WOMEN'S INTERAGENCY HIV STUDY

SECTION 26: INTENSIVE PHARMACOKINETICS (PK) STUDY PROTOCOL

A. STUDY PURPOSE

To perform intensive pharmacokinetics sampling for WIHS participants on various antiretroviral medications in order to model the factors that contribute to inter-individual variability in overall treatment exposure.

B. RESEARCH GOALS

1. HYPOTHESES

   - The principal factors that will contribute to antiretroviral exposure in the WIHS are adherence to the treatment regimen, characteristics of the regimen, and individual pharmacokinetic profiles, which include factors of drug bioavailability, metabolism and elimination.
   - The addition of factors that contribute to inter-individual variation in drug bioavailability and clearance to measures of adherence will substantially improve predictive models of highly active antiretroviral therapy (HAART) exposure on degree of virologic suppression, immunologic response, and the severity and frequency of adverse effects.

2. SPECIFIC AIMS

   - To assess exposure to antiretroviral treatment among HIV-positive women enrolled in the WIHS cohort, including indices of adherence, clearance (involving the processes of drug metabolism and elimination) and bioavailability.
   - To identify the factors that significantly influence variance in bioavailability and clearance of the new integrase inhibitor among WIHS women, including use of other drugs and medications, ethnicity, multidrug-resistance transporter 1 genotype, body mass, liver and renal function, diet, symptoms such as diarrhea and fever, smoking and concurrent infections such as hepatitis C.
   - To identify the factors that significantly influence variance in bioavailability and clearance of the three most commonly used protease inhibitors and the two most commonly used non-nucleoside reverse transcriptase inhibitors among WIHS women, including use of other drugs and medications, ethnicity, multidrug-resistance transporter 1 genotype, body mass, liver and renal function, diet, symptoms such as diarrhea and fever, smoking and concurrent infections such as hepatitis C. **(NOTE: This aim has been completed.)**

C. BACKGROUND

One of the key questions currently being addressed in the WIHS is how highly active combinations of antiretroviral medications (HAART) affect the outcome of HIV infection. Widespread use of HAART in the United States began in 1996, and increasing use of these combinations has occurred among participants in the WIHS cohort. Once available, HAART usage became the most influential factor in determining the outcome of HIV infection among treated individuals. However, issues such as participant non-adherence with complex chronic regimens, the acquisition of virologic resistance over time, and the frequency of adverse effects have eroded the initial optimism regarding the durability of response to HAART. Large prospective cohort studies, including the WIHS, have now demonstrated that up to 50% of antiretroviral recipients will not achieve suppression of viremia with a given regimen. In addition, both adverse effects and treatment discontinuation occur frequently among HAART recipients in the WIHS.

Since nonadherence to treatment continues to be recognized as the major contributor to suboptimal exposure to antiretroviral drugs and treatment failure, measures of adherence are often included in studies of HAART outcome, including in the WIHS. However, unexpectedly low bioavailability and variations in clearance of drugs also contribute to suboptimal drug exposure, and these effects are far less commonly studied outside of clinical trial settings. The potential impact of individual variability in pharmacokinetics (PK) and pharmacodynamics (PD) on the effectiveness of HAART has not been substantially explored. Clinical trials of antiretroviral agents have described exposure-outcome relationships, but tend to systematically exclude individuals who have risk factors for low drug exposure. The lack of clinically applicable methods for determining exposure to antiretrovirals over long periods of time remains a significant challenge in the clinical management of their use.

High inter-individual variability in the pharmacokinetics of antiretrovirals results in substantial differences among individuals in total drug exposure and the minimum concentration of drug. Dosing adjustments may be required to compensate for marked differences in the bioavailability, metabolism and elimination of drugs among individuals on antiretroviral therapy (ART) to ensure adequate treatment exposure. A more accurate assessment of treatment exposure may provide new insights into the occurrence of adverse effects, as well as virologic and immunologic outcomes of ART. Total drug exposure will most likely be a combination of individual participant adherence and individual pharmacokinetic parameters of bioavailability and clearance. Population pharmacokinetic methods can help determine the statistical distribution of individual PK estimates and identify sources of intra- and inter-individual variability. Population pharmacokinetic methods permit measurement of serum drug concentration and then recalculation of parameters to produce an individualized model of drug exposure in a given individual. The proposed research will perform intensive PK sampling (over 12 hours) for a key antiretroviral agent in a group of treated WIHS participants, and collect data on factors that may influence exposure to this drug. These data will then be used to construct population pharmacokinetic models to assess the contributions of these factors to variance in drug exposure among participants in the WIHS.

D. OVERVIEW OF STUDY

1. Phase 1: NNRTI and PI enrollment

NOTE: enrollment into Phase 1 was discontinued during visit 25.

480 participants total (participants on each of five antiretroviral agents) were enrolled from five WIHS sites for this substudy, encompassing WIHS visits 18 to 25. The San Francisco site will enroll 120 participants over the two years; Chicago, 120 participants over two years; Washington D.C., 80 participants over two years; Bronx, 60 participants over two years; and Brooklyn, 100 participants over two years. A single participant on more than one of the five target antiretroviral medications can receive PK testing for each of the drugs she is taking, and thus may be counted more than once towards a single site's goal number of participants. An enrollment report is available in Apollo to provide real-time statistics regarding the number of participants enrolled at each site for each medication.

2. Phase 2: *Isentress* enrollment

NOTE: enrollment into Phase 2 will begin during visit 30.

120 participants total on *Isentress* will be enrolled from five WIHS sites over three years for this substudy, encompassing WIHS visits 30-36. The San Francisco site will enroll 40 participants over the three years; Chicago, 20 participants over three years; Washington D.C., 20 participants over two years; Bronx, 20 participants over two years; and Brooklyn, 20 participants over two years. When additional antiretroviral medications to be studied are added to the intensive PK list, a single participant on more than one of the target antiretroviral medications can receive PK testing for each of the drugs she is taking. An enrollment report is available in Apollo to provide real-time statistics regarding the number of participants enrolled at each site for each medication.

