Protocol

Protocol for: Chen D, Berona J, Chan Y-M, et al. Psychosocial functioning in transgender youth after 2 years of hormones. N Engl J Med 2023;388:240-50. DOI: 10.1056/NEJMoa2206297

This trial protocol has been provided by the authors to give readers additional information about the work.

This supplement contains the following items:

1. Original protocol, including statistical analysis plan
2. Final protocol, including statistical analysis plan
3. Summary of IRB amendments

The Impact of Early Medical Treatment in Transgender Youth

(Trans Youth Care)

Version 1.0

April 18, 2016

Sponsored by:
The Eunice Kennedy Shriver
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STUDY MANAGEMENT

Before the recruitment and enrollment of participants, the participating R01 study sites must have the protocol and consent form approved by their local Institutional Review Boards (IRBs). All original, approved documents must be maintained at each study site.

All queries for this protocol should be sent to Children's Hospital Los Angeles (CHLA), via email at TransYouthCare@chla.usc.edu. The appropriate team member will respond to queries generally within 48 hours via email and copy the other team members as needed.

Dr. Olson-Kennedy will be responsible for answering general protocol implementation, eligibility, study and participant management, exemptions and/or adverse event queries, although the response may be sent via another team member.

The CHLA Data Manager, with the help of other study personnel, if necessary, will answer general data management, data entry, and case report form (CRF) completion queries.

This study will use an Audio Computer-Assisted Self-Interview (ACASI) to collect study data. Additional psychosocial data will be collected during in-person interviews conducted by research staff at each study site.

LIST OF ABBREVIATIONS

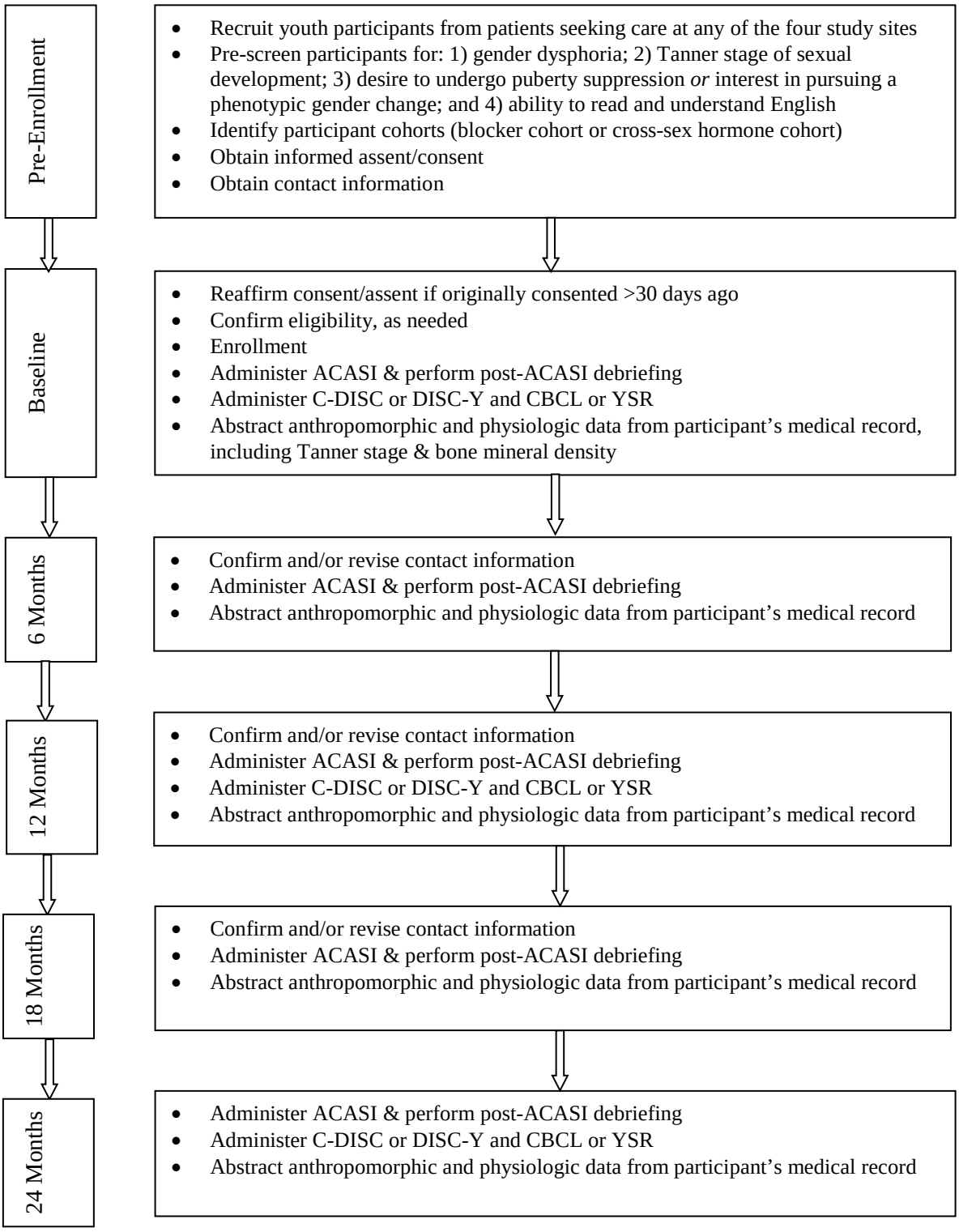
ASAI	Adolescent Sexual Activity Index
ACASI	Audio Computer-Assisted Self-Interview
ASSIST	Alcohol, Smoking and Substance Involvement Screening Test
BDI	Beck Depression Inventory
C-DISC	Computerized Diagnostic Interview Schedule for Children
CBCL	Child Behavior Checklist
CI	Confidence Interval
CRF	Case Report Form
DHHS	U.S. Department of Health and Human Services
DISC-Y	Youth-Informant Diagnostic Interview Schedule for Children
FIPS	Federal Information Processing Standards Publication
GCP	Good Clinical Practices
GnRH agonists	Gonadotropin-releasing hormone agonists
HRQoL	Health-Related Quality of Life
HIPAA	Health Insurance Portability and Accountability Act
IOM	Institute of Medicine
IRB	Institutional Review Board
NICHD	*The Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NIDA	National Institute on Drug Abuse
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
PI	Principal Investigator
PID	Participant Identification Number
SCARED	Screen for Child Anxiety Related Disorders
STS	Social Transitioning Scale
STI	Sexually Transmitted Infection
YSR	Youth Self Report

STUDY ABSTRACT

DESIGN:	The study will use a longitudinal observational design to examine the outcomes of existing medical treatment protocols for gender dysphoria in two distinct cohorts: youth initiating puberty suppression and youth pursuing a phenotypic gender change. Routinely collected anthropometric and physiologic parameters will be collected through chart abstraction throughout the 24 month study period. Audio computer-assisted self-interview (ACASI) and individual interview survey instruments will be used to collect demographic, mental health, psychosocial, and behavioral data from parents and youth in the blocker cohort and only youth in the cross-sex hormone cohort at baseline and 6, 12, 18, and 24 months.
SAMPLE SIZE :	Participants will be recruited from those patients seeking medical care for gender dysphoria at any of the four study sites. 88 youth who are initiating pubertal suppression with GnRH agonists and their parent/legal guardian will be enrolled in the blocker cohort. 300 youth who are initiating cross-sex hormones will be enrolled in the cross-sex hormone cohort. While patients between 8 and 20 years inclusive will be eligible for enrollment in one or the other cohorts depending on their Tanner stage and medical intervention plan, the focus of this investigation is *early treatment*. Therefore, no more than 20% of youth age 19 or older will be enrolled in the cross-sex hormone cohort. In addition, due to the expected impact of previous blocker experience on study results, no more than 60 youth enrolling in the cross-sex cohort will have had previous blocker experience. Total accrual is expected to be 388 youth and 88 parents/guardians.
POPULATION:	Blocker Cohort – Youth • Presence of gender dysphoria; • Tanner stage 2 or 3 of sexual development; • Aged 8 to 16 years inclusive; • Appropriate to undergo puberty suppression; • Ability to read and understand English; and • Receiving or planning to receive services at a study site clinic. Blocker Cohort – Parent/Caretaker/Legal Guardian • Parent, caretaker, or legal guardian aged 18 or older of a child who meets the Blocker Cohort Inclusion/Exclusion Criteria, and • Ability to read and understand English. Cross-Sex Hormone Cohort – Youth • The presence of gender dysphoria; • Aged 8 to 20 years inclusive; • Appropriate to pursue phenotypic gender change with cross-sex hormones; • Ability to read and understand English; and • Receiving or planning to receive services at a study site clinic.
INTERVENTION:	There is no intervention in this study.
DURATION:	Accrual is expected to last approximately 2 years starting from the first participant enrolled. The total duration of study participation for each subject is 24 months.
PRIMARY OBJECTIVES:	1. To investigate the impact of medical treatments for gender dysphoria in two developmentally distinct and multi-ethnic cohorts of transgender youth recruited from across the nation via a network of Gender Centers dedicated to their care.

	2. To evaluate the impact of GnRH agonists administered for puberty suppression, on mental health, psychological well-being, physiologic parameters, and bone health as well as document the safety of GnRH agonists in a cohort of children and adolescents with gender dysphoria. 3. To evaluate the impact of cross-sex hormones administered for phenotypic gender transition on mental health, psychological well-being, and metabolic/physiologic parameters as well as document the safety of cross-sex hormones in a cohort of adolescents with gender dysphoria.
SECONDARY/ EXPLORATORY OBJECTIVE:	Based on evidence of high rates of substance use and HIV infection in some transgender adolescents (specifically, young transgender women), we will measure substance use and sexual risk behavior over time.
DATA COLLECTION:	Routinely collected anthropometric and physiologic parameters will be collected through chart abstraction throughout the 24 month study period. Items abstracted include lab results, height, weight, BMI, blood pressure, diagnoses, and prescription medications. At baseline, 12, and 24 months, Tanner stage and bone mineral density results will be collected. Audio computer-assisted self-interviewing (ACASI) survey instruments will be used to collect demographic, mental health, psychosocial, and behavioral data from parents/legal guardians and youth in the blocker cohort and only youth in the cross-sex hormone cohort. Survey data are from 4 domains: 1) demographic; 2) transgender-specific experiences including gender dysphoria; 3) mental health and trauma assessments; and 4) additional psychosocial information including quality of life and relationships with parents and peers. These data will be collected at baseline and 6, 12, 18, and 24 months. For youth in the blocker cohort, mental health diagnoses will be assessed by administration of the parent-informant Computerized Diagnostic Interview Schedule for Children (C-DISC) at baseline, 12 months, and 24 months. The Child Behavior Checklist (CBCL) will also be administered to parents/caretakers at baseline, 12 months, and 24 months to assess behavioral and emotional problems in blocker cohort youth. For youth in the cross-sex hormone cohort, mental health diagnoses will be assessed by administration of the Youth-Informant Diagnostic Interview Schedule for Children (DISC-Y) at baseline, 12 months, and 24 months. Behavioral and emotional problems will be assessed by administration of the Youth Self Report (YSR) at baseline, 12 months, and 24 months.
MONITORING:	Routine team monitoring of untoward events identified during the study will rely on site staff notification via email to the CHLA PI and data manager. Sites will also record and enter in the study database, untoward events occurring during study participation, which will be reviewed by the PIs and reported to the IRBs.

PROTOCOL SCHEMA



1.0 INTRODUCTION

1.1 Background and Significance

"Transgender" is an umbrella term that is used to describe individuals whose gender self-identification or expression transgresses established gender norms. Specifically, it is the state of one's "gender identity" (self-identification as male, female, both or neither) not matching one's "assigned gender" (identification by others as male or female based on natal sex).[1] The identity and behavior of transgender individuals are socially and medically stigmatized, resulting in a grossly underserved population at high risk for significant morbidity and mortality. Transgender people are often diagnosed with gender dysphoria when they are experiencing dissonance between their birth sex and their gender identity. The Diagnostic and Statistical Manual of Mental Disorders (DSM 5) identifies gender dysphoria in the following manner: the presence of a marked incongruence between one's experienced/expressed gender and assigned gender, of at least six month's duration, as manifested by at least two of the following:

1. A marked incongruence between one's experienced/expressed gender and primary and/or secondary sex characteristics (or in young adolescents, the anticipated secondary sex characteristics).
2. A strong desire to be rid of one's primary and/or secondary sex characteristics because of a marked incongruence with one's experienced/expressed gender (or in young adolescents, a desire to prevent the development of the anticipated secondary sex characteristics).
3. A strong desire for the primary and/or secondary sex characteristics of the other gender (or a gender different from that assigned at birth).
4. A strong desire to be of the other gender (or a gender different from that assigned at birth).
5. A strong desire to be treated as the other gender (or a gender different from that assigned at birth).
6. A strong conviction that one has the typical feelings and reactions of the other gender (or a gender different from that assigned at birth).

The condition is associated with clinically significant distress or impairment in social, occupational or other important areas of functioning.[2]

Transgender children and adolescents are a poorly understood and a distinctly understudied population in the United States. The limited available data suggest that transgender youth who are gender dysphoric are at increased risk for anxiety, depression, suicide, and substance use compared to their peers.[3-6] The development of undesired secondary sex characteristics during puberty intensifies the distress associated with gender incongruence and increases the risk for these conditions. Current clinical practice guidelines aim to decrease gender dysphoria and ameliorate potential negative health outcomes. Treatment recommendations vary depending on the age and developmental stage of youth with gender dysphoria. For those youth in the earliest stages of pubertal development (Tanner stages 2-3), treatment with gonadotropin-releasing hormone (GnRH) agonists is recommended in order to suppress endogenous puberty and avoid the development of undesired secondary sex characteristics. In older adolescents in the later stages of pubertal development (Tanner stages 4-5), treatment with cross-sex hormones is recommended to induce desired masculine or feminine features.[7] While these guidelines have been used at academic and community centers across the U.S., they are based on very limited data. Furthermore, there are no available data examining the physiologic and metabolic consequences of cross-sex hormone treatment in youth. This represents a critical gap in knowledge that has significant implications for clinical practice across the U.S. In 2011, a report of the Institute of Medicine (IOM) called for the development of rigorous research aimed at understanding the health implications of hormone use and other transgender-specific issues.[8] The objective of the proposed research is to provide evidence-based data to inform clinical care for transgender youth. The study will leverage the partnership between four, university-affiliated, gender clinics across the U.S. to recruit two developmental cohorts and conduct a multi-site, observational study examining the safety of hormonal interventions and the physiological and psychosocial outcomes associated with these treatments.

