to $60.00 added to the Visa® gift card you received at your last visit. If you no longer have the card, we can provide you a new one by mail or in-person when you visit UNC Chapel Hill.

What are some general things you should know about research studies?

You are being asked to take part in a research study. Participating in this part of the study is voluntary. You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina at Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

Our study team is developing a new test called MedViewer. MedViewer measures the amount of antiretroviral (ART) medicine in a patient's body each day, which can show how a patient takes their ART over time. Antiretroviral therapy, also known as ART, is medicine used to treat and suppress the Human Immunodeficiency Virus (HIV).

This study is part of a larger study at the University of North Carolina (UNC) Infectious Disease (ID) Clinic. You are already enrolled in this larger study, which involves having a hair sample collected and tested by MedViewer and receiving the results at your clinic visit with your medical provider. Because MedViewer is still being tested and finalized, your medical provider will not make any clinical decisions based on your MedViewer results.

During this additional part of the study, we are conducting follow-up surveys and in-depth interviews with about 30 individuals who participated in the larger study. By participating in the additional part of this study, we will be able to gather more information about your experience with the MedViewer test. The information you share with us will be used to improve MedViewer and help us learn how it can be used in the clinic with patients and medical providers. Participating in this part of the study is voluntary.

Are there any reasons you should not be in this study?

You should not participate in this study if you are not enrolled in the main ENLIGHTEN study or if you were enrolled in the main study and did not have a hair sample collected and tested by MedViewer. You should also not participate in this part of the study if you do not have 1.0 cm of caput hair.

How many people will take part in this study?

You are one of about 50 patients participating in the main ENLIGHTEN study. If you decide to participate in this part of the study, you will be one of about 30 patients in this part of the study.

We will also interview about 15-30 medical providers who discussed MedViewer results with their patients.

How long will your part in this study last?
Today's interview and survey will take approximately 1.5 to 2 hours.

What will happen if you take part in this part of the study?
This part of the study will include the following activities:

- **In- depth interview:** A trained member of our research team will conduct an individual, in-depth interview with you either over the phone or over videoconferencing. During the interview, the research staff member will ask you some questions about your experience with MedViewer. The goal of these questions is to understand how you felt about having your hair sample collected, receiving the MedViewer report, and talking about the report with your medical provider. There are no right or wrong answers. We simply want to know what you thought of your experience with MedViewer so we can improve it for potential future use.
    - To help assure that we get the best understanding possible from your answers, the interview will be audio-recorded with your permission. You will be asked at the end of this consent form to indicate if you give your permission to be recorded during the interview.
    - If you do not want us to audio record the interview, we will take typed notes instead. The typed notes will be stored on a password-protected server. A printed copy of the notes will be stored with your other study materials in your study chart in a locked drawer in a secure room to which only the research team has access. We will not write your name - we will only write your unique ID number but not any information that can identify you.
    - If you do give us permission to audio record, after the interview is finished, all audio files will be stored on the same secure internal computer network that can only be accessed using a password-protected computer by members of the study team. This audio recording will be transcribed (typed) by a secure professional transcription service. All identifying information will be removed from the transcript. Your name will not be included on the transcript - only a unique participant ID number (an identification number unique to you that only contains your initials and a set of numbers) will be used. Once analysis of the interviews is completed, the digital audio file will be stored on a password-protected secure server.
- **Survey:** During the survey, you will answer a set of questions about your experiences with MedViewer and asked to mark your responses on an electronic survey form sent to you through a secure email link. Alternatively, we can read the questions to you over the phone or videoconferencing and enter your responses on the electronic form. Your responses will only be recorded with your unique ID number. Any information that can identify you will not be recorded.

What are the possible benefits from being in this study?
Research benefits society by providing new knowledge. Although there may be no direct benefits

from being in the study, we believe your participation will help us improve the MedViewer test so that it can be used to support people living with HIV in the future with taking their ART medicine as prescribed by their medical provider.

What are the possible risks or discomforts involved from being in this study?

Survey and in-depth interview:
Immediate known risks to participating in the surveys and in-depth interviews include the potential for you to feel uncomfortable or experience anxiety when being asked questions about how you take your ART medicine or your MedViewer results. To minimize these risks, the study team will conduct the surveys and interviews in a neutral and non-judgmental manner. You can also stop participating at any time or skip any questions that make you feel uncomfortable.

Social Harms
There may be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions you will be asked, some of the procedures that will be done, or some of the test results that you will receive. It is possible that you may also experience stigma as a result of being involved in a clinical research study.

Confidentiality
Participation in clinical research includes the risks of loss of confidentiality and discomfort with the personal nature of questions. Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that a participant's involvement in the study could become known to others and that social harms may result from a loss of confidentiality.

There may be uncommon or previously unknown risks. You should report any problems to the researcher.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will information about you be protected?
Your privacy will be protected in the following ways:

- Interview notes and all other study records will be stored in a locked drawer in a designated, secure room or electronically on a secure internal computer network or server that can only be accessed using a password-protected computer by members of the study team.
- Audio files and notes from the interviews will be stored on a secure internal computer network or server that can only be accessed using a password-protected computer by members of the study team.
- Only the study team will have access to any personally identifiable information about you.

- No study records will contain your name. You will be assigned a unique study ID number that only contains your initials and a set of numbers. One study investigator will keep the file that connects this number to you on a secure internal computer network. It will be kept separate from the audio files, interview notes, transcripts, surveys, and other study records.

- All interviews will be conducted securely over videoconferencing or by telephone. Study staff will be in a private area with a closed door and will ask that you find a quiet, private area in which to participate in the interview and survey.

Participants will not be identified in any report or publication about this study.

Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC at Chapel Hill will take steps allowable by law to protect your personal information. In some cases, your information in this study could be reviewed by representatives of the University, research sponsors, or government agencies (such as the National Institute of Health (NIH)). This would be for purposes such as quality control or safety.

Under North Carolina law, confidentiality does not extend to certain communicable diseases, such as syphilis, gonorrhea, and chlamydia or other illnesses that put others at risk. If the researchers become aware that participants have such an illness, they are required to report it to state authorities.

The study coordinator will keep the signed original consent form and a copy will be given to you. A copy of this informed consent will also be placed in your medical record.

What is a Certificate of Confidentiality?

To help us protect your privacy, the study team has obtained a Certificate of Confidentiality from the NIH. With this certificate, the researchers cannot be forced to disclose any information that can be used to identify you, even by court subpoena, in any federal, state, local or civil, criminal, administrative, legislative or other proceedings. The researchers would use the certificate to resist any demands for information that would identify you, as described.

The Certificate cannot be used to resist a demand for information from personnel of the United States government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an employer, insurer, or other person receives your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate does not limit the researchers from disclosing information that may include details about child abuse, homicidal or suicidal intent, or other information deemed appropriate. The researchers will not voluntarily release any information about you under this Certificate.

What will happen if you are injured by this research?
All research involves a chance that something bad might happen to you. If you are hurt, become sick, or develop a reaction from something that was done as part of this study, the researcher will help you get medical care, but the University of North Carolina at Chapel Hill nor the NIH has not set aside funds to pay you for any such injuries, illnesses or reactions, or for the related medical care. Any costs for medical expenses will be billed to you or your insurance company. You may be responsible for any co-payments and your insurance may not cover the costs of study related injuries. If you think you have been injured from taking part in this study, call Dr. Cindy Gay at 919-216-2156. She will let you know what you should do. By signing this form, you do not give up your right to seek payment or other rights if you are harmed as a result of being in this study.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
You will receive up to $60.00, in the form of a Visa® gift card, for participating in this part of the study. You will only be paid for the parts of the study that you complete. If you do not complete the study, your payment will be adjusted based on the parts you complete:

- You will receive $30.00 for the in-depth interview conducted today.
- You will receive $10.00 for the survey conducted today.

You will also receive $20.00 on your Visa® gift card to cover any telephone minutes or internet data you may have purchased to complete this study visit.

Will it cost you anything to be in this study?
It will not cost you anything to be in this study except your time.

What if you are a UNC student?
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by NIH National Institute of Allergy and Infectious Diseases (NIAID). This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What if you have questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

IRB Study # 18-3360
Title of Study: Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)

Participant's Agreement:
I have asked all the questions I have at this time. By giving my verbal consent, I voluntarily agree to participate in this research study. I understand that my participation is voluntary and I can withdraw my consent to participate at any time.

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

Participant's Agreement to be Audio Recorded

_____ OK to record me during the interview
(Research Team Member Initials)

_____ NOT OK to record me during the interview
(Research Team Member Initials)

B.3 PROVIDER INFORMED CONSENT FORM

IRB TEMPLATE
Version 2.0-12/5/2018

****DO NOT CHANGE THIS FIELD-IRB USE ONLY****

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Verbal Consent - Adult Participants – Provider participants

Consent Form Version Date: Version 2.0, 18 August 2020
IRB Study # 18-3360
Title of Study: Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)

Principal Investigator: Angela Kashuba
Principal Investigator Department: UNC Eshelman School of Pharmacy-Division of Pharmacotherapy and Experimental Therapeutics
Principal Investigator Phone number: (919) 966-9998
Principal Investigator Email Address: akashuba@unc.edu
Funding Source and/or Sponsor: NIH National Institute of Allergy and Infectious Diseases (NIAID)

CONCISE SUMMARY

The purpose of this research study is to determine the feasibility, acceptability and appropriateness of using an investigational clinical test, called MedViewer, as a clinical adherence-monitoring tool. The MedViewer test, being developed by our research team at UNC, measures the concentration of antiretroviral medication in a patient's hair over time. MedViewer results have the potential to impact adherence counseling in a clinical setting in the future; however, since MedViewer is currently for investigational use only, no clinical decisions should be made using MedViewer results. This is research, and participation is completely voluntary. You may not receive a direct benefit. As part of this study, patient participants will provide a hair sample within three days before a routine in-person or telehealth clinic visit scheduled with their provider. Prior to the appointment time, the hair sample will be analyzed by the MedViewer test with the aim of producing the MedViewer reports and delivering the reports to the provider before the patient's appointment. For each participating patient, providers will receive a copy of their patient's MedViewer reports to view and discuss with the patient during the appointment. Following the appointment, the patient and provider will each complete a brief post-visit questionnaire. Before seeing any participating patients for the study, provider participants must attend a study training session. After the training, providers will complete a baseline

questionnaire. Part way through the study, providers will complete an in-depth interview, and before study close, providers will complete a brief endline questionnaire. If you choose to participate, study activities may occur via videoconferencing, email, telephone, or in-person. In total, your active participation in the study may last up to 10 months. The greatest risks of participation include a loss of confidentiality or feeling stressed or anxious about discussing MedViewer results with patients or possible disruptions to your schedule related to study procedures. If you are interested in learning more about this study, please continue to read below.

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher or the University of North Carolina at Chapel Hill.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to investigate the feasibility, acceptability, and appropriateness of using a clinical test called MedViewer as a clinical adherence-monitoring tool. The MedViewer test, being developed by our study team here at UNC, ultimately aims to:

a) longitudinally quantify, in easy to understand patient and provider reports, antiretroviral (ARV) concentrations in patient hair; and

b) promote enhanced adherence counseling conversations between patients living with HIV and their medical providers through review and discussion of the MedViewer report during routine clinic visits.

Although MedViewer is still in investigative stages, formative work with MedViewer has shown that MedViewer is able to quantify adherence over longer time periods and on a more granular level than other measures of adherence using blood samples. Yet, because MedViewer is still in investigative stages, no clinical decisions can be made based on MedViewer results at this time. Results from this study will help to determine how, once fully validated, MedViewer could be used in a clinical setting.

You are being asked to be in this research study because you are a health care provider in the UNC Chapel Hill Infectious Diseases (ID) Clinic and you see HIV-positive patients at least 1 half-day per week (i.e., at least 4 hours per week) for the UNC ID Clinic.

Are there any reasons you should not be in this study?

You should not be in this study if you are:

1. Not a health care provider (including attending physician, ID fellow, nurse practitioner, physician assistant, or a designated HIV Care pharmacist) who sees HIV-positive patients at least 1 half-day per week for the UNC ID Clinic; or
2. Not willing or able to participate in any provider training sessions for this study or any form of make-up training session with the research team.

How many people will take part in this study?

If you choose to participate in this study you will be 1 of approximately 15-30 health care providers participating in this study. Additionally, approximately 50 patients from the UNC ID Clinic will participate.

How long will your part in this study last?

Your active participation in this study will be up to 10 months, including:

- *Up to one month between the date of your enrollment and the date of the first provider training session:* Providers who enroll prior the first provider training session may be enrolled for up to one month before the date of the first provider training, at which point patient enrollment for the study will begin. After patient enrollment begins providers who have completed a study training can begin seeing patient participants for their MedViewer study visits.
- *Up to 7.5 months of patient study visits between completion of a provider study training session and the close of patient enrollment*. Patients will be enrolled in the study over 7.5 months. Provider participants may see patients for their study visits for up to the full 7.5 months if they have enrolled in the study prior to the start of patient enrollment. If a provider has not enrolled prior to initiation of patient enrollment, they may enroll in the study at any point over the course of the 7.5 months of patient study visits. We expect provider participants will see an average of 2-3 patient participants throughout the duration of the study though the exact number of patients may vary for each provider participant. During each patient study visit, you will receive the patient's MedViewer results report to discuss with your patient during the routine clinic visit. Receipt of the MedViewer report, the MedViewer discussion with the patient, and completion of a 5-item questionnaire after the patient visit is expected to add only several extra minutes to the routine clinic visit. You may also be invited to participate in an in-depth interview during this time period after you have seen at least two patient participants for the study.
- *Approximately 1.5 months to complete endline questionnaire and in-depth interview*. After the close of patient enrollment in the study there will be a 1.5-month period for you to complete the endline questionnaire. During this time, you will also complete an in-depth interview, if you have not already done so during the 7.5-month period of patient visits. The in-depth interview will take place after you have seen at least two patient participants or within 1.5 months of the close of patient enrollment, whichever comes first.

What will happen if you take part in the study?

This research study consists of several activities for provider participants. Study activities may occur via IRB-approved videoconferencing, email, phone, or in-person, as appropriate.

1. **Study training and post-training questionnaire:** First, you will be asked to attend an approximately 30-60-minute study training session. This training will be offered on several different dates. The training will introduce you to the study materials and procedures and provide an opportunity to practice reviewing hypothetical MedViewer reports. Throughout the training, research staff will reiterate that MedViewer is currently for investigational use only and no clinical decisions can be made based on MedViewer results at this point. At the conclusion of the study training, you will be asked to complete a brief post-training baseline questionnaire (paper or electronic), assessing your satisfaction with the training, understanding of the MedViewer test and study procedures, and comfort reviewing MedViewer reports with patients. Supplemental training will be offered to providers, as needed.
2. **Receiving and discussing MedViewer results reports with patients:** After you complete a study training session, your patients can begin being recruited and enrolled in the study, at which point you will start to have clinic visits with patient participants. We expect during the approximately 7.5-month period of recruitment and enrollment of patients in this research study, each provider will see, on average, 2-3 patient participants. Before their scheduled visit with you, a trained clinical research team member will collect a hair sample (and in some cases a blood sample) from the patient participant. A team member will take the blood and hair samples to the MedViewer lab for testing. Once MedViewer results are generated, the trained team member will deliver either a printed copy of the MedViewer reports to you at the ID Clinic or an electronic copy via secure email. You should not make clinical changes or decisions based on the results of the MedViewer test. During the regularly scheduled clinic appointment you and your patient will be able to review the MedViewer reports together. This discussion is expected to take several minutes. If for any reason you do not discuss the MedViewer reports with your patient during the routine clinic visit, you will have the option to schedule a follow-up appointment to discuss the MedViewer reports within four weeks of hair sample collection.
3. **Completing a brief post-visit questionnaire:** After your clinic visit with each patient participant you will be asked to complete a brief 5-item questionnaire that can be completed immediately or at any point during that workday. The purpose of the post-visit questionnaire is to identify whether you were able to discuss the MedViewer results with your patient and, if so, some aspects of that experience.
4. **In-depth interview:** All participating providers will also be asked to complete an in-depth interview that will last approximately 30-45 minutes. This will be scheduled at your convenience after you have received MedViewer reports for at least 2 patient participants or after the close of patient enrollment in the study, whichever comes first. The interview will be conducted via videoconferencing or phone call by a trained member of the research team, who will ask you questions about your experience receiving the MedViewer reports and discussing them with patient participants. Specifically, interview topics will explore how useful and feasible you perceived MedViewer to be, your view of patient's reactions to MedViewer, your satisfaction with different aspects of MedViewer, and its effects on clinic flow and your relationship with

patients. The information you provide will be used to improve MedViewer so that it can help other patients in the future.

- To help assure that we get the best understanding possible from your answers, the interview will be audio-recorded with your permission. You will be asked at the end of this consent form to indicate if you give your permission to be recorded during the interview.
- If you do not want us to audio record the interview, we will take notes that will be stored with your other study materials in your study chart instead. We will not write your name or any information that can identify you - we will only write your unique ID number. The notes will be typed and stored on a password-protected server or in in a locked drawer in a secure room to which only the research team has access.
- If you do give us permission to audio record, after the interview is finished, all audio files will be stored on the same secure internal computer network that can only be accessed using a password-protected computer by members of the study team. This audio recording will be transcribed (typed) by a secure professional transcription service. All identifying information will be removed from the transcript. Your name will not be included on the transcript - only a unique participant ID number (an identification number unique to you that only contains your initials and a set of numbers) will be used. Once analysis of the interviews is completed, the digital audio file will be stored on a password-protected secure server.

5. **Endline questionnaire:** At the conclusion of patient enrollment in this study, we will ask you to complete an endline questionnaire. In the event that you must end your participation in the study before the close of patient enrollment, the endline questionnaire may be completed at that time. The questionnaire will assess your thoughts about recommending MedViewer to future patients; your perceived level of confidence to use the MedViewer report; your satisfaction and perceived usefulness for different aspects of the report; and who you think is most appropriate for delivering future MedViewer counseling.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

What are the possible risks or discomforts involved from being in this study?

Questionnaires:

Immediate known risks to participating in the questionnaires include the potential for you to feel uncomfortable or experience anxiety when being asked questions about your patients' medication adherence or about your adherence counseling practices. To minimize these risks the questionnaires are self-administered. You can also stop participating at any time or skip any questions that make you feel uncomfortable.

MedViewer test and report:

It is possible that discussing the MedViewer report may alter patient-provider communication during the clinic visit, such that you or your patient may feel uncomfortable. You may also end up spending extra time with patients in order to discuss MedViewer report, which may prolong the appointment and delay your schedule for the day. As an objective of this study is to assess whether the use of the report will prolong patient visits and whether it proves to be a feasible, useful, and acceptable tool, we may not be able to fully prevent this risk. To minimize it, however, we will obtain feedback after each patient visit about both patient and provider experiences with the tool.

In-depth interviews:

Risks relating to participation in the interviews includes the potential for you to feel uncomfortable or experience anxiety when being asked about your patients' medication adherence, your adherence counseling practices, and your opinions about your experiences using the MedViewer with your patients. To minimize these risks, the study team will conduct the interviews in a neutral and non-judgmental manner. Also, you may stop participating at any time or skip any questions that make you feel uncomfortable.