Each eligible participant will be offered PK substudy enrollment into Phase 2 at the time of her core WIHS visit during visits 30 through 36 (during visits 18 through 25 for Phase 1). If she qualifies and is interested in participating, the participant will be scheduled for a 12-hour (or 24-hour, during Phase 1) stay at the site's GCRC (or similar clinical center) within one month of her core visit. At the PK visit, the participant will be consented and have an intravenous catheter placed for multiple blood draws over the 12-hour (or 24-hour, during Phase 1, depending upon the drug being studied) visit to perform a full pharmacokinetic analysis of the antiretroviral medication(s) of interest. WIHS core data and additional PK visit data will then be used to model the factors that influence drug exposure in these intensively sampled women.

Study forms to be administered for the PK substudy will include:

- PK01: *Eligibility Form* (administered at core WIHS visit; not data entered)
- PK-diet: *Dietary Assessment* (administered via telephone interview when GCRC visit is scheduled; not data entered)
- PKNOTI: *Participation Notification*
- PK02: *Current Antiretroviral Medication Use*
- PK02a: *Antiretroviral Adherence* (complete one form for each target antiretroviral)
- PK03: *Recent Illnesses, Concurrent Medications and Ob/Gyn History*
- PK04: *Recent Substance Use*
- PK05a: *Weight/Specimen Collection Form for Group A Participants*
- PK05b: *Weight/Specimen Collection Form for Group B Participants* (not used in Phase 2)
- PK05c: *Weight/Specimen Collection Form for Group C Participants* (not used in Phase 2)
- PK06: *Dosing of Antiretroviral Medications*
- PK08: *Dietary Fat Percentage Questionnaire*

E. PARTICIPANT ELIGIBILITY AND ENROLLMENT

Eligible women include WIHS participants at five clinical sites (Bronx, Brooklyn, Chicago, San Francisco and Washington D.C.) who meet the following eligibility criteria:

1. The participant should be currently taking the target antiretroviral medication under study, which, in Phase 2, is *Isentress* (raltegravir). (Target antiretrovirals for Phase 1 included *Viracept*, *Kaletra*, *Sustiva*, *Viramune*, and *Reyataz*.)
2. The participant should not have undergone intensive pharmacokinetic sampling for the same medication at a previous PK visit.

Each participant who qualifies for the PK substudy will be enrolled at core WIHS visits 30 through 36, as outlined below in **Section F**.

F. STUDY PROCEDURES

1. CORE VISIT PROTOCOL (PHASE 2 ENROLLMENT)
   a. At the core WIHS visit, complete the ***Eligibility Form*** (PK01) for the PK substudy.
   b. If the participant is on the Phase 2 target antiretroviral medication for the PK Substudy [*Isentress* (raltegravir)], show her the PK Substudy flyer (**Appendix A**) and offer her enrollment into the substudy. As written on the flyer, explain that this substudy will involve the participant being scheduled for a visit to the GCRC (or similar clinical center) within one month of her core visit. The visit will be 12 hours long; sleeping arrangements and all meals

will be provided. She will require an intravenous catheter to be placed in her arm to draw blood every 30 minutes to four hours during that time, and reimbursement is $150.

c. If the participant is eligible and wants to enroll, or just has further questions about the study, circle "1" under question **B4** on form ***PK01*** (eligible, okay to call to schedule appointment). Explain to the participant that a staff member will be contacting her to schedule her appointment for the GCRC visit.

2. SCHEDULING THE PK VISIT

For those women who agree to participate in the substudy, the PK Substudy visit should ideally be scheduled to take place within one month of the core visit (and no more than six weeks after the core visit). If possible, the GCRC where the PK visit will occur should have an idea of the participant's basic dietary patterns before she presents for the PK visit so that the GCRC dieticians can simulate the participant's usual meals during her stay. The staff member scheduling the participant's GCRC visit should perform a quick dietary assessment (using ***PK-diet*** form) over the phone and provide that assessment to the site's GCRC beforehand for meal planning. For those sites where the GCRC will not be utilized for the PK visit, meal planning may not be possible and the dietary assessment need not be administered.

Participants who qualify for the PK Substudy will fall into three separate groups with different procedures required for each:

a. ***GROUP A:*** **Phase 2: Participants who are on *Isentress* (raltegravir). Phase 1 (discontinued): Participants who are on *Kaletra, Viracept,* and/or *Viramune*, but not on *Sustiva* or *Reyataz.***

Group A participants have the option of choosing a 12-hour or a 24-hour stay in the GCRC. If a participant in Group A chooses a 24-hour stay, she should be instructed not to consume her usual evening medications prior to presentation to the GCRC in the evening. Upon presentation, she will take her evening medications at the GCRC (which will be witnessed and recorded by GCRC staff), sleep overnight at the center, and have the pharmacokinetic sampling performed over 12 hours the next day. If she chooses a 12-hour stay, she will be contacted the night before her visit to the GCRC in order to confirm the time when she took her evening doses of medication. She will then present to the GCRC the following morning for a 12-hour stay for pharmacokinetic sampling.

For participants with poor venous access, sites may want to arrange for the participant to have a PICC line placed by local services on the day prior to or day of the PK visit. Arrange for transportation to the GCRC per site's usual protocol.

