Title: Acute HIV Infection in Youth: Protocol for the Adolescent Trials Network 147 Comprehensive Adolescent Research and Engagement Studies (CARES) Study

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ABSTRACT

Background: Early treatment studies have shown that prompt treatment of HIV with combination antiretroviral therapy (cART) can limit the size of latent viral reservoirs, thereby providing clinical and public health benefits. Studies have demonstrated that adolescents have a greater capacity for immune reconstitution than adults. Nevertheless, adolescents who acquired HIV through sexual transmission have not been included in early treatment studies due to challenges in identification and adherence to cART.

Objectives: Adolescent Trials Network 147: Comprehensive Adolescent Research and Engagement Studies: Acute HIV Infection in Youth is a longitudinal strategic prospective treatment study aimed to identify and promptly treat with cART recently diagnosed youth aged 12 to 24 years of age in Los Angeles and New Orleans who have acute, recent or established HIV infection, as determined by Fiebig stages I to VI on HIV-1 antibody western blot. Surveillance and dedicated behavioral strategies are used to retain them in care and optimize adherence. Through serial follow-up, HIV biomarkers and response to ART are assessed. The study aims to assess viral dynamics, decay and persistence of viral reservoirs over time and correlate this data with duration of viral suppression.

Methods: A total of 72 youth (36 acutely infected and 36 treatment naïve controls) are enrolled across clinical sites using a current community-based strategy and direct referrals. Youth are prescribed ART according to standard of care HIV-1 management and followed for a period of two years. Assessments are conducted at specific time points throughout these two years of follow-up for monitoring of adherence to ART, virus load, magnitude of HIV reservoirs and presence of co-infections.
Results: The study began enrolling youth in July 2017 across study sites in Los Angeles and New Orleans. As of February 8, 2018, a total of 11 youth were enrolled, 3 with recently acquired and 8 with established HIV infection as determined by Fiebig staging. Recruitment and enrollment are ongoing.

Conclusions: We hypothesize that the size of the HIV reservoir and immune activation markers will be different across groups treated with cART, i.e., those with acute or recent HIV infection and those with established infection. Adolescents treated early who are virally suppressed will have diminished HIV reservoirs than those with established infection. These youth may be potential candidates for a possible HIV vaccine and additional HIV remission intervention trials. Our study will inform future studies of viral remission strategies.

KEYWORDS: HIV infected youth, acute HIV infection, viral reservoirs, CARES
INTRODUCTION

Of the roughly 50,000 people infected with HIV each year, about 25% are youth aged 13 to 24 years old and more than half of them are not aware of their status [1] [2]. Runaway and homeless youth are particularly susceptible to substance abuse, survival sex (prostitution due to extreme need), contact with the juvenile justice system and sexually transmitted infections (STIs) putting them at a higher risk of acquiring HIV [3], [4]. Specifically, African American males engaging in male-to-male contact continue to have the highest risk of HIV [2]. Although the level of HIV incidence in adolescents between 13 and 24 years of age remains unknown, one early study found the seroprevalence of HIV within this group to be as high as 5.3% [5].

It is believed that individuals who are acutely infected with HIV play a disproportionate role in the transmission of HIV [6] [7]. Acute HIV infection is defined as the 4 - 5 week time period [8] which occurs between initial HIV-1 exposure and development of HIV-1 specific antibodies (seroconversion). During this time, there is first a burst of viremia which allows for the detection of p24 antigen, a core viral protein, and HIV RNA, but not HIV specific antibodies. Many patients experience a variety of non-specific flu-like symptoms in this phase, which bear resemblance to mononucleosis-like syndrome. During this time, patients have significantly elevated HIV burden in the plasma and genital secretions, thus increasing the likelihood of transmission [9, 10]. According to the commonly employed Fiebig staging classification system for HIV infection, acutely infected patients do not have any detectable HIV antibodies (Fiebig stages 1 to 2). Recent infection is defined as the next phase in HIV
infection when HIV antibodies become detectable by immunoassays but the HIV-specific western blot can range from negative to indeterminate to incomplete (missing p31 band), which corresponds to Fiebig stages 3 to 5. This can last anywhere from 30 to 90 days post initial infection until a full set of HIV antibodies are present [9, 11]. Established infection is considered to be the time in which an immune response is fully mounted and is characterized by a plateau in HIV viremia also known as Fiebig 6 (fig 1). Data show that acute HIV infectivity is about five-fold higher than established HIV infectivity [12, 13].