Social Harms:

There may be social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions you will be asked or discussing the results of the procedures and tests with your patient participants. You may also experience stigma from participating in this research study which could alter your relationships with patients and peers.

Confidentiality:

Participation in research includes the risks of loss of confidentiality. Although the study site makes every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others. We discuss this in more detail below.

There may be risks unknown to the study researchers. Because of this, you are encouraged to report any problems.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will information about you be protected?
Your privacy will be protected in the following ways:

- No study records will contain your name. You will be assigned a unique participant ID number that only contains your initials and a set of numbers. One study investigator will keep the file that connects this number to you on a secure internal computer network. It will be kept separate from the audio files, transcripts, questionnaires and other study records.

- Only the study team will have access to any personally identifiable information about you.

- All interviews will be conducted securely over videoconferencing or by telephone. Study staff will be in a private room with a closed door and will ask that you find a quiet, private area in which to participate in the interview.
- All study records will be stored in a locked drawer in a designated, secure room or electronically on a secure internal computer network or server, that can only be accessed using a password-protected computer by members of the study team
- Audio files from the interviews will be stored on a secure internal computer network or server, accessed using a password-protected computer, that can only be accessed by members of the study team.
- All identifying information will be removed from the transcript. Your name will not be included on the transcript, only a unique participant identification (ID) number will be used.
- If you choose only to have notes taken during the interview, we will only write a unique participant ID number that does not contain any of your identifying information, including your name on the notes. These notes will be stored in a locked drawer in a secure room to which only the research team has access or electronically on a secure internal computer network or server, that can only be accessed using a password-protected computer by members of the study team.
- Neither you, nor any other participant, will be identified in any report or publication about this study.

Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect your personal information. In some cases, your information in this study could be reviewed by representatives of the University, research sponsors, or government agencies such as the National Institutes of Health. This would be for purposes such as quality control or safety.

The study coordinator will keep the signed original consent form and a copy will be given to you.

What is a Certificate of Confidentiality?

To help us protect your privacy, the study team has obtained a Certificate of Confidentiality from the National Institutes of Health (NIH). With this certificate, the researchers cannot be forced to disclose any information that can be used to identify you, even by court subpoena, in any federal, state, local or civil, criminal, administrative, legislative or other proceedings. The researchers would use the certificate to resist any demands for information that would identify you, as described.

The Certificate cannot be used to resist a demand for information from personnel of the United States government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

You should understand that a Certificate of Confidentiality does not prevent you or a member of your family from voluntarily releasing information about yourself or your involvement in this research. If an employer, insurer, or other person receives your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate does not limit the researchers from disclosing information that may include details about child abuse, homicidal or suicidal intent, or other information deemed appropriate. The researchers will not voluntarily release any information about you under this Certificate.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?
At the end of your total participation in the study, you will receive up to $80.00, in the form of a Visa® gift card, for taking part in this study. You will only be paid for the portions of the study that you complete. If you do not complete all of the study activities, your payment will be adjusted as follows:

- You will receive $20.00 for attending the provider training session and completing the baseline questionnaire.
- You will receive $30.00 total for completing the post-visit questionnaires for all MedViewer patient visits you conduct over the duration of the study.
- You will receive $20.00 for completing the in-depth interview.
- You will receive $10.00 for completing the endline questionnaire.

So, if you complete all components of this study you would receive $80.00 in the form of a Visa® gift card, in total.

Will it cost you anything to be in this study?
There will be no cost to you to participate in the study, other than your time.

What if you are a UNC employee?
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

Who is sponsoring this study?
This research is funded by NIH National Institute of Allergy and Infectious Diseases (NIAID). This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

What if you have questions about this study?
You have the right to ask, and have answered, any questions you may have about this research. If

you have questions about the study, complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

What if you have questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

IRB Study # 18-3360
Title of Study: Establishing Novel Antiretroviral Imaging for Hair to Elucidate Non-Adherence (ENLIGHTEN)

Participant's Agreement: I have asked all the questions I have at this time. By giving my verbal consent, I voluntarily agree to participate in this research study. I understand that my participation is voluntary and I can withdraw my consent to participate at any time.

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

Participant's Agreement to be Audio Recorded:

_____ OK to record me during the interview
(Research Team Member Initials)

_____ Not OK to record me during the interview
(Research Team Member Initials)

APPENDIX C. DATA COLLECTION INSTRUMENTS

C.1 PATIENT BASELINE QUESTIONNAIRE

Participant ID __ __ __ __ / __ __ __

PATIENT BASELINE QUESTIONNAIRE

ENLIGHTEN PILOT STUDY

Introduction

Thank you for joining us in the ENLIGHTEN Study!

This survey will ask questions about your health, taking your Human Immunodeficiency Virus (HIV) medicine, called antiretroviral therapy (ART), and your views of MedViewer. When we say MedViewer we are talking about the new investigational test that looks at the amount of ART in your body using hair and the report you will get from your medical provider.

This survey should take about 20 minutes to complete. The information you provide will be kept private. Your name will not be reported alongside any of the information you give us. Please feel free to be open and honest in responding to the questions - there are no right or wrong answers. If at any time you feel uncomfortable, you may skip questions or stop the survey. Please let study staff know if you have questions or need help.

Instructions: First, we'd like to start by asking some general questions about your health. Please mark your responses to the following questions.

SECTION 1: GENERAL HEALTH

1. In general, how would you rate your health?

- ○ Poor
- ○ Fair
- ○ Good
- ○ Very good
- ○ Excellent

- ○ I prefer not to answer the question

2. How long does it take you to get to the UNC ID clinic from your home by your typical mode of transportation (e.g. car, bus, walking, Medicaid van)?

- ○ Less than 30 minutes
- ○ Between 30 minutes and 1 hour
- ○ Between 1 and 2 hours
- ○ More than 2 hours

- ○ I prefer not to answer the question

SECTION 2: YOUR VIEWS ON THE MEDVIEWER VIDEO

Now we'll ask you some questions about the information in the MedViewer video you just watched and your views of the video.

3. Please mark the best answer for each question about the MedViewer test.

a. What is the MedViewer test designed to measure?

i. The amount of ART medicine in the body over time
ii. The amount of HIV virus in the body over time

iii. Don't know
iv. I prefer not to answer the question

b. What kind of sample does the MedViewer test use?

i. A blood sample from your arm
ii. A hair sample from the back of your head

iii. Don't know
iv. I prefer not to answer the question

c. How will your sample be labeled?

i. A unique ID number
ii. Your name

iii. Don't know
iv. I prefer not to answer the question

d. What will happen to your hair sample after it has been analyzed?

i. It will be kept by the research team for additional testing
ii. It will be permanently destroyed

iii. Don't know
iv. I prefer not to answer the question

e. How can you and your provider use your MedViewer report?

i. To see how you are doing with taking your ART medicine
ii. To know how much HIV virus is in your body
iii. Don't know
iv. I prefer not to answer the question

f. Now we want to ask you some questions about how helpful you found the video.
In terms of <u>how much the video helped you understand what would happen</u> to you if you took the MedViewer test, would you say the video was:

- ○ Extremely helpful
- ○ Very helpful
- ○ Moderately helpful
- ○ A little helpful
- ○ Not at all helpful

i. I prefer not to answer the question

g. In terms of how much the video <u>helped you decide whether or not you wanted to take the MedViewer test</u>, would you say the video was:

- ○ Extremely helpful
- ○ Very helpful
- ○ Moderately helpful
- ○ A little helpful
- ○ Not at all helpful

i. I prefer not to answer the question

h. In terms of how much the video <u>helped you decide whether or not to be in this study</u>, would you say the video was:

- ○ Extremely helpful
- ○ Very helpful
- ○ Moderately helpful
- ○ A little helpful
- ○ Not at all helpful

i. I prefer not to answer the question

i. In terms of how much the video <u>helped you understand how to interpret your MedViewer report,</u> would you say the video was:

- ○ Extremely helpful

- ○ Very helpful
- ○ Moderately helpful
- ○ A little helpful
- ○ Not at all helpful

i. I prefer not to answer the question

j. In terms of how much the video <u>helped you feel more comfortable about having the MedViewer test</u>, would you say the video was:

- ○ Extremely helpful
- ○ Very helpful
- ○ Moderately helpful
- ○ A little helpful
- ○ Not at all helpful

i. I prefer not to answer the question

k. In terms of how much you enjoyed watching the video, would you say you:

- ○ Enjoyed it A Lot
- ○ Enjoyed it Some
- ○ Enjoyed it A Little
- ○ Did not Enjoy it at All

- ○ I prefer not to answer the question

4. How confident are you that you will be able to understand the information on your MedViewer report?

- ○ Not at all confident
- ○ A little bit confident
- ○ Moderately confident
- ○ Very confident
- ○ Completely confident

- ○ I prefer not to answer the question

<u>SECTION 3: ABOUT YOU</u>

5. How would you describe your sexual orientation?

- ○ Straight or heterosexual
- ○ Lesbian, gay, or homosexual
- ○ Bisexual
- ○ Queer

- O Asexual
- O Pansexual
- O Other (please specify): _____

- O I prefer not to answer the question.

6. What is your CURRENT marital status?

- O Married
- O Widowed
- O Divorced or separated
- O Living together, but not married
- O Single, never married

- O I prefer not to answer the question.

7. What is the HIGHEST grade or level of school you have completed or the highest degree you have received?

- O Middle school (junior high school) or less
- O Some high school, no diploma
- O High school graduate / GED or equivalent
- O Junior (2-year) college
- O Technical / Trade / Vocational school
- O Some college (4-year college or university)
- O College graduate (4-year college or university)
- O Advanced Degree (After a 4-year college)

- O I prefer not to answer the question.

8. Which of the following best describes the total yearly income from all legal sources before taxes in your household IN THE PAST YEAR?

- ○ Under $5,000
- ○ $5,000-10,000
- ○ >$10,000-20,000
- ○ >$20,000-$50,000
- ○ >$50,000-$100,000
- ○ >$100,000
- ○ I prefer not to answer the question.

9. What is your current work status? (Check ALL that apply)

- ☐ Employed part-time
- ☐ Employed full-time
- ☐ Unemployed
- ☐ Take care of the home and/or family
- ☐ Student
- ☐ Retired
- ☐ Unable to work due to disability
- ☐ Other (specify) _____
- ○ I prefer not to answer the question.

10. What kind of health insurance coverage do you have? (Check ALL that apply)

- ☐ Medicaid
- ☐ Medicare
- ☐ Military insurance coverage

☐ Private insurance provided by employer or spouse/partner's employer

☐ Private insurance purchased by you directly (from an insurance company or through a health insurance exchange created as part of the Affordable Care Act/Obamacare)

☐ None

☐ Other: _____

○ I prefer not to answer the question.

11. How do you pay for your ART medicines? (Check ALL that apply)

☐ AIDS Drug Assistance Program (ADAP)/ Ryan White

☐ Medicaid

☐ Medicare Part D

☐ Military insurance coverage

☐ A private health insurance plan (provided by an employer or purchased by you directly, either from an insurance company or through a health insurance exchange)

☐ I pay for my ART medicines partly out-of-pocket

☐ I pay for my ART medicines completely out-of-pocket

☐ Other: _____

○ I prefer not to answer the question.

SECTION 4: Taking Your ART

12. Now thinking about the ART medicine you are taking…

a. How sure are you that you can or cannot take the right amounts of medicine at the right times over the next 30 days?

i. Very sure I cannot
ii. Somewhat sure I cannot
iii. Neither sure nor unsure
iv. Somewhat sure I can
v. Very sure I can

vi. I prefer not to answer the question

b. How sure are you that you can or cannot do better with taking the right amounts of medicine at the right times over the next 30 days?

i. Very sure I cannot
ii. Somewhat sure I cannot
iii. Neither sure nor unsure
iv. Somewhat sure I can
v. Very sure I can

vi. I do not think I need to do better
vii. I prefer not to answer the question

c. How sure are you that you can or cannot take the right amounts of your medicine at the right times even if you were very tempted not to over the next 30 days?

i. Very sure I cannot
ii. Somewhat sure I cannot
iii. Neither sure nor unsure
iv. Somewhat sure I can
v. Very sure I can

vi. I prefer not to answer the question

13. Would you say that in the next 30 days, taking your ART is:

- ○ Not at all important to you
- ○ A little important to you
- ○ Very important to you
- ○ More important to you than just about anything

- ○ I prefer not to answer the question

14. **The following question relates to the reasons why you would take your ART every day at the right times.**

Different people have different reasons for taking their ART every day at the right times. We want to know how true each of the following reasons is for you. Please indicate the extent to which each reason is true for you, using the 7-point scale provided, where 1 means not true at all and 7 means very true.

The reason I would take my ART every day at the right times is:	**1 not true at all**	**2**	**3**	**4 somewhat true**	**5**	**6**	**7 very true**
a. Because I feel that I want to take responsibility for my own health.	○	○	○	○	○	○	○
b. Because I would feel guilty or ashamed of myself if I did not take my ART regularly.	○	○	○	○	○	○	○
c. Because I personally believe it is the best thing for my health.	○	○	○	○	○	○	○
d. Because others would be upset with me if I did not.	○	○	○	○	○	○	○
e. I really don't think about it.	○	○	○	○	○	○	○
f. Because I have carefully thought about it and believe it is very important for many aspects of my life.	○	○	○	○	○	○	○
g. Because I would feel bad about myself if I did not take my ART regularly.	○	○	○	○	○	○	○
h. Because it is an important choice I really want to make.	○	○	○	○	○	○	○
i. Because I feel pressure from others to do so.	○	○	○	○	○	○	○
j. Because it is easier to do what I am told than think about it.	○	○	○	○	○	○	○
k. Because it is consistent with my life goals.	○	○	○	○	○	○	○
l. Because I want others to approve of me.	○	○	○	○	○	○	○
m. Because it is very important for being as healthy as possible.	○	○	○	○	○	○	○
n. Because I want others to see I can do it.	○	○	○	○	○	○	○
o. I don't really know why.	○	○	○	○	○	○	○

Instructions for Question 15 (remote): *Please think of all the ART medicines you take together to answer the next question.*

15 (Remote). Percentage antiretroviral (ART) medicine taken in the past 30 days

Please tell us your best guess about how much of your current antiretroviral medication you have taken in the **past 30 days**. Please write the percentage you have taken — you can write any number from **0% to 100%.**

0% means you have **taken none of your current antiretroviral medication**, **50%** means you have **taken half your current antiretroviral medication**, **100%** means that you have **taken every single dose** of your current antiretroviral medication in the past 30 days.

Percentage ART medicine taken in the past 30 days: _____%

C.2 PATIENT POST-VISIT QUESTIONNAIRE

Participant ID __ __ __ __ / __ __ __

Patient Post-Visit Questionnaire

ENLIGHTEN Pilot Study

Instructions: ***Please read each question and mark your response(s).***

1. **Who <u>showed</u> you a copy of your MedViewer report at your visit?**

   ○ No one, I did not see a copy of my MedViewer report→ Skip to Question 2

   ○ My usual HIV medical provider

   ○ The clinic pharmacist

   ○ A clinic nurse

   ○ Someone else, please specify _____

   a. **Did this person <u>discuss</u> your MedViewer report with you at your visit today?**

○ Yes

○ No

b. How satisfied were you with the discussions you had with this provider during today's MedViewer visit, compared with a usual visit?

○ Much less satisfied than usual

○ Somewhat less satisfied than usual

○ Neither more nor less satisfied than usual

○ Somewhat more satisfied than usual

○ Much more satisfied than usual

○ I prefer not to answer the question.

2. How likely would you be to use MedViewer again in the future if your medical provider offered it to you?

- ○ Definitely would not use
- ○ Probably would not use
- ○ Probably would use
- ○ Definitely would use

- ○ Prefer not to answer

3. How likely would you be to recommend using MedViewer to others if it were available?

- ○ Definitely would not recommend
- ○ Probably would not recommend
- ○ Probably would recommend
- ○ Definitely would recommend

- ○ Prefer not to answer

Now we'd like to ask you some questions about how you felt about your MedViewer report. (Skip this section (questions 4 to 6) if you did not receive the MedViewer report.)

4. How difficult or easy was it to understand the information in the MedViewer report?

- ○ Very difficult
- ○ Somewhat difficult
- ○ Somewhat easy
- ○ Very easy
- ○ I prefer not to answer the question

5. How closely did the information in the MedViewer report line up with how you remember taking your ART medicine?

- ○ Did not line up at all with how I remember taking my ART medicine
- ○ Lined up a little with how I remember taking my ART medicine
- ○ Lined up pretty well with how I remember taking my ART medicine
- ○ Lined up exactly with how I remember taking my ART medicine
- ○ I prefer not to answer the question

6. How useful do you think the MedViewer report is for helping you <u>feel motivated</u> to take your ART medicine over the next 30 days?

- ○ Not at all useful to you
- ○ A little bit useful
- ○ Somewhat useful
- ○ Very useful
- ○ Extremely useful to you
- ○ I prefer not to answer the question

Now we'd like to ask you a couple questions about your satisfaction with different aspects of the MedViewer test. Please tell us how you would rate the following parts of the MedViewer experience:

7. **How satisfied or unsatisfied are you with the <u>waiting time</u>, from when your hair sample was collected to when you saw your medical provider?**

   - ○ Very unsatisfied
   - ○ Somewhat unsatisfied
   - ○ Neither satisfied nor unsatisfied
   - ○ Somewhat satisfied
   - ○ Very satisfied
   - ○ I prefer not to answer the question.

8. **How satisfied or unsatisfied are you with the <u>experience of having your hair plucked</u>?**

   - ○ Very unsatisfied
   - ○ Somewhat unsatisfied
   - ○ Neither satisfied nor unsatisfied
   - ○ Somewhat satisfied
   - ○ Very satisfied
   - ○ I prefer not to answer the question.

9. **How satisfied or unsatisfied are you with the <u>discussion you had with your medical provider today about your ART adherence</u> using the MedViewer report? *(Skip if you did not receive the MedViewer report.)***

   - ○ Very unsatisfied
   - ○ Somewhat unsatisfied
   - ○ Neither satisfied nor unsatisfied
   - ○ Somewhat satisfied

○ Very satisfied

○ I prefer not to answer the question.

10. How satisfied or unsatisfied are you with the <u>format of the MedViewer report?</u> *(Skip if you did not receive the MedViewer report.)*

○ Very unsatisfied

○ Somewhat unsatisfied

○ Neither satisfied nor unsatisfied

○ Somewhat satisfied

○ Very satisfied

○ I prefer not to answer the question.

Finally, we have a couple of questions about how you feel about taking your ART.

11. Thinking about the ART you are taking…

a. How sure are you that you can or cannot take <u>the right amounts of medicine at the right times</u> over the <u>next 30 days</u>?

○ Very sure I cannot

○ Somewhat sure I cannot

○ Neither sure nor unsure

○ Somewhat sure I can

○ Very sure I can

○ I prefer not to answer the question.

b. How sure are you that you can or cannot <u>do better</u> with taking the right amounts of medicine at the right times over the <u>next 30 days</u>?