1.1.2 Transgender Youth Are an Underserved, Understudied Population

Transgender adolescents and children, those who experience incongruence between assigned birth sex and internal gender identity, are a poorly understood and understudied population in the United States. As detailed in the May 2011 IOM report, "The Health of Lesbian, Gay, Bisexual, and Transgender People," the existing body of scientific evidence documenting health and well-being of transgender individuals is sparse. The report explicitly calls for NIH-supported research on transgender health needs, including the development of evidence-based data for providing transgender-specific health care to address gender dysphoria and rigorous research aimed at understanding the health implications of hormone use and other transgender-specific issues. In addition, the IOM report calls for longitudinal and cohort studies that incorporate a life course perspective to examine the specific experiences of transgender individuals across different chronological ages.[8]

Research on transgender youth has historically focused on the disproportionate morbidity and mortality among transgender individuals in comparison to the population at large. One study of 55 transgender youth in New York City reported that 45% had seriously thought about suicide, and 26% had attempted suicide at least once, indicating that transgender youth specifically are at increased risk for anxiety, depression, social isolation, and suicide compared to non-transgender peers.[1,9,10] A recent study examining baseline characteristics of 101 multi-ethnic transgender youth at Children's Hospital Los Angeles (CHLA), supports these numbers, with 52% participants reporting thoughts of suicide and 30% reporting having attempted suicide at least once.[6]

1.1.3 High Risk Behavior

Studies have shown that self-identified transgender women have a significantly higher rate of HIV than other individuals considered "at risk" for HIV. In a systematic review of all studies on transgender and HIV, prevalence rates of HIV among male to female transgender people (6.3%) were found to exceed that of men who have sex with men (4.2%).[11] In one study of 400 transgender women, the overall prevalence of HIV was found to be exceptionally high at 35% with African American transgender women being nearly six times more likely than Caucasian transgender women to be HIV infected.[12] In Wilson's study of 151 female transgender youth, 67% had participated in survival sex.[13] Studies of high-risk transgender male-to-female adolescents found that 19-22% reported being HIV positive.[9,13,14] Furthermore Garofalo found an extremely high prevalence of history of incarceration (37%), homelessness (18%), sex in exchange for resources (59%), forced sexual activity (52%), difficulty finding a job (63%) and difficulty accessing health care (41%).[9] Due to societal marginalization and transphobia, many transgender youth and adults resort to "survival sex" (the trading of sexual acts for money, food, clothing or a place to live) in order to subsist on their own.[12]

Without appropriate care, as they age, transgender youth are likely to face economic and societal marginalization, incarceration, and physical abuse leaving them at significantly higher risk for drug abuse, violence, HIV acquisition, other sexual transmitted infections, and homelessness.[9,10,13,14]

1.1. The "Dutch Model"

Over the past 30 years, a team of specialists in the Netherlands at the Amsterdam Center of Expertise on Gender Dysphoria observed that transgender individuals who underwent hormonal gender transition at earlier ages assimilated easier into their "new gender" roles because of improved physical outcomes.[15] Additionally, transgender youth suffering through an undesired endogenous puberty experience distress, and this "wrong puberty" has a strong negative impact on their emotional, academic, and family functioning.[15,16] Based on these clinical observations, Dutch clinician investigators initiated early treatment of transgender youth aimed at suppressing undesired puberty with gonadotropin-releasing hormone agonists (GnRH agonists). GnRH agonists have previously been used as the primary strategy for the suppression of puberty in children experiencing precocious puberty.[17] Early results from the first 70

gender dysphoric youth undergoing puberty suppression with GnRH agonists in the Netherlands showed a decrease in behavioral and emotional problems, as well as a decrease in depressive symptoms. Improved general functioning for these youth was also reported.[16] By blocking the progression of puberty, the distress associated with full development of adult sexual characteristics incongruent with an internal gender identity is prevented.[18] A single study examining the physiologic impact of puberty suppression with GnRH agonists reported that the first 21 patients undergoing this treatment had adequate suppression of their pituitary gonadal axis and no progression of their endogenous puberty. While on GnRH agonists, height standard deviation scores decreased, and bone density remained in the same range for patients experiencing suppression. Compared to age-matched peers, bone density z-scores went down while patients were being suppressed.[18] In 2006, the "Dutch Model" was introduced, outlining this new approach to the care of transgender youth. Gender dysphoric adolescents 12 years and older are given GnRH agonists to prevent (or minimize in those already undergoing puberty) the development of undesired secondary sexual characteristics. For youth on GnRH agonists who continue to experience gender dysphoria through adolescence, appropriate cross-sex hormones (estrogen for the development of female characteristics and testosterone for the development of male characteristics) are added to the regimen to feminize or masculinize accordingly. The Dutch model recommends the addition of cross-sex hormones to allow the body to be brought into closer alignment with the identified internal gender when youth reach age 16. A recent follow up study from the Dutch team examining the impact of puberty suppression followed by cross sex hormones and gender reassignment surgery in 55 transgender young adults showed alleviation of gender dysphoria and steady improvement of psychological functioning.[19]

One important limitation of the Dutch model is the chronological age criterion that requires gender dysphoric children be at least age 12 before initiating suppression of puberty; however, by that age, it is well documented that many children in the United States are already well into their puberty.[20,21] In 2010, Biro et al. reported that across three metropolitan areas of the U.S., 42.1% of girls had Tanner 2 breast development by the age of eight years.[22] Relying on this chronological age criterion rather than sexual developmental stage decreases early intervention potential and may increase risk for negative mental health outcomes. Additionally, only a few studies describe the physiological[18] and psychosocial impact of this treatment protocol for puberty suppression.[19] Finally, the recommendations from the Dutch model are based on data collected from a homogenous population of white, European youth living in relatively supportive environments and are not necessarily generalizable to multi-ethnic transgender youth in the U.S.

1.1.4 The Endocrine Society Clinical Practice Guidelines

In 2009, using the best available evidence, the Endocrine Society incorporated the Dutch model into the clinical guidelines "Endocrine Treatment of Transsexual Persons," which includes recommendations for treatment of transgender youth.[7] In contrast to the Dutch model, the Endocrine Society recommends starting treatment with GnRH agonists for puberty suppression based on sexual development (Tanner staging) rather than chronological age. These guidelines recommend puberty blocking medications for youth with gender dysphoria at the beginning stages of puberty (Tanner stage 2 or 3), followed by appropriate cross-sex hormone therapy at around age 16. Since the introduction of these guidelines, no data have been collected in the U.S. on the physiologic and mental health impact, safety, or tolerability of pubertal-blocking medical interventions with GnRH agonists for transgender youth, particularly in children younger than age 12, leaving a gap in the evidence for this practice. Furthermore, the impact of GnRH agonists on the bone health of transgender children, specifically in those younger than 12 years, remains unknown. This study will investigate the physiologic and mental health impact of GnRHa administration as well as document the safety of GnRH agonists in a large cohort of transgender children and adolescents in the early stages of puberty.

The Endocrine Society Clinical Practice Guidelines include recommendations to initiate cross-sex hormones for gender dysphoric, late pubertal adolescents at around the age of 16. For those youth who are on GnRH agonists, cross-sex hormones are added to the regimen. For those new to clinical care, cross-sex hormones are prescribed without GnRH agonists, a protocol commonly used in both adolescents and adults. Studies in adult transgender populations have reported on the physiologic impact of cross-sex hormones,[23-25] but no studies to date have detailed the physiological impact of cross-sex hormone administration in transgender adolescents. This study will investigate both the physiologic and the mental health impact of cross-sex hormone administration as well as document the safety of cross-sex hormones in transgender adolescents in the later stages of puberty.

1.2 Rationale

The lack of data supporting medical interventions for transgender youth combined with a shortage of providers knowledgeable in the complex psychosocial risk factors facing these young people contributes to a health disparity and public health crisis of considerable magnitude. This research is highly significant in scope as it is the first longitudinal study collecting data - assessing both physiologic and mental health outcomes - to evaluate commonly used clinical guidelines for transgender youth in the U.S. In addition, we will do this work in four geographically distinct sites, in part because of the relative rarity of these conditions and also to increase the generalizability of the work. Results from this study have the potential to significantly impact the medical and mental health services provided to transgender youth in the U.S. by making available rigorous scientific evidence outlining the impact and safety of early treatment based on sexual development stage. Available data about the impact of the recommended treatment protocols come from a primarily white, European cohort in the Netherlands. This study examines the impact of treatment on diverse, multiethnic, transgender youth more representative of the U.S. population.

There are providers scattered around the U.S. (and the world) utilizing the Endocrine Society Clinical Practice Guidelines, but there are no formal empirical studies of related clinical outcomes in transgender children and adolescents. This project creates a network of four academic hospitals (i.e., Children's Hospital Los Angeles/University of Southern California, Boston Children's Hospital, Lurie Children's Hospital of Chicago/Northwestern University, and the Benioff Children's Hospital/University of California San Francisco) strategically situated across the country with strong histories of clinical service in this area to investigate the impact of the treatment on multi-ethnic transgender youth. All four sites have dedicated transgender youth clinics, employ a similar model of care that includes medical and mental health professionals, and are considered the national leaders in the care of transgender children and adolescents. The involvement of these four sites provides the experience, expertise, and clinic populations for a research endeavor of this magnitude and importance. In addition to the significant combined clinical experience of these four sites, all of the sites have strong and deep-rooted ties to academic research.

This longitudinal, observational study will collect critical data on the existing models of care for transgender youth that have been commonly used in clinical settings for close to a decade, although with very limited empirical research to support them. The gap in existing knowledge about the impact of these practices leaves providers and caretakers uncertain about moving forward with the recommended medical interventions for transgender youth seeking phenotypic transition. This research is a direct response to the IOM report calling for such studies, as well as the needs of clinicians and patients. The findings from this research have the capacity to substantially expand treatment across the country by providing rigorous evidence to demonstrate the benefits of early treatment and ultimately decrease the health disparity currently existing for transgender youth.

Avoiding the development of undesired secondary sex characteristics by starting puberty suppression at the earliest stages of puberty is recommended in the Endocrine Society Clinical Practice Guidelines, but has never been comprehensively studied in the U.S. and has never been studied in children under the age

of 12 years. This study will enroll transgender children eight years or older in the earliest stages of puberty to start treatment with GnRH agonists for puberty suppression, which will provide a critically important extension to the base of empirical knowledge about treatment outcomes. Despite the knowledge that transgender identity is stable by the time youth reach adolescence, The Endocrine Society Clinical Practice Guidelines recommend introducing cross-sex hormones "around" the age of 16. This study will evaluate the effects and document the tolerability and safety of cross-sex hormones in youth, including those younger than 16 years. While it is common for this team of experts to initiate cross-sex hormone therapy in transgender youth younger than age 16, there are no available data on this younger population.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

- To investigate the impact of medical treatments for gender dysphoria in two developmentally distinct and multi-ethnic cohorts of transgender youth recruited from across the nation via a network of Gender Centers dedicated to their care.
- To evaluate the impact of GnRH agonists administered for puberty suppression on mental health, psychological well-being, physiologic parameters, and bone health as well as document the safety of GnRH agonists in a cohort (Tanner stages 2-3) of children and adolescents with gender dysphoria, comparing baseline and follow-up assessments .
- To evaluate the impact of cross-sex hormones administered for phenotypic gender transition on mental health, psychological well-being, and metabolic/physiologic parameters as well as document the safety of cross-sex hormones in a cohort of gender dysphoric adolescents, comparing baseline and follow up assessments.

2.2 Secondary/Exploratory Objectives

- Based on evidence of high rates of substance use and HIV infection in some transgender adolescents (specifically, young transgender women), substance use and sexual risk behavior will be measured over time.

3.0 STUDY DESIGN

The study has a longitudinal, observational design for both the blocker and the cross-sex hormone cohorts. Data will be collected on age and Tanner staging to be able to examine if early vs. delayed treatment in these young people affects health outcomes. The anthropometric and physiologic parameters in the study are those routinely collected within the constructs of the clinical visit at each site. At baseline, 6 months, 12 months, 18 months and 24 months, audio computer-assisted self-interview (ACASI) survey instruments, will be used to collect demographic, mental health, psychosocial, and behavioral data from: 1) youth initiating pubertal suppression with GnRH agonists (blocker cohort) and their parent/caretaker/legal guardian, and 2) youth initiating hormone treatment for phenotypic gender transition (cross sex hormone cohort). At baseline, 12 months, and 24 months, parents/caretakers/legal guardians of blocker cohort participants will be asked to complete: 1) the Child Behavior Checklist (CBCL) to assess for emotional and behavioral problems among blocker cohort youth, and 2) the parent-informant version of the Diagnostic Interview Schedule for Children (C-DISC) to assess for mental health diagnoses. Participants in the cross-sex hormone cohort will be asked, at baseline and 12 and 24 months, to complete: 1) the Youth Self Report (YSR) to assess for emotional and behavioral problems, and 2) the youth-informant Diagnostic Interview Schedule for Children (DISC-Y) to assess for mental health diagnoses.

3.1 Study Population

Youth participants will be recruited from patients seeking care at any of the four study sites (Benioff Children's Hospital, Boston Children's Hospital, Children's Hospital Los Angeles, or Lurie's Children's Hospital of Chicago). Patients between 8 and 16 years old inclusive and their parents/caretakers/legal guardians will be eligible for enrollment in the blocker cohort. Patients less than 21 years old will be eligible for enrollment in the cross-sex hormone cohort. The focus of this investigation is early treatment; therefore, enrollment of youth aged 19 or 20 in the cohort of youth initiating treatment with cross-sex hormones will be limited to not more than 20% of the cohort. In addition, due to the expected impact of previous experience with blockers, no more than 60 participants in the cross-sex hormone cohort may be blocker experienced. Enrollment of approximately equal numbers of each gender for both cohorts will be targeted. Clinical observation indicates that an equal number of girls and boys seek care for gender dysphoria in the specialty clinics; thus, overrepresentation of either gender is not anticipated.

3.2 Sample Size

Target enrollment is 88 youth and 88 parents/caretakers/legal guardians in the blocker cohort. In the cross-sex hormone cohort, 300 participants will be enrolled. Total enrollment is 388 youth participants and 88 parent/caretakers/legal guardians across all sites.

4.0 SELECTION AND ENROLLMENT OF STUDY PARTICIPANTS

4.1 Blocker Cohort Youth Inclusion Criteria

To be considered eligible for enrollment, an individual must meet all the criteria listed below.

4.1.1 Presence of gender dysphoria as determined by a clinician;

4.1.2 Tanner stage 2 or 3 of sexual development;

4.1.3 Appropriate to undergo puberty suppression with GnRH agonists;

4.1.4 Ages 8 through 16 years inclusive;

4.1.5 Ability to read and understand English;

4.1.6 Receiving or planning to receive services at a study site clinic; and

4.1.7 Willing and able to provide signed informed assent.

NOTE: If assent is obtained > 30 days prior to the enrollment date, assent must be verbally reaffirmed prior to starting the baseline visit.

4.2 Blocker Cohort Youth Exclusion Criteria

To be considered eligible for enrollment, an individual must *not* meet any of the criteria listed below.

4.2.1 Prior utilization of GnRH agonists;

4.2.2 Precocious puberty (natal males younger than 9 years or natal females younger than 8 years);

4.2.3 Pre-existing osteoporosis;

4.2.4 Presence of serious psychiatric symptoms (e.g., active hallucinations, thought disorder) that would impair the individual's ability to provide true informed consent or participate in the baseline ACASI*;

4.2.5 Visibly distraught (e.g., suicidal, homicidal, exhibiting violent behavior) at the time of consent or the baseline ACASI*;

4.2.6 Intoxicated or under the influence of alcohol or other substances to such an extent that in the opinion of the study staff, the ability to give true informed consent or to understand and answer the questions is impaired*.

***NOTE: If consent is obtained prior to the enrollment date, assessment for these exclusion criteria must be performed again prior to administration of the baseline ACASI (Enrollment). If any are present during the baseline visit, the participant cannot be enrolled; do not administer the ACASI. Reassessment of eligibility and enrollment may take place at a later date per the discretion of the treating clinician.*

4.3 Parent/Caretaker of Blocker Cohort Youth Inclusion Criteria

To be considered eligible for enrollment, an individual must meet all the criteria listed below.

4.3.1 Parent or caretaker of a child who meets the Blocker Cohort Youth Inclusion/Exclusion Criteria,

4.3.2 Ages 18 and above;

4.3.3 Ability to read and understand English; and

4.3.4 Willing and able to provide signed informed consent.

NOTE: If consent is obtained > 30 days prior to the enrollment date, consent must be verbally reaffirmed prior to starting the baseline visit.

4.4 Parent/Caretaker of Blocker Cohort Youth Exclusion Criteria

To be considered eligible for enrollment, an individual must not meet any of the criteria listed below.

4.4.1 Presence of serious psychiatric symptoms (e.g., active hallucinations, thought disorder) that would impair the individual's ability to provide true informed consent or participate in the baseline ACASI*;

4.4.2 Visibly distraught (e.g., suicidal, homicidal, exhibiting violent behavior) at the time of consent or the baseline ACASI*;

4.4.3 Intoxicated or under the influence of alcohol or other substances to such an extent that in the opinion of the study staff, the ability to give true informed consent or to understand and answer the questions is impaired*.

**NOTE: If consent is obtained prior to the enrollment date, assessment for these exclusion criteria must be performed again prior to administration of the baseline ACASI (Enrollment). If any are present during the baseline visit, the participant cannot be enrolled; do not administer the ACASI.*

4.5 Cross-Sex Hormone Cohort Youth Inclusion Criteria

To be considered eligible for enrollment, an individual must meet all the criteria listed below.

4.5.1 The presence of gender dysphoria as determined by a clinician;

4.5.2 Appropriate for initiating phenotypic gender change with cross-sex hormones;

4.5.3 Ages 8 through 20 years inclusive;

4.5.4 Ability to read and understand English;

4.5.5 Receiving or planning to receive services at a study site clinic; and

4.5.6 Willing and able to provide signed informed consent or assent.

NOTE: If consent is obtained > 30 days prior to the enrollment date, consent must be reaffirmed prior to starting the baseline visit.

4.6 Cross-Sex Hormone Cohort Youth Exclusion Criteria

To be considered eligible for enrollment, an individual must *not* meet any of the criteria listed below.

4.6.1 Prior utilization of cross-sex hormones;

4.6.2 Previously or currently enrolled in the Blocker Cohort;

4.6.3 Presence of serious psychiatric symptoms (e.g., active hallucinations, thought disorder) that would impair the individual's ability to provide true informed consent or participate in the baseline ACASI*;

4.6.4 Visibly distraught (e.g., suicidal, homicidal, exhibiting violent behavior) at the time of consent or the baseline ACASI*;

4.6.5 Intoxicated or under the influence of alcohol or other substances to such an extent that in the opinion of the study staff, the ability to give true informed consent or to understand and answer the questions is impaired*.

***NOTE: If consent is obtained prior to the enrollment date, assessment for these exclusion criteria must be performed again prior to administration of the baseline ACASI (Enrollment). If any are present during the baseline visit, the participant cannot be enrolled; do not administer the ACASI.*

4.7 Recruitment and Pre-Screening

Potential participants will be receiving services at one of the four sites (Benioff Children's Hospital, Boston Children's Hospital, Children's Hospital Los Angeles, or Lurie's Children's Hospital of Chicago) and seeking hormonal intervention to either delay the progression of puberty with GnRH agonists or begin phenotypic gender transition with cross-sex hormones. Site care members will recruit participants for the study by speaking with patients and their parents/caretakers/legal guardians face-to-face or by telephone. Information regarding the study will be provided and interest in participation will be assessed. A Pre-Screening Worksheet will be completed by the medical provider to assess whether the potential participant may be eligible for study participation. The medical provider must be approved by the site's IRB as a study team member. The Pre-Screening Worksheet will be signed and dated by the medical provider in order for it to be used for source documentation by the study staff when completing the Eligibility and Enrollment case report form (CRF).