NOTE: There will be no Group B or Group C PK study visits scheduled at this time.

b. ***GROUP B:*** **Phase 1 (discontinued): Participants who are on *Sustiva* and/or *Reyataz,* but who are NOT taking any other target antiretroviral medication.**

Group B participants will require a 24-hour stay in the GCRC. Please remind the participant to bring her usual antiretroviral medications with her when she presents for her scheduled PK visit. The participant can be admitted either in the morning or the evening for the 24-hour *Sustiva* or *Reyataz* visit, depending on the time of day that she usually takes her ART doses.

c. ***GROUP C:*** **Phase 1 (discontinued): Participants who are on *Sustiva* and/or *Reyataz,* and who are ALSO taking one or more of the other three target antiretroviral medications.**

Group C participants will require a 24-hour stay in the GCRC. Please remind the participant to bring her usual antiretroviral medications with her when she presents for her scheduled PK visit. The participant can be admitted either in the morning or in the evening for the 24-hour *Group C* visit, depending on the time of day that she usually takes her ART doses.

3. DAY BEFORE PK VISIT

   a. ***GROUP A:*** **Phase 2: Participants who are on *Isentress* (raltegravir). Phase 1 (discontinued): Participants who are on *Kaletra, Viracept,* and/or *Viramune,* but not on *Sustiva* or *Reyataz.***

      WIHS staff must call each Group A participant scheduled for a 12-hour stay in the GCRC the evening before her PK visit to verify that she consumed her evening dose of the target antiretroviral medication. For these "12-hour stay" participants, please record the time she reported consuming her target medication the night before the PK visit on form ***PK05a*** (*Weight/Specimen Collection Form For Group A Participants)*, question **D1**. Then, under question **D1a**, circle "1" (telephone interview). Participants in Group A scheduled for 24-hour stays in the GCRC should receive a reminder call about the visit the day before. Please remind participants to bring their usual antiretroviral medications to the PK visit for both 12-hour and 24-hour stays.

      NOTE: There will be no Group B or Group C PK study visits scheduled at this time.

   b. ***GROUP B:*** **Phase 1 (discontinued): Participants who are on *Sustiva* and/or *Reyataz* (if patient is on both meds, must take them simultaneously), but who are NOT taking any other target antiretroviral medication.**

      Participants in Group B should receive a reminder call the day before the PK visit. Please remind participants to bring their usual antiretroviral medications to the PK visit.

   c. ***GROUP C:*** **Phase 1 (discontinued): Participants who are on *Sustiva* and/or *Reyataz* (if patient is on both meds, must take them simultaneously), and who are ALSO taking one or more of the other three target antiretroviral medications.**

      WIHS staff must call each Group C participant scheduled to be admitted in the morning to the GCRC the evening before her PK visit to verify she consumed her evening doses of the target antiretroviral medication(s). WIHS staff must call each Group C participant scheduled to be admitted in the evening to the GCRC the morning of her PK visit to verify she consumed her morning doses of the target antiretroviral medication(s). Please record the time the participant reported consuming her target medication(s) the night before or the morning of her PK visit on form ***PK05c*** (*Weight/Specimen Collection Form For Group C Participants)*, question **D1**.

4. PK VISIT PROCEDURES

   The visit protocols for participants in each group (A, B and C) are detailed below; however, Group A only will participate in this phase (2) of the substudy. When a participant presents to the GCRC, if she reports that she is no longer taking the target medication(s) that made her eligible for enrollment in the PK Substudy, she will not be eligible for participation at that time. However, if she reports use of a target medication(s) at a later core WIHS visit, she may be enrolled for that visit. Of note, meals and snacks in the GCRC should be provided at the approximate times the participant consumes her meals/snacks during a routine day. The participant should be allowed to consume her usual medications, herbal supplements, vitamins, etc., per her routine during her stay on the GCRC.

   a. ***Group A:*** **Phase 2: Participants who are on *Isentress* (raltegravir). Phase 1 (discontinued): Participants who are on *Kaletra, Viracept* and/or *Viramune,* but who are NOT on *Sustiva* and/or *Reyataz*.**

      1. Verify that the participant brought her usual antiretroviral medications with her to the PK visit. If she did not bring in her medications, the PK visit will need to be rescheduled.

2. Obtain informed consent for the study (please see **PK protocol consent** in **Appendix B** for template of consent form, which should be modified for each individual site in terms of CHR contact information and name(s) of principal investigator). Complete form ***PKNOTI***.

3. Fill out form ***PK02*** (*Current Antiretroviral Medication Use)*. Show the participant the **Antiretroviral Medication Cards** to help fill out question **B2**. Then, fill out Section A of form ***PK05a*** and circle the names(s) of the target antiretroviral medication(s) under study.

4. If the participant was scheduled for a 12-hour visit for PK sampling for one or more of the target drugs, she should have been called the night before her PK visit and question **D1** on form ***PK05a*** should have been filled out, with "1" circled under **D1a.** For the 12-hour stay, admit participant to the GCRC in the morning and skip to step 6 of this list.

5. If the participant was scheduled for a 24-hour visit for PK sampling for one or more of the target drugs, admit her to the GCRC in the evening. Allow the participant to take her usual evening medications. Record the exact time the participant took her evening dose of the target antiretroviral medication(s) on form ***PK05a,*** question **D1**. Then, under question **D1a**, circle "2" (witnessed dose in clinical center).

6. Measure and record weight of the participant in pounds (round to nearest 1.0 pound) in question **B1** on form ***PK05a***.

7. Perform urine pregnancy test for participant and record result on question **C1** on form ***PK05a***. Circle "3" (not done) if the test is not performed.

8. Place a saline lock catheter. (Catheter can be placed in the evening or the morning for 24-hour stay participants, per staff and participant preference/convenience. However, the first time point of PK sampling – time point '0' – should be drawn in the morning prior to the ingestion of the morning doses of medications.). See **Section G** for instructions on placement of the catheter and blood collection.