○ Very sure I cannot

○ Somewhat sure I cannot

○ Neither sure nor unsure

- ○ Somewhat sure I can
- ○ Very sure I can

- ○ I do not think I need to do better
- ○ I prefer not to answer the question

c. How sure are you that you can or cannot take the right amounts of your medicine at the right times <u>even if you were very tempted not</u> to over the <u>next 30 days</u>?

- ○ Very sure I cannot
- ○ Somewhat sure I cannot
- ○ Neither sure nor unsure
- ○ Somewhat sure I can
- ○ Very sure I can

- ○ I prefer not to answer the question

12. Would you say that in the next 30 days, taking your ART is:

- ○ Not at all important to you
- ○ A little important to you
- ○ Very important to you
- ○ More important to you than just about anything

- ○ I prefer not to answer the question.

13. Imagine that the MedViewer test became available as a routine clinical test (in addition to viral load testing). What is the most you would be willing to pay out of pocket to get the MedViewer test?

- ○ None. I am not willing to pay out of pocket for MedViewer.
- ○ $10.00
- ○ $25.00
- ○ $50.00
- ○ $100.00
- ○ $150.00
- ○ $250.00

○ Other

○ Don't know

○ I prefer not to answer the question.

You have reached the end of the survey. Thank you for your time and attention.

C.3 PATIENT IN-DEPTH INTERVIEW GUIDE

Participant ID __ __ __ __ / __ __ __

Patient In-Depth Interview Guide

ENLIGHTEN Pilot Study

Introduction: Thank you for meeting with me today. During our discussion, I would like to ask your opinions about MedViewer and about your first MedViewer study visit. As you may remember, MedViewer is the new test to measure the amount of ART medicine in the body by testing hair. There are no right or wrong answers to the questions I will ask you. Your opinion will help us learn more about how MedViewer can help doctors and people living with HIV. Everything you say here will be kept anonymous, and nothing you say during this interview will affect your care at the clinic. Please feel free to interrupt me or let me know if you have questions at any time. This discussion should last about 45-60 minutes.

Interview Questions

1. To begin, please tell me about the day you came in for your MedViewer visit. What was that like?

Section 1: Intervention components

As I you may remember from your first study visit, there were a few study activities you did on the day you had the MedViewer test. You watched a short video to give you information about MedViewer. You gave a hair sample for MedViewer testing. And you probably received the MedViewer report and talked about it with your provider. I'd like to ask you about each of those things in more detail.

2. Let's begin with the video since it was the first thing you did. Generally, what did you think about the video?

a. What did you like about it?
b. What would you change about it?
c. How useful or not useful do you think the video was?
d. What additional information would you have wanted in the video?

Thank you. Now, let's talk about how it was to have a hair sample collected for MedViewer testing and the time it took to receive the results.

3. Generally, what did you think about having your hair sample collected for MedViewer testing?

    a. How did it feel to have your hair plucked?
    b. What would you change about the collection procedure, if anything?
    c. How did you feel about having your hair sent to the lab for testing?
    d. How willing or unwilling would you be to provide a hair sample for MedViewer in the future? Please explain.
    e. What concerns did you have about hair collection and MedViewer testing, if any?

4. How did you feel about the amount of time you had to wait to receive your MedViewer results? Please explain.

    a. How long did you wait?
    b. How willing or unwilling would you be to wait that long again in the future for MedViewer results? Please explain.
    c. Is there anything that would make it easier for you to wait that long?
    d. If dissatisfied, how long would you be willing to wait?

Section 2: MedViewer results report

Thank you. Now, I'd like to spend some time talking about the MedViewer report.

5. In your own words, what is the purpose of the MedViewer report?

6. What did you like about the report?

7. What did you not like about the report?

8. In the MedViewer report you received from your provider, how much do you feel the information in the report lined up with how you actually took your ART medicine?
    a. Did the MedViewer report show what you expected it would? How/why?
    b. In what ways was it different than how you actually took your ART medicine?
    c. How much did specific dates marked as missed doses on the report match up with the dates you remembered missing ART doses?

This is a sample report – these are not yours or anyone else's actual results. But this report does have the same features. (*Show sample report but don't describe the report – let participant describe it first*.)

9. Using your own words, please describe this SAMPLE results report to me.

    a. What do the colors mean?
    b. What do the dates represent?
    c. What are these boxes of text?

10. Now I'd like to hear about your opinion of the report.
    a. What parts of the report are easy to understand?
    b. What parts of the report are more difficult to understand? Why?
    c. What changes would you make to the way the report looks or the information provided? (*Specify if need be that we are asking about the REPORT not these specific RESULTS.*)

Section 3: Results discussion and effects

Thank you. Let's talk about what it was like to discuss your MedViewer results report with your provider and any changes in medication-taking strategies this discussion might have led to.

11. Who provided your MedViewer report to you?
    a. How did you feel about having this person deliver the results to you?
    b. Is there someone else you would have preferred? Please explain.
    c. In what ways was your conversation with your provider different than a typical visit?

12. Tell me about the conversation you and your provider had about your MedViewer report.

    a. What did you and provider talk about?
    b. How was your conversation different or the same as a typical visit?
    c. How much did you like or not like having this conversation with your provider?
    d. What about the MedViewer conversation was helpful? What was not helpful?
    e. What did you learn about how adherent you were to your ART medicine?
    f. Were there any missed days/periods that surprised you?
    g. How much did MedViewer results help you remember reasons you missed doses?
    h. What were reasons for missing doses that you and your provider talked about?

13. How much did you and your provider talk about strategies or ways you could make sure you take your ART medicine at the right times?
    a. Tell me about the strategies you identified?
    b. Have you followed the strategies you identified with your provider? Why or why not?
    c. Do you think having more MedViewer tests, like the one you had that day, could help you to improve/continue these using these strategies? Please explain.

14. In general, how did MedViewer affect your motivation to take your ART medicine at the right days/times, if at all?

    a. How did the MedViewer report contribute your motivation?
    b. How did the discussion with your provider, using the MedViewer report, contribute to your motivation?

c. How would MedViewer affect your motivation if you used it on a regular basis?
d. How could the MedViewer report or discussion be more motivating for you?

15. How have you changed how you take your ART medicine since the MedViewer test?
    a. How useful do you think MedViewer has been in helping actually take your doses regularly and on time? Please explain (why/how).

Section 4: Additional thoughts about future MedViewer use
Thank you. Let's talk now about your thoughts about using MedViewer in the future.

16. Imagine that a medical provider recommended MedViewer to you again in the future, what are the main reasons you would or wouldn't take the MedViewer test?

17. If MedViewer became a regular part every visit with your provider, how would that affect your relationship with your provider? Please explain.

18. If you get the MedViewer test and report regularly during your visits, what effect do you think it would have on your adherence to your ART medicine?

Closing question:

19. Do you have anything else you'd like to share regarding your thoughts about MedViewer?

Conclusion: Thank you for your time and thoughts today. This has been very helpful.

C.4 PATIENT ENDLINE QUESTIONNAIRE

Participant ID __ __ __ __ / __ __ __

PATIENT ENDLINE QUESTIONNAIRE

ENLIGHTEN PILOT STUDY

Instructions: Please read each question and mark your response(s) in the appropriate space.

1. **How useful do you think having the MedViewer test was for:**
    a. **Helping you <u>feel motivated</u> to take your ART medicine over the LAST 30 days?**

○ Not at all useful to you

○ A little bit useful

○ Somewhat useful

○ Very useful

○ Extremely useful to you

○ I prefer not to answer the question

b. Helping you take your ART medicine as prescribed over the LAST 30 days?

○ Not at all useful to you

○ A little bit useful

○ Somewhat useful

○ Very useful

○ Extremely useful to you

○ I prefer not to answer the question

c. Helping you to develop skills and strategies to take your ART medicine over the LAST 30 days?

○ Not at all useful to you

○ A little bit useful

○ Somewhat useful

○ Very useful

○ Extremely useful to you

○ I prefer not to answer the question

2. **Thinking about the ART you are taking…**
   a. **How sure are you that you can take <u>the right amounts of medicine at the right times</u> over the <u>next 30 days</u>?**

- O Very sure I cannot
- O Somewhat sure I cannot
- O Neither sure nor unsure
- O Somewhat sure I can
- O Very sure I can
- O I prefer not to answer the question.

   b. **How sure are you that you can <u>do better</u> with taking the right amount of medicine at the right times over the <u>next 30 days</u>?**

- O Very sure I cannot
- O Somewhat sure I cannot
- O Neither sure nor unsure
- O Somewhat sure I can
- O Very sure I can
- O I do not think I need to do better
- O I prefer not to answer the question

   c. **How sure are you that you can take the right amount of your medicine at the right times <u>even if you were very tempted not</u> to over the <u>next 30 days</u>?**

- O Very sure I cannot
- O Somewhat sure I cannot
- O Neither sure nor unsure
- O Somewhat sure I can
- O Very sure I can
- O I prefer not to answer the question

3. **Would you say that in the next 30 days, taking your ART is:**

- O Not at all important to you
- O A little important to you
- O Very important to you

○ More important to you than just about anything

○ I prefer not to answer the question.

4. **Imagine that the MedViewer test became available as a routine clinical test (in addition to viral load testing). What is the most you would be willing to pay out of pocket to get the MedViewer test?**

○ None. I am not willing to pay out of pocket for MedViewer.

○ $10.00

○ $25.00

○ $50.00

○ $100.00

○ $150.00

○ $250.00

○ Other

○ Don't know

○ I prefer not to answer the question.

Instructions for Question 5 (remote): Please think of all the ART medicines you take together to answer the next question.

5 (Remote). Percentage antiretroviral (ART) medicine taken in the past 30 days

Please tell us your best guess about how much of your current antiretroviral medication you have taken in the **past 30 days**. Please write the percentage you have taken — you can write any number from **0% to 100%.**

0% means you have **taken none of your current antiretroviral medication**, **50%** means you have **taken half your current antiretroviral medication**, **100%** means that you have **taken every single dose** of your current antiretroviral medication in the past 30 days.

Percentage ART medicine taken in the past 30 days: _____%

C.5 PROVIDER POST-TRAINING (BASELINE) QUESTIONNAIRE

Participant ID __ __ __ __ / __ __ __

Provider Post-Training Questionnaire

ENLIGHTEN Pilot Study

Instructions: Please respond to each question by selecting or writing in your response(s) in the appropriate space.

1. **How would you rate the overall quality of this training on a scale of 1 (poor) to 5 (excellent)?**
   - ○ 1- Poor
   - ○ 2 - Fair
   - ○ 3 - Good
   - ○ 4 - Very Good
   - ○ 5 - Excellent

2. **How would you describe the amount of time used for the training?**
   - ○ Too little
   - ○ Just right
   - ○ Too much

2. **After completing this training, <u>how confident are you in your ability to</u>:**
   a. **Accurately provide a brief description of the ENLIGHTEN pilot study?**
      - ○ Not at all confident
      - ○ Slightly confident
      - ○ Moderately confident
      - ○ Very confident
      - ○ Completely confident

   b. **Interpret patient and provider MedViewer reports?**
      - ○ Not at all confident
      - ○ Slightly confident
      - ○ Moderately confident
      - ○ Very confident
      - ○ Completely confident

   c. **Explain MedViewer reports to patients?**

- ○ Not at all confident
- ○ Slightly confident
- ○ Moderately confident
- ○ Very confident
- ○ Completely confident

d. Utilize MedViewer reports to counsel patients on adherence?

- ○ Not at all confident
- ○ Slightly confident
- ○ Moderately confident
- ○ Very confident
- ○ Completely confident

3. **After completing this training, how would you rate your level of knowledge of:**

a. Study activities for <u>providers?</u>

- ○ Not at all knowledgeable
- ○ Slightly knowledgeable
- ○ Moderately knowledgeable
- ○ Very knowledgeable
- ○ Fully knowledgeable

b. Study activities for <u>patients?</u>

- ○ Not at all knowledgeable
- ○ Slightly knowledgeable
- ○ Moderately knowledgeable
- ○ Very knowledgeable
- ○ Fully knowledgeable

4. What did you like about the training?

_____
_____
_____
_____

5. **What about the training could have been improved?**

_____
_____
_____
_____

6. **Would you like to receive additional information about MedViewer or the ENLIGHTEN Pilot Study prior to seeing patients for their study visits?**
   - ○ Yes…Go to 6a and 6b
   - ○ No…END SURVEY

   a. **What additional information would you like to receive? (Select ALL that apply)**

      Information about the MedViewer technology
      Information about the purpose of the ENLIGHTEN Pilot Study
      Guidance on interpreting patient and provider MedViewer reports
      Suggestions for using MedViewer reports to counsel patients on adherence
      Guidance on using the communication aids
      Information on patient study activities
      Information on provider study activities
      Other: _____

   b. **In what format would you like to receive that information? (Select ALL that apply)**

      Additional group training session
      One-on-one training/conversation
      Other: _____

C.6 PROVIDER POST-VISIT QUESTIONNAIRE

Participant ID __ __ __ __ / __ __ __

Provider Post-Visit Questionnaire

ENLIGHTEN Pilot Study

Instructions: Thinking about the most recent patient you saw for a MedViewer visit, please respond to the following questions using a BLACK-INK PEN. Place the completed questionnaire in the blue deposit box. Thank you!

Date of patient MedViewer visit: ____ /____ /_____

dd *mmm* *yyyy*

1. **Did you show your patient his/her/their MedViewer report during the visit?**
   - ○ No….Go to Question 1a
   - ○ Yes…Go to Question 2

   1a. What were the reasons you did not show your patient his/her/their MedViewer report? (Mark all that apply)

   Did not receive a MedViewer report for this patient before the visit
   No time to show the MedViewer report during the visit
   The patient had other more pressing medical issues to address
   I did not think it would be beneficial to show the report. Please explain:
   _____
   Technical difficulties. Please explain: _____
   Other: _____

   END SURVEY

2. **How much time did you spend during the visit discussing the MedViewer report with your patient?**
   a. 0 minutes - I did not discuss the MedViewer report with the patient…Go to Question 2a
   b. < 1 minute…Go to Question 3
   c. 2-5 minutes…Go to Question 3
   d. 5-10 minutes…Go to Question 3
   e. 10-15 minutes…Go to Question 3
   f. >15 minutes…Go to Question 3

   2a. What were the reasons you did not discuss the MedViewer report with your patient during the visit? (Mark all that apply)

   No time to discuss the MedViewer report during the visit
   The patient was not interested in discussing the MedViewer report
   The patient had other more pressing medical issues to address

I did not think it would be beneficial to discuss the report. Please explain: _____

Technical difficulties. Please explain: _____

Other: _____

END SURVEY

3. **How useful would you say the MedViewer report was for helping you <u>understand this patient's ART adherence</u>?**
    a. Not at all useful
    b. Slightly useful
    c. Moderately useful
    d. Very useful
    e. Extremely useful

    f. I prefer not to answer the question

4. **How useful would you say the MedViewer report was for helping you <u>have a productive conversation with this patient about their ART adherence</u>?**
    a. Not at all useful
    b. A little bit useful
    c. Moderately useful
    d. Very useful
    e. Extremely useful

    f. I prefer not to answer the question

5. **How well would you say your patient understood the information in their MedViewer report?**
    a. Not at all well
    b. Slightly well
    c. Moderately well
    d. Very well
    e. Excellently

    f. I prefer not to answer the question

Participant ID __ __ __ __ / __ __ __

Provider In-Depth Interview Guide

ENLIGHTEN Pilot Study

Introduction

Thank you for meeting with me today to discuss your experience with the MedViewer intervention and using MedViewer reports with patients at the ID Clinic. Specifically, I am interested in hearing your thoughts on: 1) the usefulness of the MedViewer reports and discussions with your patients using the reports; 2) the extent to which the MedViewer intervention fits within routine clinical practice; and 3) future MedViewer use. Please feel free to ask questions at any time. There are no right or wrong answers. Your feedback will help us determine the future direction of MedViewer.

Do you have any questions before we get started?

Note: **Bold** indicates essential topics to be covered during the interview.

Interview Questions

Section 1: Overall opinions

First, I am interested in hearing your overall experience using MedViewer.

1. **To start, please walk me through a typical visit with a patient you saw for a MedViewer visit.**

Section 2: Use of MedViewer reports

Thank you. Now, we'll talk more about the MedViewer reports. You may remember that you received two versions of the report for each patient. One was the "provider" report in a

bar graph format showing the concentration of ART medication detected each day. The other was the "patient" report that was in a calendar format showing whether or not a missed dose of medication was detected.

2. **What do you think of the MedViewer reports for *providers?*** *(Show or describe these to the provider as a reminder.)*

   Probes:
   - **a. How did you use these, if at all?**
   - **b. What aspects of the report were helpful?**
   - **c. What aspects of the report were difficult to understand or use?**
   - **d. What changes would you suggest?**

3. **What do you think about the MedViewer reports for *patients?*** *(Show or describe these to the provider as a reminder.)*

   Probes:
   - **a. How did you use these reports, if at all?**
   - **b. How well did you think your patients generally understood the reports?**
   - **c. From your perspective, what aspects of these reports were helpful for patients?**
   - **d. From your perspective, what aspects of these reports were difficult to understand for patients?**
   - **e. What changes would you suggest to further facilitate patient understanding of the report?**

<u>Section 3: Impact of MedViewer report on adherence counseling discussions</u>

Now I'd like to learn about the discussions you had with your patients using the MedViewer reports.

4. **Please tell me about the discussions you had with patients using the MedViewer reports.**

   Probes:
   - **a. How did you explain the results to your patients?**
   - b. How did patients respond to seeing their MedViewer results?
   - c. Was there any aspect of the results you had difficulty discussing with your patients?
   - d. If applicable, how did your discussions differ for patients whose results showed missed doses vs. patients whose results showed none?

5. **In what ways did your discussions using MedViewer differ from typical discussions you have with patients about adherence?**

   Probes:
   a. What things did you discuss with the MedViewer results that you might not otherwise have discussed?
   b. Do you feel like visits using MedViewer were more beneficial or less beneficial for your patients than normal visits? What are the reasons for that?

6. **Now thinking specifically about using MedViewer reports for adherence counseling, overall, how did you find the MedViewer reports for counseling your patients about their adherence?**
   a. **How did you use the MedViewer results to encourage adherence, if at all, in terms of problem solving, motivation, or behavioral skills?**

7. **If applicable, if you have seen any of your MedViewer patients since they had a MedViewer study visit, did you observe or discuss any changes in the patient's adherence related to their receipt of MedViewer results?**

   Probes:
   a. Any observed or reported changes in adherence?
   b. Any observed or reported changes in motivation or adherence strategies that you talked about?

<u>Section 4: Potential impacts of routine/future MedViewer use</u>

Thank you. I'd like to hear your thoughts about using MedViewer if it became available for *routine* use.

8. **If MedViewer were available for routine use in the future, to what extent do you think it could be a useful tool to help your patients with adherence? What are the reasons for that?**

   Probes:
   a. **How would you decide whether to recommend MedViewer to patients?**
   b. **Are there patients for whom you would be more likely to recommend MedViewer? Please explain.**
   c. How useful would routine use be for promoting adherence *motivation* among your patients?
   d. How useful would routine use be for promoting *skills* to avoid missed doses? Are there certain types of patients for whom it might be more or less useful?