Each individual youth participant who has their medical records reviewed to assess potential eligibility, is approached for recruitment, and/or consented for study participation will have the following information entered on the Study Recruitment Log, which will be maintained in a secure area at the site: name or initials, date of birth, age, sex assigned at birth, race, ethnicity and enrollment status. Individuals that were assessed as ineligible for enrollment will have the reasons for ineligibility recorded. Individuals who are approached, but do not consent to participate (or whose parent/legal guardian refuses to provide permission, if applicable), will be asked if they are willing to supply their reason(s) for declining participation; responses will be recorded. Information collected on the Study Recruitment Log, excluding names or initials and dates of birth, will be entered in the study-specific database for individuals that do not enroll in the study as instructed on the Study Recruitment Log.

For those individuals who were not eligible or did not consent to participate in the study, the Pre-Screening Worksheet will be destroyed immediately after having the information recorded on the Study Recruitment Log. When study accrual ends, project staff will obliterate on the Study Recruitment Log, all names or initials and dates of birth belonging to individuals who did not consent to participate in the study.

4.8 Informed Consent

Once it is determined that the individual may qualify for the study, details will be discussed and all questions answered during the informed consent process. Signed informed consent from the individual (or assent with signed parent/legal guardian permission as determined by the local IRB) will be obtained (see Appendix IV, V, VI, and VII for sample informed consent/assent/permission forms and assent forms (for younger children). The informed consent/permission/assent form covers information about the overall purpose of the study, what the study entails, potential risks, potential benefits to participating individuals and society, the confidentiality of data, and contact information for the Principal Investigator and the IRB. Once informed consent/permission/assent has been obtained, the research staff will have the form reviewed by a fellow research team member, who will confirm that it is fully and accurately completed before it is filed in a secure location under double-lock when not in use and with restricted access during work hours and/or when unattended. Consent/Assent/Permission must be obtained within 30 days prior to study entry. If more than 30 days has elapsed since consent/assent/permission was obtained, consent/assent/permission must be verbally reaffirmed on the day of study entry prior to implementing any study activities.

4.9 Contact Information

Once participants have provided informed consent/assent/permission, research staff will complete the Locator Form with the study participants. Study participants will be asked to provide a working phone number and/or valid email address through which they can be reached. Participants will also be asked to provide valid contact information for a family member and/or friend who can be called in the event the participant cannot be reached by phone or email. Participants will be asked if text and/or voice messages can be left at the numbers provided and which name and pronouns to use for the participant. Study staff will not leave messages unless expressly permitted to do so by the participant, which will be documented on this form. If permission is given to leave messages, site staff will assure participants that messages left with a family member or friend will only ask the participant to contact study staff and will not include any protected health information or information related to study participation. As the Locator Form will include names and contact information, it must be stored under double-lock and separate from any documents that utilize the participant's ID.

4.10 Co-Enrollment Guidelines

Co-enrollment in other studies may be considered at the discretion of the PIs. Permission may be requested through the TransYouthCare@chla.usc.edu email. Participants enrolled currently or previously in the Blocker Cohort may not be enrolled in the Cross-Sex Hormone Cohort.

5.0 STUDY PROCEDURES

5.1 Enrollment/Study Entry Procedures

An Eligibility and Enrollment CRF will be completed by research staff prior to the start of the baseline ACASI. If needed, research staff can verify that information has not changed between the completion of the Pre-Screening Worksheet and the Baseline Visit. If more than 30 days has passed between the date of the Pre-Screening Worksheet and the baseline visit, documentation of eligibility verification is required prior to baseline ACASI.

A unique ID will be assigned to the youth and/or parent/guardian. The identifiers consist of the designated site code (i.e., Benioff Children's Hospital = 1, Boston Children's Hospital = 2, Children's Hospital Los Angeles = 3, or Lurie's Children's Hospital of Chicago = 4) followed by the participant's enrollment number. Youth participant enrollment numbers for the Blocker Cohort will start with 0001 for each site; and parent/caretaker/legal guardian enrollment numbers will start with 1001 with the final digit matching the corresponding youth participant. The parent/caretaker/legal guardian participant cannot change

throughout the study. For example, if parent 1 completes the baseline survey, the same parent must complete all future study surveys. The adolescent enrollment numbers for the Cross-Sex Hormone Cohort will start with 2001. For example, the first Blocker participant for Boston Children's Hospital will be 20001, and the corresponding parent/guardian will be 21001. The first Cross-Sex Hormone participant at Boston Children's Hospital will be 22001.

For the purpose of this study, enrollment/study entry is equivalent to the participant completing the baseline ACASI. The Eligibility and Enrollment Form must be entered into the database with 2 working days after the Baseline Visit.

6.0 EVALUATIONS AND MEASURES

See Appendix I. for the Schedule of Evaluations

6.1 Baseline

6.1.1 Blocker Cohort

6.1.1.1 Psychosocial Assessments – Baseline: Blocker Cohort – Youth

Study participants will be asked to complete a series of questionnaires via the ACASI at the baseline visit, including measures to evaluate mental health, gender dysphoria, the experience of life stressors, parental and peer relationships, and quality of life. Participants will also be asked demographic questions. The ACASI will take approximately 2 to 2.5 hours to complete.

The measures in the blocker cohort ACASI survey fall into four domains: Demographics, transgender specific experiences, mental health and trauma and additional psychosocial information.

- Demographics: including age, ethnicity, educational level, and birth city/country
- Transgender-specific experiences including gender dysphoria: age of realization of transgender status, age of first living in the desired gender role, and domains where they are living in their desired gender role, if any.
    - Utrecht Gender Dysphoria Scale (UGDS) – a dimensional scale designed specifically to measure gender dysphoria. The adolescent version of the Utrecht Gender Dysphoria scale consists of 12-items to which individuals rate their level of agreement on a 5-point Likert scale.[26]
- Mental health and trauma assessments:
    - Revised Children's Manifest Anxiety Scale: Second Edition (RCMAS-2) – A measure used in both clinical and educational settings to determine levels of anxiety in young children and adolescents aged 6 to 19 years old. This measure is designed to capture levels of: physiological anxiety; worry; social anxiety; and defensiveness.[27]
    - Beck Depression Inventory (BDI-Y) - The BDI-Y contains 20 statements about thoughts, feelings, and behaviors associated with depressive symptomology within the last 2 weeks. Items are rated from 0 (Never) to 3 (Always).[28] (for those youth 8-11 years)
    - Suicidal Ideation Scale - Eight yes/no questions will be asked to capture participants' suicide ideation and attempts.[29]
    - Connor-Davidson Resilience Scale (CD-RISC) – A self-report metric used in clinical practice and research studies to assess characteristics of resilience when internal and external stressors arise.[30]
- Additional psychosocial information including body image, quality of life and relationships with parents and peers:

- Body Esteem Scale (8-10.5) - Body Esteem Scale is a questionnaire that has 3 subscales: BE–Appearance (general feelings about appearance), BE–Weight (weight satisfaction), and BE–Attribution (evaluations attributed to others about one's body and appearance).[31]
- Body Image Scale - As an assessment tool designed to evaluate requests for sex-reassignment surgery and treatment, the Body Image scale is a 30-item measure that is used to determine body attitudes among transgender men and women, by asking that participants rate their satisfaction with 30 body features on a Likert-type scale[32]
- Parental Support Scale – Youth Version - Items designed to assess the level of support that transgender/gender dysphoric youth perceive from their parent(s)
- Social Relationships – Emotional support/Friendship/loneliness/perceived hostility/perceived rejection – NIH Toolbox[33]
- Stress/Self-efficacy – Self efficacy (CAT 8-12 / CAT 13-17) – NIH Toolbox[33]
- Harter's Self-Perception Profiles for Adolescents & Children – This is a metric designed to capture an individual's definition of self by assessing self-image and self-esteem using five domains of perceived competence (scholastic competence, social competence, athletic competence, physical appearance, and behavioral conduct).[34]
- Pediatric Quality of Life Inventory (PedsQL) v4.0 (Child Report) – This instrument is used to assess physical, emotional, social, and school functioning in children ages 8 to 12 years old. This measure is designed to be sensitive to children's perceptions of the described domains.[35]
- Physical Activity Questionnaire – A 7-day recall instrument used to assess levels of weight-bearing exercise among school-aged youth[36]

6.1.1.2 Psychosocial Assessments – Baseline: Blocker Cohort – Parent/Guardian

- Demographics: including assigned sex at birth, age, ethnicity, educational level, socioeconomic status, birth city/country; religiosity and spirituality (Modified Duke University Religion Index)[37]; child's service utilization; relationship to child (adoptive parent, biological parent, foster parent)
- Transgender-specific experiences: child's gender identity, child's age of realization of transgender status, child's age of first living in the desired gender role, domains where the child is living in their desired gender role, social transitioning, parental/guardian support, and disclosure of child's transgender status to others.
  - Parent Report Gender Identity Questionnaire – An assessment tool that is utilized to identify children with gender dysphoria among gender-referred probands.[38]
  - Diagnostic and Statistical Manual of Mental Disorders (DSM-5) – Criteria for gender dysphoria will be assessed by parent report.[39]
  - Social Transitioning Scale – assesses domains in which a child's asserted gender identity is affirmed.[40]
- Mental health and trauma assessments: parent reports of child's anxiety, child's experience of trauma and trauma symptoms (including those associated with impending or beginning pubertal development), child's suicide (ideation and attempts), and self-harm.
  - Parent Report Diagnostic Interview Schedule for Children – The Diagnostic Interview Schedule for Children (DISC) is a structured psychiatric diagnostic interview for children and adolescents aged 6 to 18, or their parents. It was developed primarily for epidemiological research but is also useful in clinical settings. The most recent version of the DISC (DISC-IV) is able to address more than 30 psychiatric diagnoses that occur in children and adolescents based on DSM-IV criteria. [41]
  - Autism Spectrum Quotient - 10 (AQ-10) Child version – A short questionnaire for parents to complete about a child 4-11 years old with suspected autism who does not have a learning disability.[42]

  - Child Behavioral Checklist - The CBCL/6-18 yields scores on internalizing, externalizing, and total problems as well as scores on DSM-IV related scales. [43]
  - Suicidality - Eight parent-proxy items will be used to capture children's suicide ideation and attempts.[29]

- Additional psychosocial and physiologic measures:

  - Parental Support Scale – Parent Version– Questions related to the parent's level of support around their transgender/gender dysphoric child
  - Parenting Stress Index – Short Form (PSI-SF) – A metric designed to capture the stress within a parent-child dyad. This 36-item scale, adapted from the full-length form, is used to assess levels of stress within the following domains: child characteristics, parent characteristics, and situational/demographic life stress[44]
  - Social Relationships – Empathic Behaviors/Peer Rejection/Positive Peer Interactions/Social Withdrawal (Parent Report) – NIH Toolbox[33]
  - Negative affect – Anger/Fear/Sadness (Parent Report) – NIH Toolbox[33]
  - Psychological well-being – General life satisfaction/positive affect (Parent Report) – NIH Toolbox[33]
  - Stress/Self efficacy – Self efficacy (Parent Report) – NIH Toolbox[33]
  - Pediatric Quality of Life Inventory (PedsQL) v4.0 (Parent Report) – The parent-proxy report PedsQL questionnaire is used to examine parents' perceptions of their children's physical, emotional, social, and school functioning.[35]
  - Daily calcium intake

- The Child Behavior Checklist and the Parent-Informant Diagnostic Interview Schedule for Children (DISC) will be completed by parents of blocker cohort participants after the Baseline ACASI on the same day or during a second visit within two weeks of the Baseline/Enrollment Visit. The DISC is conducted through a face to face interview and the CBCL will be completed via an ACASI. These items will take 1.5 to 2.25 hours to complete.

6.1.1.3 Medical Record Abstraction – Baseline – Blocker Cohort

Research staff will obtain information from the participant and/or parent/caretaker/legal guardian and abstract data from the medical record. Data items to be collected are:

- Ongoing prescription medications (may be asked directly of the participant and/or parent and/or abstracted from the medical record)
- Clinically significant diagnoses (may be asked directly of the participant and/or parent and/or abstracted from the medical record)
- Anthropometric measures (height, weight, BMI, blood pressure, Tanner stage) – most recent results
- Physiologic parameters – most recent results:
  - Hormone levels (ultrasensitive luteinizing hormone, ultrasensitive follicle stimulating hormone, ultrasensitive serum estradiol or ultrasensitive serum testosterone, based on assigned sex at birth)
  - Bone health (mineral bone density (BMD), bone age, 25-hydroxy vitamin D, calcium, serum phosphorus, and alkaline phosphatase)
- Date of insertion of GnRH agonist. This information will be entered into the database after the baseline ACASI visit, once the insertion has been completed.
- Insurance status.

6.1.2 Cross-Sex Cohort

6.1.2.1 Psychosocial Assessments- Baseline: Cross-Sex Cohort

Study participants in the cross-sex hormone cohorts will be asked to complete a series of questionnaires via the ACASI at the baseline visit, including measures to evaluate mental health, gender dysphoria, the experience of life stressors, parental and peer relationships. Participants will also be asked demographic questions, substance use behaviors, and sexual risk questions. The ACASI will take approximately 2 to 2.5 hours to complete. Measures utilized in the ACASI survey fall into five domains:

- Demographics: including assigned sex at birth, age, ethnicity, race, sexual orientation educational level, socioeconomic status; birth city/country; religiosity and spirituality (Modified Duke University Religion Index)[37]; and service utilization
- Transgender-specific experiences including gender dysphoria:
    - The Gender Identity/Gender Dysphoria Questionnaire for Adolescents and Adults – A dimensional assessment tool designed to determine the degree of gender dysphoria or gender uncertainty among adolescents and young adults.[45]
    - Transgender Congruence Scale –A construct of congruence to conceptualize the degree to which transgender individuals feel genuine, authentic, and comfortable with their gender identity and external appearance.[46]
    - Diagnostic and Statistical Manual of Mental Disorders (DSM-5) - *see description in section 6.1.1.2*
    - Utrecht Gender Dysphoria Scale (UGDS) - *see description in section 6.1.1.1*
- Mental health and trauma assessments:
    - Youth Informant Diagnostic Interview Schedule for Children - The Youth - Diagnostic Interview Schedule for Children (DISC-Y) is a structured psychiatric diagnostic interview for youth informants between the ages of 9 and 17 years old. It was developed primarily for epidemiological research but is also useful in clinical settings. The most recent version of the DISC (DISC-IV) is able to address more than 30 psychiatric diagnoses that occur in children and adolescents based on DSM-IV criteria.[41]
    - Autism-Spectrum Quotient -10 (AQ-10) Adult Version - A self-administered questionnaire for adults (16 years and older) with normal intelligence. The AQ-10 is an adaptation of the original 50-item Autism Spectrum Quotient (AQ) questionnaire. Items are used to assess social skills, attention, communication, and imagination.[42]
    - Revised Children's Manifest Anxiety Scale: Second Edition (RCMAS-2 - What I Think and Feel) – A measure used in both clinical and educational settings to determine levels of anxiety in young children and adolescents aged 6 to 19 years old. This measure is designed to capture levels of: physiological anxiety; worry; social anxiety; and defensiveness.[27]
    - Youth Self Report - The YSR was designed to assess the emotional and behavioral problems in adolescents in a standardized format. It assesses internalizing (i.e., anxiety, depression, and overcontrolled) and externalizing (i.e., aggressive, hyperactivity, noncompliant, and undercontrolled) behaviors.[47]
    - Beck Depression Inventory II – BDI-II (Adolescent) – A screening tool used to identify symptoms and severity of depression among adolescents and adults with or without self-reported depression.[48]
    - Suicidal Ideation Scale - *see description in section 6.1.1.1*
    - Gender Minority Stress and Resilience (GMSR) Measure – The Gender Minority Stress and Resilience questionnaire is a measure that consists of subscales (i.e., pride, internalized transphobia, non-affirmation, etc.) designed to assess mental health outcomes, such as depression and social anxiety among transgender and gender-nonconforming individuals.[49]
    - Connor-Davidson Resilience Scale (CD-RISC) – *see description in section 6.1.1.1*
- Additional psychosocial information including quality of life and relationships with parents and peers
    - Body Esteem - *see description in section 6.1.1.1*

  - Body Image Scale - *see description in section 6.1.1.1*
  - Parental Support Scale – Youth Version– *see description in section 6.1.1.1*
  - Negative affect – Anger/Fear/Sadness NIH Toolbox[33]
  - Psychological well-being – General life satisfaction/positive affect NIH Toolbox[33]
  - Social Relationships – Emotional support/Friendship/loneliness/perceived hostility/perceived rejection – NIH Toolbox[33]
  - Stress/Self efficacy – Self efficacy (CAT 13-17)– NIH Toolbox[33]
  - Health Related Quality of Life – A scale adapted from the HIV-specific health related quality of life measure. This measure includes an assessment of perceived burden of being transgender, measured as the degree of satisfaction with domains of life that are potentially affected by being transgender (e.g. social relationships, family burden, medical management), interference with life goals and daily activities, and related worries.[50]

- Behavior risk including alcohol/drug use[41], sex work and high-risk sexual activities.
  - The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) – The revised version of the ASSIST v2.0 consists of eight sections covering use of the following substances: tobacco, alcohol, cannabis, cocaine, amphetamine-type stimulants (including ecstasy), inhalants, sedatives, hallucinogens, opioids, and 'other drugs'.[51]
  - Sexual Risk Behavior – A measure modified after the work of Jemmott *et al.*[52] Items are used to assess an individual's sexual activity in the three months prior to completion of the scale to determine the level of HIV risk-associated sexual activity.
  - History of sexually transmitted infections (STIs)

- The Youth Self Report (YSR) and the Youth-Informant Diagnostic Interview Schedule for Children will be completed after the ACASI on the same day or during a second visit within two weeks of the Baseline/Enrollment Visit. The DISC is conducted through a face to face interview and the YSR will be completed via an ACASI. These items will take 1.5 to 2.25 hours to complete.