9. In the morning, which would be upon admission for the "12-hour stay" participants, draw 5ml of blood into an EDTA tube using the methods described in **Section G** of the protocol. This will serve as the 0-minute time sample. Label the specimen as specified in **Section H** and place the EDTA tube on ice. Record the exact time of specimen collection in question **D2** of form ***PK05a.***

10. Instruct the participant to take her antiretroviral medicines as she does at home (with food if that is her routine). Record the time the participant takes her medicines in question **D3**, form ***PK05a***. Indicate whether the participant took her target medication(s) with or without food in question **D4**.

11. Set an electronic timer for each of the subsequent blood draws. At 30, 60, 120 (2 hours), 150 (2.5 hours), 180 (3 hours), 240 (4 hours), 300 (5 hours), 360 (6 hours), 480 (8 hours), and 720 minutes (12 hours) after the participant takes her medications, collect one 5ml EDTA tube of blood from the saline lock, using the methods described in **Section G**. Record the exact time of each of these blood draws on form ***PK05a*** **(**questions **D5-D14)**. If you draw the blood specimen a few minutes early or late, please be sure to record the actual time at which you obtained the blood, not the time for which the draw was scheduled. Place all EDTA tubes on ice after collection. Label all specimens as specified in **Section H**.

12. Blood samples should be kept on ice, and undergo processing, aliquoting and freezing, as specified in **Section I**, within six hours of collection.

13. Please use the time between blood draws to administer forms ***PK02a*** (*Antiretroviral Adherence)*, ***PK03*** (*Recent Illnesses, Concurrent Medications And Ob/Gyn History)*, ***PK04*** (*Recent Substance Use)*, ***PK06*** (*Dosing Of Antiretroviral Medications)*, and ***PK08*** *(Dietary Fat Percentage Questionnaire)*, as outlined in steps 14-18.

14. Administer one form ***PK02a*** for each target antiretroviral medication(s) checked on form ***PK02***. As prompted on form, please use **response cards PK02A-6, PK02A-7** and **PK02A-8** when asking questions **B6, B7** and **B8** to help the participant keep track of the possible answers.

15. Administer form ***PK03***. Please use **PK03 medication cards A-I** to show the participant pictures of the medications being queried about in questions **D1-D12**.

16. Administer form ***PK04***. As prompted on the form, use r**esponse card PK04** to help the participant keep track of the possible answers.

17. Administer form ***PK06***.

18. Administer Sections A and B of form ***PK08***. As prompted on the form, please use **response card PK08** when asking questions **B1-B17**. After completing the form, go to Berkeley Nutrition Services, on-line Fat Screener form: http://www.nutritionquest.com/fat_screener.html. Enter the responses indicated by the participant for questions **B1-B17** into the form and click "submit questionnaire." The first paragraph of the "Fat Screener Results" will contain an estimate of the participant's fat intake. Circle the participant's estimated fat intake in question **C1**.

19. Once all the blood samples have been collected and recorded on form ***PK05a,*** and all other study forms have been completed, please remove the intravenous catheter and discharge the participant from the GCRC.

NOTE: If a blood draw time point is missed during a PK visit, this should be recorded on form ***PK05*** (a, b or c, as applicable) using the standard WIHS notation for missing data. For example: If the participant refused to allow blood to be drawn at a particular time point, enter "-7" and note that the participant refused. If a time point is missed (e.g., staff forgets, staff is busy elsewhere and misses time point), enter "-9" and note the reason for the missed time point on the form.

NOTE: There will be no Group B or Group C PK study visits scheduled at this time.

b. ***Group B*:** **Phase 1 (discontinued): Participants who are on *Sustiva* and/or *Reyataz* (if patient is on both meds, must take them simultaneously), but who are NOT taking any other target antiretroviral medication.**

1. Verify that the participant brought her usual antiretroviral medications with her to the PK visit. (If she did not bring in her medications, the PK visit will need to be rescheduled.) Remember that the participant should present in the morning to the GCRC if she usually takes her target medications in the morning; she should present to the GCRC in the evening if she routinely takes her target medications in the evening.

2. Obtain informed consent for the study (please see the **PK protocol consent** in **Appendix B** for a template of consent form, which should be modified for each individual site in terms of CHR contact information and name(s) of principal investigator). Complete form ***PKNOTI*.**

3. Fill out form ***PK02*** (*Current Antiretroviral Medication Use).* Show the participant the **Antiretroviral Medication Cards** to help fill out question **B2**. Then, fill out Section A of form ***PK05b*** (*Weight/Specimen Collection Form For Group B Participants)*.

4. Take weight of the participant and record in pounds (round to nearest 1.0 pound) in question **B1** on form ***PK05b***.

5. Perform urine pregnancy test for participant and record result on question **C1** on form ***PK05b***. Circle "3" (not done) if the test is not performed.

6. Place a saline lock catheter. See **Section G** for instructions on placement of the catheter and blood collection.

7. Draw 5ml of blood into an EDTA tube. This will serve as the 0-minute time sample. Label the specimen as specified in **Section H** and place the EDTA tube on ice. Record the exact time of specimen collection in question **D1** on form ***PK05b.***

8. Instruct the participant to take her target medications along with any other medications that she usually takes at home at the same time (Target medications should be taken with food if that is her routine). Record the time the participant took her target medications in question **D2**, form ***PK05b***. Indicate whether the participant took her target medications with or without food in question **D3**.

9. Set an electronic timer for each of the subsequent blood draws. At 1, 2, 4, 6, 8, 12, 15, 18, 21 and 24 hours after the participant takes her target medications, collect one 5ml EDTA tube of blood from the saline lock, using the methods described in **Section G** of the protocol. Record the exact time of each of these blood draws on form ***PK05b*** **(**questions **D4-D13)**. If you end up obtaining the blood specimen a few minutes early or late, please be sure to record the actual time at which you obtained the blood, not the time for which the draw was scheduled. Place all EDTA tubes on ice after collection. Label all specimens as specified in **Section H**.