9. **If MedViewer were to be used routinely in the clinic, how do you anticipate it would affect your communication or relationships with patients, if at all?**

   Probes:
   - **a. For what types of patients might it enhance communication or relationships?**
   - b. Are there specific patients for whom it is less worthwhile or even harmful? If so, please explain.

10. **If MedViewer became available for routine use, what type of provider (such as the physician, pharmacist, nurse or social worker, etc.) do you think should deliver the MedViewer report to patients for the purposes of adherence counseling? Why?**

Section 5: Training session and user support

Next, I'd like to hear your thoughts on the provider training session and communication aid and FAQ you received.

11. **Now that you have used the MedViewer report with patients, how well do you think the study training session prepared you to use MedViewer reports with patients?**

    Probes:
    - **a. What changes would you suggest for the training session, if any?**
    - b. How well did the training prepare you to interpret the MedViewer results?
    - c. What further training/information would you have desired, if anything?

12. **How did you use the communication aid and FAQ sheet, if at all?** (*Show or describe these to the provider as a reminder.*)

    Probes:
    - a. To what extent did these materials help you interpret MedViewer reports and explain them to patients?
    - b. How helpful were these materials as a guide for counseling patients on adherence using MedViewer reports?
    - c. What aspects of the communication aid and FAQ were helpful?
    - d. What aspects about the communication aid and FAQ were difficult to understand or use?
    - e. What would you change about the communication aid and FAQ?

Section 6: MedViewer impact on clinic flow

Next, I'd like to hear your thoughts on the impact of the MedViewer intervention on clinic flow.

13. **On days when you had MedViewer visits with patients, how did MedViewer activities affect your overall clinic flow for the day?**

    Probes:
    a. How easy or difficult was it to integrate MedViewer into your activities for the day?
    b. Did you experience or hear about any instances where the MedViewer intervention interrupted clinic flow? If yes, can you tell me more about that?

14. **Logistically speaking, how well did discussing the MedViewer report with your patients fit into visits with them?**

    Probes:
    a. How about for visits conducted in the clinic versus virtually?
    b. What made it easy to deliver and discuss reports with patients? What made it challenging?

Section 6: Concluding remarks

Just a few final questions:

15. **Are there any other changes or improvements we have not discussed yet that could be made to MedViewer reports or how they are used?**

16. **From your perspective, is there anything else promising or beneficial about continued MedViewer use that we have not previously discussed?**

17. **Do you have any other questions or final comments about the MedViewer intervention that you would like to share?**

Conclusion: Thank you for sharing your thoughts and time today.

C.8 PROVIDER ENDLINE QUESTIONNAIRE

ENLIGHTEN Pilot Study

Instructions: Please indicate your responses to the following questions.

1. **After completing your MedViewer visits with patients, how confident are you in your ability to:**
   a. **Explain the MedViewer report to patients?**
      - ○ Not at all confident
      - ○ Slightly confident
      - ○ Moderately confident
      - ○ Very confident
      - ○ Completely confident
      - ○ Prefer not to answer

   b. **Use the MedViewer report to counsel patients on adherence?**
      - ○ Not at all confident
      - ○ Slightly confident
      - ○ Moderately confident
      - ○ Very confident
      - ○ Completely confident
      - ○ Prefer not to answer

2. **How did using MedViewer with patients affect your relationships with them?**
   - ○ Very negatively
   - ○ Somewhat negatively
   - ○ No change
   - ○ Somewhat positively
   - ○ Very positively
   - ○ Prefer not to answer

3. **How would you rate your overall satisfaction with <u>adherence counseling discussions</u> using MedViewer?**
   - ○ Very dissatisfied
   - ○ Somewhat dissatisfied

- ○ Neither satisfied nor dissatisfied
- ○ Somewhat satisfied
- ○ Very satisfied

- ○ Prefer not to answer

4. In general, how useful would you say MedViewer is for the following things?

a. Promoting <u>ART adherence</u> among your patients

- ○ Not at all useful
- ○ Slightly useful
- ○ Somewhat useful
- ○ Very useful
- ○ Extremely useful

- ○ Prefer not to answer

b. <u>Developing strategies</u> with your patients to overcome adherence challenges

- ○ Not at all useful
- ○ Slightly useful
- ○ Somewhat useful
- ○ Very useful
- ○ Extremely useful

- ○ Prefer not to answer

c. <u>Motivating</u> your patients to adhere to ART after a clinic visit

- ○ Not at all useful
- ○ Slightly useful
- ○ Somewhat useful
- ○ Very useful
- ○ Extremely useful

- ○ Prefer not to answer

5. How satisfied are you with the <u>content</u> of the MedViewer report for *providers*?

- ○ Very dissatisfied
- ○ Somewhat dissatisfied
- ○ Neither satisfied nor dissatisfied

- ○ Somewhat satisfied
- ○ Very satisfied

- ○ Prefer not to answer

6. How satisfied are you with the format of the MedViewer report for *patients*?

- ○ Very dissatisfied
- ○ Somewhat dissatisfied
- ○ Neither satisfied nor dissatisfied
- ○ Somewhat satisfied
- ○ Very satisfied

- ○ Prefer not to answer

7. If MedViewer were available for routine use, how likely would you be to recommend it to some of your patients in the future?

- ○ Definitely would not recommend
- ○ Likely would not recommend
- ○ Likely would recommend
- ○ Definitely would recommend

- ○ Prefer not to answer

8. If MedViewer were available for routine use, what would be your recommendation for the type of clinician that should discuss MedViewer results with patients? (Mark all that apply)

MD/NP/PA-C
Pharmacist
Nurse
Other: _____

APPENDIX D. BENCHMARKING

D.1 BENCHMARKING OF IR-MALDESI MSI LONGITUDINAL ARV PROFILING IN HAIR STRANDS

Benchmarking of IR-MALDESI MSI Longitudinal ARV Profiling in Hair Strands

As part of NCT03218592, we conducted structured dose proportionality studies to develop mathematical benchmarks of real-time IR-MALDESI MSI hair adherence monitoring for both PrEP and HIV treatment applications. We enrolled 12 healthy volunteers into one of 3 drug arms. Our study schema is outlined in **Figure 1**. Subjects gave informed consent and then underwent laboratory screening for safety. Subjects will return to the clinic within 2 weeks to start drug dosing. Dosing was divided into three 28 day phases: single dose, daily dose, and dose proportionality. Subjects were randomly assigned to receive one of 3 drug regimens: TFV+FTC (Truvada®; TDF 300mg/FTC 200mg), DTG 50mg once daily, and MVC 300 mg once daily. Subjects visited the research center for hair collection (10 strands), blood collection (3mL), and PBMC collection (for the Truvada arm only) on days 3, 7, 14, 21, and 28 of each phase. After the single dose phase, study subjects initiated daily dosing with their assigned ARV. One tablet per day was administered by directly observed therapy. After the daily dosing phase, subjects were randomly assigned to one of three arms for final dose proportionality assessments. They either: 1) stopped their medication (0 doses/week), 2) switched to 1 dose/week, or 3) took 3 doses/week. This approach allowed evaluation of dose-response in hair strands for 1, 4, 12, and 28 doses per month (28 days). Here, we summarize benchmarking results for TFV+FTC and DTG.

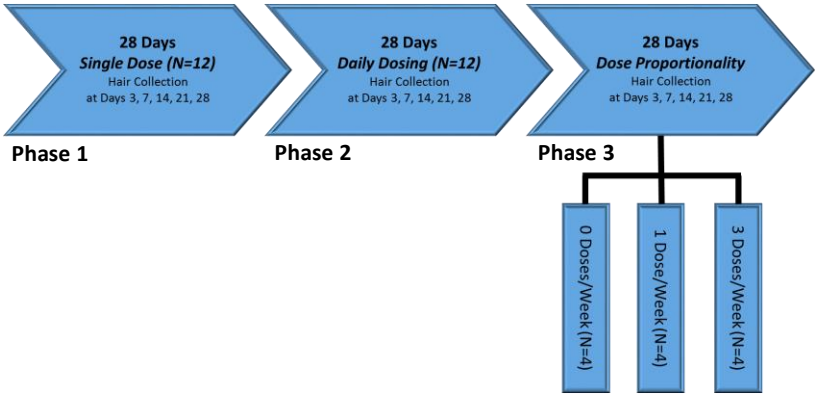


Figure 1 – Study schema for NCT03218592, a single-center, open-label, 3-arm, directly observed therapy, triple phase study.

Sample Evaluation and Determination of Longitudinal ARV Profiles

An example of IR-MALDESI MSI evaluation of ARV disposition in hair strands can be seen in **Figure 2** for a sample collected on Phase 3 Day 21 from a volunteer receiving 3 doses/week. Four hair strands from each volunteer were adhered to a glass slide and analyzed by IR-MALDESI (**Figure 2A**). MS images in **Figure 2B and C** show heatmaps corresponding to the measured signal abundance of an endogenous lipid and ARV, respectively. The endogenous lipid provides a chemical signature delineating the boundaries of each hair strand, and the IR-MALDESI response is consistent over the entire length of each hair strand in the region of interest interrogated, corresponding to the proximal 15 mm of the cut hair strands. Unlike the uniformity of the endogenous response, the ARV heatmap shows distinct and localized bands of higher abundance within

each strand. Average hair growth is approximately 10 mm/month, such that the 15 mm long region of interest investigated in this example reflects roughly one and a half months of hair growth. This period of time captures the Phase 3 dosing response and the Phase 2 daily dosing response, along with the 7 day gap between study phases as denoted in Figure 2C. These features can be seen more clearly in the accompanying profiles associated with each hair strand shown in **Figure 2D**.

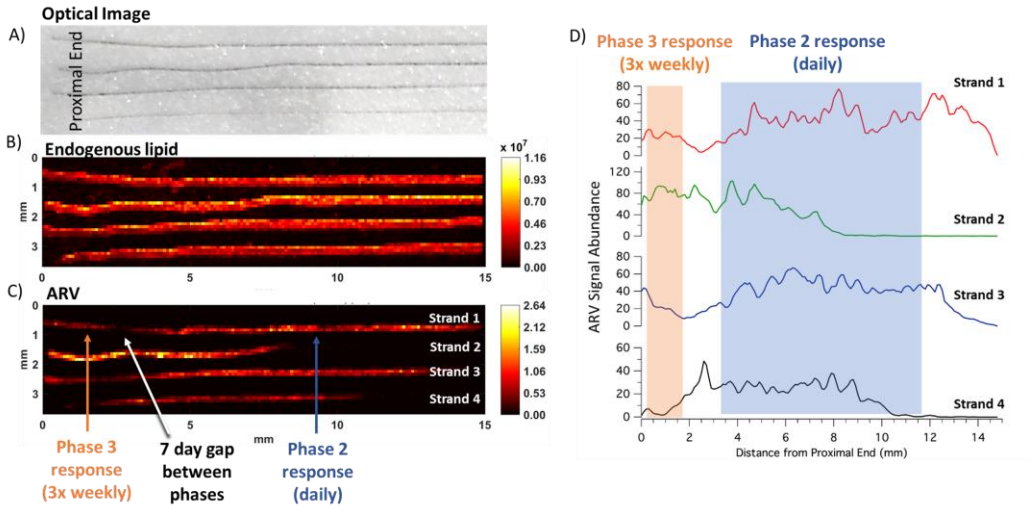


Figure 2 – IR-MALDESI MSI evaluation of ARVs in hair strands. A) Optical image of four hair strands prepared for analysis. B) Endogenous lipid response from sampled hair strands. C) ARV signal abundance, showing characteristic patterns associated with transition between daily and 3x per week dosing. D) Longitudinal profiles of ARV for each strand.

Features of the ARV profiles are not in exactly the same position on each strand relative to the proximal strand end due to variability in the angled cutting of hair by scissors during sampling. We align profile features across strands using built-in algorithms of our custom MATLAB analysis software before evaluating a composite average profile for a sample.

Assessment of Dose-Response in Hair Strands from Composite Profiles

Summary ARV profiles for all 12 volunteers in each arm of NCT03218592 are shown in **Figures 3 and 5** for FTC and DTG, respectively. Profiles are shown in blue with accompanying 95% confidence intervals. Shaded regions denote portions of the longitudinal profile attributed to daily and differentiated dosing phases. Average signal abundances associated with each identified region from these profiles are shown in **Figures 4 and 6** for FTC and DTG, respectively. For FTC and DTG, incorporation rates in hair were rapid enough to clearly discern regions of the Phase 3 Day 28 profiles associated with the Phase 3 differentiated dosing. For these two ARVs, receiver operating characteristic (ROC) curves were determined for classification of adherence based on average signal abundances. Sensitivity and selectivity are separately plotted as a function of IR-MALDESI signal abundance cutpoint. Based on cutpoint selection, adherence can be evaluated for the longitudinal profiles. An example report for an individual on the DTG study transitioning from daily to 1x-week dosing (RSAU) is shown in **Figure 7**, where an adherence cutpoint IR-MALDESI signal abundance of 2000 has been selected. **Figure 7A** shows the original profile for RSAU shown in Figure 5 for comparison to the bar graph format of the provider report (**Figure 7B**) and the calendar format of the patient report (**Figure 7C**).

Emtricitabine

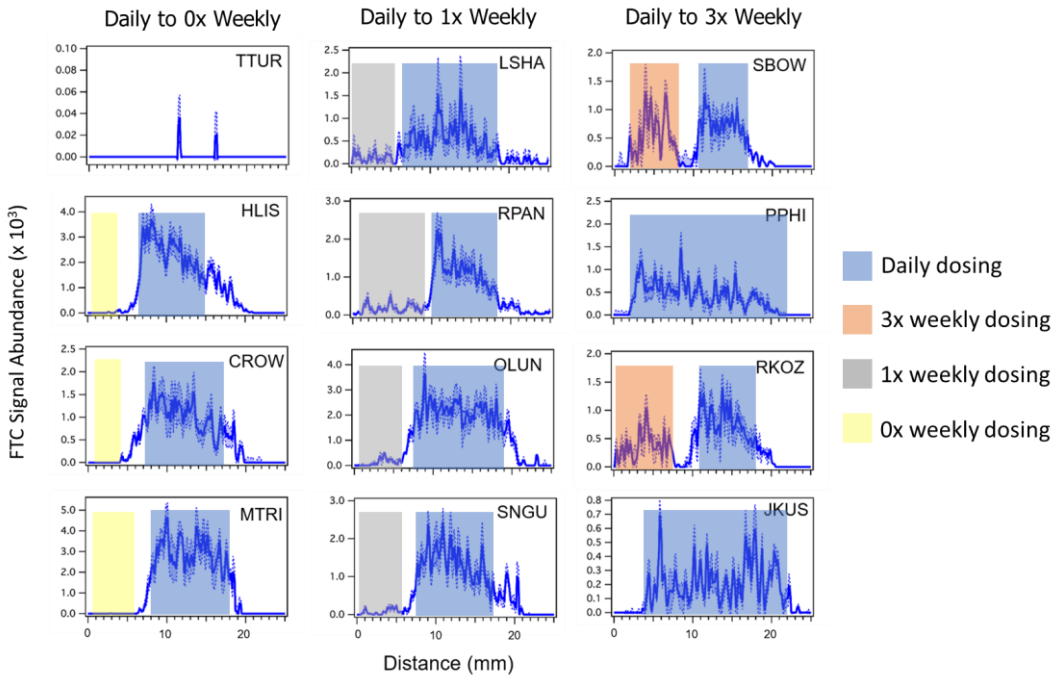


Figure 3 – Phase 3 Day 28 longitudinal profiles for FTC measured by IR-MALDESI.

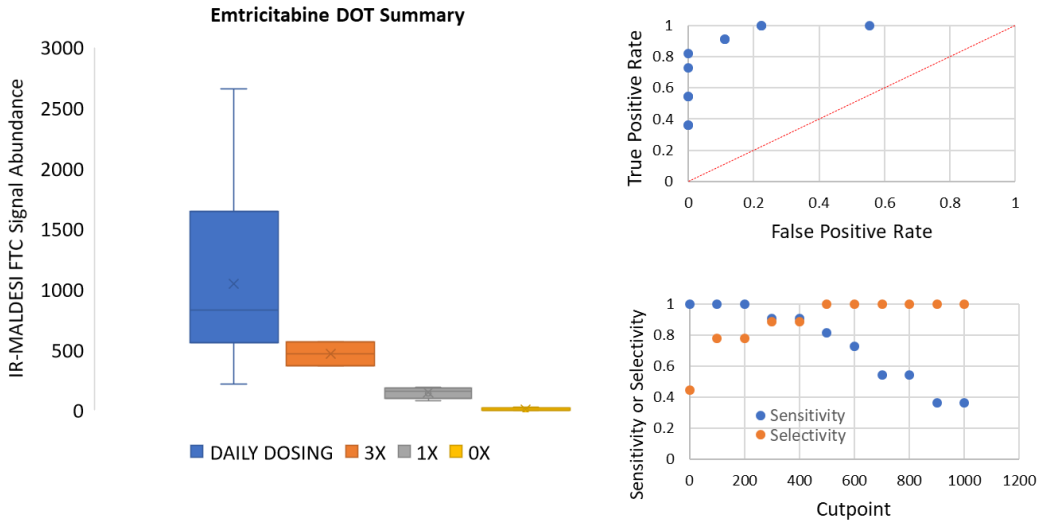


Figure 4 – Dose-Response Summary for FTC based on IR-MALDESI longitudinal profiles.

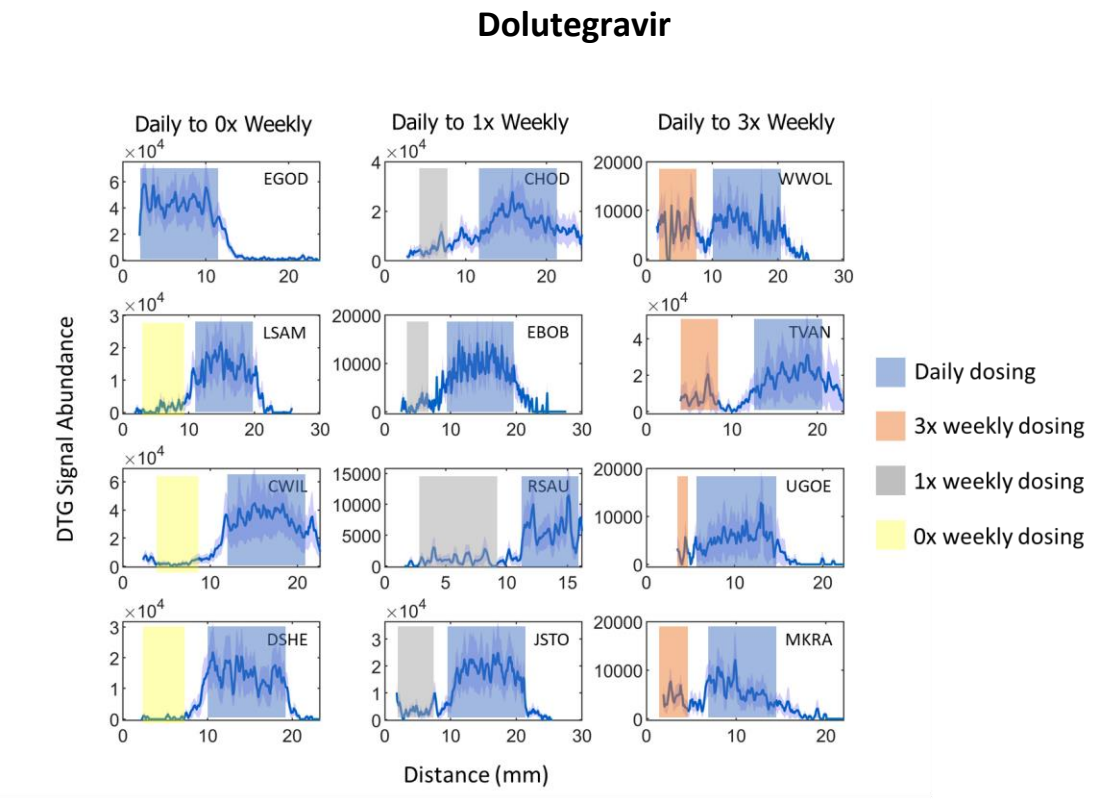


Figure 5 – Phase 3 Day 28 longitudinal profiles for DTG measured by IR-MALDESI.