6.1.2.2 Medical Record Abstraction – Baseline – Cross-Sex Cohort

Research staff will obtain information from the participant and abstract data from the medical record. Data items to be collected are:

- Ongoing prescription medications (may be asked directly of the participant and/or abstracted from the medical record)
- Clinically significant diagnoses (may be asked directly of the participant and/or abstracted from the medical record)
- Anthropometric measures (height, weight, BMI, blood pressure, Tanner stage) – most recent results
- Physiologic parameters – most recent results:
  - Hormone levels (serum estradiol and prolactin or serum testosterone and free testosterone, based on assigned sex at birth).
  - White blood cell count, hemoglobin, hematocrit, platelets, glycosylated hemoglobin, sodium, potassium, chloride, CO2 Total, BUN, creatinine, fasting glucose, AST, ALT, Triglycerides, cholesterol, cholesterol HDL, cholesterol LDL, and cholesterol VLDL.
- Insurance status.

6.2 Study Follow-Up: Medical Record Abstraction

6.2.1 Blocker Cohort – Follow-up

Project staff at each site will abstract the following medical chart data at 6 months, 12 months, 18 months, and 24 months. Data abstracted will be from the previous visit date through the 6, 12, 18, and 24 month target dates based on the date the blocker was inserted. Data to be collected are:

- Ongoing prescription medications (may be asked directly of the participant and/or parent and/or abstracted from the medical record)
- Clinically significant diagnoses (may be asked directly of the participant and/or parent and/or abstracted from the medical record)
- Anthropometric measures (height, weight, BMI, blood pressure) – most recent results prior to and including the target date
- Physiologic parameters:
  - Hormone levels (ultrasensitive luteinizing hormone, ultrasensitive follicle stimulating hormone, ultrasensitive serum estradiol or ultrasensitive serum testosterone, based on assigned sex at birth)
  - Bone health (25-hydroxy vitamin D, calcium, phosphate, and serum bone-specific alkaline phosphatase)

Additional measures related to bone health will be collected at 12 and 24 months from the date the blocker was inserted. Data to be collected are:

- mineral bone density (BMD) measured via a DXA or QCT
- bone age

6.2.2 Cross-Sex Hormone Cohort – Follow-up

Project staff at each site will abstract the following medical chart data at 6 months, 12 months, 18 months, and 24 months. Data abstracted will be from the previous visit date through the 6, 12, 18, and 24 month target dates based on the baseline visit. Data to be collected are:

- Ongoing prescription medications (may be asked directly of the participant and/or abstracted from the medical record)
- Clinically significant diagnoses (may be asked directly of the participant and/or abstracted from the medical record)
- Anthropometric measures (height, weight, BMI, blood pressure) – most recent results
- Physiologic parameters:
  - Hormone levels (serum estradiol and prolactin or serum testosterone and free testosterone, based on assigned sex at birth).
  - White blood cell count, hemoglobin, hematocrit, platelets, glycosylated hemoglobin, sodium, potassium, chloride, CO2 Total, BUN, creatinine, fasting glucose, AST, ALT, Triglycerides, cholesterol, cholesterol HDL, cholesterol LDL, and cholesterol VLDL.

6.3 Study Follow-Up: Psychosocial Assessments

6.3.1 Psychosocial Assessments for the Blocker Cohort - Follow-up

Child and parent participants will be asked to complete a series of questionnaires via the ACASI at 6, 12, 18, and 24 months from the date the blocker was inserted +/- 14 days. The ACASIs will be identical to the ACASI conducted at the baseline visit and include demographics and measures to evaluate mental health, gender dysphoria, the experience of life stressors and traumatic events, parental and peer relationships. These measures are described above in sections 6.1.1.1 and 6.1.1.2. The ACASI should take no more than 2 to 2.5 hours to complete.

Parents of participants will also complete the CBCL and the Parent-Informant DISC at the 12 and 24 month visits. This will be completed either after the ACASI on the same day or at a separate visit within 2 weeks after the completion of the ACASI. These items will take an additional 1.5 to 2.25 hours complete.

6.3.2 Psychosocial Assessments for the Cross-Sex Hormone Cohort - Follow-up

Cross-sex hormone study participants will be asked to complete ACASI questionnaires at the 6, 12, 18, and 24 months from the baseline/entry visit +/- 14 days. The ACASI will be identical to the ACASI conducted at the baseline visit and will include measures to evaluate mental health, gender dysphoria, the experience of life stressors and traumatic events, parental and peer relationships, and quality of life as described above. Participants will also be asked demographic questions, substance use behaviors, and sexual risk questions. The ACASI should take no more than 2 to 2.5 hours to complete.

The participants will also complete the YSR and the Youth-Informant DISC at the 12 and 24 month visits. This will be completed either after the ACASI on the same day or at a separate study visit within 2 weeks after the completion of the ACASI. These items will take an additional 1.5 to 2.25 hours to complete.

7.0 DATA COLLECTION AND SITE MONITORING

7.1 Data Records

Participants will be assigned a unique identification number code. No personal identifying information will be used on the CRFs, ACASIs, or Diagnostic Interview data records. Consent forms will be filed and stored separate from the raw data in a secured location under double-lock when not in use and with restricted access during work hours and/or when unattended. A key file that matches the ID number to the participant and organization will be maintained in a secure data repository within the project offices at each of the four sites. Data will be kept strictly confidential, except as required by law, and stored on a secure network, with password protection such that only authorized users will have access to the file server.

Data will remain on the CHLA server during data collection, verification, cleaning, and analysis. At the closure of the study, electronic and hardcopy data will be maintained for a minimum of six years from the closure of the study per CHLA IRB policy. The local site data will be retained at the local site for the length of time as defined the local site's IRB policy. Project binders at CHLA containing archival information will be stored a minimum of six years from the closure of the study and will then be eligible to be destroyed.

7.2 Data Collection and Submission

7.2.1 Case Report Forms

Anthropometric measures, physiologic parameters, hormone levels, and measures related to bone health will be collected on CRFs created by CHLA. All case report forms (CRFs) will be entered into password protected databases at the site and will be transmitted with an ID securely to CHLA via secure file transfer. A password will be used to access survey data and chart abstraction files and will be made accessible only to the Principal Investigators and study staff. These data will be archived in a password-protected database on the local network on a daily basis. Hard copies of all CRFs will be securely filed at each respective study site. Study-wide data management procedures, including integration and verification of multi-site data, will take place at CHLA. All CRFs collecting participant-related data must have corresponding source documentation at the clinical site to substantiate all submitted data.

7.2.2 Data Collection Methods

All data collected using an ACASI will be through software installed on a portable laptop computer. For the ACASI baseline and follow-up surveys, each participant will be assigned a code and their baseline and follow up surveys will be linked via this code. A master list of the codes assigned to participants will be kept in a secure, password protected file or in a secured location at each site under double-lock when not in use and with restricted access during work hours and/or when unattended. ACASI data collected at

the collaborating sites will be encrypted and transferred to CHLA. All data collected from the administration of the CBCL and YSR will be accessed through password protected Achenbach System of Empirically Based Assessment (ASEBA)-PC Software. All data obtained from ASEBA-PC software will be securely exported, encrypted, and transferred to CHLA. Upon completion of the Diagnostic Interview Schedule for Children (parent *and* youth-informant DISC), the DISC program will be used to prepare ASCII data files in which participant responses will be saved for analysis. All data collected from participants through the Diagnostic Interview Schedule for Children will be electronically stored, encrypted, and transferred to CHLA. Upon transfer of the encrypted data to CHLA, the data will be stored on CHLA's secured network (with firewall protection), which cannot be accessed by anyone outside of CHLA.

7.2.2.1 Data Security

As each section of the ACASI survey is completed, the section will be saved and encrypted so that no one is able to look at previous screens to view the data. Only authorized users with a login name and password will be able to open the ACASI survey on the laptop. All diagnostic interviews will be conducted in a private office to ensure privacy and confidentiality of participant responses. If the participant completing either the ACASI survey or the diagnostic interview requires a short break, it is possible to stop and return later to complete it. The ACASI must be completed within 7 days from when it is initiated and within the study visit window.

After a participant completes the ACASI survey or DISC at a site, the data will be secured by being saved in a password-protected compressed file. All data collected using ASEBA-PC software is electronically stored, under policies compliant with the Health Information Portability and Accountability Act (HIPAA) and the Health Information Technology for Economic and Clinical Health Act (HITECH).

All computers will be located in locked facilities, and consent forms will be filed and stored separate from the raw data in a secured location under double-lock when not in use and with restricted access during work hours and/or when unattended. Any temporary data files kept on removable storage devices, as well as printouts derived from data analysis, will be stored in a locked compartment when not in use.

7.3 Data Quality Assurance

Investigators receiving federal funding must adhere to the Code of Federal Regulations (CFR) to protect research participants and produce reliable study information. The data manager at each study site will work with site staff to facilitate the receipt of data, provide technical assistance as necessary, and meet regularly via telephone with the staff person responsible for site-specific quality assurance. Data edits through range checks and field inconsistencies will be built into the database to enable real time correction of key entries and CRF completion errors.

7.4 Data Management

Data must be cleaned and stored uniformly in order to perform useful analyses and generate meaningful conclusions. A data coordination staff at CHLA will conduct trainings of data collection personnel at each site concerning appropriate procedures for recruitment, enrollment, and use of computer assisted data collection. Additionally, CHLA staff will be responsible for verifying that data from all sites are comparable. The CHLA data manager will be responsible for ensuring that site data are safely transferred via secure protocols to CHLA for cleaning and merging. The CHLA data manager will generate tracking reports regarding the accrual of participants at each site, as well as specific characteristics of the emerging cross-site data file (such as balance of male and female transgender respondents, previous blocker use for cross sex hormone cohort, or age categories, etc.).

7.5 Study Site Monitoring and Record Availability

Each of the participating study sites will review a selected portion of the individual subject records, including assent/consent forms, CRFs and supporting source documentation to ensure the protection of

study subjects, compliance with the protocol, and accuracy and completeness of records. Regulatory files, as required, will also be inspected to ensure that regulatory requirements are being followed.

The primary investigators at each site will make study documents (e.g., consent forms, case report forms) *and pertinent hospital or clinic records* readily available for inspection by the local IRB, CHLA staff, the NICHD, the Office of Human Research Protection (OHRP), or the sponsor's designee for confirmation of the study data.

8.0 PARTICIPANT MANAGEMENT

8.1 Tracking Participants/Follow-up

All participants will be contacted before each follow-up study visit (i.e., 6, 12, 18, and 24 months after baseline visit for the cross-sex hormone cohort or after blocker insertion date for the blocker cohort). Multiple contact methods will be used for participants who are difficult to reach (e.g., phone, email, text message). Participants will be asked whether or not messages can be left for each of the phone numbers that they provide. They will be informed that messages will not contain any information regarding the nature of the project.

8.2 Study Visit Management

All study visits are to be conducted according to the Schedule of Evaluations in Appendix I. The preferred timeframe for all follow-up visits including dates of physiologic and anthropomorphic data is within 14 days prior to or after the target study visit date. If the participant is unable to attend a visit within this timeframe, the site staff should work with the participant to identify a day closest to the scheduled visit to perform the visit. Extension of the visit window must first be communicated to and approved by the CHLA team via TransYouthCare@chla.usc.edu. If not approved, the visit will be considered a "missed visit." Scheduling of study visits will not be recalibrated based on the actual date that a visit was made. All follow-up study visits must be made based on the elapsed time from the date of enrollment.

8.2.1 Completing the ACASI Survey, DISC, and YSR or CBCL

Participants should be provided with a quiet, private area to complete the survey or interview. Prior to starting the survey or interview, the participant should be reminded of their right to discontinue at any time with no penalty and the right to leave unanswered, any questions that make them feel uncomfortable. Project staff at each study site will assist with ACASI tutorials, if required. If the participant requires breaks, project staff at each site will make sure the computer program is exited and re-entered properly, so that the participant's confidentiality is maintained. The CBCL/YSR and Diagnostic Interview Schedule for Children (DISC) must be completed after the ACASI either on the same day or on a separate day. If completed on the same day as the ACASI, participants must be given an adequate break in between.

8.2.2 Debriefing and Referral Procedures for ACASI Participants

Because responding to questions in the surveys or interviews may be distressing to participants, a short debriefing of all participants will be conducted upon completion of the ACASI/interview to ensure that study staff has an opportunity to assess the participant's reaction to the survey or interview, whether the survey was wholly or partially completed. Study staff will ask participants if there is any part of the ACASI that was upsetting to them or that they would like to discuss. Further, if participants indicate current suicidal thoughts on the ACASI, the ACASI program will flag those responses. At the completion of the ACASI, a flag will show up for study staff indicating follow-up is needed. The study staff will then determine whether the participant currently presents a danger to self or others. If the participant's response to the debriefing interview or suicide assessment indicates a potential risk to the participant's safety and an urgent need of mental health assistance, site staff should follow their individual site procedures for acute mental health referrals. Site staff should contact a supervisor immediately and stay

with the study participant until the supervisor, mental health professional or emergency services, if needed, arrives. Results of the debriefing interview are kept at the sites; however, sites are instructed to inform the CHLA team whether a referral was made and complete and submit the Monitoring Untoward Event Form.

If a participant wholly or partially completes the survey, becomes distraught, and leaves the clinic without the debriefing interview being conducted, the site staff will report the protocol deviation to their local IRB and the CHLA team. In addition, they will complete a Monitoring Untoward Event Form and submit it to the CHLA team.

Participants who do not appear distressed and do not want to discuss anything about the survey or interview will be informed that they can contact study personnel for referrals in the event that issues or concerns arise later.

8.3 Compensation

The decisions around compensation will be determined separately by each site and approved by each site's IRB.

8.4 Intervening on "Social Harms"

The research staff and protocol will be certified through the local IRB at each of the four sites to conduct research on human participants, particularly as related to children. This observational study is 46.404, research not involving greater than minimal risk, and 46.408 requirements for permission by parents or guardians and for assent by children will be followed. All sites have specific policies governing the treatment of human participants. These policies specify that medical and psychological assistance will be available in the immediate environment in the event a participant should experience any adverse reactions resulting from study procedures.

While participants will be informed that they may refuse to answer any question at any time, responses or reactions to certain questions may indicate distress on the part of the participants. If at any time during the study, a participant divulges being at risk for harm, including but not limited to being abused or experiencing violence, if harm is suspected or likely, or if the participant reports suicidal/homicidal intentions, measures will be taken to ensure the participant's safety per each site's IRB requirements and safety protocol. Reporting will be done as appropriate to the specific situation and the local legal statues, including reporting to child protection agencies, or other appropriate agencies and referrals will be provided to appropriate support, counseling, or treatment resources. In addition, social harms will be reported to the CHLA Team as part of study conduct.

8.5 Premature Discontinuation from the ACASI or the DISC, CBCL, or YSR Interview

If a participant appears to be distressed while completing the ACASI or participating in an interview, research staff will ask the participant if there is anything bothering them and if they would like to stop the survey or interview. If they choose to stop the survey or interview, the research staff will follow their local site procedures for assessing and intervening. Medical and psychological professionals on the project team will be available to provide support where necessary. Participants will continue on study, unless the participant requests to end study participation as discussed in the Premature Study Discontinuation.

8.6 Premature Study Discontinuation

Participants will be prematurely discontinued from the study if any of the following occurs:

- The participant withdraws consent (or assent and/or parent/legal guardian withdraws permission, if applicable);
- Participant is diagnosed with osteoporosis at baseline via bone densitometry;

- The participant is lost to follow-up;
- The participant experiences an untoward event that warrants discontinuation form the study;
- The participant develops a health problem and needs treatment that would affect the results of this study;
- The study is cancelled by *The Eunice Kennedy Shriver* NICHD;
- The study is cancelled for other administrative reasons; and
- Death of the participant.