Figure 1. Fiebig Staging

Figure Legend:
Trajectories of HIV-RNA viremia, CD4 T cells, p24 antigen and HIV antibody over the early phase of HIV infection. Sequence of appearance of different generations of HIV diagnostic assays is presented. Fiebig staging which represents a mean estimation of time from viral acquisition, divided into six phases, has also been superimposed. Eclipse phase is defined by the absence of any marker including p24 and viral RNA. Units for p24 antigen and HIV antibody are not mentioned due to the difference in magnitude.

Studies have shown that early detection and treatment of HIV infection has many clinical and public health benefits [14]. Studies of perinatally infected infants have been able to provide important insights about the pathogenesis of acute HIV infection and the need for prompt initiation of antiretroviral therapy. Molecular assays have made it possible to rapidly identify HIV in infants who have been exposed and estimate the duration of infection. Based on the time of detection, mother to child transmission of HIV can be classified as in utero (during gestation), intrapartum (during labor or delivery), or via breastfeeding [15, 16]. Studies in which cART (combined antiretroviral therapy) was administered to mothers intrapartum, and to the newborn shortly after birth showed that HIV mother-to-child transmission was significantly reduced by two thirds [17] [18]. HIV acquisition was also significantly reduced when ART was administered to infants in the first 48 hours of life as opposed to previous standards of 3 days or older [18].

To further demonstrate the effectiveness of early antiretroviral therapy on infants, a large multicenter phase 3 trial, NICHD HPTN 040, conducted by our team of investigators, revealed that cART reduced intrapartum HIV transmission by 50% [19] in high risk HIV-exposed infants whose mothers did not receive cART during pregnancy. A few perinatally infected infants have been able to obtain a period of drug free remission (plasma HIV undetectable following cessation of cART for more than one year). One example is that of a French child born in 1996 who began cART at 3 months of age with treatment for several years and, after drug interruption, experienced long term drug-free remission that lasted over 12 years [20]. The well described “Mississippi baby” case began ART 30 hours after birth following high-risk maternal exposure and continued treatment until 18 months of age; this infant experienced drug free remission for 27
months [21]. Likewise a recent report of an African 9 year-old who was treated as an infant for a limited period of time around 7 weeks of age as part of the CHER clinical has subsequently been in HIV drug free remission for almost 9 years [22]. These reports provide important information of potentially advances in the field in infants and children while little is known about adolescence.

The biggest barrier to HIV remission and cure in children and adults is the presence of latent HIV reservoirs (resting memory T cells and other sites which contain integrated proviral DNA) [23]. These reservoirs usually reach a set point within the first two months of infection and serve as predictors of long term HIV control [10] [24]. When ART is discontinued, these HIV latent reservoirs allow for viral rebound to occur [25] [26]. However, if cART is initiated during the acute phase of infection, it is possible to preserve the CD4 T cells and decrease the size of HIV reservoirs [24] [27] [19]. A period of drug free remission may then be possible [21]. The ANRS Visconti trial identified 14 adults that were treated during early acute infection, and were able to maintain undetectable viral levels for several years after discontinuing cART [28]. Unfortunately, cART initiated after HIV has become established is not associated with a limit in viral reservoir size or attainment of remission after cessation of cART [29] [30].

Traditionally, adolescents who acquired HIV through sexual transmission have not been included in early treatment studies. Identification and adherence to ART and study visits are some of the many challenges associated with enrolling this population. However, data has shown that adolescents retain more residual thymic tissues than
adults, giving them a greater capacity for immune reconstitution and CD4 T cell recovery than adults [31] [32]. Therefore, it has been suggested that adolescents may be more responsive to early cART than adults with better chances of obtaining drug free remission [24]. By identifying this population early and promptly initiating potent ART, with adequate surveillance and dedicated behavioral strategies to retain them in care and optimize ART adherence, it may be possible to significantly limit the size of their latent viral reservoirs and preserve their immune systems. This may enable them to better control HIV persistence long term and allow them the opportunity to participate in additional strategies to induce HIV drug free remission and/or become elite post-treatment controllers.