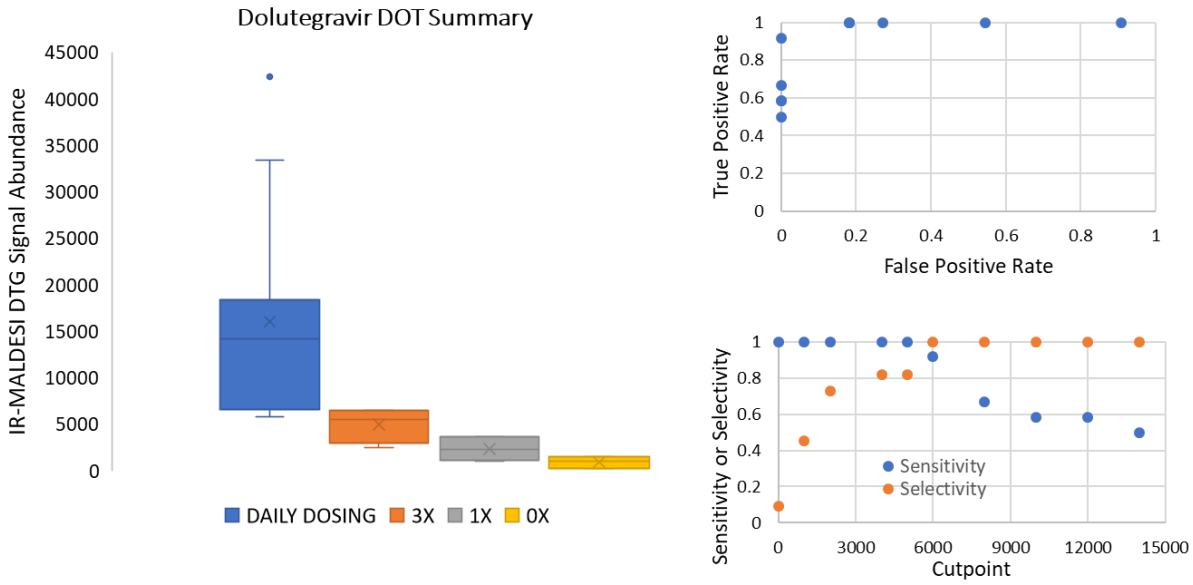


Figure 6 – Dose-Response Summary for DTG based on IR-MALDESI longitudinal profiles.

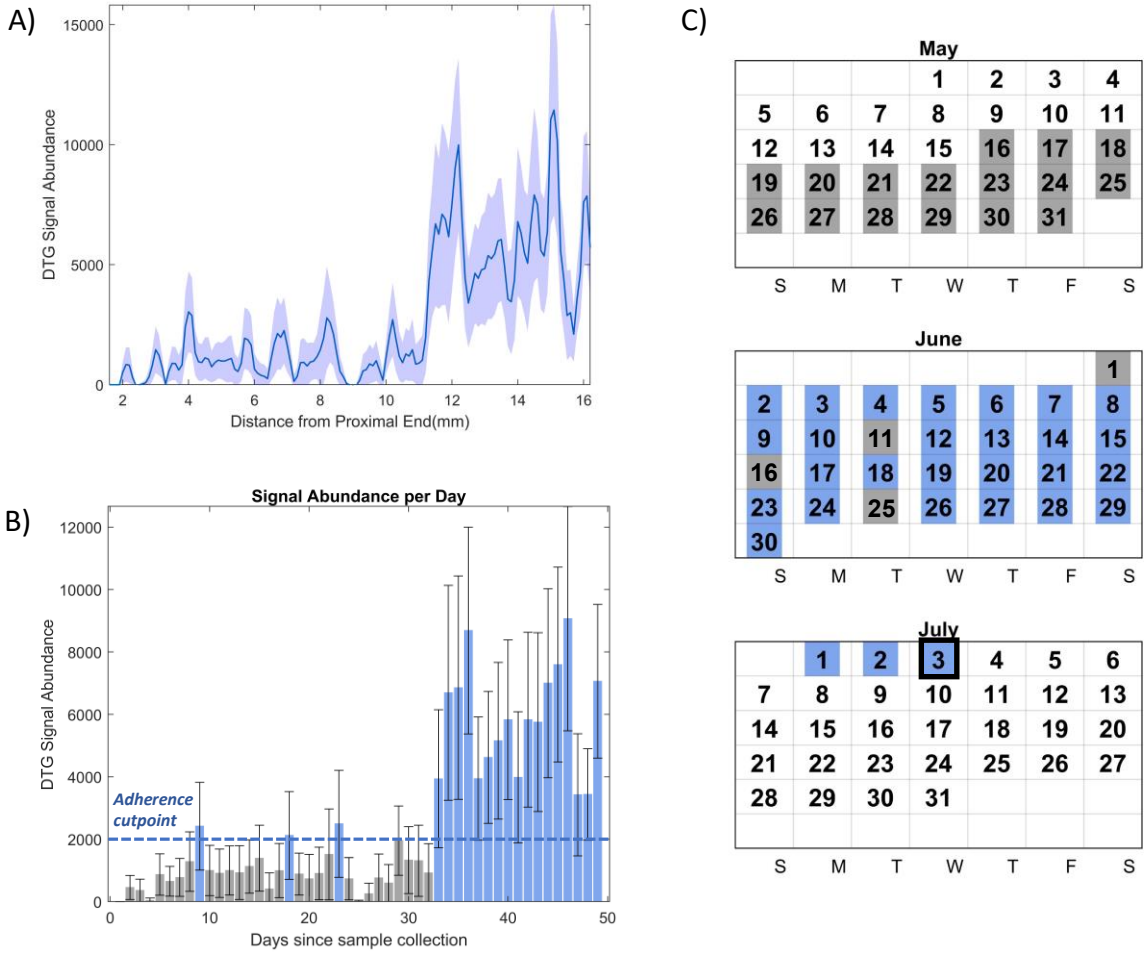


Figure 7 –Example adherence assessment to DTG regimen in samples collected from the volunteer RSAU. A) IR-MALDESI DTG profile in strands collected from RSAU. B) Bar graph format of the provider report based on an adherence cutpoint IR-MALDESI signal abundance of 2000. C) Calendar format of the adherence assessment for the patient report.

APPENDIX E. IR-MALDESI METHOD VALIDATION

Laboratory Name: Clinical Pharmacology and Analytical Chemistry Laboratory

Laboratory address: School of Pharmacy
1100 Genetic Medicine Building, CB 7361
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Approved by: _____

Title: Laboratory Director

Reviewed by:_____

Assistant Laboratory Director

Reviewed by:_____

Quality Assurance Auditor

LIST OF TABLES AND FIGURES

E.1 INTRODUCTION

A method for quantifying the levels of emtricitabine (FTC), and dolutegravir (DTG) in single strands of human hair has been validated using infrared matrix assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging (MSI). An analyte-specific linear response range has been established. The analytical range was selected based on screening of hair samples collected from subjects adherent to HIV treatment regimens containing FTC or DTG. Concentrations expected to be found in clinical hair samples are anticipated to fall within the selected analytical range.

IR-MALDESI MSI is an analytical technique that is based on the ambient ionization of material directly desorbed from the solid matrix of an individual hair strand without any extraction or analytical separation steps. Creating analytical standards for unique, solid matrices such as hair represents a considerable challenge. The following is a brief description of the method used to validate the quantification of FTC or DTG in human hair:

Calibration standards were prepared from blank (drug-free) hair matrix by two approaches: 1. spray deposition, and 2. incubation. In the former approach, a template was placed on top of blank hair adhered to a glass slide to mask deposition of sprayed standards into discrete wells and total concentration of drug in each well was controlled based on the number of passes of a moving sprayer head. In the latter approach, standards were prepared by transferring drug-free hair (approximately 10 mg) into a vial containing 20 mL of analyte and solvent (50:50 Methanol:Water), and incubating for approximately 24 hours in a reciprocal shaking bath. Incubated standards were then rinsed with fresh solvent and stored at -20°C and used as needed for analysis.

All hair samples were prepared for analysis by mounting hair strands onto a glass slide by VHB tape. For quantitative analysis, samples were sprayed with isotopically labeled internal standard solution containing FTC-d_3 (FTC-IS). After air drying, samples were placed into the IR-MALDESI MSI source for evaluation. Target compounds generated by IR-MALDESI sample interrogation were detected on a Thermo Q Exactive Plus mass spectrometer. Acquisition was conducted using electrospray ionization in the positive ion mode with a solvent of 0.2% Formic Acid in 50:50 Methanol:Water. The following ions were monitored:

Analyte	Analysis Mode	Target peak	Peak identity	
FTC	MS/MS	130.0412	[F+H]+	protonated fragment ion
FTC-d3	MS/MS	133.0384	[F+H]+	protonated fragment ion
DTG	Full MS	420.136555	[M+H]+	protonated parent ion

Quantitation is based on the peak area of each sampling point associated with a hair strand defined during post-processing analysis in MSiReader software (**Figure 1**).

E.2 MATERIALS AND METHODS

E.2.1 REFERENCE STANDARDS

The following reference standards were used for the preparation of calibration standards, quality control samples, and internal standard solutions:

Compound	Source	Lot No.	Purity	Salt Correction
FTC	Toronto Research Chemicals, Inc.	8-SSR-128-1	0.980	1.00
FTC-d3	Moravek	390-095-000-B-20150316-PVA	0.980	1.00
DTG	Toronto Research Chemicals, Inc.	5-RTU-102.1	0.980	1.00

E.2.2 Reagents

Description	Specification
Acetonitrile (ACN)	HPLC grade
Formic Acid	Certified ACS, 88%
Water (H_2O)	Hydro UltraPure H_2O System or HPLC grade
Methanol (MeOH)	HPLC grade
Human Hair	BioreclamanationIVT

E.2.3 INSTRUMENTATION AND SUPPLIES

Description	Supplier
Q-Exactive Plus Mass Spectrometer	ThermoElectron
TM Sprayer	HTX Technologies
Pipettor	5000 µL adjustable; 1000 µL adjustable; 200 µL adjustable; 100 µL adjustable
Reciprocal shaking bath	Precision

E.2.4 Preparation of Stock and Internal Standard Solutions, and Calibration Standards

E.2.4.1 PREPARATION OF INTERNAL STANDARD STOCK SOLUTIONS

Typically, a 1 mg/mL stock solution of internal standard is prepared. Due to the cost associated with isotopically-labeled internal standards, these compounds are typically ordered in 1mg amounts. The internal standard is then solvated by adding the appropriate amount of solvent directly to the vial received from the vendor. The purity and salt corrections are figured into the calculation of the amount of solvent (50:50 Methanol:Water) required to result in a 1mg/mL solution for FTC-IS. These compounds are not weighed in our lab. A completely accurate weighing is not critical for the internal standard component as it is only used as a constant mass addition to each sample. The exact concentration of internal standard has no impact on the quantitation of DTG.

E.2.4.2 PREPARATION OF PRIMARY ANALYTE STANDARD STOCK SOLUTIONS

Typically, 1 mg/mL stock solutions are prepared for FTC or DTG. The purity and any salt correction (listed on the certificate of analysis) are figured into the calculation of the amount of solvent (50:50 Methanol:Water) required to result in a 1mg/mL solution.

E.2.4.3 PREPARATION OF CALIBRATION STANDARDS BY TM SPRAYER

The amount of standard deposited onto the hair sample surface during spraying is determined by the following equation:

$$\text{Sample Concentration (ng/mm}^2\text{)} = \frac{\#\ \text{Spray Passes} \times \text{Flow Rate} \times \text{Standard Concentration}}{\text{Spray Head Velocity} \times \text{Track Spacing}}$$

The surface concentration is then converted to a per mass basis ARV concentration (ng/mg) hair based on estimates for the depth of laser desorption and density of hair. Optical inspection of ablated hair strands yielded an estimated depth of 0.05 mm. Hair density was evaluated by placing 95 mg of blank hair in a glass graduated cylinder and adding 5 ml of water. The displacement of 1 ml resulted in an estimated hair density of 95 mg/ml.

The primary stock solutions were used to make spraying solutions of 1μM in polypropylene plastic. The diluent used for this preparation was 50:50 Methanol:Water. Solutions are stored at -80 °C and are stable for up to 91 days. Spraying of calibration standards was performed with the following conditions:

Flow Rate	0.02	ml/min
Concentration	1	µM
Head velocity	600	mm/min
Track width	2	mm

The number of passes was varied in each well of the templated mask, as shown in **Figure 2**, to generate the desired range of concentrations for calibration.

E.2.4.4 PREPARATION OF CALIBRATION STANDARDS BY INCUBATION

The primary stock solutions were used to make incubation solutions in polypropylene plastic. The diluent used for this preparation was 50:50 Methanol:Water. Solutions are stored at -80 °C and are stable for up to 91 days. Standards were prepared by transferring drug-free hair (approximately 10 mg) into a vial containing 20 mL of spiked calibration solution and incubating for approximately 24 hours in a reciprocal shaking bath. Incubated standards were then rinsed with fresh solvent and allowed to dry for 10 minutes before being stored at -20°C and used as needed for analysis.

E.2.4.5 PREPARATION OF HAIR SAMPLES WITH INTERNAL STANDARDS USING THE TM SPRAYER

Stable isotope labelled internal standards were sprayed onto prepared hair samples using the TM Sprayer with the following conditions:

Flow Rate	0.02	ml/min
Concentration	4	µM
Head velocity	600	mm/min
Track width	2	mm
# passes	6	
Spray configuration	Criss-cross	

E.3 VALIDATION

E.3.1 EXPERIMENTAL DESIGN

The validation procedure for FTC consisted of triplicate analytical runs to evaluate assay performance. The standard curves were constructed from at least five calibration standard levels and analyzed using a linear regression algorithm with $1/x^2$ weighting to plot the peak area ratio of analyte to internal standard versus concentration.

The analytical procedure consisted of the following steps:

Blank hair strands were adhered to a glass microscope slide and placed in the TM sprayer with a templated mask. FTC standards were sprayed over the sample, increasing the numbers of spray passes in each well of the mask. After 10 minutes of drying, FTC-IS were sprayed onto the sample. An additional 10 minutes of drying was provided prior to analysis.

Based on the observation that adherence of more hydrophyllic analytes (FTC, DTG) to blank hair strands was unacceptably variable between tests, particularly at lower calibration concentrations, calibration of DTG response was performed using incubated hair strands. LC-MS/MS evaluation of DTG concentrations in incubated hair strands was conducted to determine absorbed concentrations within the hair matrix.

Typical mass spectra and extracted ion chromatograms from FTC and DTG from incurred samples, illustrating relevant concentration levels, are shown in **Figure 3**.

E.4 RESULTS

E.4.1 VERIFICATION OF CALIBRATION MODEL

All validations used peak area ratios of calibration standard to internal standard to construct calibration curves. All calibration curves employed linear regression with $1/x^2$ weighting. All correlation coefficients exceeded 0.95. Analytical results are reported in ng/mg.

See **Figure 4** for an example calibration curve for each analyte.

Calibration curve details from individual validation experiments are presented below:

Analyte	Calibration Range (ng/mg)	Calibration Curve Table	Regression Table
FTC	1.4 to 111	1	2
DTG	0.04 to 6.6	1	2

E.4.2 LOWER LIMIT OF QUANTITATION (LLOQ)

The LLOQ has been imputed at half the signal abundance of the lowest level of calibration based on the calibration slope and intercept.

	IS-corrected	Slope	Intercept	R-squared	LLOQ (ng/mg)
FTC	Yes	0.0063	0.0051	0.9971	0.270
DTG	No	6.12E+04	-1.45E+03	0.9527	0.040

As seen in the example chromatograms of **Figure 3**, analysis shows no off-strand background response and no signal carryover. With little baseline noise present, a raw signal abundance of 1e3 represents a standard limit of detection for the Q-Exactive Plus. For FTC and DTG, half the signal abundance of the lowest level of calibration exceeded 1e3.

E.4.3 PRECISION

Precision was determined by triplicate analysis of human hair matrix calibration concentrations representing the entire calibration range. Precision is expressed as relative standard deviation (%RSD).

$$\%\ \text{RSD} = \frac{\text{standard deviation}}{\text{mean of found values}} x\,100\%$$

Data for IS-normalized FTC and DTG are summarized in the following table. Individual results can be found as indicated in the table listed.

Analyte	%RSD	Table
FTC	11.3% to 63.1%	1
DTG	40% to 41%	1

E.4.4 FTC AND DTG ANALYSIS OF FIVE INCURRED SAMPLES

Also included in the evaluation of the method were replicate analyses of hair samples from six separate subjects. Hair from two subjects on MVC regimens, three subjects on FTC, and one subject on a DTG regimen was utilized. LC-MS/MS concentrations were also determined for all but one sample for which insufficient hair was available.

IR-MALDESI MSI detected measurable signal abundance for all incurred samples within the linear calibration range, as shown in **Figure 5**, and samples were quantified based on these calibrations.

E.4.4.1 ACCURACY OF IR-MALDESI QUANTITATIVE ANALYSIS OF INCURRED SAMPLES

Accuracy, expressed as %Bias (mean % difference from nominal), was determined according to the following equation:

$$\% \text{ Bias} = \frac{\text{mean of found values - theroretical value}}{\text{theoretical value}} x\,100\%$$

A summary of quantitative evaluation by LC-MS/MS and IR-MALDESI for incurred samples is provided in **Table 5.** Bias ranged from -5 to 65%, but should be treated with some caution because analysis by each method cannot be conducted on truly matching samples. LC-MS/MS analysis was performed on 2-5 mg of hair comprising 10s of strands evaluated in aggregate. IR-MALDESI MSI is performed on individual hair strands that may reflect differing levels of pigmentation or growth phase than the average over many strands. Nonetheless, the results of this test demonstrate good agreement between measurement approaches. No systematic bias in response is apparent over the relatively small number of samples investigated.

E.4.4.2 PRECISION OF IR-MALDESI ANALYSIS OF INCURRED SAMPLES

Additionally, precision was evaluated for repeated analysis of hair strands from two incurred samples.

Analyte	Sample	%Intra-day Precision	%Inter-day Precision	Table
FTC	UCSF003	20% to 76%	35%	4
DTG	DK010	25% to 42%	22%	4

As incurred samples, these are not true replicate analyses due to expected biological variability but the measured precision demonstrates the reproducibility between separate hair samples from the same subject.