If a youth participant in the Blocker Cohort is prematurely discontinued from participating in the study, the parent, legal guardian, caretaker participant will also be prematurely discontinued from the study.

Complete the Off Study Form when the decision is made to permanently discontinue the participant from the study and no further data collection will occur. Data through the time that the participant is taken off study will still be used for study purposes.

9.0 MONITORING UNTOWARD EVENTS

Site staff must first follow their own IRB's procedure for reporting and managing untoward events. Project staff will record any untoward event experienced by the participant. Reporting is required for occurrences including social harms, psychological distress and serious life threatening events such as suicide attempts. These may be immediately apparent to the study staff, such as the participant's emotional upset requiring referral for counseling; or they may be delayed and reported later to study staff, such as physical harm to an individual for having participated in the study. Study staff will notify CHLA of these untoward events as soon as possible, but no later than 48 hours after awareness of the event. In addition, study staff will complete the Untoward Event Form and enter it into the study database within three working days after awareness of the event. Study staff will be briefed during the training on the scope of possible untoward events and instructed to report them.

10.0 STATISTICAL/ANALYTIC CONSIDERATIONS

10.1 Introduction

This is a longitudinal observational multi-site study to study the impact of medical treatments for gender dysphoria in youth who are initiating puberty suppression *or* pursuing a phenotypic gender change with cross sex hormones. Participants will be studied prospectively over a 24-month period.

10.1.1 Population for Analysis

Gender dysphoric youth ages 8 through 20, seeking care at one of the participating sites,
All participants with available endpoint data will be included in the analysis.

10.1.2 Study Hypotheses

Effects of Hormonal Interventions on Mental Health and Psychological Well-Being:

- Hypothesis 1a: Patients treated with GnRH agonists will exhibit decreased symptoms of gender dysphoria, depression, anxiety, trauma symptoms, self-injury, and suicidality and increased body esteem and quality of life over time
- Hypothesis 2a: Patients treated with cross-sex hormones will exhibit decreased symptoms of anxiety and depression, gender dysphoria, self-injury, trauma symptoms, and suicidality and increase body esteem and quality of life over time

Safety of Hormonal Interventions:

- Hypothesis 1b: GnRH agonists are tolerable and safe for transgender youth in Tanner stage 2 or 3 of sexual development, i.e., lipids, glucose, liver enzymes, electrolytes, and HgbA1c will not increase above clinically safe ranges

- Hypothesis 2b: Cross-sex hormones are tolerable and safe to use with transgender youth initiating phenotypic transition, i.e., will not increase lipids, glucose, liver enzymes, electrolytes, hemoglobin A1c and hemoglobin above clinically safe ranges

Bone Density in Blocker Cohort:

- Hypothesis 1c: Raw bone density scores will remain stable for youth receiving GnRH agonists; however, age-matched z-scores may decrease

10.1.3 Exploratory Aim

Risk Behavior in Cross Sex Hormone Youth:

- Based on evidence of high rates of substance use and HIV infection in some transgender adolescents, we will measure substance use and sexual risk behaviors over time

10.2 Study Endpoints

The primary study endpoint for each participant will be reached at the end of the observational period – two years after the initiation of treatment. However, it is the intention of the PIs to extend this study at a future time point. At that time, IRB approval of the extension will be obtained.

10.3 Sample Size and Power Analysis

10.3.1 Blocker Cohort – Power Analysis

Unique to the blocker cohort is the need to assess the effect of GnRH agonists on bone health (Hypothesis 1c). Although we hypothesize that there is no net change in raw bone density over time (precluding power analysis), it is important to assess nontrivial lags in development compared to age-matched peers. Using G*Power version 3.1.3 to conduct an a priori power analysis in a repeated measures MANOVA framework with effect size f=.20 (a moderate effect size equivalent to Cohen's d=.40), alpha=.05, adequate statistical power=.80, and 4 measurement time points, a sample of 73 participants would be sufficient to detect significant decrease in age-matched z-scores over time. Thus, we will recruit a sample of 80 evaluable participants, across all study sites, in the blocker cohort, which will yield comparable power to detect moderate changes in mental health outcomes over time (Hypothesis 1a) and good (.89) power to detect significant differences in metabolic and physiologic lab values of one-third of a standard deviation from clinical cutoffs (Hypothesis 1b).

10.3.2 Cross Sex Hormone Cohort – Power Analysis

In the absence of available longitudinal metabolic and physiological data, the study is powered to assess changes in mental health and psychological well-being (Hypothesis 2a) based on evidence from our preliminary data. Using G*Power version 3.1.3 and conducting an a priori power analysis in a repeated measures MANOVA framework with effect size f=.11 (a small effect size equivalent to Cohen's d=.22), alpha=.05, adequate statistical power=.80, and 4 measurement time points, with a small natural correlation among repeated measures of r=.15, a total sample of 196 participants is needed for adequate power to detect multivariate significance. This sample will generate adequate (.80) power to detect effects as small as d=.17, or less than a fifth of a standard deviation from clinical cutoffs, in the safety analyses of Hypothesis 2b. Therefore, we will recruit a total sample of 200 evaluable participants, across all study sites, in the cross sex hormone cohort to ensure adequate statistical power to test the two hypotheses of Aim 2 and to conduct the exploratory analysis.

10.4 Randomization Procedure

This is an observational study and participants will not be randomly assigned to treatment.

10.5 Statistical Analysis

10.5.1 Primary Objective: *Effects of Hormonal Interventions on Mental Health and Psychological Well-Being:*

Hypotheses under the primary objective will be tested in each cohort, respectively, using repeated measures multivariate analysis of variance (MANOVA) to assess the trajectories of continuous mental health outcomes and psychological well-being over time within each cohort. The MANOVA approach will preserve statistical power to detect significant effects among this set of related continuous outcomes without the inflated Type I error rates associated with a series of individual ANOVA or regression analyses. The MANOVA analyses will investigate the changes over time in gender dysphoria, depression, anxiety, trauma symptoms, self-injury, suicidality, body esteem, and quality of life. The model will incorporate time (i.e., measurement time point: baseline, 6-month, 12-month, 18-month or 24-month survey) as a within-participants factor. Asserted gender, age, ethnicity, and other socio-demographic variables may additionally be entered as possible covariates (i.e., ANCOVA) to improve statistical power to detect significant time effects. However, we do not propose any a priori hypotheses about demographic effects on these outcomes, and any demographic variables that do not contribute significantly to the model will be removed from the analysis in order to preserve power and increase model parsimony.

In keeping with conventional practice, analysis will first proceed with a review of Box's test for the equality of covariance matrices. Violations of this assumption would require the use of Pillai's trace, as opposed to Wilks' Lambda, to determine multivariate statistical significance. If, as hypothesized, the within-participants time variable demonstrates significant multivariate effects, the follow-up univariate results will be inspected as appropriate. The assumption of sphericity via Mauchly's test will be checked for each measured outcome; if sphericity is violated, the Huyhn-Feldt correction for degrees of freedom will be applied to that outcome. Finally, for outcomes showing significant time effects, linear and quadratic contrasts will be checked for significance and marginal means will be computed and plotted to create a visual display of significant trajectories. An a priori p-value of 0.05 will be applied as the criterion for statistical significance in all analyses.

10.5.2 Secondary Objectives

Safety of Hormonal Interventions:

Unlike the mental health and psychological well-being measures, the question of interest for these metabolic and physiological parameters is not whether they show significant fluctuation over time (which may or may not be meaningful), but rather whether development after initiation of hormonal interventions pushes any physiological indicator above the clinically safe range for that indicator, i.e., above predetermined safety cutoff values based on previous literature and clinical guidelines. Safety will be assessed cross-sectionally with one-sided one-sample t-tests comparing cohort mean scores to the cutoff value. We hypothesize that the cohort means will be significantly lower than the cutoff score. We will use the Benjamini-Hochberg procedure to account for inflated family-wise alpha due to multiple comparisons at each time point.

Additionally, ranges of raw scores from all patient labs will be computed at each time point as part of the preliminary data cleaning and descriptive analysis phase. This will provide an immediate assessment whether the indicator value for any individual patient has crossed the safety threshold for that indicator as data are collected at each time point. In the event any patient experiences an individual increase in laboratory values above the threshold, medication adjustments will be made to protect the well-being of the patient according to the discretion of the medical provider at the site where they are receiving care regardless of the whole-cohort significance test results for that time point.

Bone Density in Blocker Cohort:

We will use repeated measures ANOVA to estimate trajectories of raw and age-matched bone density scores over time in blocker cohort youth. As before, asserted gender and socio-demographic variables may be entered as possible covariates, linear and quadratic contrasts will be assessed, and marginal means will be computed and plotted to create a visual display of trajectories for both outcomes. We hypothesize that for raw scores, the linear term will not differ significantly from zero, indicating net stability in bone density over time. However, for age-matched z-scores, the linear term may be negative as gender non-conforming youth receiving GnRH agonists fail to add bone density at a rate comparable to their age-matched peers.

10.5.3 Exploratory Objective: *Risk Behavior in Youth Initiating Cross Sex Hormones*

We will conduct an exploratory assessment of sexual risk and substance use behavior in the cross sex hormone cohort, using repeated measures MANOVA to model trajectories of these risk outcomes over time. As before, asserted gender and socio-demographic variables may be entered as possible covariates. Given that sexual risk and substance use behaviors increase during adolescence in normative samples, we do not specify a priori hypotheses regarding the impact of hormone treatment on these risk outcomes in our transgender population. However, linear and quadratic contrasts will be assessed. Significant positive terms (indicating increased risk over time) would be indicative of a typical adolescent risk trajectory, whereas significant negative terms (indicating decreasing engagement in risky behaviors) or non-significant time effects (suggesting no net change in risk) would instead support a "treatment-as-prevention" explanation. Again, Box's test will be reviewed for equality of covariance matrices and multivariate test statistic determined accordingly, and sphericity will be assessed via Mauchly's test with the Huyhn-Feldt correction applied as needed.

10.5.4 Additional Analytic Considerations: Site Clustering Effects

Although the observational study will take place at four sites nationwide, we do not anticipate substantial site effects. To verify this, a group identifier for each participant is included in the merged analytic dataset, and the intra-class correlation (ICC) for each outcome will be calculated prior to conducting multivariate analyses. If, as anticipated, no significant variance is carried at the group level, we will reduce the model to a traditional one-level model. If significant group-level variance does emerge, dummy codes to control site-specific variance will be used to enhance statistical power.

10.6 Missing Data

CHLA will conduct missing data analyses in order to differentiate between data that are missing at random (MAR) and data that are missing related to gender or aspects of the treatment plan (e.g., hormone dosing). If missing data can be regarded as MAR, multiple imputations may be used. If the MAR assumption is not plausible, sensitivity analyses will be conducted to evaluate the impact of MAR violations on analyses by specifying models for non-ignorable missing data mechanisms.

11.0 HUMAN SUBJECTS

This study is being conducted in compliance with the protocol, International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, and 45 Code of Federal Regulations (CFR) §46.

11.1 Participants' Confidentiality

No personal identifying information (e.g., names) of the participants will appear in any computer files associated with this research project in any location. Participants will be assigned a unique identification number code. A key file that matches the ID number to the participant and organization will be maintained in a secure data repository within the project offices at each of the four sites. Data will be kept strictly confidential, except as required by law, and stored on a secure network, with password protection

such that only authorized users will have access to the file server. All computers will be located in locked facilities, and consent forms will be filed and stored separate from the raw data in a secured location under double-lock when not in use and with restricted access during work hours and/or when unattended. Any temporary data files kept on removable storage devices, as well as printouts derived from data analysis, will be stored in a locked compartment when not in use.

11.2 Certificate of Confidentiality

To further protect the privacy of the study participants, a Certificate of Confidentiality will be obtained from the U.S. Department of Health and Human Services (DHHS). With this Certificate in place, the researchers at the study sites cannot be forced to turn over identifying information about a study participant in any Federal, State, or local criminal, administrative, legislative, or other proceedings. This Certificate does not prevent a study participant from volunteering to turn over their research information nor does it prevent researchers from providing research-related information to others when requested by the study participant, or when required by law such as in cases of suspected or actual harm to or by the study participant.

11.3 Risks and Benefits

11.3.1 Risks

The Principal Investigators have determined that this study does not involve greater than minimal risk (45 CFR §46.404 and 21 CFR §50.51). Participation in this study poses no more harms or discomforts to participants than they may experience in normal daily life or during routine psychological examinations or tests.

Due to the personal nature of the information being collected in this study, there is some risk of emotional discomfort or distress. Participants will be informed that they are free to decline to answer any questions. Furthermore, participants will be informed that at any point they may stop if they do not wish to continue the questionnaire/interview. In the event of discomfort or upset, there are counselors at study sites with whom participants can talk and who can provide ongoing support as needed. Every effort will be made to keep the participant's participation in the study and personal information private and confidential, but absolute confidentiality cannot be guaranteed.

As this is an observational study, there are no alternative treatments or procedures.

11.3.2 Benefits

There may be no direct benefit to the participants for their participation in this study, but information learned from this study may benefit other youth, now or in the future. This research provides the opportunity to obtain a better understanding of transgender youth, improve their care, and share information on a local and national level about how to provide care and hormone therapy for gender dysphoric children and adolescents. The information that is learned from this project will support innovative approaches to identifying, understanding, and providing optimal care for multi-ethnic transgender youth.

11.4 Institutional Review Board Review and Informed Consent

The Institutional Review Boards requires that all research participants review and sign an informed consent/permission/assent form. The informed consent/permission/assent form covers information about the overall purpose of the study, what the study entails, potential risks, potential benefits to participating individuals and society, the confidentiality of data, and contact information for the Principal Investigator and the IRB. Once informed consent/permission/assent has been obtained, the research staff will have the form reviewed by a fellow research team member, who will confirm that it is fully completed before it is filed in a secure location under double-lock when not in use and with restricted access during work hours and/or when unattended.

For participants aged 7 to 13 years old, the participant will sign an age-appropriate assent form, and the parent/legal guardian will sign a consent/permission/assent form. For participants aged 14 to 17 years old, the participant and the parent/legal guardian will sign a consent/permission/assent form. For participants aged 18 years or older, the participant will sign a consent/permission/assent form.

11.5 Requirement for Consenting Participants Enrolled as Minors Who Reach Age of Majority While on Study

Pursuant to guidance requested from OHRP, when a minor participant is enrolled with parental permission into the study, and the study will extend beyond the participant's age of legal majority, research staff must establish a mechanism to track the participant to obtain a legally effective consent when the participant reaches majority to remain on study.

11.6 Prisoner Participation

The Principal Investigators and NICHD have concluded that this protocol does not meet Federal requirements governing prisoner participation in human participant research and should not be considered by local IRBs for the recruitment of prisoners. Participants may not engage in study activities if they become incarcerated or are placed in detention. In addition, research staff will not collect study-related data during the time that the participant is incarcerated or placed in detention.

11.7 45 CFR Parts 160 and 164 Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule" Pursuant to the Health Insurance Portability and Accountability Act - HIPAA)

Each site is responsible for adherence to their individual institution's HIPAA policies and procedures.

11.8 Study Discontinuation

This study may be discontinued at any time by *The Eunice Kennedy Shriver* NICHD.

12.0 PUBLICATION OF RESEARCH FINDINGS

Data will be made available to other NIH investigators under the data-sharing agreement with NICHD after a reasonable time period that includes enough opportunity to prepare and have submitted for publication four manuscripts presenting the basic outcomes of the project. Beginning in Year 3, peer-reviewed publications will be developed pertaining to cross-sectional hypotheses and research questions found in primary and secondary objectives, though the bulk of publications are longitudinal in nature and will be developed in Year 5. Dissemination of findings to State and County officials, policy makers, and organizations will begin in Year 3, when preliminary data become available.

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APPENDIX I: SCHEDULE OF EVALUATIONS

	Pre-screening Worksheet [1]	Participant Consent/ Assent[2]	Locator Form[3]	Eligibility Confirmation [4]	ACASI & Debriefing [5]	DISC/ CBCL/ YSR[6]	Chart Abstraction
Pre-Screening	X						
Baseline	X	X	X	X	X	X	X[7]
6 Months			X		X[8]	X	X[9]
12 Months			X		X[8]	X	X[9]
18 Months			X		X[8]	X	X[9]
24 Months			X		X[8]	X	X[9]
Premature Discontinuation			X		X	X	X[9]

1 Pre-Screening Worksheet must be completed by an IRB-approved medical provider.

2 Participant consent/assent must be obtained within 30 days prior to enrollment (baseline ACASI). Consent/assent must be reaffirmed prior to enrollment if > 30 days has elapsed.

3 The Locator Form data should be confirmed to be correct at every visit.

4 If eligibility is confirmed prior to the baseline visit date, eligibility must be reconfirmed at the baseline visit before administering the ACASI.

5 Participation in the Baseline ACASI = Enrollment. Ideally, the ACASI should be completed in a single day. If extenuating circumstances prevent completion in a single day, the subject may return within the next 7 days to retake it from the beginning again. If retaken, the subject's first ACASI will be deleted.

6 The DISC and CBCL or YSR may be completed either on the same day as the ACASI or in a separate visit within 2 weeks after the ACASI. Blocker Child participants do not complete.