10. Blood samples should be kept on ice, and undergo processing, aliquoting and freezing, as specified in **Section I**, within six hours of collection.

11. Please use the time between blood draws to administer forms ***PK02a*** (*Antiretroviral Adherence*), ***PK03*** (*Recent Illnesses, Concurrent Medications And Ob/Gyn History*), ***PK04*** (*Recent Substance Use*), ***PK06*** (*Dosing Of Antiretroviral Medications*), and ***PK08*** (*Dietary Fat Percentage Questionnaire*), as outlined in steps 12-16.

12. Administer one form ***PK02a*** for each target antiretroviral medication(s) checked on form ***PK02***. As prompted on form, please use r**esponse cards PK02A-6, PK02A-7** and **PK02A-8** when asking questions **B6, B7** and **B8** to help the participant keep track of the possible answers.

13. Administer form ***PK03***. Please use **PK03 medication cards A-G** to show the participant pictures of the medications being queried about in questions **D1-D10**.

14. Administer form ***PK04***. As prompted on the form, use r**esponse card PK04** to help the participant keep track of the possible answers.

15. Administer form ***PK06***.

16. Administer Sections A and B of form ***PK08***. As prompted on the form, please use **response card PK08** when asking questions **B1-B17**. After completing the form, go to Berkeley Nutrition Services, on-line Fat Screener form: http://www.nutritionquest.com/fat_screener.html. Enter the responses indicated by the participant for questions **B1-B17** into the form and click "submit questionnaire." The first paragraph of the "Fat Screener Results" will contain an estimate of the participant's fat intake. Circle the participant's estimated fat intake in question **C1**.

17. Once all the blood samples have been collected and recorded on form ***PK05b*** and all other study forms have been completed, please remove the intravenous catheter and discharge the participant from the GCRC.

NOTE: If a blood draw time point is missed during a PK visit, this should be recorded on form ***PK05*** (a, b or c, as applicable) using the standard WIHS notation for missing data. For example: If the participant refused to allow blood to be drawn at a particular time point, enter "-7" and note that the participant refused. If a time point is missed (e.g., staff forgets, staff is busy elsewhere and misses time point), enter "-9" and note the reason for the missed time point on the form.

c. ***Group C:*** **Phase 1 (discontinued): Participants who are on *Sustiva* and/or *Reyataz* (if patient is on both meds, must take them simultaneously), and who are ALSO taking one or more of the other three target antiretroviral medications.**

1. Verify that the participant brought her usual antiretroviral medications with her to the PK visit. If she did not bring in her medications, the PK visit will need to be rescheduled.
2. Obtain informed consent for the study (please see **PK protocol consent** in **Appendix B** for template of consent form, which should be modified for each individual site in terms of CHR contact information and name(s) of principal investigator). Complete form ***PKNOTI*.**
3. Fill out form ***PK02*** (*Current Antiretroviral Medication Use)*. Show the participant the **Antiretroviral Medication Cards** to help fill out question **B2**. Then, fill out Section A of form ***PK05c*** and circle the names(s) of the target antiretroviral medication(s).
4. The participant should have been called the night before or the morning of her PK visit and question **D1** on form ***PK05c*** should have been completed at this time.
5. Take weight of the participant and record in pounds (round to nearest 1.0 pound) in question **B1** on form ***PK05c***.
6. Perform urine pregnancy test for participant and record result in question **C1** on form ***PK05c***. Circle "3" (not done) if the test is not performed.
7. Place a saline lock catheter. See **Section G** for instructions on placement of the catheter and blood collection.
8. Draw 5ml of blood into an EDTA tube using the methods described in **Section G** of the protocol. This will serve as the 0-minute time sample. Label the specimen as specified in **Section H** and place the EDTA tube on ice. Record the exact time of specimen collection in question **D3** on form ***PK05c*.**
9. Instruct the participant to take her antiretroviral medicines as she does at home (with food if that is her routine). Record the time the participant takes her medicines in question **D3**, form ***PK05c***. Indicate whether participant took her target medication(s) with or without food in question **D4**.
10. Set an electronic timer for each of the subsequent blood draws. At 30, 60, 120 (2 hours), 150 (2.5 hours), 180 (3 hours), 240 (4 hours), 300 (5 hours), 360 (6 hours), 480 (8 hours), 720 (12 hours), 900 (15 hours), 1080 (18 hours), 1260 (21 hours), and 1440 minutes (24 hours) after the participant takes her medications, collect one 5ml EDTA tube of blood from the saline lock, using the methods described in **Section G**. Record the exact time of each of these blood draws on form ***PK05c* (**questions **D5-D18)**. If you end up obtaining the blood specimen a few minutes early or late, please be sure to record the actual time at which you obtained the blood, not the time for which the draw was scheduled. Place all EDTA tubes on ice after collection. Label all specimens as specified in **Section H**.

11. Blood samples should be kept on ice, and undergo processing, aliquoting and freezing, as specified in **Section I**, within six hours of collection.

12. Please use the time between blood draws to administer forms ***PK02a*** (*Antiretroviral Adherence*), ***PK03*** (*Recent Illnesses, Concurrent Medications And Ob/Gyn History*), ***PK04*** (*Recent Substance Use*), ***PK06*** (*Dosing Of Antiretroviral Medications*), and ***PK08*** (*Dietary Fat Percentage Questionnaire*), as outlined in steps 13-17.

13. Administer one form ***PK02a*** for each target antiretroviral medication(s) checked on form ***PK02***. As prompted on form, please use r**esponse cards PK02A-6, PK02A-7** and **PK02A-8** when asking questions **B6, B7** and **B8** to help the participant keep track of the possible answers.