STUDY AIMS

ATN147: Acute HIV Infection in Youth (CARES) is aimed to identify and promptly initiate potent combination antiretroviral therapy in acutely/recently/established HIV infected youth aged 12 to 24 years of age in Los Angeles and New Orleans. We hypothesize that the size of the HIV reservoir and immune activation markers will be different across groups treated with cART: those with acute/recent infection and established infection. We predict that adolescents who are treated early and are virally suppressed during acute/recent infection will have a decreased quantity of HIV reservoirs compared to those with established HIV infection. These youth with low reservoirs and preserved immune systems may be potential candidates for a possible HIV vaccine and additional HIV remission intervention trials.
We expect to recruit youth with acute and recent HIV infection from a large prospective HIV negative high-risk youth cohort as well as from numerous community clinics. The negative HIV high-risk youth cohort are enrolled in a CARES partner study, where youth at risk for HIV acquisition are followed periodically for a period of two years with point of care (POC) periodic HIV testing performed, including 4th generation HIV-1 assays (Alere Determine), GeneXpert qualitative HIV assays (Cepheid), plasma PCR assays and detuned HIV-1/2 EIA. Serial POC testing for syphilis, GC and CT are done at the same time points.

Upon initial identification of HIV infection, following written informed consent, youth are enrolled into the study, with confirmatory HIV RNA performed, as well as a standard HIV fourth generation antigen antibody assays. Study youth have a clinical visit with history, physical exam, and detailed medical/behavioral questionnaire performed. Youth have HIV genotypic susceptibility testing performed, and basic hematologic and chemistry panels assessed. They are evaluated for concurrent infections including syphilis, hepatitis A, B and C, chlamydia, gonorrhea, tuberculosis and CMV. Potent cART is prescribed at the enrollment visit (according to standard of care procedures/ DHHS guidelines and preferably using a single daily pill) and whole blood is collected to perform tests to determine Fiebig staging [11]. Serial follow up blood samples are obtained in subsequent visits for HIV biomarkers and to monitor response to cART. The aim is to describe and compare viral dynamics, viral decay and persistence of viral reservoirs in individuals who are acutely, recently or chronically infected over time, with results correlated with duration of viral suppression. These
assays used to measure these parameters include quantitative HIV RNA PCR, measurement of proviral DNA by digital droplet PCR and studies of HIV specific immunity, including cellular immunity, cytotoxic T cell responses, immune activation markers and HIV neutralizing antibody.

Due to the relatively short study duration of two years and the need for continuous prolonged viral suppression following cART, we have not included a planned treatment interruption protocol. It is expected that some youth may stop or interrupt therapy due to unanticipated reasons. In these cases, we will strive to re-implement therapy as soon as possible, and, meanwhile, will make every effort to closely monitor clinical outcomes and track disease progression. We will definitely recommend re-initiation of cART based on any clinical symptoms or presence of detectable plasma viremia > 1000 copies/ml. The duration of ART-free HIV remission will be assessed in this subpopulation and repeat studies of HIV viral reservoirs will be done at baseline and every 2 to 4 months during the ART free period. Ultimately, in addition to quantifying the viral reservoirs of this unique population, our hope is that our overall study will lead to the development of a prolonged HIV remission strategy.

**DESIGN & POPULATION**

ATN147: Acute HIV Infection In Youth (CARES) is a longitudinal strategic prospective treatment study that identifies, promptly treats and follows a cohort of adolescent/young adults aged 12 to 24 years who have acute, recent or established HIV infection. It will measure the effects of early intensive antiretroviral therapy on the
establishment and persistence of HIV-1 reservoirs and HIV-1-specific immunity in acutely/recently HIV infected youth as compared to newly diagnosed youth with established infection lasting over 6 months. All youth will be treated according to standard of care and followed for a period of 24 months.

This study is the first of its kind to enroll a highly at risk population of HIV infected adolescents/young adults in the United States. In 2015, among youth ages 13-24 diagnosed in the United States, 81% were gay and bisexual males. Out of newly diagnosed male youth, 55% were black and 24% were Latino [33]. Among youth, an HIV seroprevalence study showed a 5.3% homelessness rate and considered homeless youth to be at highest risk of infection [5]. We have elected to target and enroll youth from two HIV epicenters with large populations of infected and at risk youth: Los Angeles (LA) and New Orleans (NO).