7 The Baseline Chart Abstraction is for data from the most recent previous medical visit and labs.

8 For the Blocker Cohort, the timeline for the follow-up ACASIs is based on the date of the insertion of the GnRH agonist. For the Cross-Sex Hormone Cohort, the timeline for the follow-up ACASI is based on the date of the Baseline ACASI.

9 The follow-up Chart Abstractions should collect data from the previous the visit through the date of the ACASI.

APPENDIX II: MEASURES

Blocker Cohort – Youth Survey Measures	
ACASI Measures: Time of Completion	Baseline, 6-month, 12-month, 18-month, & 24-month follow-up periods
Construct	**Measure**
Weight Bearing Exercise	Physical Activity Questionnaire
Demographics	Demographic questions for Blocker Cohort Youth
Gender Dysphoria	Utrecht Gender Dysphoria Scale (UGDS)
Depression	BDI-Y
Anxiety	Revised Children's Manifest Anxiety Scale: Second Edition (RCMAS-2 – What I Think and Feel)
Quality of Life	Pediatric Quality of Life Inventory – Child Report (PedsQL–CH)
Suicidality	Suicidal Ideation Scale
Body Esteem	Body Esteem Scale
Body Image	Body Image Scale
Social Relationships	Emotional Support / Friendship / Loneliness / Perceived Hostility / Perceived Rejection – NIH Toolbox
Self-Efficacy	Self-Efficacy (CAT 8-12 / CAT 13-17)– NIH Toolbox
Perceived Parent Support	Parental Support Scale – Youth Version
Resiliency	Connor-Davidson Resilience Scale
Self-Perception	Harter's Self-Perception Profiles for Adolescents & Children

Blocker Cohort – Parent Survey Measures	
ACASI Measures: **Time of Completion**	Baseline, 6-month, 12-month, 18-month, & 24-month follow-up periods
Construct	**Measure**
Demographics	Demographic questions for Blocker Cohort Parents
Service Utilization	Service Utilization Questions
Socio-Economic Status	Socioeconomic Status Questions (for Adults)
Religiosity & Spirituality	Modified Duke University Religion Index (DUREL)
Calcium Intake	Daily Calcium Intake Form
Gender Identity	Parent Report Gender Identity Questionnaire (GIQC)
Social Transitioning	Social Transitioning Scale
Gender Dysphoria	DSM 5 – Chicago adapted
Quality of Life	Pediatric Quality of Life Inventory - Parent Report (PedsQL – PC)
Suicide attempts	Suicidality Questions
Parent distress/stress	Parenting Stress Index
Social Relationships	Empathic Behaviors / Peer Rejection / Positive Peer Interactions / Social Withdrawal (Parent Report) – NIH Toolbox
Negative Affect	Anger / Fear / Sadness (Parent Report) – NIH Toolbox
Psychological well-being	General Life Satisfaction / Positive Affect (Parent Report)– NIH Toolbox
Self-Efficacy	Self-Efficacy (Parent Report) – NIH Toolbox
Perceived Parent Support	Parental Support Scale – Parent Version
Autism	Autism-Spectrum Quotient (AQ-10) – Child
Additional Assessments: **Time of Completion**	Baseline, 12-month, & 24-month follow-up periods
Construct	**Measure**
Depression/Externalization	Child Behavior Checklist (CBCL)
DSM diagnoses	Parent Report Diagnostic Interview Schedule for Children (C-DISC IV)

Cross Sex Hormone Cohort Survey Measures	
ACASI Measures: **Time of Completion**	Baseline, 6-month, 12-month, 18-month, & 24-month follow-up periods
Construct	**Measure**
Demographics	Demographic questions for Cross Sex Hormone Cohort
Religiosity & Spirituality	Modified Duke University Religion Index (DUREL)
Socio-Economic Status	Socioeconomic Status Questions (for Adolescents & Young Adults)
Gender Identity	Transgender Congruence Scale
Gender Dysphoria	Utrecht Gender Dysphoria Scale (UGDS)
	Gender Identity/Gender Dysphoria Questionnaire Adolescents & Adults
	DSM 5 – Chicago adapted
Service Utilization	Dr. Olson's Service Utilization Questions
Depression	BDI-II
Anxiety	Revised Children's Manifest Anxiety Scale: Second Edition (RCMAS-2 – What I Think and Feel)
Quality of Life	Health-Related Quality of Life Scale (modified HIV QOL)
Suicidality	Suicidal Ideation Scale
Body Esteem	Body Esteem Scale
Body Image	Body Image Scale
Social Relationships	Emotional Support / Friendship / Loneliness / Perceived Hostility / Perceived Rejection – NIH Toolbox
Negative Affect	Anger / Fear / Sadness – NIH Toolbox
Psychological well-being	General Life Satisfaction / Positive Affect – NIH Toolbox
Self-Efficacy	Self-Efficacy (CAT 13-17)– NIH Toolbox
Perceived Parent Support	Parent Support Scale – Youth Version
Resiliency	Gender Minority Stress and Resilience Scale
	Connor-Davidson Resilience Scale
Sexual Behavior	Sexual Risk Behavior Questions
STI history	STI Questions
Alcohol/Drug Use	Youth Informant DISC (DISC-Y)
	Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST)
Autism	Autism-Spectrum Quotient (AQ-10) – Adult

Additional Assessments: Time of Completion	**Baseline, 12-month, & 24-month follow-up periods**
Construct	**Measure**
Internalizing/Externalizing	Youth Self-Report (YSR)
DSM diagnoses	Youth Informant Diagnostic Interview Schedule for Children (DISC-Y)

The Impact of Early Medical Treatment in Transgender Youth

(Trans Youth Care)

Version 5

May 11, 2021

Sponsored by:
The Eunice Kennedy Shriver
National Institute of Child Health and Human Development (NICHD)

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STUDY MANAGEMENT

Before the recruitment and enrollment of participants, the participating R01 study sites must have the protocol and consent, assent, and permission forms approved by their local Institutional Review Boards (IRBs). All original, approved documents must be maintained at each study site.

All queries for this protocol should be sent to Children's Hospital Los Angeles (CHLA), via email at TransYouthCare@chla.usc.edu. The appropriate team member will respond to queries generally within 48 hours via email and copy the other team members as needed.

Dr. Olson-Kennedy or her designee will be responsible for answering general protocol implementation, eligibility, study and participant management, exemptions and/or adverse event queries.

The CHLA Data Manager or Research Manager, with the help of other study personnel, if necessary, will answer general data management, data entry, and case report form (CRF) completion queries.

This study will use web-based software to collect study survey data. Additional psychosocial data will be collected during in-person or remote interviews conducted by research staff at each study site.

LIST OF ABBREVIATIONS

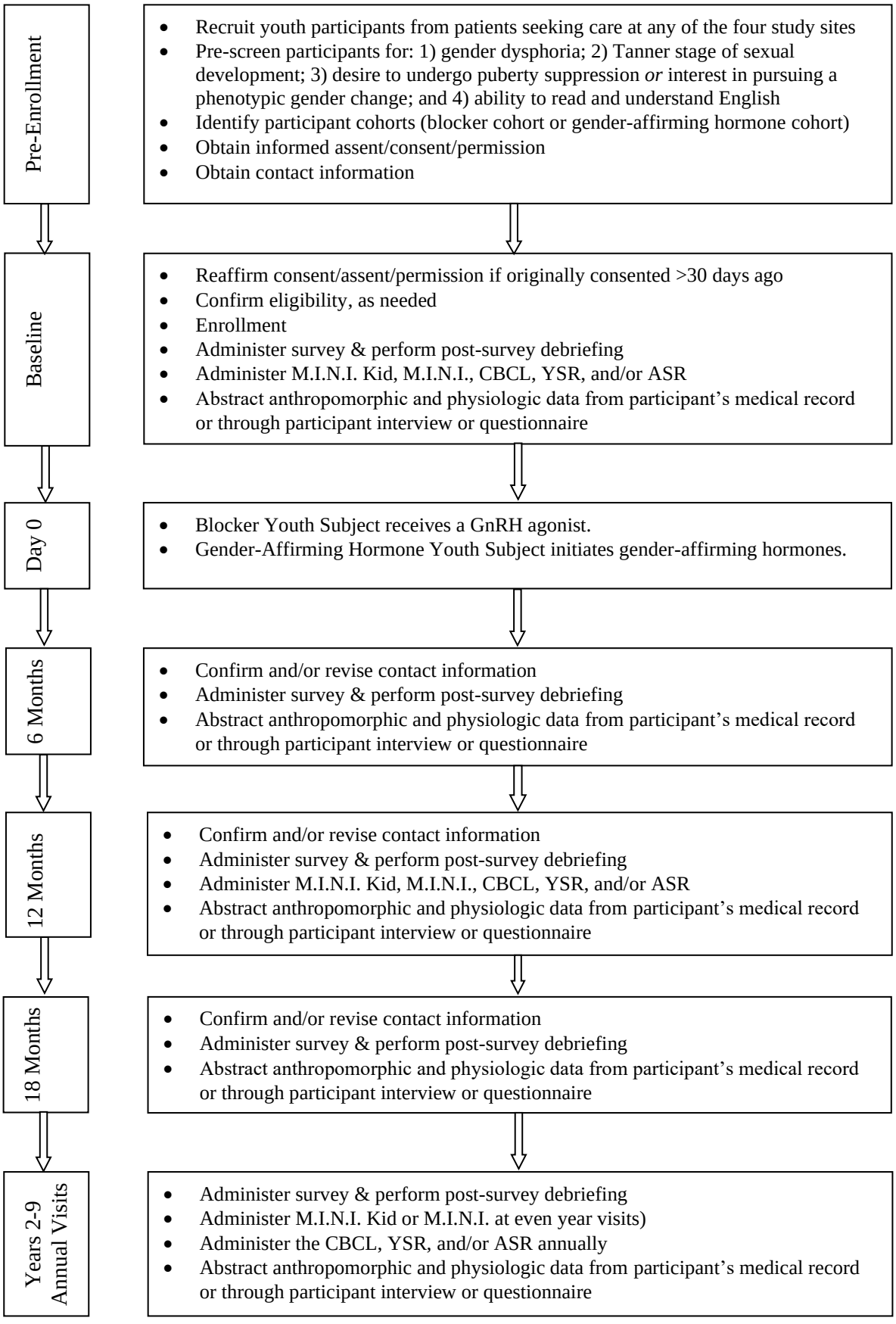
ASEBA	Achenbach System of Empirically Based Assessment
ASR	ASEBA Adult Self-Report
ASSIST	Alcohol, Smoking and Substance Involvement Screening Test
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
AST	Aspartate Aminotransferase
BDI	Beck Depression Inventory
BE	Body Esteem
BUN	Blood Urea Nitrogen
CBCL	ASEBA Child Behavior Checklist
CFR	Code of Federal Regulations
CHLA	Children's Hospital Los Angeles
Cholesterol HDL	High Density Lipoprotein Cholesterol
Cholesterol LDL	Low Density Lipoprotein Cholesterol
Cholesterol VLDL	Very-Low Density Lipoprotein Cholesterol
CO2 Total	Carbon Dioxide/Bicarbonate Test
COVID-19	SARS-CoV-2 Coronavirus
CRF	Case Report Form
DHHS	U.S. Department of Health and Human Services
DSM-5	Diagnostic and Statistical Manual of Mental Disorders - 5
DXA	Dual-energy X-ray Absorptiometry
GnRH agonists	Gonadotropin-Releasing Hormone agonists
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Syndrome
IOM	Institute of Medicine
IRB	Institutional Review Board
MANOVA	Multivariate Analysis Of Variance
MAR	Missing At Random
M.I.N.I.	Mini International Neuropsychiatric Interview
M.I.N.I. Kid	Mini International Neuropsychiatric Interview for Children and Adolescents
NICHD	*The Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PI	Principal Investigator
PID Number	Participant Identification Number
QCT	Quantitative Computed Tomography
REDCap	Research Electronic Data Capture
STI	Sexually Transmitted Infection
YSR	ASEBA Youth Self Report

STUDY ABSTRACT

DESIGN:	The study will use a longitudinal observational design to examine the outcomes of existing medical treatment protocols for gender dysphoria in two distinct cohorts: youth initiating puberty suppression and youth pursuing a phenotypic gender change. Routinely collected anthropometric and physiologic parameters will be collected through chart abstraction, online surveys, and interviews throughout the 9-year study period. These instruments will be used to collect demographic, mental health, psychosocial, and behavioral data from parents/caregivers and youth in the blocker cohort and only youth/young adults in the gender-affirming hormone cohort at baseline and 6, 12, 18, and 24 months and annually thereafter.
SAMPLE SIZE:	Participants will be recruited from those patients seeking medical care for gender dysphoria at any of the four study sites. 120 youth who are initiating pubertal suppression with GnRH agonists and their parent/caregiver will be enrolled in the blocker cohort. 400 youth who are initiating gender-affirming hormones will be enrolled in the gender-affirming hormone cohort. While patients between 8 and 20 years inclusive will be eligible for enrollment in one or the other cohorts depending on their Tanner stage and medical intervention plan, the focus of this investigation is *early treatment*. Therefore, no more than 20% of youth age 19 or older will be enrolled in the gender-affirming hormone cohort. In addition, due to the expected impact of previous blocker experience on study results, no more than 60 youth enrolling in the gender-affirming cohort will have had previous blocker experience from when they started puberty. Total accrual is expected to be 520 youth/young adults and approximately 135 parents/caregivers.
POPULATION:	Blocker Cohort – Youth • Presence of gender dysphoria; • Tanner stage 2, 3, or 4 of sexual development; • Aged 8 to 16 years inclusive; • Appropriate to undergo puberty suppression; • Ability to read and understand English; and • Receiving or planning to receive services at a study site clinic. Blocker Cohort – Parent/Caretaker/Legal Guardian • Parent, caretaker, or legal guardian aged 18 or older of a child who meets the Blocker Cohort Inclusion/Exclusion Criteria, and • Ability to read and understand English. Gender-Affirming Hormone Cohort – Youth • The presence of gender dysphoria; • Aged 8 to 20 years inclusive; • Appropriate to pursue phenotypic gender change with gender-affirming hormones; • Ability to read and understand English; and • Receiving or planning to receive services at a study site clinic.
INTERVENTION:	There is no intervention in this study.
DURATION:	Accrual is expected to last approximately 8 years starting from the first participant enrolled. The total duration of study participation for each subject ranges from 12 months to 9 years from initiation of a GnRH agonist (Blocker Cohort) or gender-affirming hormones (Gender-Affirming Hormone Cohort)

PRIMARY OBJECTIVES:	1. Evaluate the longer-term physiological and psychosocial impact of GnRHa initiated in early puberty on youth with gender dysphoria by extending follow-up for an additional 4 years (3, 4, 5 and 6 years after initiation of puberty blockers). 2. Evaluate the longer-term physiological and psychosocial impact of GAH (estrogen or testosterone) in later puberty in youth with gender dysphoria by extending follow-up for an additional 4 years (3, 4, 5 and 6 years after initiation of gender-affirming hormones). 3. Compare effects of gender-affirming hormones within and outside the context of prior pubertal suppression.
SECONDARY/ EXPLORATORY OBJECTIVE:	Characterize emerging sub-cohorts.
DATA COLLECTION:	Routinely collected anthropometric and physiologic parameters will be collected through chart abstraction, questionnaires, and interviews throughout the study period. Items include lab results, height, weight, blood pressure, diagnoses, prescription medications, Tanner stage, physiologic changes, and bone mineral density results. Online surveys will be used to collect demographic, mental health, psychosocial, behavioral, and physiologic data from parents/caregivers and youth in the blocker cohort and only youth/young adults in the gender-affirming hormone cohort. Survey data are from 4 domains: 1) demographic; 2) transgender-specific experiences including gender dysphoria; 3) mental health and trauma assessments; and 4) additional psychosocial information including quality of life and relationships with parents and peers. These data will be collected at baseline and 6, 12, 18, and 24 months and annually thereafter. For youth and young adults in the blocker and gender-affirming hormone cohorts, mental health diagnoses will be assessed by administration of the Mini International Neuropsychiatric Inventory for Children and Adolescents (M.I.N.I. Kid) for those participants 17 and younger at administration and the Mini International Neuropsychiatric Inventory (M.I.N.I.) for those participants 18 and older at administration. It will be conducted at baseline, years 1 and 2, and every two years thereafter. For youth 11-17 in both the blocker and gender-affirming hormone cohorts, behavioral and emotional problems will be assessed by administration of the ASEBA Youth Self Report (YSR) at baseline and annually thereafter. Young adult participants aged 18 and older will complete the ASEBA Adult Self-Report (ASR). The ASEBA Child Behavior Checklist (CBCL) will also be administered to parents/caregivers at baseline and annually thereafter to assess behavioral and emotional problems in blocker cohort youth.
MONITORING:	Routine team monitoring of untoward events identified during the study will rely on site staff notification via email to the CHLA PI, clinical research manager, and data manager. Sites will also record and enter in the study database untoward events occurring during study participation, which will be reviewed by the PIs and reported to the appropriate IRB(s).