14. Administer form ***PK03***. Please use **PK03 medication cards A-G** to show the participant pictures of the medications being queried about in questions **D1-D10**.

15. Administer form ***PK04***. As prompted on the form, use r**esponse card PK04** to help the participant keep track of the possible answers.

16. Administer form ***PK06***.

17. Administer Sections A and B of form ***PK08***. As prompted on the form, please use **response card PK08** when asking questions **B1-B17**. After completing the form, go to Berkeley Nutrition Services, on-line Fat Screener form: http://www.nutritionquest.com/fat_screener.html. Enter the responses indicated by the participant for questions **B1-B17** into the form and click "submit questionnaire." The first paragraph of the "Fat Screener Results" will contain an estimate of the participant's fat intake. Circle the participant's estimated fat intake in question **C1**.

18. Once all the blood samples have been collected and recorded on form ***PK05c*** and all other study forms have been completed, please remove the intravenous catheter and discharge the participant from the GCRC.

NOTE: If a blood draw time point is missed during a PK visit, this should be recorded on form ***PK05*** (a, b or c, as applicable) using the standard WIHS notation for missing data. For example: If the participant refused to allow blood to be drawn at a particular time point, enter "-7" and note that the participant refused. If a time point is missed (e.g., staff forgets, staff is busy elsewhere and misses time point), enter "-9" and note the reason for the missed time point on the form.

G. INSTRUCTIONS FOR PLACING THE CATHETER AND DRAWING BLOOD

1. Ready an 18- or 20-gauge intravenous catheter.
2. Have three syringes, each filled with 5cc of normal saline, ready.
3. **Optional:** Have three syringes, each filled with 1cc of heparin flush, ready.
4. Place the IV catheter into a large vein (antecubital if nothing else is available). If trouble is encountered in finding a vein, putting a heating pad around the arm may help; in addition, finding a vein in a well-hydrated patient is easier, so have the patient drink a lot of water prior to a repeat attempt.
5. Attach extension tubing to the catheter.
6. Flush the catheter with 5cc of normal saline. Clamp extension tubing just prior to finishing the flush.
7. **Optional:** Unclamp the tubing and inject 1cc of heparin flush into the line; clamp tubing just prior to finishing the flush.

8. At the appropriate time points, discard 1-3 cc of blood prior to drawing the new PK sample, or hang a saline bag to keep the line open during the duration of PK sampling. Then draw 5ml of blood into an EDTA tube. EDTA tubes are typically lavender-topped and should be large enough to contain 5ml. The catheter should be flushed with 5cc of normal saline (**optional:** flush catheter with 1cc heparin after saline flush) after each blood draw. All EDTA tubes should be filled to capacity and gently inverted several times immediately after blood collection to ensure that the anticoagulant mixes evenly with the blood. Place each EDTA tube on ice.
9. Once all blood samples have been collected, remove the IV catheter.

H. SPECIMEN LABELING

Each site will make its own special set of labels for the PK Substudy. Given the number of timepoints in this study, it is generally best to prepare labels ahead of time. Each label should include the WIHSID, date of collection, WIHS S-code and material type (see **Manual of Operations, Section 10**), time point of collection, the core visit number and the PK visit number. The date and times of specimen collections must be written onto the pre-printed labels. It is crucial that the specimens be labeled immediately after collection and that the correct label is placed on each timed sample.

I. LABORATORY PROCEDURES

Specimens can be processed at the GCRC if lab services are available; otherwise they should be delivered to the local processing lab within six hours of the blood draw, counting from time "0," for processing. The PK specimens should be kept on ice (4°C) during transport. Steps for processing are as follows:

Specimen processing and storage

1. Centrifuge EDTA tubes at 1,200 x g for 10 minutes (can be performed at room temperature or at 4^{o}C).
2. Sub-aliquot 1.2 ml of plasma into 2 ml cryotubes for a total of two aliquots in two different tubes. Screw-cap crytotubes are preferred to snap-top cryotubes. One will be sent to Dr. Benet's laboratory for testing; the other held locally until it is verified that the Benet lab received and was able to analyze the first aliquot. At this point, the second aliquot can be donated to the ACSR (See **Section L**).
3. Sub-aliquot remaining plasma in 1 ml or 0.5 ml aliquots, depending on the amount remaining, into cryotubes for shipment to the WIHS central repository. (Example: if 2 ml remain, aliquot 2 x 1 ml; if 1.5 ml remain, aliquot 1 x 1ml and 1 x 0.5 ml.) The aliquots should be labeled the same as the EDTA tubes used in collection.
4. PK sampling is done at 11 different time points. It is extremely important to know when each sample was drawn. All aliquots must be labeled immediately after processing with the following:

    Participant WIHSID

    Date of specimen collection

    S-code (see WIHS **Manual of Operations, Section 10.VIII.A**)

    WIHS alpha code (see WIHS **Manual of Operations, Section 10.VIII.A**)

    Core visit #

5. Polyester tape should then be placed over the labels.

6. Freeze plasma at –70°C.

7. Plasma for PK testing is to be stored at the local lab until shipment to Dr. Yong Huang's laboratory as in **Section J**. Specimens must be kept frozen at –80°C prior to shipment.

J. SHIPPING

1. Specimens should be batch-shipped to the Huang lab on the first Monday or Tuesday of each month. On the day of shipment, the lab manager of Huang's lab (Winnie Gee; winnie.gee@ucsf.edu) must be emailed with the tracking number to inform her that the shipment will be expected in the lab later in the week. In addition, each site should email Winnie Gee the invoice that will be included in the shipment (as detailed in #2).