In 2013, a total of 1,820 Los Angeles County residents were reported as newly diagnosed with HIV infection, more than that of other urban cities including Cook County, New York County, Miami-Dade County and Harris County [34]. Los Angeles contains 6 geographic "hotspots" which include the following areas: Metro, San Fernando Valley, South LA, East LA, San Gabriel and South Bay [34]. According to a 2015 surveillance report, the New Orleans Metairie area was ranked 3rd based on rate (per 100,000) of HIV diagnosis [33]. The Adolescent Trials Network (ATN) 110 Study in New Orleans screened a total of 200 gay, transgender, bisexual youth (GTBY) between
January and September 2013 and found 9 to be acutely infected, demonstrating the magnitude of the problem in this population [35].

Over a 2 year duration, ATN147 is attempting to enroll a total of 72 youth across clinical sites in Los Angeles and New Orleans. Criteria for study recruitment include: a) age of 12 to 24 years, b) a positive HIV result (Alere rapid test, GeneXpert HIV qualitative PCR) c) ability and willingness to provide written informed consent, d) Willingness to initiate cART. In order for a physician to treat a participant, they must be willing to follow DHHS guidelines for antiretroviral naïve adolescents and adults (https://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-arv-guidelines/11/what-to-start) including management of acutely HIV infected adolescents and adults as outlined in the guidelines.

Youth can be excluded from the study according to the following criteria: a) prior ART use (> 1 week, b) active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements, c) any acute, chronic, or recent and clinically significant medical condition that, in the opinion of the site investigator, would interfere with adherence to study requirements or jeopardize the safety or rights of the participant, d) chronic or recurrent use of medications that modify host immune response, e.g., oral or parenteral steroids, cancer chemotherapy, e) clinical treatment with an ART regimen less effective than those recommended by DHHS HIV clinical guidelines, f) enrollment on an experimental ART regimen.
STUDY SITES AND RECRUITMENT

Participating ATN 147 study sites include David Geffen UCLA School of Medicine, Department of Pediatrics, Division of Infectious Diseases, the Los Angeles LGBT Center, and Tulane University School of Medicine, Department of Adolescent Medicine. Identification and enrollment of acutely or recently infected adolescents is challenging. To address this challenge, we have two distinct methods of enrollment: ATN149: Cost-efficient Interventions for Youth at Risk for HIV (CARES) and Direct Referrals as illustrated in Figure 2.

Figure 2: Recruitment Study Flow

Method 1: ATN149: Cost-efficient Interventions for Youth at Risk for HIV (CARES)

ATN149: Cost-efficient Interventions for Youth at Risk for HIV (CARES) is a study of high risk HIV seronegative youth who are followed prospectively as part of a

1. Enrollment in Study 3
   - High Risk Cohort
     - Estimate: 1500
     - Every 4 months at Study 3 Recruitment Sites
     - No Acute Symptoms/High Risk Exposure
     - ASAP: Acute Symptoms/High Risk Exposure

2. At Study 3 Recruitment Site: ASAP
   - Alere q HIV-1/2 Detect
   - GeneXpert HIV-1 Viral Load Qualitative Assay
   - Confirmed to be treatment naive
   - Candidate for Study 1 Established Cohort

3. HIV Quant PCR within 24-48 hours
   - Direct Referral
   - HIV Diagnostic Assay at Clinic Outside Study

4. Alere q HIV-1/2 Detect
   - Cepheid GeneXpert HIV-1 Viral Load Qualitative Assay
   - Cepheid, Negative Alere
   - Candidate for Study 1 Acute Cohort