PROTOCOL SCHEMA



1.0 INTRODUCTION

1.1 Background and Significance

"Transgender" is an umbrella term that is used to describe individuals whose gender self-identification or expression transgresses established gender norms. Specifically, it is the state of one's "gender identity" (self-identification as male, female, both or neither) not matching one's "assigned gender" (identification by others as male or female based on natal sex).[1] The identity and behavior of transgender individuals are socially and medically stigmatized, resulting in a grossly underserved population at high risk for significant morbidity and mortality. Transgender people are often diagnosed with gender dysphoria when they are experiencing dissonance between their birth sex and their gender identity. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) identifies gender dysphoria in the following manner: the presence of a marked incongruence between one's experienced/expressed gender and assigned gender, of at least six month's duration, as manifested by at least two of the following:

1. A marked incongruence between one's experienced/expressed gender and primary and/or secondary sex characteristics (or in young adolescents, the anticipated secondary sex characteristics).
2. A strong desire to be rid of one's primary and/or secondary sex characteristics because of a marked incongruence with one's experienced/expressed gender (or in young adolescents, a desire to prevent the development of the anticipated secondary sex characteristics).
3. A strong desire for the primary and/or secondary sex characteristics of the other gender (or a gender different from that assigned at birth).
4. A strong desire to be of the other gender (or a gender different from that assigned at birth).
5. A strong desire to be treated as the other gender (or a gender different from that assigned at birth).
6. A strong conviction that one has the typical feelings and reactions of the other gender (or a gender different from that assigned at birth).

The condition is associated with clinically significant distress or impairment in social, occupational or other important areas of functioning.[2d]

Transgender children and adolescents are a poorly understood and a distinctly understudied population in the United States. The limited available data suggest that transgender youth who are gender dysphoric are at increased risk for anxiety, depression, suicide, and substance use compared to their peers.[3-6] The development of undesired secondary sex characteristics during puberty intensifies the distress associated with gender incongruence and increases the risk for these conditions. Current clinical practice guidelines aim to decrease gender dysphoria and ameliorate potential negative health outcomes. Treatment recommendations vary depending on the age and developmental stage of youth with gender dysphoria. For those youth in the earliest stages of pubertal development (Tanner stages 2-3), treatment with gonadotropin-releasing hormone (GnRH) agonists is recommended in order to suppress endogenous puberty and avoid the development of undesired secondary sex characteristics. In older adolescents in the later stages of pubertal development (Tanner stages 4-5), treatment with gender-affirming hormones is recommended to induce desired masculine or feminine features.[7] While these guidelines have been used at academic and community centers across the U.S., they are based on very limited data. Furthermore, there are no available data examining the physiologic and metabolic consequences of gender-affirming hormone treatment in youth. This represents a critical gap in knowledge that has significant implications for clinical practice across the U.S. In 2011, a report of the Institute of Medicine (IOM) called for the development of rigorous research aimed at understanding the health implications of hormone use and other transgender-specific issues.[8] The objective of the proposed research is to provide evidence-based data to inform clinical care for transgender youth. The study will leverage the partnership between four, university-affiliated, gender clinics across the U.S. to recruit two developmental cohorts and conduct a multi-site, observational study examining the safety of hormonal interventions and the physiological and psychosocial outcomes associated with these treatments.

1.1.2 Transgender Youth Are an Underserved, Understudied Population

Transgender adolescents and children, those who experience incongruence between assigned birth sex and internal gender identity, are a poorly understood and understudied population in the United States. As detailed in the May 2011 IOM report, "The Health of Lesbian, Gay, Bisexual, and Transgender People," the existing body of scientific evidence documenting health and well-being of transgender individuals is sparse. The report explicitly calls for NIH-supported research on transgender health needs, including the development of evidence-based data for providing transgender-specific health care to address gender dysphoria and rigorous research aimed at understanding the health implications of hormone use and other transgender-specific issues. In addition, the IOM report calls for longitudinal and cohort studies that incorporate a life course perspective to examine the specific experiences of transgender individuals across different chronological ages.[8]

Research on transgender youth has historically focused on the disproportionate morbidity and mortality among transgender individuals in comparison to the population at large. One study of 55 transgender youth in New York City reported that 45% had seriously thought about suicide, and 26% had attempted suicide at least once, indicating that transgender youth specifically are at increased risk for anxiety, depression, social isolation, and suicide compared to non-transgender peers.[1,9,10] A recent study examining baseline characteristics of 101 multi-ethnic transgender youth at Children's Hospital Los Angeles (CHLA), supports these numbers, with 52% participants reporting thoughts of suicide and 30% reporting having attempted suicide at least once.[6]

1.1.3 High Risk Behavior

Studies have shown that self-identified transgender women have a significantly higher rate of HIV than other individuals considered "at risk" for HIV. In a systematic review of all studies on transgender and HIV, prevalence rates of HIV among male to female transgender people (6.3%) were found to exceed that of men who have sex with men (4.2%).[11] In one study of 400 transgender women, the overall prevalence of HIV was found to be exceptionally high at 35% with African American transgender women being nearly six times more likely than Caucasian transgender women to be HIV infected.[12] In Wilson's study of 151 female transgender youth, 67% had participated in survival sex.[13] Studies of high-risk transgender male-to-female adolescents found that 19-22% reported being HIV positive.[9,13,14] Furthermore Garofalo found an extremely high prevalence of history of incarceration (37%), homelessness (18%), sex in exchange for resources (59%), forced sexual activity (52%), difficulty finding a job (63%) and difficulty accessing health care (41%).[9] Due to societal marginalization and transphobia, many transgender youth and adults resort to "survival sex" (the trading of sexual acts for money, food, clothing or a place to live) in order to subsist on their own.[12]

Without appropriate care, as they age, transgender youth are likely to face economic and societal marginalization, incarceration, and physical abuse leaving them at significantly higher risk for drug abuse, violence, HIV acquisition, other sexual transmitted infections, and homelessness.[9,10,13,14]

1.1.4 The "Dutch Model"

Over the past 30 years, a team of specialists in the Netherlands at the Amsterdam Center of Expertise on Gender Dysphoria observed that transgender individuals who underwent hormonal gender transition at earlier ages assimilated easier into their "new gender" roles because of improved physical outcomes.[15] Additionally, transgender youth suffering through an undesired endogenous puberty experience distress, and this "wrong puberty" has a strong negative impact on their emotional, academic, and family functioning.[15,16] Based on these clinical observations, Dutch clinician investigators initiated early treatment of transgender youth aimed at suppressing undesired puberty with gonadotropin-releasing hormone agonists (GnRH agonists). GnRH agonists have previously been used as the primary strategy for the suppression of puberty in children experiencing precocious puberty.[17] Early results from the first 70 gender dysphoric youth undergoing puberty suppression with GnRH agonists in the Netherlands showed a decrease

in behavioral and emotional problems, as well as a decrease in depressive symptoms. Improved general functioning for these youth was also reported.[16] By blocking the progression of puberty, the distress associated with full development of adult sexual characteristics incongruent with an internal gender identity is prevented.[18] A single study examining the physiologic impact of puberty suppression with GnRH agonists reported that the first 21 patients undergoing this treatment had adequate suppression of their pituitary gonadal axis and no progression of their endogenous puberty. While on GnRH agonists, height standard deviation scores decreased, and bone density remained in the same range for patients experiencing suppression. Compared to age-matched peers, bone density z-scores went down while patients were being suppressed.[18] In 2006, the "Dutch Model" was introduced, outlining this new approach to the care of transgender youth. Gender dysphoric adolescents 12 years and older are given GnRH agonists to prevent (or minimize in those already undergoing puberty) the development of undesired secondary sexual characteristics. For youth on GnRH agonists who continue to experience gender dysphoria through adolescence, appropriate gender-affirming hormones (estrogen for the development of female characteristics and testosterone for the development of male characteristics) are added to the regimen to feminize or masculinize accordingly. The Dutch model recommends the addition of gender-affirming hormones to allow the body to be brought into closer alignment with the identified internal gender when youth reach age 16. A recent follow up study from the Dutch team examining the impact of puberty suppression followed by gender-affirming hormones and gender reassignment surgery in 55 transgender young adults showed alleviation of gender dysphoria and steady improvement of psychological functioning.[19]

One important limitation of the Dutch model is the chronological age criterion that requires gender dysphoric children be at least age 12 before initiating suppression of puberty; however, by that age, it is well documented that many children in the United States are already well into their puberty.[20,21] In 2010, Biro et al. reported that across three metropolitan areas of the U.S., 42.1% of girls had Tanner 2 breast development by the age of eight years.[22] Relying on this chronological age criterion rather than sexual developmental stage decreases early intervention potential and may increase risk for negative mental health outcomes. Additionally, only a few studies describe the physiological[18] and psychosocial impact of this treatment protocol for puberty suppression.[19] Finally, the recommendations from the Dutch model are based on data collected from a homogenous population of white, European youth living in relatively supportive environments and are not necessarily generalizable to multi-ethnic transgender youth in the U.S.

1.1.5 The Endocrine Society Clinical Practice Guidelines

In 2009, using the best available evidence, the Endocrine Society incorporated the Dutch model into the clinical guidelines "Endocrine Treatment of Transsexual Persons," which includes recommendations for treatment of transgender youth.[7] In contrast to the Dutch model, the Endocrine Society recommends starting treatment with GnRH agonists for puberty suppression based on sexual development (Tanner staging) rather than chronological age. These guidelines recommend puberty blocking medications for youth with gender dysphoria at the beginning stages of puberty (Tanner stage 2 or 3), followed by appropriate gender-affirming hormone therapy at around age 16. Since the introduction of these guidelines, no data have been collected in the U.S. on the physiologic and mental health impact, safety, or tolerability of pubertal-blocking medical interventions with GnRH agonists for transgender youth, particularly in children younger than age 12, leaving a gap in the evidence for this practice. Furthermore, the impact of GnRH agonists on the bone health of transgender children, specifically in those younger than 12 years, remains unknown. This study will investigate the physiologic and mental health impact of GnRH agonist administration as well as document the safety of GnRH agonists in a large cohort of transgender children and adolescents in the early stages of puberty.

The Endocrine Society Clinical Practice Guidelines include recommendations to initiate gender-affirming hormones for gender dysphoric, late pubertal adolescents at around the age of 16. For those youth who are on GnRH agonists, gender-affirming hormones are added to the regimen. For those new to clinical care, gender-affirming hormones are prescribed without GnRH agonists, a protocol commonly used in both

adolescents and adults. Studies in adult transgender populations have reported on the physiologic impact of gender-affirming hormones,[23-25] but no studies to date have detailed the physiological impact of gender-affirming hormone administration in transgender adolescents. This study will investigate both the physiologic and the mental health impact of gender-affirming hormone administration as well as document the safety of gender-affirming hormones in transgender adolescents in the later stages of puberty.

1.2 Rationale

The lack of data supporting medical interventions for transgender youth combined with a shortage of providers knowledgeable in the complex psychosocial risk factors facing these young people contributes to a health disparity and public health crisis of considerable magnitude. This research is highly significant in scope as it is the first longitudinal study collecting data - assessing both physiologic and mental health outcomes - to evaluate commonly used clinical guidelines for transgender youth in the U.S. In addition, we will do this work in four geographically distinct sites, in part because of the relative rarity of these conditions and also to increase the generalizability of the work. Results from this study have the potential to significantly impact the medical and mental health services provided to transgender youth in the U.S. by making available rigorous scientific evidence outlining the impact and safety of early treatment based on sexual development stage. Available data about the impact of the recommended treatment protocols come from a primarily white, European cohort in the Netherlands. This study examines the impact of treatment on diverse, multiethnic, transgender youth more representative of the U.S. population.

There are providers scattered around the U.S. (and the world) utilizing the Endocrine Society Clinical Practice Guidelines, but there are no formal empirical studies of related clinical outcomes in transgender children and adolescents. This project creates a network of four academic hospitals (i.e., Children's Hospital Los Angeles/University of Southern California, Boston Children's Hospital, Lurie Children's Hospital of Chicago/Northwestern University, and the Benioff Children's Hospital/University of California San Francisco) strategically situated across the country with strong histories of clinical service in this area to investigate the impact of the treatment on multi-ethnic transgender youth. All four sites have dedicated transgender youth clinics, employ a similar model of care that includes medical and mental health professionals, and are considered the national leaders in the care of transgender children and adolescents. The involvement of these four sites provides the experience, expertise, and clinic populations for a research endeavor of this magnitude and importance. In addition to the significant combined clinical experience of these four sites, all of the sites have strong and deep-rooted ties to academic research.

This longitudinal, observational study will collect critical data on the existing models of care for transgender youth that have been commonly used in clinical settings for close to a decade, although with very limited empirical research to support them. The gap in existing knowledge about the impact of these practices leaves providers and caretakers uncertain about moving forward with the recommended medical interventions for transgender youth seeking phenotypic transition. This research is a direct response to the IOM report calling for such studies, as well as the needs of clinicians and patients. The findings from this research have the capacity to substantially expand treatment across the country by providing rigorous evidence to demonstrate the benefits of early treatment and ultimately decrease the health disparity currently existing for transgender youth.

Avoiding the development of undesired secondary sex characteristics by starting puberty suppression at the earliest stages of puberty is recommended in the Endocrine Society Clinical Practice Guidelines but has never been comprehensively studied in the U.S. and has never been studied in children under the age of 12 years. This study will enroll transgender children eight years or older in the earliest stages of puberty to start treatment with GnRH agonists for puberty suppression, which will provide a critically important extension to the base of empirical knowledge about treatment outcomes. Despite the knowledge that transgender identity is stable by the time youth reach adolescence, The Endocrine Society Clinical Practice Guidelines recommend introducing gender-affirming hormones "around" the age of 16. This study will evaluate the effects and document the tolerability and safety of gender-affirming hormones in youth,

including those younger than 16 years. While it is common for this team of experts to initiate gender-affirming hormone therapy in transgender youth younger than age 16, there are no available data on this younger population.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

- **Aim 1: Evaluate the longer-term physiological and psychosocial impact of GnRHa initiated in early puberty on youth with gender dysphoria by extending follow-up for an additional 4 years (3, 4, 5 and 6 years after initiation of puberty blockers). Aim 1a:** Examine the trajectories of height, weight, and bone mineralization as youth continue treatment with pubertal suppression and initiate treatment with gender-affirming hormones. **Aim 1b:** Continue assessment of mental health and psychosocial well-being with the existing measures and with the addition of new measures as these children progress from preteen to adolescent development. **Aim 1c:** Selectively enroll more non-Hispanic ethnic/racial minority youth (n=43) to better reflect the increasingly diverse clinical population.
- **Aim 2: Evaluate the longer-term physiological and psychosocial impact of GAH (estrogen or testosterone) in later puberty in youth with gender dysphoria by extending follow-up for an additional 4 years (3, 4, 5 and 6 years after initiation of gender-affirming hormones). Aim 2a:** Evaluate longer-term effects of sex steroids on cardiovascular risk factors such as obesity, blood pressure, and lipids. **Aim 2b**: Employ a developmental framework to assess mental health and psychosocial well-being with the existing measures and with the addition of new measures as youth move into later adolescence and early adulthood. **Aim 2c:** Selectively enroll more transfeminine (assigned male at birth) and/or ethnic/racial minority youth into the GAH cohort (n=156) to better reflect the increasingly diverse clinical population.
- **Aim 3: Compare effects of gender-affirming hormones within and outside the context of prior pubertal suppression.** Many within GnRHa cohort will begin GAH for induction of masculinization or feminization over time. In addition, a subset of GAH cohort was receiving GnRHa at enrollment. These two sets together can be considered a GnRHa + GAH group. We propose to longitudinally examine across 2-3-year follow-up this GnRHa + GAH group with age- and sex assigned at birth-matched GAH only group to compare psychosocial well-being between GAH youth with and without a history of GnRHa use.

2.2 Secondary/Exploratory Objectives

- **Exploratory Aim 4: Characterize emerging sub-cohorts.** Examination of emerging sub-groups from the first five years will inform optimal care models for the increasing diversity of youth with gender dysphoria. We propose to explore the demographics, challenges, and medical needs of non-binary youth (i.e., youth who identify outside of a male/female binary) as well as those youth who discontinue GnRHa or GAH.

3.0 STUDY DESIGN

The study has a longitudinal, observational design for both the blocker and the gender-affirming hormone cohorts. Data will be collected on age and Tanner staging to be able to examine if early vs. delayed treatment in these young people affects health outcomes. Day 0 for this study is the insertion of the GnRH agonist (blocker cohort) or the initiation of gender-affirming hormones (gender-affirming hormone cohort). Baseline may be from Day 0 to Month -3 prior to Day 0. Those youth who are enrolled in the gender-affirming hormone cohort and receive a blocker first will still have a Day 0 of initiation of gender-affirming hormones. If participants do not have their blocker implanted (blocker cohort) or do not initiate gender-affirming hormones (gender-affirming hormone cohort) within 3 months of their baseline survey, they will

need to retake the baseline survey. If participants decide not to initiate treatment, we will continue following them in 6-month intervals (starting from their baseline survey date). All study activities may be conducted by in-person or via remote study visits.