2. Each shipment to a testing lab should be accompanied by an invoice indicating its contents (specimen list), date of shipment, conditions and destination. The specimen list should be prepared in the same format used for shipments to SeraCare, and printed out in a column format (e.g., Excel). Columns 4 through 9 (FREEZER and RACK) and 14 through 23 (FREEZER ID) should be left blank. Additionally, a cover letter should be sent with the specimens stating that they are PK specimens, the total number of specimens included in the shipment, and a contact person with phone and pager number. Samples should be sent on dry ice in a box with the samples put in order of time point. Please be sure to include enough dry ice in the shipments to cover unexpected courier delays. Box should be marked on outside as "Infectious Material," and the following information should also be included: the city of origin, the date of shipment, whether the samples are WIHS PK or VRS.

   PK samples:

Amount to be sent:	1 x 1.2ml aliquots of plasma per time point per visit per person.
Address:	Yong Huang PhD Drug Studies Unit (DSU) 347 Littlefield Avenue South San Francisco, CA 94080 Phone number of lab: 415-476-5220 Alternative phone number: 415-476-8191
Notation:	WIHS PK specimens

As described in **Section I**, #2, above, once it has been verified that the Huang lab received and was able to analyze the first 1.2 ml aliquot, the second 1.2 ml aliquot may be donated to the ACSR. In addition, any other remaining aliquots (as described in **Section I**, #3, above) can be donated to the ACSR. These samples must be labeled as indicated above, and include the appropriate s-code and alpha code. Shipments to the ACSR should be made as described, in **Section L**, below.

K. DATA REPORTING

PK testing results will be transmitted from the Huang laboratory to WDMAC via an Excel file at the end of each visit window.

L. DONATION OF REMAINING ALIQUOTS FROM PK AND METABOLIC STUDIES TO ACSR

1. STORAGE AND SHIPMENT OF SPECIMENS

   Any aliquots not sent for testing should be held locally until test results have been received in case back up specimens are needed at the testing lab. After test results have been received, extra aliquots not needed for testing may be donated to the ACSR. Specimens from the San Francisco

and Chicago sites should be sent to the UCSF Specimen Resource. The UCSF ACSR address is the following:

Leanne C. Huysentruyt, Ph.D.
Project Manager
AIDS and Cancer Specimen Resource / ACSR
McGrath Labs
University of California San Francisco
1001 Potrero Avenue
Bldg 100, Room 333E
San Francisco, CA 94110

Email: leanne.huysentruyt@ucsf.edu
Tel: 415-206-5510
Fax: 415-206-6625

Prior to shipping specimens to UCSF, please call the lab at (415) 206-5510 and e-mail Leanne Huysentruyt at Leanne.huysentruyt@ucsf.edu to inform the lab of the pending shipment and tracking number. In addition, an electronic manifest in Excel format should be e-mailed to Eileen along with the notification of shipment. See **Section L.2** for details on manifest preparation. Also see **Appendix C** for notification format.

In case of problems, call Leanne Huysentruyt at (415) 206-5510, Ron Honrada at 415-206-5434, Alanna Morris at 415-206-5434, or Melissa Ancheta at (415) 206-3858.

Specimens from the Bronx, Brooklyn and Washington DC sites should be sent to the East Coast AIDS and Cancer Specimen Bank at George Washington University Medical Center. The EC ACSB address is the following:

East Coast AIDS and Cancer Specimen Bank
Department of Pathology
George Washington University Medical Center
2300 I Street, NW

Phone: (202) 994-0434 or (202) 994-2530

No specimens should be shipped on Friday. Prior to shipping specimens to GWUMC, please call the Bank at (202) 994-0434 or (202) 994-2530 to inform them of the pending shipment and tracking number.

2. SHIPMENT MANIFESTS

Each shipment must be accompanied by a manifest indicating its contents (specimen list), date of shipment, conditions and destination. The specimen list should be prepared in the same format used for shipments to SeraCare, and printed out in a column format (e.g., Excel). Columns 4 through 9 (FREEZER and RACK) and 14 through 23 (FREEZER ID) should be left blank. Boxes should be numbered consecutively, starting with the first shipment and continuing in subsequent shipments. For example, if the first shipment consists of box 1 and box 2, the box number in the second shipment should start with 3. Additionally, a cover letter should be sent with the specimens stating that these specimens are being donated to the ACSR, the total number of specimens included in the shipment, and a contact person with phone and pager number.

3. SPECIMEN PACKING, BOX LABELING AND INVOICES

Samples should be sent on dry ice in a specimen storage box. The name of shipping site should be clearly marked on the outside of the specimen storage box.

All specimens should be packed with enough absorbent material to contain the sample volume in case of a leak. Each box should be rubberbanded. Box(es) should then be placed in a leak proof bag and then packed in dry ice. The fiberboard boxes containing the specimens should be labeled with the following information:

- the city of origin
- the date of the shipment
- the study name: WIHS PK specimens
- Box #

Specimens should be packed and shipped according to IATA regulation 650 for diagnostic specimens packed on dry ice.

APPENDIX A: PK FLYER

WIHS INTENSIVE PHARMACOKINETICS (PK) STUDY

ARE YOU

HIV positive AND on the following antiretroviral medication?

***Isentress* (Raltegravir)**

If so, you may qualify for the PK substudy. The purpose of PK is to try to figure out why different people get different responses (in terms of side effects and success) from antiretroviral medications by measuring drug levels on these meds over 12-24 hours. This study would involve –

- You being scheduled to come into the research center within 1 month of this core visit for either a 12- or 24-hour visit (depending on the med). Your bed and meals will be provided for the entire stay.
- Placement of a catheter in your arm to draw blood every 1 to 4 hours during that time
- **$150 reimbursement**

If interested or if you have questions, please let us know and WIHS staff will contact you within the next 1-2 weeks to give you more information and/or to schedule the substudy visit.