E.5 CONCLUSION

A method to quantify the levels of emtricitabine (FTC) and dolutegravir (DTG) in human hair has been validated over a linear concentration range relevant to incurred clinical samples.

The method uses a linear regression algorithm with $1/x^2$ weighting. During the validation, the correlation coefficient (r^2) for all calibration curves exceeded 0.95. The responses of blank human hair analyzed showed no interferences.

Quantitative analysis of incurred samples by IR-MALDESI MSI showed good agreement with LC-MS/MS and consistency in repeated measures of analytes in clinical samples.

Based on these results, the method is available for use in determining the concentrations of emtricitabine and dolutegravir in human hair.

E.6 REFERENCES

- FDA Guidance for Industry Bioanalytical Method Validation, May 2001
- CPQA Guidelines for Chromatographic Method Development and Validation Based on (and including) FDA Guidelines Dated May 2001, Version 4, 2012
- SOP-0342 Guidelines for performing bioanalytical assays
- SOP-0343 Guidelines for validating bioanalytical methods
- SOP-0346 Data reporting, review and archiving

TABLE 1.CALIBRATION STANDARD CURVE SUMMARY

FTC

	Std Level 1	Std Level 2	Std Level 3	Std Level 4	Std Level 5	Std Level 6	Std Level 7
	ng/mg	ng/mg	ng/mg	ng/mg	ng/mg	ng/mg	ng/mg
Theoretical conc.	1.4	2.8	5.6	11.1	27.8	55.5	111.1
Raw IR-MALDESI Response							
Run 1	2.50E+03	2.80E+03	1.35E+04	1.22E+04	4.65E+04	9.18E+04	1.45E+05
Run 2	1.72E+03	1.81E+03	1.48E+04	1.82E+04	6.47E+04	1.40E+05	2.16E+05
Run 3	6.03E+03	5.56E+03	6.70E+03	2.50E+04	6.22E+04	1.26E+05	2.36E+05
Mean	3.42E+03	3.39E+03	1.17E+04	1.84E+04	5.78E+04	1.19E+05	1.99E+05
SD	2.30E+03	1.94E+03	4.35E+03	6.39E+03	9.87E+03	2.47E+04	4.77E+04
%RSD	67.3%	57.3%	37.3%	34.7%	17.1%	20.7%	24.0%
IS-normalized IR-MALDESI Response							
Run 1	0.017	0.035	0.055	0.065	0.156	0.348	0.710
Run 2	0.005	0.009	0.023	0.039	0.136	0.269	0.541
Run 3	0.018	0.044	0.050	0.046	0.125	0.288	0.754
Mean	0.013	0.029	0.043	0.050	0.139	0.302	0.668
SD	0.007	0.018	0.017	0.014	0.016	0.041	0.113
%RSD	54.3%	63.1%	40.4%	27.4%	11.3%	13.6%	16.9%

DTG

	Std Level 1	Std Level 2	Std Level 3	Std Level 4	Std Level 5	Std Level 6	Std Level 7
	ng/mg	ng/mg	ng/mg	ng/mg	ng/mg	ng/mg	ng/mg
Theoretical conc.	0.041	0.129	0.345	0.411	0.685	1.887	3.000
Raw IR-MALDESI Response							
Run 1	1.38E+04	2.02E+04	2.70E+04	4.62E+04	8.25E+04	1.94E+05	3.44E+05
Run 2					8.78E+04	1.42E+05	
Mean					8.52E+04	1.68E+05	
SD					3.44E+04	6.89E+04	
%RSD					40%	41%	

TABLE 2. REGRESSION SUMMARY

	IS-corrected	Slope	Intercept	R-squared	LLOQ (ng/mg)
FTC	Yes	0.0063	0.0051	0.9971	0.270
DTG	No	6.12E+04	-1.45E+03	0.9527	0.040

TABLE 3. QUANTITATIVE EVALUATION OF INCURRED SAMPLES

Sample ID	Target	IR-MALDESI Concentration	LC-MS/MS Concentration	LC-MS/MS Sample Mass	Bias
		ng/mg	ng/mg	mg	%
UCSF003	FTC	0.93	0.56	5.12	0.656
UCSF023	FTC	1.88	1.23	5.10	0.528
UCSF030	FTC	0.64	0.55	5.10	0.167
DK010	DTG	1.07	1.23	5.77	-0.13

TABLE 4. INTRA- & INTER-DAY PRECISION OF INCURRED SAMPLES

FTC UCSF 003

Strand	Date of Analysis	IR-MALDESI Signal Abundance	Intraday Statistics	
1	3/14/2019	BLD	Mean	9.85E+03
2	3/14/2019	BLD	SD	4.82E+03
3	3/14/2019	9.10E+03	%RSD	49%
4	3/14/2019	1.06E+04	n	2
5	3/19/2019	7.84E+03	Mean	7.84E+03
6	3/19/2019	BLD	SD	NA
7	3/19/2019	BLD	n	1
8	3/20/2019	6.63E+03	Mean	6.48E+03
9	3/20/2019	6.72E+03	SD	1.67E+03
10	3/20/2019	7.30E+03	%RSD	26%
11	3/20/2019	5.26E+03	n	4
Interday Statistics				
	Mean	7.64E+03		
	SD	3.04E+03		
	%RSD	40%		
	n	7		

DTG DK010

Strand	Date of Analysis	IR-MALDESI Signal Abundance	Intraday Statistics		
			Mean	SD	%RSD
1	4/11/2019	2.39E+04			
2	4/11/2019	2.06E+04	2.22E+04	9.26E+03	42%
3	4/12/2019	2.53E+04			
4	4/12/2019	2.21E+04			
5	4/12/2019	2.46E+04	2.40E+04	6.08E+03	25%

Interday Statistics	Mean	2.33E+04
	SD	5.20E+03
	%RSD	22%
	n	5

FIGURE 1. EXTRACTION OF HAIR-ASSOCIATED ION PEAKS USING MSiREADER

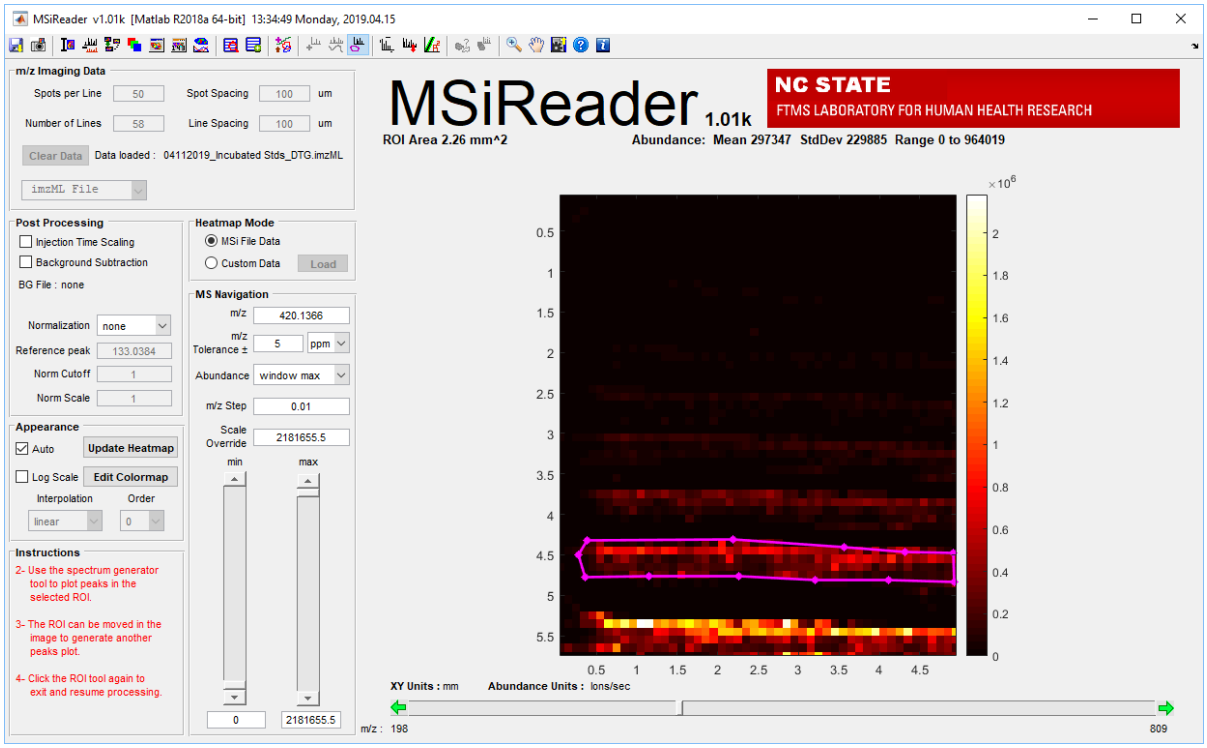


FIGURE 2. GENERATION OF CALIBRATION STANDARDS USING THE TM SPRAYER

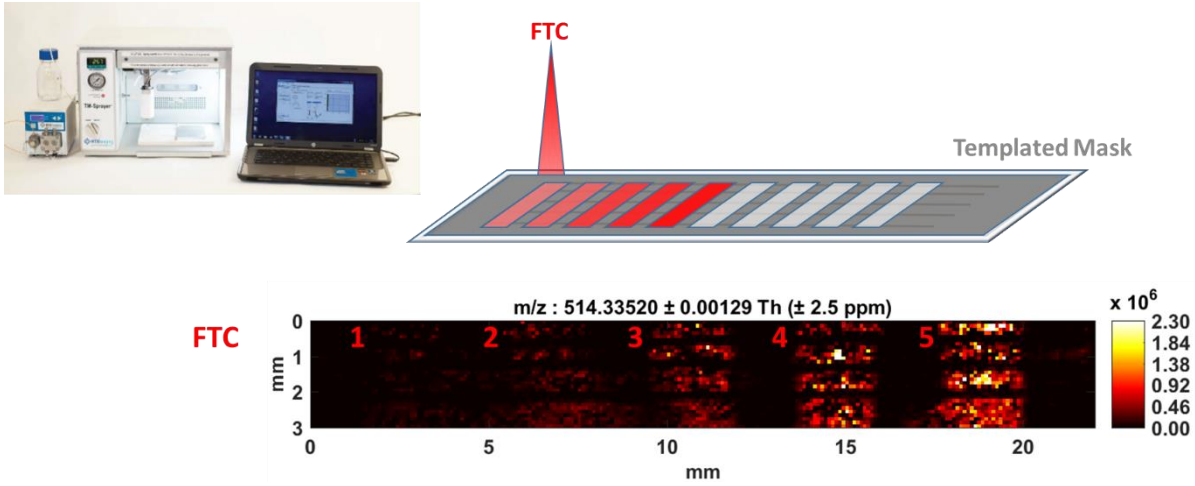
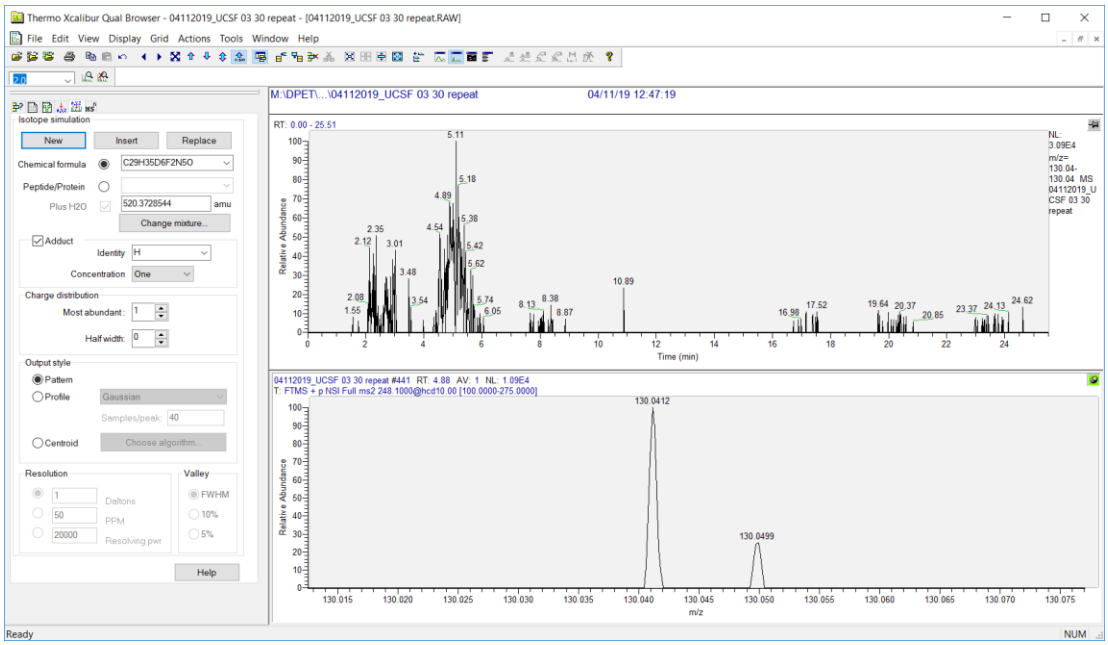


FIGURE 3. REPRESENTATIVE MASS SPECTRA AND EXTRACTED ION CHROMATOGRAMS OF TARGETED ANALYTES IN INCURRED HAIR STRANDS

FTC



DTG

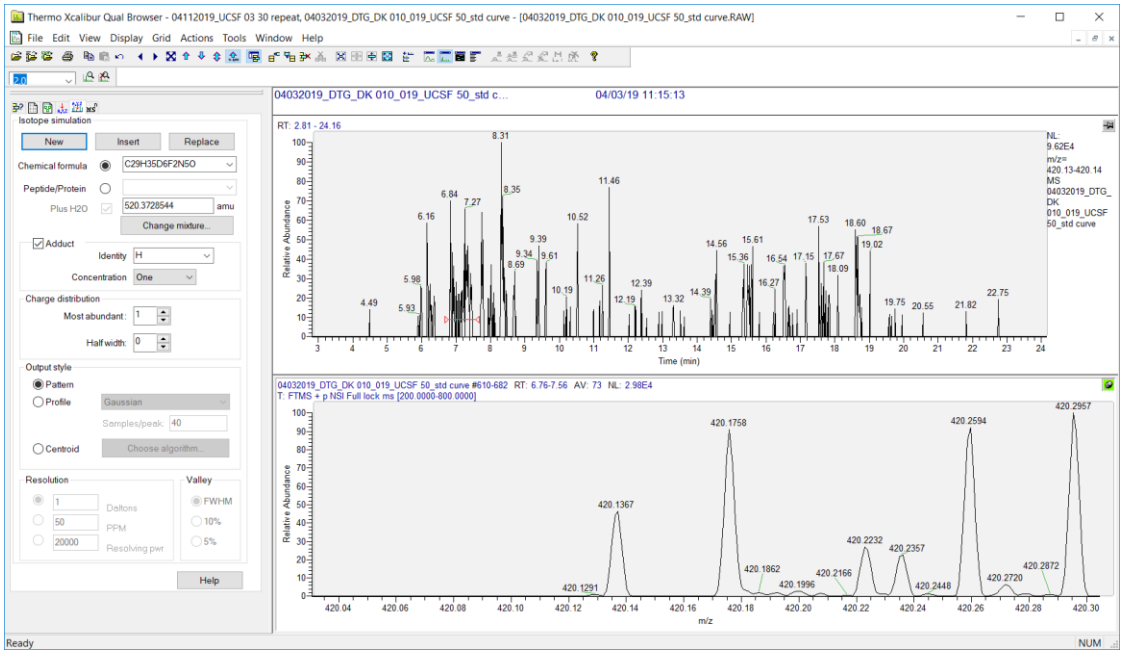
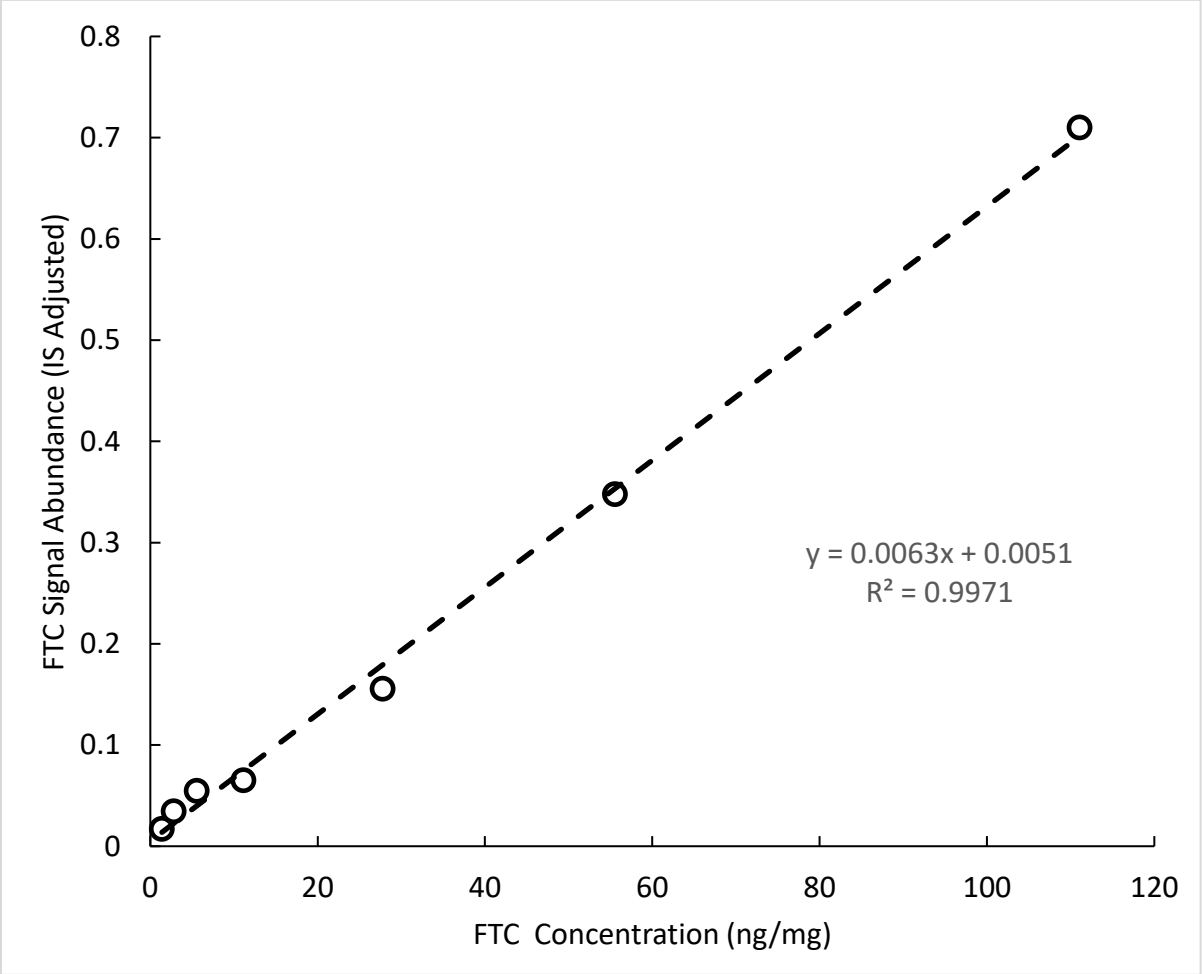