5. Cepheid GeneXpert HIV-1 Viral Load Qualitative Assay
   - HIV Quant PCR within 24-48 hours
community-based strategy conducted by our group of investigators in U19 HD089886-02, a set of coordinated study that concurrently addressed youth living with HIV and seronegative youth. The study initially screens 4,500 at risk youth between the ages of 13-24 years at recruitment sites in Los Angeles and New Orleans for clinical and laboratory evidence of HIV using POC testing such as the Alere HIV-1/2 rapid test which indicates the presence of HIV-1 antibody and/or antigen. In this initial screening phase, if youth are found to have a positive Alere test result and are treatment naïve, they are eligible to enroll in Protocol 147 and are thus referred for enrollment. Fiebig staging performed at enrollment will determine the phase of HIV infection these youth are in. Youth who happen to have a positive antigen but a negative antibody test result will undergo additional assessments to determine if they are more acutely infected and will also be referred for enrollment to Protocol 147. Youth who are found to be seronegative and are assessed as high-risk for HIV acquisition based on detailed questionnaires and sexually transmitted infection testing are eligible for enrollment into Protocol 149. The estimated sample size of the prospective cohort is 1500 youth who will undergo qualitative and quantitative testing every 4 months for HIV and other STIs over a 2 year period. If they test positive or report symptoms of acute HIV infection, they become eligible for enrollment into the acute infection Protocol 147.

**Method 2: Direct Referrals**

Eligible youth referred to the acute infection Protocol 147 from urgent care sites, emergency departments or other clinics can also be enrolled into either the
Acutely/Recently Infected Cohort or the Control Cohort based on confirmatory diagnostic testing as long as all eligibility requirements are met.

**DIAGNOSIS OF HIV INFECTION AND FIEBIG STAGING:**

When potential youth are referred for enrollment screening, they may have already tested positive for HIV previously through a point of care diagnostic assay. Confirmation of HIV infection is performed with a 4th generation Ag/Ab combo assay followed by a rapid molecular based test such as the GeneXpert HIV qualitative test and/or Alere q HIV-1/2 Detect along with quantitative HIV RNA and HIV genotypic susceptibility assays. Additional diagnostic testing includes CMV PCR of blood, STI testing including GC/CT PCR of urine, RPR, Hepatitis panel which includes hepatitis A, B and C diagnostic testing and TB gold quantiferon testing. Clinical labs include a complete blood count with differential and platelets and a chemistry panel including liver and kidney function tests.

Once enrolled, youth will eventually fall into different categories according to the Fiebig Stage Classification System based on western blot results (Table 1) which characterizes the progression of HIV-1 infection from exposure to seroconversion. This staging determines acute/recent or established HIV infection status. Enrollment visit plasma samples (which are prior to cART initiation) are used to determine Fiebig staging. Based on these stages, youth are placed into one of two cohorts which can be further divided into groups as shown in Table 1. We expect to enroll 36 youth in each cohort over a two year period. Our acutely/recently infected cohort consists of Fiebig
stages 1-5 and can be identified based on HIV-1 antibody diagnostic profile. Our control cohort consists of individuals in Fiebig Stage 6 who are identified by a positive Western blot with a p31 band.

**Table 1: Acute, Recent and Established HIV Infection as per Fiebig Staging**

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Group</th>
<th>Fiebig Stage</th>
<th>Est. Time from Infection (days)</th>
<th>HIV-1 antibody diagnostic profile</th>
<th>Est. Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acutely/Recently Infected</td>
<td>1</td>
<td>I/II</td>
<td>0 - 20</td>
<td>Non-reactive HIV-1 antibody</td>
<td></td>
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<td></td>
<td>2</td>
<td>III/IV</td>
<td>20 - 30</td>
<td>Reactive HIV-1 Antibody; negative or indeterminate results on the Western blot</td>
<td>36</td>
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<td></td>
<td>3</td>
<td>V</td>
<td>30 - 90</td>
<td>Reactive HIV-1 Antibody; positive Western blot without p31 band</td>
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<tr>
<td>Control/Established</td>
<td>4</td>
<td>VI</td>
<td>90+</td>
<td>Reactive HIV-1 Antibody; positive Western blot with p31 band</td>
<td>36</td>
</tr>
</tbody>
</table>

Adapted from: Fiebig EW, Wright DJ, Rawal BD, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. AIDS 2003; 17:1871. Graphic 96506 Version 3.0