APPENDIX B: SAMPLE INFORMED CONSENT FORM

Consent to be a Research Subject

WIHS Antiretroviral Treatment Exposure Study

Women's Interagency HIV Study (WIHS)

A. PURPOSE AND BACKGROUND

Ruth Greenblatt MD and colleagues at the University of California, San Francisco are conducting a study to assess the actual level of exposure to anti-HIV therapy in women. This study may help to identify reasons for success and failure of anti-HIV therapies. I am being asked to participate in this study because I am a woman, because I have HIV infection, and because I am taking the HIV medication named raltegravir (*Isentress).*

B. PROCEDURES

If I agree to participate in this substudy, the following will happen:

1. For this stay at the hospital, I have been asked to bring in all my usual medications with me. I will have all meals provided for me with my usual food preferences (no food restrictions) and will sleep in the hospital if on an overnight stay. Transportation back home will be provided.

2. I understand that there are no restrictions to my usual activities prior to my hospital visit; I will be asked about drug use or other habits prior to my visit to help the researchers understand factors that may influence HIV medication exposure, but there are no restrictions on any of my usual activities (smoking, drinking, etc.) prior to the hospital visit.

3. I will have an intravenous (IV) catheter placed in a vein in my arm or other site upon coming to the hospital. This catheter will be used to draw a series of blood samples over the next 12 to24 hours so that only one needle stick will be required. A urine sample for a pregnancy test will be collected. .

4. I will be asked a series of questions about recent habits and recent patterns of medication use. I will also be asked about recent substance use.

5. I will take my HIV medications and other medications as usual during my 12 or 24 hour stay in the hospital; the time of my medication doses will be recorded by the hospital nurses.

6. Over the next 12 or 24 hours, I will have a series of blood samples drawn from the IV catheter for testing for HIV medication drug levels. Since I am on *Isentress* taken twice a day, the blood samples will only be collected after 7am in the morning. The total amount of blood to be collected is 4-5 tablespoons.

7. After all blood samples have been collected, the IV catheter will be removed and I will be provided transportation back home.

8. The blood samples collected will be used to test for HIV medication levels. The blood samples may be saved and tested in the future for medication levels as well. This will help determine how much HIV medication actually gets into the system of women such as myself and will help researchers figure out why some women have side effects on the HIV therapies.

C. RISKS AND DISCOMFORTS

1. The risk of having an IV catheter placed and my blood drawn includes temporary discomfort from the needle stick, bruising, and rarely, infection.

2. The interview includes questions that are personal (for example, do I use any recreational drugs). I am free to decline to answer, and I may stop the interview at any time.

3. Participation in research may involve a loss of privacy. Records of my participation in the study will be kept as confidential as possible. My identity will not be used in any reports or publications generated by the study. Study information will be coded on to forms which identify me by study number only, and not by name. These forms will be kept in locked files at all times. Only study personnel will have access to the files.

4. The Department of Health and Human Services has issued a Certificate of Confidentiality to this project. The Certificate of Confidentiality insures that all confidential and personal information I provide for this study remains confidential, and cannot be requested or utilized by other official governmental and local agencies. The Certificate further insures that research records cannot be subpoenaed by a court of law.

5. If I am injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, depending on a number of factors. The University does not normally provide any form of compensation for injury. For further information about his, I may call or write the Committee on Human Research (telephone: (415) 476-1814, mail: Box 0962, UCSF, San Francisco, CA 94143).

D. BENEFITS

By participating in this study I may gain knowledge about my HIV and help investigators to learn more about HIV.

E. ALTERNATIVES

I am free to choose not to participate in this study. If I decide not to participate in this study, I can still participate in the remainder of the WIHS Natural History of HIV in Women Study.

F. COSTS

There are no costs to me as a result of taking part in this study.

G. REIMBURSEMENT

I will be reimbursed $150 for this overnight visit. $50 cash will be given to me at the end of the overnight visit and a $100 check will be sent to me within the following month.

H. QUESTIONS

This study has been explained to me by _____ and my questions have been answered. If I have further questions about the study, or I wish to express a complaint or comment, I can contact Dr. Monica Gandhi via telephone at (415) 502-6290. If I do not wish to do this, I may contact the Committee on Human Research, which is concerned with protection of volunteers in research projects. *I may reach the Committee on Human Research by phone at (415) 476-1814, or by mail at Suite 11, Laurel Heights Campus, Box 0962, University of California, San Francisco, CA 94143.*

I. CONSENT

I will be given a copy of this consent form to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY. I am free to decline to participate in this study or to withdraw from it at any point. My decision as to whether or not to participate in this study will have no influence on my present or future status as a WIHS study participant, patient, student, or employee at UCSF.

I agree to participate in this study by signing below.

Participant's Signature	Date
Signature of Person Obtaining Consent	Date

APPENDIX C: NOTIFICATION OF SHIPMENT: INTENSIVE PK DONATIONS TO ACSR

WOMEN'S INTERAGENCY HIV STUDY

Notification of Shipment: Intensive PK donations to ACSR

I. Sender Details

(Fax this form one day prior to shipment)

Site Name: _____
Contact Person: _____
Phone #: _____
Fax #: _____
Email address: _____
Project: _____

II. Recipient Details

Leanne Huysentruyt
ACSR / McGrath Labs
1001 Potrero Avenue
Bldg 100, Room 333E
San Francisco, CA 94110

415-206-5510
415-206-6625 (FAX)
Leanne.huysentruyt@ucsf.edu

III. Shipment Details

(To be completed by sender)

Courier: _____
Airbill #: _____
Date shipped: _____
Number of Boxes: _____
Number of Vials: _____
Amount of Refrigerant: _____ kg

IV. Comments

_____
_____
_____
_____
_____
_____
_____