FIGURE 4. EXAMPLE OF A CALIBRATION CURVE

FTC



DTG

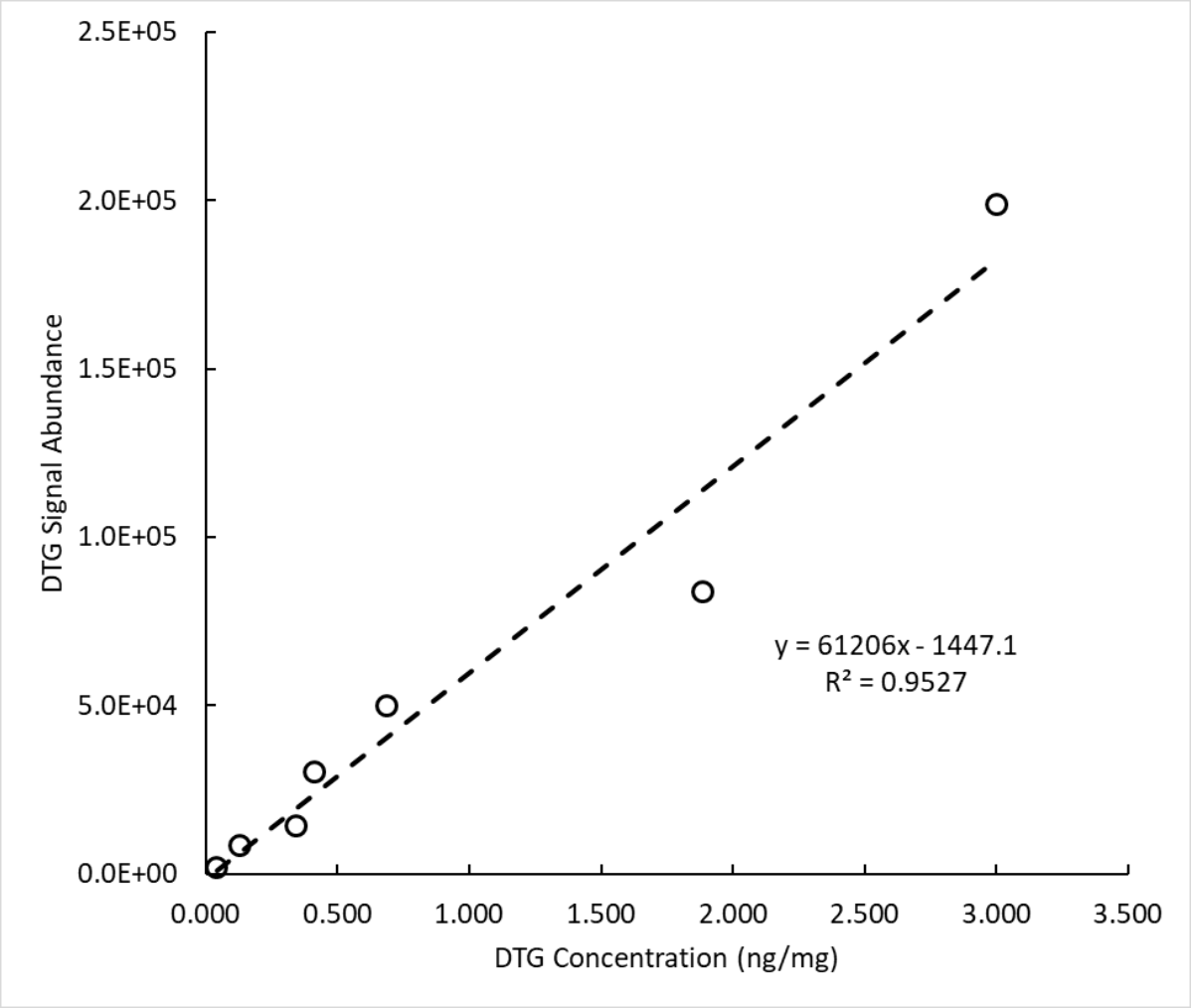
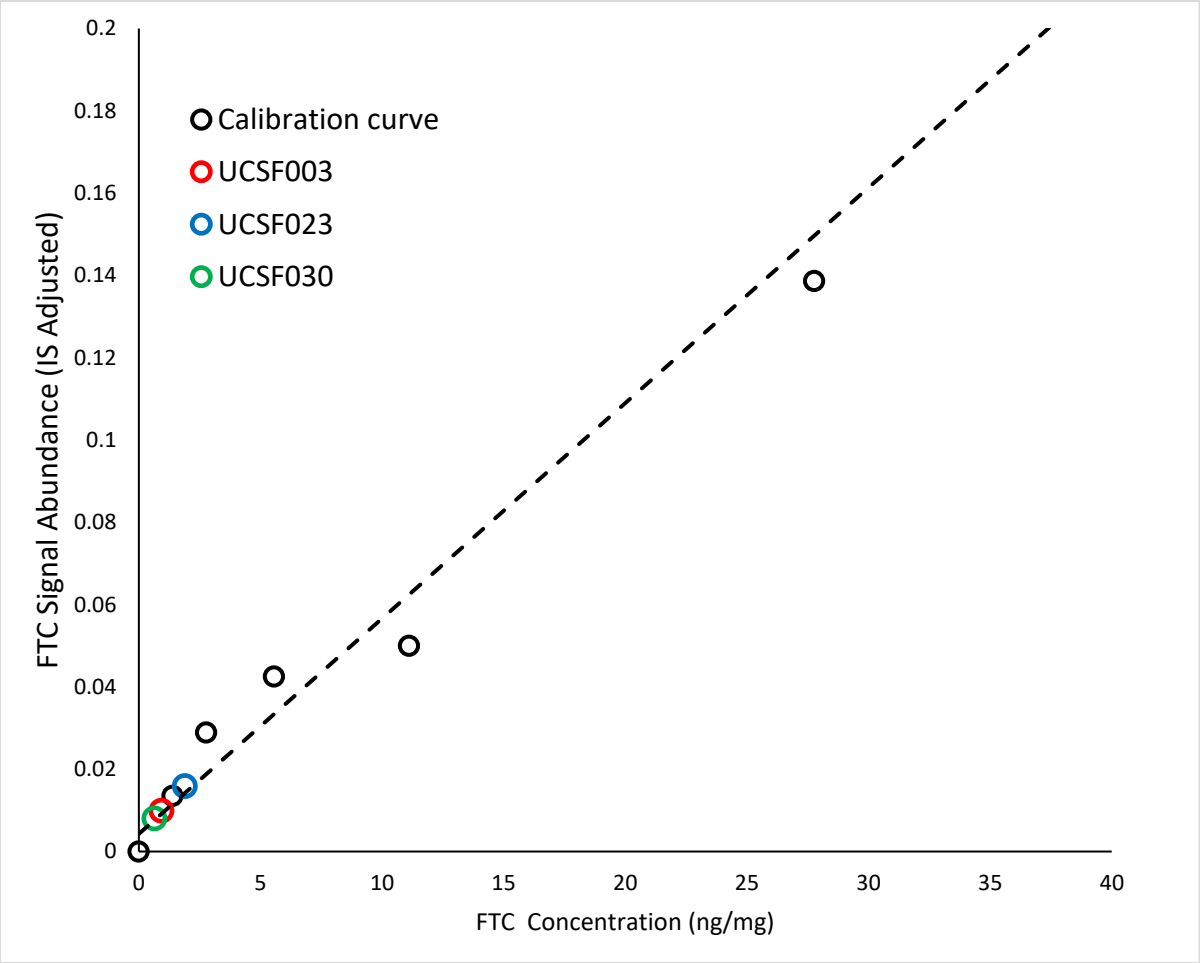
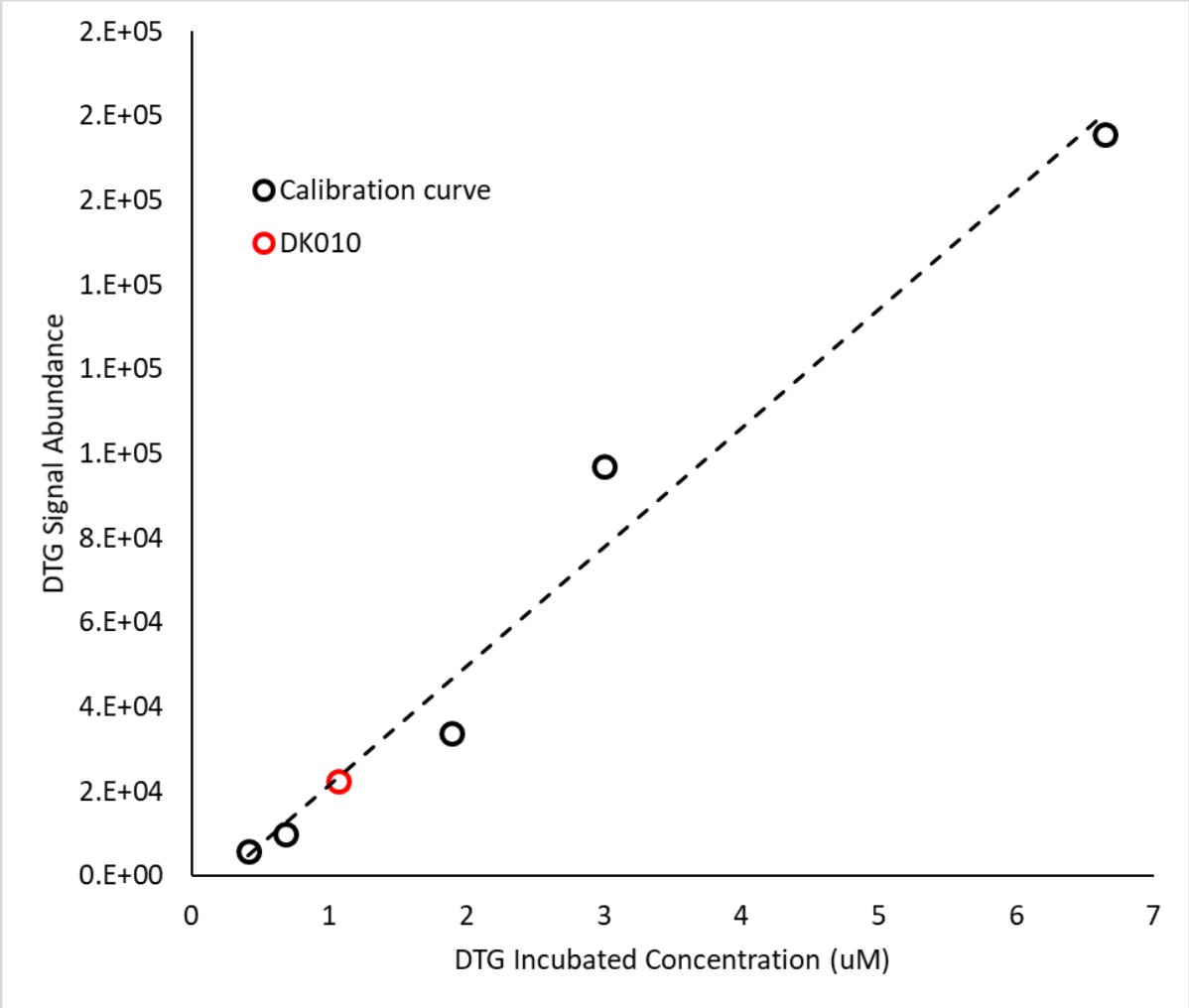

2.5E+05
2.0E+05
1.5E+05
1.0E+05
5.0E+04
0.0E+00
DTG Signal Abundance
0.000
0.500
1.000
1.500
2.000
2.500
3.000
3.500
DTG Concentration (ng/mg)
y = 61206x - 1447.1
R² = 0.9527

FIGURE 5. EVALUATION OF INCURRED SAMPLES BASED ON CALIBRATION CURVES





APPENDIX F. IR-MALDESI EVALUATION OF HAIR COLOR AND TREATMENT

F.1 IR-MALDESI EVALUATION OF HAIR COLOR AND TREATMENT

Influence of Hair Color and Treatments on Detection of Antiretrovirals by IR-MALDESI Mass Spectrometry Imaging

1. Correlation Between Hair Color and ARV Response

Hair samples collected from the 3 phase DOT study (NCT03218592) were evaluated for emtricitabine (FTC) and dolutegravir (DTG) in combination with the melanin biomarker pyrrole-2,3,5-tricarboxylic acid (PTCA). Four hair strands/subject were fixed to glass slides with double-sided tape, oxidized for 10 min in 1M NH_4OH in 10/45/45 H_2O_2/H_2O/MeOH (v/v/v) before analysis by IR-MALDESI MSI. Results are shown in Figure 1A. Relationships between the ARVs and the melanin biomarker were evaluated by

Pearson correlation coefficient, which indicated positive correlation for DTG with melanin (r_{DTG}=0.42) and negative correlation for FTC with melanin (r_{FTC}=-0.29). Scaling of the raw ARV IR-MALDESI response by PTCA response is shown in Figure 1B. For each of the investigated ARVs, the raw ARV response scaled by maximum value was compared to the PTCA-normalized ARV response scaled by maximum value. Based on the relative standard deviation (RSD) of these distributions, PTCA scaling increased variability for IR-MALDESI measurement of FTC and DTG.

Overall, PTCA correction of IR-MALDESI measurements in hair is not effective for reducing IR-MALDESI measurement variability of these ARVs.

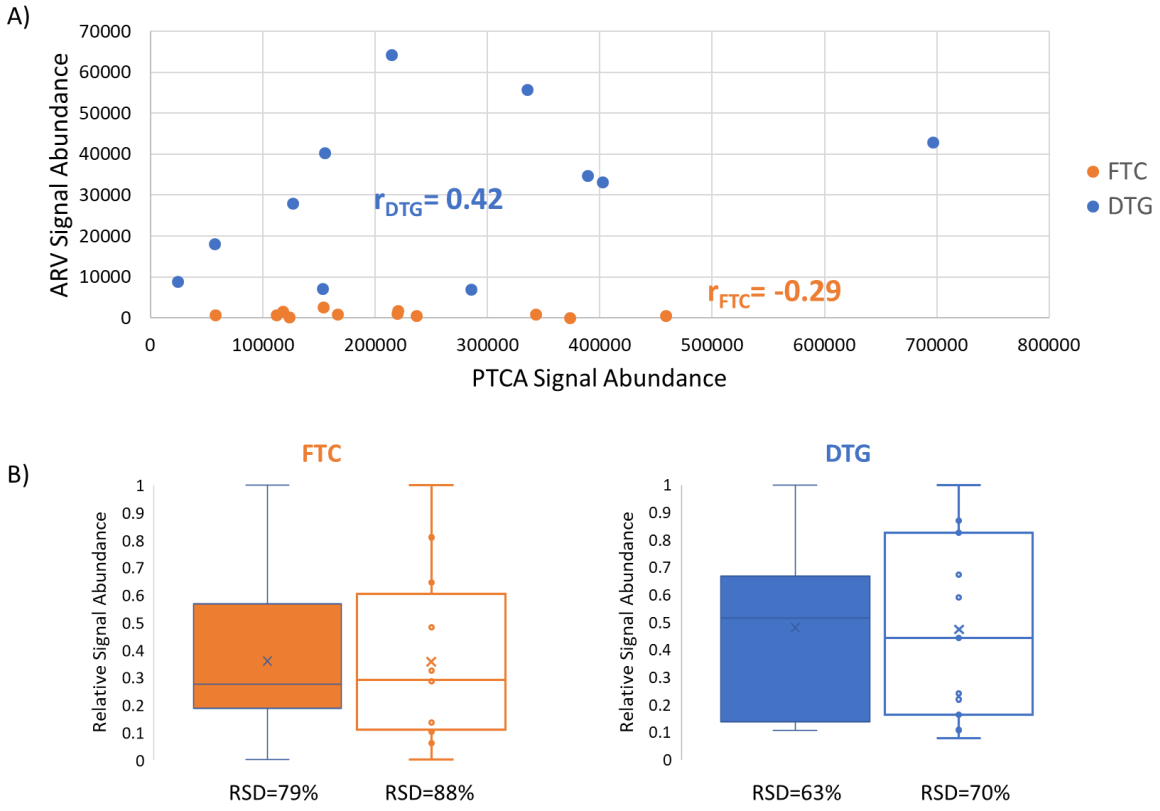


Figure 1- A) IR-MALDESI measurements of ARV and melanin signal abundance in hair strands collected during NCT03218592. B) Comparison of raw (solid) and PTCA-normalized (outlined) ARV IR-MALDESI measurements, scaled by measurement maxima.

2. Hair Treatment Influence on ARV Response

Manual Manipulation of Hair Treatments.

Hair strands collected from participants of NCT02768779, classified as adherent to varied ARV regimens based on plasma levels of <50 copies HIV RNA/mL, were used to test the influence of benchtop treatment with bleach (powder lightener from Wella), dye (brown hair dye from Wella), and relaxer (medium relaxer from Mizani). A cream developer was used with the bleach and dye (20 Volume,

ColorCharm, Wella) before application to hair strands. Prior to treatment, samples were fixed to a glass microscope slide by taping one end of the hair strand. Bleach powder and dye were mixed in a 1:2 ratio with the cream developer in a centrifuge tube prior to application to the hair strands. The compounds were then applied to the hair strands using a pipette or spatula. The contact times were 30 min for the bleach and dye and 15 min for the relaxer. The hair strands were then washed thoroughly with water and dried with nitrogen. Finally the hair strands were transferred to double-sided tape (VHB Tape, 3M, St. Paul, MN) on a microscope slide for MSI analysis.

MS images for hair strands from an individual on ART combining MVC and FTC are shown in **Figures 2 and 3**. A total of 12 strands were analyzed: 3 strands each were treated with bleach (B), dye (D), and relaxer (R), with 3 strands left untreated (C). **Error! Reference source not found.A** shows an image c orresponding to the endogenous ion cholesterol (*m/z* 369.3516), and Error! Reference source not found.**B** shows the ion image corresponding to MVC (*m/z* 514.3352). Error! Reference source not found.**C** shows the average MVC intensity for each hair strand and each of the three treatments and control. The bleached strands appeared to have slightly higher MVC ion abundance than the control strands (1.5x on average), and the dyed strands appeared to have slightly lower MVC ion abundance than the controls (0.6x on average). The MVC ion abundance remains unchanged between any treatment and the control strands, indicating that the manual manipulation of these strands with these three treatments had no significant effect on the detection of MVC.