**TREATMENT**

Starting at the enrollment visit, all youth sign an informed consent, are prescribed cART and clinically treated according to standard of care HIV-1 management as defined in DHHS guidelines [9]. Genotypic drug resistance testing is performed before initiation of ART to guide the selection of the regimen. Once results of drug resistance testing are available, the treatment regimen can be modified if warranted.
Our study recommends that physicians prescribe fixed dose combination regimens, favoring once daily integrase-inhibitor based regimens. Use of complex ART regimens with the inclusion of protease inhibitors may trigger gastrointestinal symptoms and potential loss of adherence by our adolescent/young adult study youth while efficacy of integrase inhibitor (INSTI) based ARTs is successful in suppressing virus more rapidly than non-INSTITI based therapy [36]. By recommending fixed dose combination once daily integrase-inhibitor based regimens, we can minimize pill burden and the possibility of ART side effects. Our hope is that doing so will promote ART adherence and patient satisfaction in a population that is generally not amenable to medication use nor has prior experience with daily medications.

The fixed dose combination once daily integrase-inhibitor based regimens recommended may include the single tablet regimen elvitegravir 150 mg/cobicistat 150 mg/emtricitabine 200 mg/tenofovir alafenamide 10 mg(EVG/COBI/FTC/ TAF) (Genvoya® Foster City, CA: Gilead Sciences, Inc; 2016) [37] or elvitegravir 150 mg/cobicistat 150 mg/emtricitabine 200 mg/tenofovir disoproxil 300 mg (EVG/COBI/FTC/ TDF)(Stribild® Foster City, CA: Gilead Sciences, Inc; 2016.), depending on availability; or other similar regimens. EVG/COBI/FTC/TAF has been approved by the US FDA in November 2015 [38] and is similar to the approved single-tablet regimen with TDF but uses the tenofovir alafenamide (TAF) formulation of tenofovir, which appears to have distinct safety advantages. There have been many clinical trials that have included EVG/COBI/FTC/ TDF as well as EVG/COBI/FTC/TAF.
One phase II study (GS-US-292-0102) treated youth with both regimens and found that, while both groups had increased viral suppression, 88.4% of those treated EVG/COBI/FTC/TAF with had HIV-1 RNA <50 copies/mL at 48 weeks by snapshot analysis compared with 87.9% in those given EVG/COBI/FTC/TDF [39].

Other potent antiretroviral regimens may be prescribed by the physician based on availability, patient tolerability and preference. Changes to antiretroviral regimens should be performed when necessary according to HIV management guidelines. Patients who do not tolerate the ART regimens will be prescribed an alternative ART regimen as clinically indicated. The same will occur with subjects who do not achieve virus load remission, they may be prescribed an alternative ART regimen as clinically indicated.

Youth will take study medications and come for follow up visits with physicians at clinic sites for up to 24 months according to the schedule of evaluations (Table 2) for assessment of virus load and HIV reservoir assays as well as monitoring of co-infections. In addition to the enrollment visit, there will be a total of 9 follow up visits during which samples will be collected for Clinical Labs and Immune Reservoir studies. Eight of the 10 visits are scheduled during the first 12 months of ART, with two subsequent visits performed at 18 and 24 months.

Table 2: Schedule of Evaluations
The HIV WB is a part of immune reservoir studies and does not need to be repeated if it shows a full profile Fiebig stage VI until 1 year. If subjects cannot do both 2

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Screen</th>
<th>Entry</th>
<th>1 WK</th>
<th>2 WK</th>
<th>4 WK</th>
<th>8 WK</th>
<th>4 MO</th>
<th>8 MO</th>
<th>12 MO</th>
<th>18 MO</th>
<th>24 MO</th>
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<td>Documentation of Acute HIV</td>
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<td>Fiebig Staging</td>
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<td>HIV-1/2 EIA 4th Generation Assay</td>
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<td>Urine Analysis</td>
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<td>Complete Blood count with differential and platelets</td>
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<td>Liver Function Tests (AST, ALT, GGT)</td>
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<td>Renal Function Tests (BUN, Creatine)</td>
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<td>HIV-1 WB</td>
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<td>GC/CT PCR of Urine, oropharynx and rectum</td>
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<td>Pregnancy Test</td>
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<td>Reservoir Study Labs</td>
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week and 4 week follow up visit, it is acceptable to combine the two visits into one as long as all clinical labs specified for week 4 are taken. The window periods (not shown in table) are as follows: -7 days for screen; ±7 days for week 1; ±14 days for week 2 and 4; ±7 days for week 8; ±14 days for 4 month, 8 month, 12 month and 24 month. In the event of virologic failure (not shown in table), HIV-1 genotypic testing will be conducted. In the event of premature study/treatment discontinuation (not shown), our team will try to collect HIV-1 genotypic testing, T cell subsets, HIV RNA quantitative assay and 30 cc for immune reservoir studies.

**EVALUATIONS AND ACTIVITIES (Table 2)**

1) **Screening Visit: Day of Diagnosis**

   During screening, youth are asked a series of questions as part of Protocol 147 procedures. If HIV diagnosis is confirmed, a baseline interview will be conducted or, if previously completed in Study 3, a completed assessment interview will be performed. If youth are referred from other healthcare providers, the baseline assessment occurs after enrollment into the acute infection Protocol 147.

2) **Study Entry: Day 0**

   Although Screening visit and Day 0 may occur on the same day, subjects are enrolled within 48 to 72 hours of an HIV diagnosis. On this day, any necessary confirmatory diagnostic testing is performed and those who are eligible for the study will provide written informed consent for study participation. Behavioral interventions are provided and demographic and medical history are collected and recorded in case report forms. A physical exam is performed and information regarding clinical signs, symptoms, findings of acute HIV infection and clinical treatment initiation are recorded.

   A number of clinical laboratory assays are performed and include the following: a) Complete blood count with differential and platelets, b) Liver function tests (AST, ALT, GGT), renal function tests (BUN, Creatinine), c) CMV PCR blood if clinically suspected,
d) Urine analysis, e) GC/CT PCR of urine f) RPR, g) Hepatitis A, B and C panel, h) HIV-1 genotypic testing, i) T cell subsets, j) Urine pregnancy test (if female) and TB Gold quantiferon testing. In addition to these clinical labs, 30 cc of whole blood are collected for Immune Reservoir Studies. Pregnancy testing is also performed if applicable.

3) Study Visits Post-ART Initiation: Day 7, Week 8

A physical exam is performed and information regarding clinical signs, symptoms and findings of acute HIV infection or side effects from medication are recorded. Virus load testing, complete blood count and chemistries are performed. Adherence assessment is performed; pregnancy testing performed if applicable. 10 cc of whole blood is collected for immune reservoir studies.

4) Study Visits Post-ART Initiation: Week 2 and 4

A physical exam is performed and information regarding clinical signs, symptoms and findings of acute HIV infection or side effects from medication are recorded. Adherence is assessed and the following clinical laboratory assays are performed: a) Complete blood count with differential and platelets, b) Liver function tests (AST, ALT, GGT), renal function tests (BUN, Creatinine), c) HIV-1 genotypic testing (if clinically indicated/detectable viremia) d) T cell subsets, e) HIV-1 RNA PCR quantitative, f) Urine pregnancy test (if suspected). In addition to these clinical labs, 10 cc of whole blood is collected for immune reservoir studies. At week 4, an HIV-1 Western Blot (WB) is performed unless previous results showed a full profile Fiebig Stage VI (in that case, HIV-1 WB will be conducted at the one year mark).
5) Study Visits Post-ART Initiation: Month 4, 8, 12, 18, 24

A physical exam is performed and information regarding clinical signs, symptoms and findings of acute HIV infection or side effects from medication are recorded. Adherence is assessed and the following clinical laboratory assays are performed: a) Complete blood count with differential and platelets, b) Liver function tests (AST, ALT, GGT), renal function tests (BUN, Creatinine), c) HIV-1 genotypic testing (if clinically indicated/detectable viremia) d) T cell subsets, e) HIV-1 RNA PCR quantitative, f) Urine pregnancy test (if suspected). In addition to these clinical labs, 30 cc of whole blood are collected for immune reservoir studies.