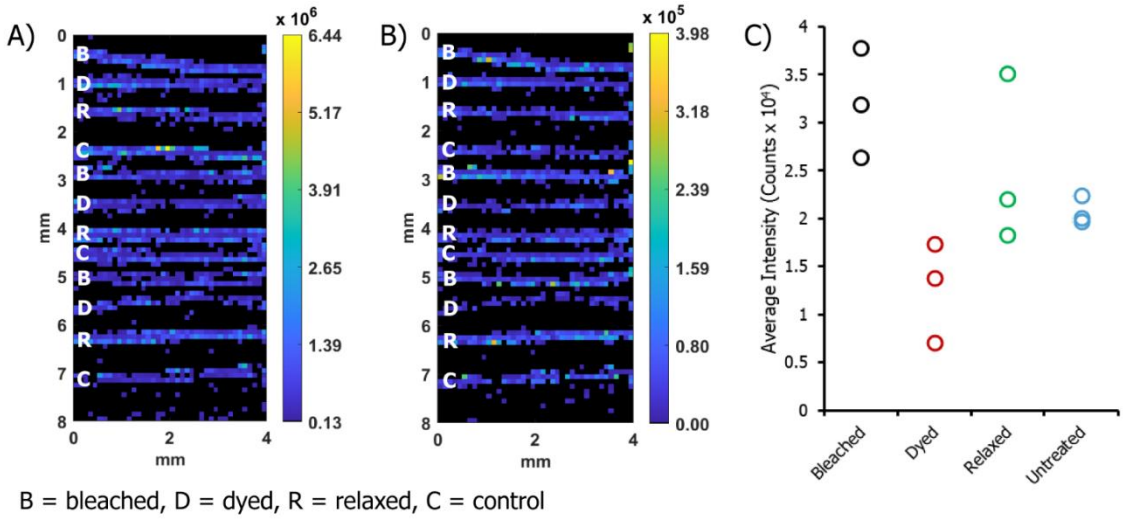


Figure 2. Effect of cosmetic hair treatment on MVC signal, with bleached, dyed, relaxed, and control hair strands. (A) IR-MALDESI MS response to cholesterol (m/z 369.3516), (B) IR-MALDESI MS response to MVC (m/z 514.3352), (C) Comparison of average MVC response in each hair strand for treated hairs and control.

Ion images from a different location on the same strands in **Error! Reference source not found.** of an u nfragmented endogenous ion (*m/z* 248.2457) and FTC (*m/z* 248.1 → *m/z* 130.041) are shown in Error! Reference source not found.**A and** Error! Reference source not found.**B**, respectively. Error! Reference source not found.**C** shows the average intensity for FTC in each of the strands and treatments in Error!

Reference source not found.**B**. While FTC was detected in control strands, no FTC was measured in any of the three treated strands indicating that any of these three treatments remove FTC from the hair strands. The difference in results between MVC and FTC may be due to the relative binding strength of MVC and FTC to melanin in hair strands. Evidence from previous research has shown that compounds with basic properties are incorporated more strongly into hair than those with non-basic properties because they bind more strongly to the melanin in hair. Because MVC is more basic than FTC (pK_a of 7.3 versus pK_a of 2.65) and therefore may bind more strongly to melanin, hair treatment may have a lesser effect on the concentration of MVC in hair relative to FTC.

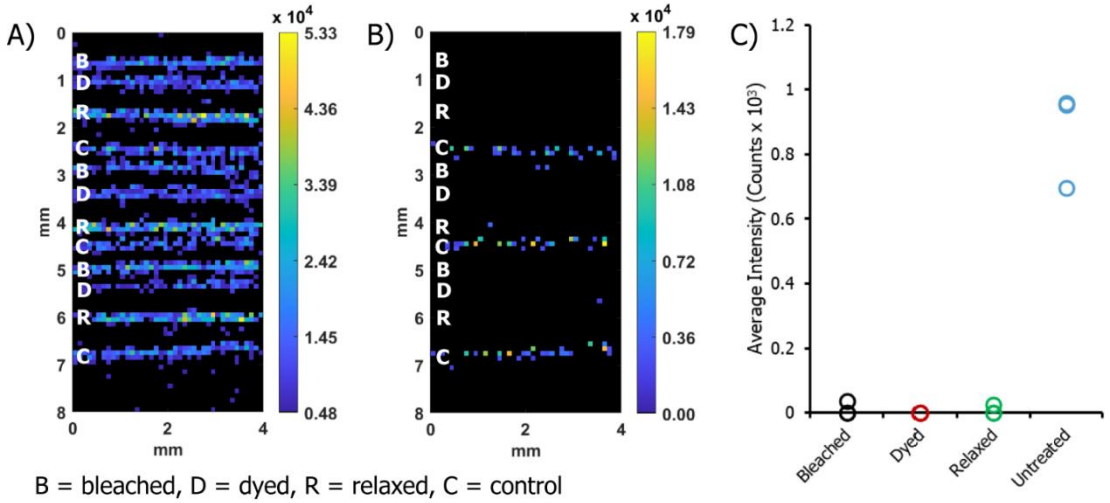


Figure 3. Effect of cosmetic hair treatment on FTC signal, with bleached, dyed, relaxed, and control hair strands. (A) IR-MALDESI MS response to an endogenous marker (m/z 248.2457), (B) IR-MALDESI MS/MS response to FTC (m/z 248.1 ± 4 → m/z 130.041), (C) Comparison of average FTC response in each hair strand for treated hairs and control.

To investigate the effect of hair treatment on other antiretrovirals, we evaluated hair strands from another patient on ARV therapy with a different drug regimen (DTG with lamivudine (3TC) and abacavir (ABC)). Plots showing the average ion abundance for hair strands with three different drugs (3TC, *m/z* 230.059; ABC, *m/z* 287.161; DTG, *m/z* 420.137) and three different manual treatments are shown in Error! Reference source not found.. **Error! Reference source not found.A, B, and C** show the intensity fo r each of the drugs in hair strands treated with and without relaxer. In each case, there was no difference between the treated strands and control strands. For the strands treated with bleach (**Error! R eference source not found.D, E, and F**), observations in bleached strands were the same or more intense than those that were untreated for all three drugs. In the case of ABC (**Error! Reference source n ot found.E**), there was a statistically significant difference between the treated and untreated strands ($p < 0.05$, paired two-tailed t-test). For the dyed strands, 3TC was undetectable in both the dyed and control indicating an experimental error. For the other two drugs, shown in Error! Reference source not f ound.**H and I**, the dyed strands had slightly lower intensities, and there was a statistically significant difference for DTG. These results indicate that relaxer does not affect the amount of drug in hair strands for 3TC, ABC, or DTG. For bleach treated strands, the treatment had no effect or, in the case of ABC, actually improved the signal response. This improvement in signal could be due to breakdown in the fidelity of the hair strand, thus reducing the energy necessary for ablation during the IR-MALDESI process and/or increasing the penetration depth of the laser. However, given that this trend was only

observed for one drug, further investigation is required. The dye appeared to lower the response to ABC and DTG. This trend may be

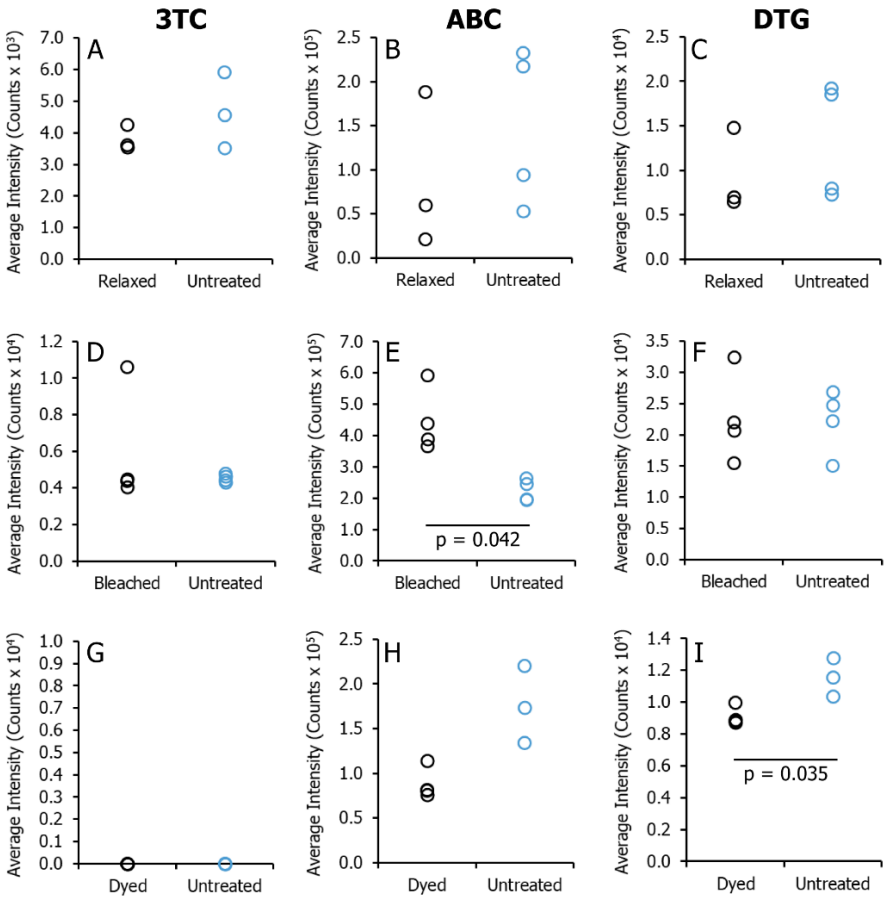


Figure 4. Comparison of relaxed (top row), bleached (middle row), and dyed (bottom row) with untreated hair strands from a patient on a regimen with 3TC (left column), ABC (middle column), and DTG (right column). Statistically significant differences are shown with a bar and p-value.

influenced by multiple factors. First, the dyeing process may extract some of the drug from the hair strand. Second, because the dye was mixed with a solution containing hydrogen peroxide, there may be reactions of the drugs with the dye solution, therefore reducing the concentration of the parent drug. Finally, the introduction of dye into the hair could increase the total number of molecules competing for ionization, resulting in ion suppression for the target of interest. Only FTC was rendered completely undetectable through mechanical treatment of individual hair strands, which may result in harsher conditions than on a full head of hair. To further assess the influence of hair treatments on FTC, we investigated clinical samples collected from donors reporting recent hair treatments.

FTC Detection in Clinical Samples with Reported Hair Treatment.

Characteristics of hair samples collected from 8 donors in NCT02768779 with recent (<8 weeks prior) is shown in Error! R eference source not found.**,** which include hair treatment including hair color, type of treatment, and time since the treatment (reported by the patient). There were 4 different hair colors and 5 different treatments sampled.

Patient ID	Targeted ARV	Hair Color	Treatment	Treatment Timeline
P-1	FTC	Brown	Perm	Previous day
P-2	FTC	Grey	Dye (brown)	Previous day
P-3	FTC	Brown	Dye	A couple of weeks ago
P-4	FTC	Brown	Bleach and Dye	3 weeks ago
P-5	FTC	Black	Relaxer	4 weeks ago
P-6	FTC	Black	Brazilian blowout	6.5 weeks ago
P-7	FTC	Blonde	Dye (red)	6 weeks
P-8	FTC	Blonde	Dye	8 weeks

Table 1- Clinical Samples with Reported Hair Treatment

Images from the proximal (closest to scalp) 7 mm of hair strands are shown in Error! Reference source n ot found., where intensity from FTC (shown in cyan) is overlaid with an endogenous ion to indicate the

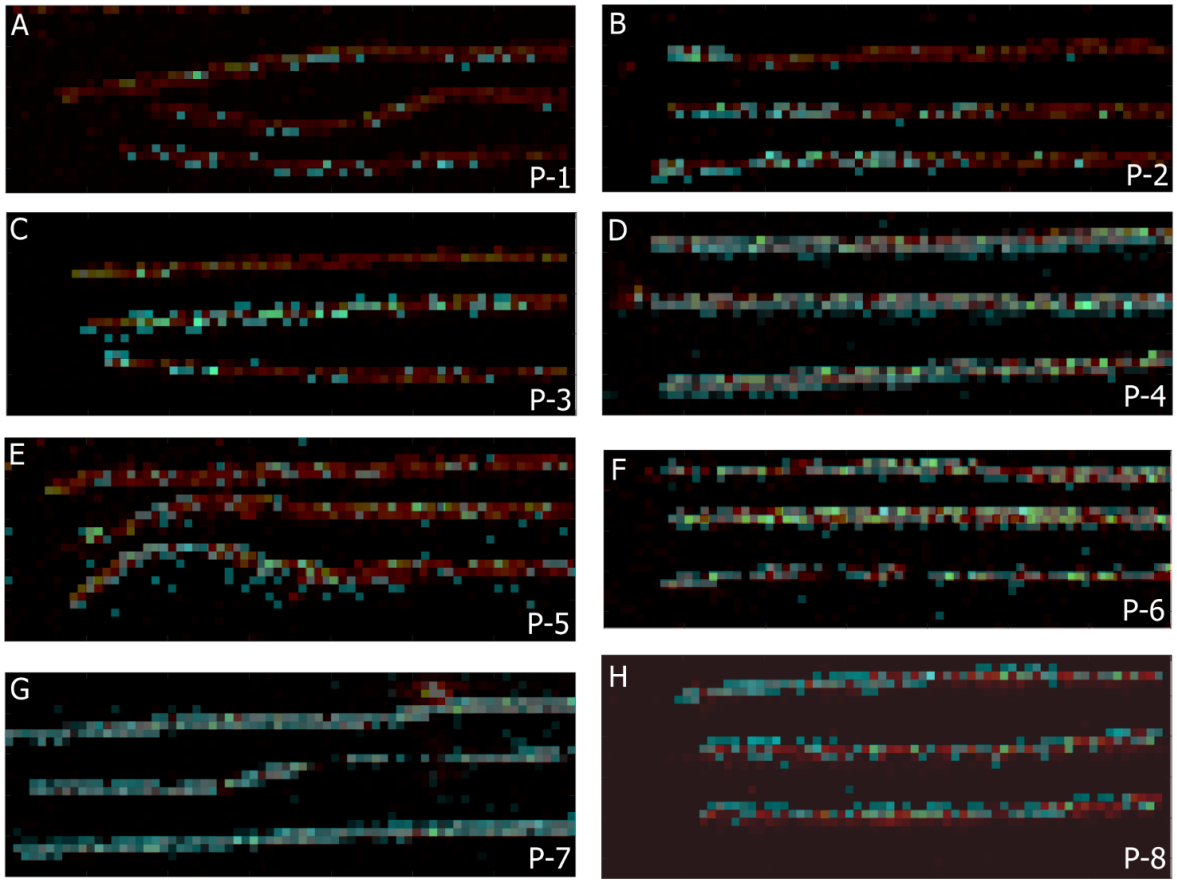


Figure 2. MS/MS images of FTC (m/z 248.1 ± 4 → m/z 130.041) from 8 patients with different cosmetic hair treatments and varying times after treatment. Patient IDs and corresponding treatments and timeline are detailed in Table 1.

location of the hair strands (shown in red/yellow). The proximal end of hair strands, reflecting more recent growth, are on the left side of the images.

Treatments from the Previous Day. The patients P-1 and P-2 had different treatments the day immediately before sampling, but some FTC signal is observed in each hair strand. In the case of P-2, only the proximal end of the hair strands contain FTC, which suggests that the hair treatment (dye) may not have reached the base of the scalp.

Treatments 2-4 Weeks Ago. For P-3, FTC was observed along the length of the middle strand, was intermittent in the bottom strand, and was undetectable in the top strand. In the case of the undetectable strand, this is likely due to this particular hair being in the dormant growth phase. For P-4, whose hair was similar in color, had similar treatment, and had hair treatment on a similar timeline, FTC was observed consistently throughout all three hair strands. P-5 was the only patient who reported using a relaxer, and FTC detection was intermittent throughout the hair strand but concentrated more toward the proximal end.

Treatments more than 4 Weeks Ago. Hair from patients P-6, P-7, and P-8 showed consistent detection of FTC throughout each of the strands.

Assuming a growth rate of ~1 cm/month, 7 mm of hair would correspond to about 3 weeks (21 days) of hair growth. Thus, we would expect to see FTC signal regardless of treatment for hair that was treated more than 3 weeks prior. However, because our method of acquiring the hair was cutting with scissors rather than plucking, approximately 3 to 5 mm of hair may remain below the scalp, and some hairs may not be cut at exactly the same point. This relative imprecision of this cutting method may account for the inter-strand variability in FTC response seen in P-3 and P-5. Nevertheless, if hair was treated very close to sample collection and FTC signal was observed, then that indicated that the hair treatment did not remove all of the drug from the hair strands, as with P-1 and P-2.

We chose to perform IR-MALDESI MSI analysis further along the length of hair strands from one patient with consistent FTC response (P-4) to evaluate a longer time period of hair growth and to observe the time point of treatment. **Error! Reference source not found.** shows the MS image of FTC overlaid with a

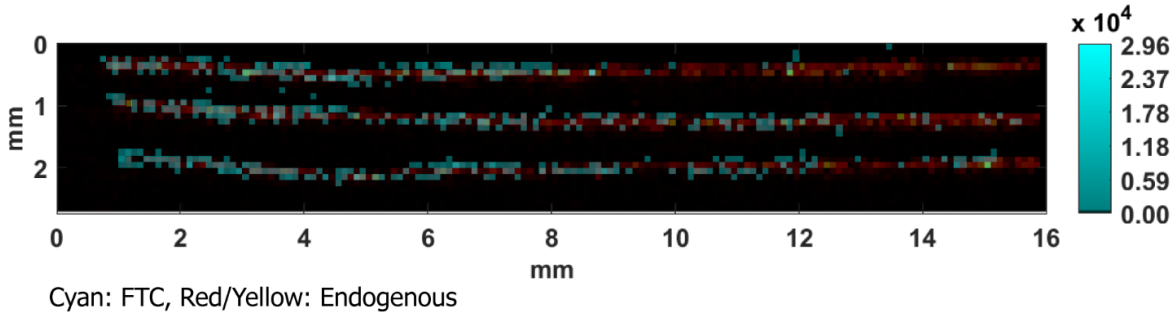


Figure 3. MS/MS image of FTC (m/z 248.1 ± 4 → m/z 130.041) and endogenous (m/z 248.2457) from P-4 sampled on the proximal 15 mm of hair.

n endogenous ion, sampled on the proximal 15 mm of the hair strand. In the top strand, a clear transition is observable at approximately 8mm from hair containing FTC to hair without any FTC. This

position, approximately 7 mm from the proximal end of the hair strand, would correspond to about 3 weeks of hair growth and is consistent with the self-reported time since treatment. The transition was less stark in the other two strands, but there was difference in FTC detection around 12 mm in the image, or about 11 mm from the proximal end of the strands. With imprecision in cutting, this indicates that the treatment may not have reached the base of the scalp, or that some hairs may be layered beneath others and protected from treatment. In the case of layering, some hairs may be unaffected by treatment. For the strands sampled here, an 8-fold decrease in FTC response was measured in the treated region of hair strands relative to the proximal untreated region.

The effect of a range of hair treatments on the detectability of several antiretrovirals was assessed by IR-MALDESI MSI. Our results indicated that a commercial relaxer, dye, and bleach had little or no effect for all but one of the drugs sampled. FTC was completely removed from hair strands following mechanical treatment of individual strands by any of the three hair treatments. Evaluation of treated hair samples from donors on FTC-based regimens indicated that IR-MALDESI MSI was capable of measuring FTC in samples undergoing treatment as recently as the previous day. FTC response was diminished in regions of hair strands exposed to treatment. To ensure fidelity of hair accumulation of FTC for adherence monitoring over a period of one month, we find that any hair treatment should have been conducted more than 4 weeks in the past.