**IMMUNE RESERVOIR STUDIES**

At enrollment, collection of 30 cc of (pre-treatment) whole blood is collected and used to determine Fiebig Staging through the following tests: a) HIV-1 POC antibody test (4th generation assay), b) HIV-1 Western blot and c) HIV-1 quantitative RNA PCR. In addition, digital droplet PCR to measure full-length and partial HIV cDNA transcripts are performed at enrollment and for each of the 9 subsequent follow up visits. These tests allow the evaluation of continued reservoir suppression and sustainability of antiretroviral effect while comparing HIV viral dynamics across subject groups. The total amount of blood to be obtained during each study visit should not exceed 30 ml for youth weighing < 50 kg.
The primary study endpoint is 24 months following enrollment, when the amount of cell-associated HIV-1 DNA (CAHD) in 5 million total peripheral blood mononuclear cells (PBMC, assayed by quantitative ddPCR [qPCR]) will be compared between individuals initiating ART at different Fiebig stages: I/II versus III/IV versus V versus established infection (Fiebig VI control arm). We will also have HIV reservoir studies assessed at 4 and 8, 12, 18, and 24 months. This data will be important to assess for studies of reservoir decay as well if there is drop out or loss to follow up or evidence of viral rebound.

**STATISTICAL CONSIDERATIONS**

Descriptive statistics: The results of this study will be primarily descriptive. In acute cases, the Fiebig score at time of initiation of ART and the time to suppression of plasma viremia will be summarized over time as mean and +/- 2 standard deviations (SD). The analyses of immune biomarker assessments follow the same analysis.

**Regression analysis:** The effects of Fiebig stage on follow-up results will be assessed in these longitudinal analyses using either numerical Fiebig stage or estimated time to initiation of ART as predictors, including stage or stage by time interaction. Drop out is also important to assess as a binary outcome and will also be assessed with the same approach. Covariates such as STI co-infection, age, race, and behavioral assessments will be considered as potential confounders, and included in the regression analysis if they confound the crude analysis. Additionally, quantity of provirus over time is a longitudinal analysis. If the data is primarily described as being
below detectable limits or not, longitudinal logistic regression will be conducted. If the outcome is numerical, a linear longitudinal analysis to assess level and time trend will be conducted. Regression analysis will be conducted using the statistical package R (R version 3.0.1, The R Foundation for Statistical Computing, www.r-project.org).

STRATEGIES FOR RETENTION AND ADHERENCE

Study youth will be receiving adherence support through a designated case manager and adherence coach, who will already be assigned to the participant in order to facilitate antiretroviral use, maintenance of appointments and facilitate overall care. All DHHS guideline recommendations for enhanced adherence will be implemented for study youth. For patients in whom adherence is identified as a major challenge, we will implement DOT. To facilitate adherence, patients will be offered free transportation to clinic and will receive continuous coaching and encouragement from a designated case manager. In addition, as this study is conducted in partnership with psychiatry and clinical psychologists, they will be available and will provide support and guidance in the management of these patients.

DISCUSSION

Acute HIV Infection in Youth is a strategic treatment study aimed to identify and promptly treat with antiretroviral therapy recently acquired HIV in youth aged 12 to 24 years of age who are enrolled in Los Angeles and New Orleans. This population is unique in that participants are of an age in which their immune systems are more mature than that of children, yet have a greater capacity for immune reconstitution and plialibility of HIV reservoirs than that of adults [24]. Our study is the first of its kind to
characterize reservoirs of an adolescent population which is generally not amenable to routine clinic visits but is at a high risk of transmission. However, through the use of a multidisciplinary approach taken in collaboration with a group of behavioral scientists, we intend to follow this cohort for 2 years and have all youth enrolled by 2019 to allow for a two year follow up.

In addition to lowering transmission in this population, our goal is to uncover new data that will inform future remission studies. The inability of cART to eradicate infected cells [40] and the fact that plasma viremia rebounds quickly after treatment discontinuation [41] are reasons why remission as a functional cure is considered a more viable goal [28]. So far, studies have shown that HIV remission is possible in both perinatally infected infants and acutely infected adults [18, 28]. We believe that this is definitely possible for acutely infected adolescents as well. By quantifying viral reservoirs of this unique population, we hope to uncover new data that will be applicable to the general HIV-infected population beyond adolescents and possibly lead to the development of a prolonged HIV remission strategy in the future.

**TRIAL REGISTRATION:** On June 20th, 2017, the protocol was registered with clinicaltrials.gov (NCT03205696)

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**CONFLICTS OF INTEREST:** None declared.
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