The anthropometric and physiologic parameters in the study are those routinely collected within the constructs of the clinical visit at each site. At baseline, 6 months, 12 months, 18 months, 24 months, and annually thereafter, REDCap survey instruments will be used to collect demographic, mental health, psychosocial, behavioral, and physiologic data from: 1) youth initiating pubertal suppression with GnRH agonists (blocker cohort) and their parent/caregiver, and 2) youth initiating hormone treatment for phenotypic gender transition (gender-affirming hormone cohort).

At baseline and annually thereafter parents/caregivers of blocker cohort participants will be asked to complete the online ASEBA Child Behavior Checklist (CBCL) to assess for emotional and behavioral problems among blocker cohort youth ages 8 to 17.

Youth and young adult participants in the blocker and gender-affirming hormone cohorts who are 11-17 will be asked at baseline and annually thereafter to complete the online ASEBA Youth Self Report (YSR) to assess for emotional and behavioral problems. At age 18, they will complete the ASEBA Adult Self Report (ASR) annually thereafter.

At baseline and years 2, 4, 6, and 8, the youth & young adult blocker and gender-affirming cohort participants, with optional parent/caregiver participation for the blocker youth cohort, will be asked to complete in person or via a HIPAA-compliant teleconference an abbreviated version of the Mini International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. Kid) or the Mini International Neuropsychiatric Interview (M.I.N.I.) to assess for mental health diagnoses. Determination of which version to be completed will be based on age (8 to 17 or 18+).

3.1 Study Population

Youth participants will be recruited from patients seeking care at any of the four study sites (Benioff Children's Hospital, Boston Children's Hospital, Children's Hospital Los Angeles, or Lurie's Children's Hospital of Chicago). Patients between 8 and 16 years old inclusive and their parents/caregivers will be eligible for enrollment in the blocker cohort. Patients 8 to 20 years old inclusive will be eligible for enrollment in the gender-affirming hormone cohort. The focus of this investigation is early treatment; therefore, enrollment of youth aged 19 or 20 in the cohort of youth initiating treatment with gender-affirming hormones will be limited to not more than 20% of the cohort. In addition, due to the expected impact of previous experience with blockers, no more than 60 participants in the gender-affirming hormone cohort may be blocker experienced from when they started puberty. Enrollment of approximately equal numbers of each gender for both cohorts will be targeted. Clinical observation indicates that an equal number of girls and boys seek care for gender dysphoria in the specialty clinics; thus, overrepresentation of either gender is not anticipated. If overrepresentation of a gender is present in either of the cohorts, enrollment will be restricted to increase the enrollment of underrepresented gender(s). In addition, if race or ethnic diversity is not achieved in the cohorts, enrollment will be restricted to support a more diverse sample. Participants who were enrolled in the study and left the study or completed the study prior to the year 3 visit may be reenrolled at the PI's discretion. At reenrollment, the participant will continue to be in the original assigned cohort.

3.2 Sample Size

Target enrollment is 120 youth and approximately 135 parents/caregivers in the blocker cohort. In the gender-affirming hormone cohort, 400 participants will be enrolled. Total enrollment is 520 youth participants and approximately 135 parent/caregivers across all sites. If a parent/caregiver is unable to continue in the study, they may be replaced by another parent/caregiver who will continue as the study participant through the end of the study.

4.0 SELECTION AND ENROLLMENT OF STUDY PARTICIPANTS

4.1 Blocker Cohort Youth Inclusion Criteria

To be considered eligible for enrollment, an individual must meet all the criteria listed below.

4.1.1 Presence of gender dysphoria as determined by a clinician;

4.1.2 Tanner stage 2, 3, or 4 of sexual development;

4.1.3 Appropriate to undergo puberty suppression with GnRH agonists;

4.1.4 Ages 8 through 16 years inclusive;

4.1.5 Ability to read and understand English;

4.1.6 Receiving or planning to receive services at a study site clinic; and

4.1.7 Willing and able to provide signed informed assent.

NOTE: If assent is obtained > 30 days prior to the enrollment date, assent must be verbally reaffirmed prior to starting the baseline visit.

4.2 Blocker Cohort Youth Exclusion Criteria

To be considered eligible for enrollment, an individual must *not* meet any of the criteria listed below.

4.2.1 Prior utilization of GnRH agonists;

4.2.2 Precocious puberty (natal males younger than 9 years or natal females younger than 8 years);

4.2.3 Pre-existing osteoporosis;

4.2.4 Presence of serious psychiatric symptoms (e.g., active hallucinations, thought disorder) that would impair the individual's ability to provide true informed consent or participate in the baseline survey*;

4.2.5 Visibly distraught (e.g., suicidal, homicidal, exhibiting violent behavior) at the time of consent or the baseline survey*;

4.2.6 Intoxicated or under the influence of alcohol or other substances to such an extent that in the opinion of the study staff, the ability to give true informed consent or to understand and answer the questions is impaired*.

***NOTE: If consent is obtained prior to the enrollment date, assessment for these exclusion criteria must be performed again prior to administration of the baseline survey (Enrollment). If any are present during the baseline visit, the participant cannot be enrolled; do not administer the survey. Reassessment of eligibility and enrollment may take place at a later date per the discretion of the treating clinician.*

4.3 Parent/Caretaker of Blocker Cohort Youth Inclusion Criteria

To be considered eligible for enrollment, an individual must meet all the criteria listed below.

4.3.1 Parent or caretaker of a child who meets the Blocker Cohort Youth Inclusion/Exclusion Criteria,

4.3.2 Ages 18 and above;

4.3.3 Ability to read and understand English; and

4.3.4 Willing and able to provide signed informed consent.

NOTE: If consent is obtained > 30 days prior to the enrollment date, consent must be verbally reaffirmed prior to starting the baseline visit.

4.4 Parent/Caretaker of Blocker Cohort Youth Exclusion Criteria

To be considered eligible for enrollment, an individual must not meet any of the criteria listed below.

4.4.1 Presence of serious psychiatric symptoms (e.g., active hallucinations, thought disorder) that would impair the individual's ability to provide true informed consent or participate in the baseline survey*;

4.4.2 Visibly distraught (e.g., suicidal, homicidal, exhibiting violent behavior) at the time of consent or the baseline survey*;

4.4.3 Intoxicated or under the influence of alcohol or other substances to such an extent that in the opinion of the study staff, the ability to give true informed consent or to understand and answer the questions is impaired*.

**NOTE: If consent is obtained prior to the enrollment date, assessment for these exclusion criteria must be performed again prior to administration of the baseline survey (Enrollment). If any are present during the baseline visit, the participant cannot be enrolled; do not administer the survey.*

4.5 Gender-Affirming Hormone Cohort Youth Inclusion Criteria

To be considered eligible for enrollment, an individual must meet all the criteria listed below.

4.5.1 The presence of gender dysphoria as determined by a clinician;

4.5.2 Appropriate for initiating phenotypic gender change with gender-affirming hormones;

4.5.3 Ages 8 through 20 years inclusive;

4.5.4 Ability to read and understand English;

4.5.5 Receiving or planning to receive services at a study site clinic; and

4.5.6 Willing and able to provide signed informed consent or assent.

NOTE: If consent is obtained > 30 days prior to the enrollment date, consent must be reaffirmed prior to starting the baseline visit.

4.6 Gender-Affirming Hormone Cohort Youth Exclusion Criteria

To be considered eligible for enrollment, an individual must *not* meet any of the criteria listed below.

4.6.1 Prior utilization of gender-affirming hormones;

4.6.2 Previously or currently enrolled in the Blocker Cohort;

4.6.3 Presence of serious psychiatric symptoms (e.g., active hallucinations, thought disorder) that would impair the individual's ability to provide true informed consent or participate in the baseline survey*;

4.6.4 Visibly distraught (e.g., suicidal, homicidal, exhibiting violent behavior) at the time of consent or the baseline survey*;

4.6.5 Intoxicated or under the influence of alcohol or other substances to such an extent that in the opinion of the study staff, the ability to give true informed consent or to understand and answer the questions is impaired*.

***NOTE: If consent is obtained prior to the enrollment date, assessment for these exclusion criteria must be performed again prior to administration of the baseline survey (Enrollment). If any are present during the baseline visit, the participant cannot be enrolled; do not administer the survey.*

4.7 Recruitment and Pre-Screening

Potential participants will be receiving services at one of the four sites (Benioff Children's Hospital, Boston Children's Hospital, Children's Hospital Los Angeles, or Lurie's Children's Hospital of Chicago) and seeking hormonal intervention to either delay the progression of puberty with GnRH agonists or begin phenotypic gender transition with gender-affirming hormones. Site care members will recruit participants for the study by speaking with patients and their parents/caregivers/legal guardians face-to-face, via teleconference, or by telephone. Information regarding the study will be provided and interest in participation will be assessed. A Pre-Screening Worksheet (see Appendix III) will be completed by the medical provider to assess whether the potential participant may be eligible for study participation. The medical provider must be approved by the site's IRB as a study team member. The Pre-Screening Worksheet will be signed and dated by the medical provider in order for it to be used for source documentation by the study staff.

Each individual youth participant who has their medical records reviewed to assess potential eligibility, is approached for recruitment, and/or consented for study participation will have the following information entered on the Study Screening Log, which will be maintained in a secure area at the site: name or initials, date of birth, age, sex assigned at birth, race, ethnicity and enrollment status. Individuals that were assessed as ineligible for enrollment will have the reasons for ineligibility recorded. Individuals who are approached, but do not consent to participate (or whose parent/legal guardian refuses to provide permission, if applicable), will be asked if they are willing to supply their reason(s) for declining participation; responses will be recorded. Information collected on the Study Screening Log, excluding names or initials and dates of birth, will be entered in the study-specific database for individuals that do not enroll in the study as instructed on the Study Screening Log.

For those individuals who were not eligible or did not consent to participate in the study, the Pre-Screening Worksheet will be destroyed immediately after having the information recorded on the Study Recruitment Log. When study accrual ends, project staff will obliterate on the Study Screening Log, all names or initials and dates of birth belonging to individuals who did not consent to participate in the study.

4.8 Informed Consent

Once it is determined that a child, adolescent, or adult may qualify for the study, details will be discussed, and all questions will be answered during the informed consent process. Youth participants under age 18 need to sign an assent form and their parent/legally authorized representative needs to sign a permission form. Youth participants aged 18 and older need to sign a consent form. Adult participants who are participating in the study as the parent/caregiver of a blocker child participant must sign a consent form for their own study participation. See Appendices VI, VII, VIII, IX and X for sample informed consent, assent, and parent/LAR permission forms. The informed consent/permission/assent forms cover information about the overall purpose of the study, what the study entails, potential risks, potential benefits to participating individuals and society, the confidentiality of data, and contact information for the Principal Investigator and the IRB. Once informed consent/assent/permission has been obtained, completed consent/assent/permission forms will be filed on a secure institutional network drive if electronic copies or under double-lock when not in use and with restricted access during work hours and/or when unattended if paper copies. Consent/Assent/Permission must be obtained within 30 days prior to study entry. If more than 30 days has elapsed since consent/assent/permission was obtained, consent/assent/permission must be verbally reaffirmed on the day of study entry prior to implementing any study activities. If a youth participant turns 18 while participating in the study, they must sign an adult consent form or consent addendum at their first study visit after they turn 18. This can be conducted in person, via e-consent, or conducted remotely if permitted by a site's IRB. When changes in the protocol occur or additional information needs to be provided to participants, the consent process can occur in person or remotely and documented on paper or via e-consent if permitted by a site's IRB. While it is preferred that a single parent/caregiver is paired with a blocker child participant throughout the study period, at times this is not

possible. If an additional parent/caregiver needs to be enrolled in the study, a new consent conference needs to occur so that the parent/caregiver is fully consented for study participation. This consent process can occur in person or remotely and document on paper or via e-consent if permitted by a site's IRB.

For participants reenrolling in the study, they will need to sign an assent, permission, and or consent form as above.

4.9 Contact Information

Once participants have provided informed consent/assent/permission, research staff will complete a paper or electronic Locator Form (see Appendix IV) with the study participants. Study participants will be asked to provide a working phone number and/or valid email address through which they can be reached. Participants will also be asked to provide valid contact information for a family member and/or friend who can be called in the event the participant cannot be reached by phone or email. Participants will be asked if text and/or voice messages can be left at the numbers provided and which name and pronouns to use for the participant. Study staff will not leave messages unless expressly permitted to do so by the participant, which will be documented on this form. If permission is given to leave messages, site staff will assure participants that messages left with a family member or friend will only ask the participant to contact study staff and will not include any protected health information or information related to study participation. As the Locator Form will include names and contact information, it must be stored under double-lock and separate from any documents that utilize the participant's identification (PID) number.

4.10 Co-Enrollment Guidelines

Co-enrollment in other studies is permitted.

5.0 STUDY PROCEDURES

5.1 Enrollment/Study Entry Procedures

The Pre-Screening Worksheet will be reviewed by research staff prior to the start of the baseline survey. If needed, research staff can verify that information has not changed between the completion of the Pre-Screening Worksheet and the Baseline Visit. If more than 30 days has passed between the date of the Pre-Screening Worksheet and the baseline visit, documentation of eligibility verification is required prior to baseline survey.

Unique PID numbers will be assigned to youth participants in both cohorts and the parent/caregiver participants in the blocker cohort. Each youth participant and parent/caregiver participant will receive a unique PID as the youth and the parent/caregiver are study participants. The identifiers consist of the designated site code (i.e., Benioff Children's Hospital = 1, Boston Children's Hospital = 2, Children's Hospital Los Angeles = 3, or Lurie Children's Hospital of Chicago = 4) followed by the participant's enrollment number. Youth participant enrollment numbers for the Blocker Cohort will start with 0001 for each site; and parent/caregiver enrollment numbers will start with 1001 with the final digit matching the corresponding youth participant. Ideally, the parent/caregiver participant should not change throughout the study. For example, if parent 1 completes the baseline survey, the same parent should complete all future study surveys. If a parent/caregiver is replaced by another parent/caregiver, then the PID assigned to the new parent/caregiver participant has a 3 for the 2nd digit (for example, parent 11001 would be replaced with 13001). If in the unlikely event a 3rd parent/caregiver is enrolled in the study to replace the 2nd parent/caregiver, then the parent/caregiver PID would have 4 as the 3rd digit, e.g., 14001 would replace 13001, and so forth.

The adolescent enrollment numbers for the Gender-Affirming Hormone Cohort will start with 2001. For example, the first Blocker participant for Boston Children's Hospital will be 20001, and the corresponding parent/guardian will be 21001. The first Gender-Affirming Hormone participant at Boston Children's Hospital will be 22001.

For the purpose of this study, enrollment/study entry is equivalent to the participant completing the baseline survey. The Pre-Screening Worksheet must be entered into the database within 2 working days after the Baseline Visit.

For participants reenrolling in the study, the original assigned PID will be used.

6.0 EVALUATIONS AND MEASURES

See Appendix I. for the Schedule of Evaluations

6.1 Baseline Psychosocial Assessments

6.1.1 Blocker Cohort – Youth Participant

Youth study participants will be asked to complete a series of questionnaires via computerized, online surveys at the baseline visit, including demographics and measures to evaluate mental health, gender dysphoria, the experience of life stressors, parental and peer relationships, and quality of life.

The measures in the REDCap blocker cohort computerized survey fall into four domains: Demographics, transgender specific experiences, mental health and trauma, and additional psychosocial information. The survey will take approximately 1 to 1.5 hours to complete.

- Demographics: including age, race, ethnicity, educational level, and birth city/country
    - Transgender-specific experiences including gender dysphoria: age of realization of transgender status, age of first living in the desired gender role, and domains where they are living in their desired gender role, if any.
- Mental health and trauma assessments:
    - Revised Children's Manifest Anxiety Scale: Second Edition (RCMAS-2) – A measure used in both clinical and educational settings to determine levels of anxiety in young children and adolescents aged 6 to 19 years old. This measure is designed to capture levels of physiological anxiety; worry; social anxiety; and defensiveness.[27]
    - Beck Depression Inventory (BDI-Y) – The BDI-Y contains 20 statements about thoughts, feelings, and behaviors associated with depressive symptomology within the last 2 weeks. Items are rated from 0 (Never) to 3 (Always).[28]
    - Suicidal Ideation Scale – Eight yes/no questions will be asked to capture participants' suicide ideation and attempts.[29]
    - Self-Harm – Questions about if and where participant has purposefully harmed themselves.
    - Connor-Davidson Resilience Scale (CD-RISC) – A self-report metric used in clinical practice and research studies to assess characteristics of resilience when internal and external stressors arise.[30]
    - COVID-19 – Portions of the COVID-19 Exposure and Family Impact Survey (CEFIS) and additional questions about the effects of COVID-19 on study participants.
    - Adolescent Life-Change Events Scale – Questions to assess the impact of life events on participants for participants ages 9+
- Additional psychosocial information including body image, quality of life and relationships with parents and peers:
    - Body Esteem Scale – Body Esteem Scale is a questionnaire that has 3 subscales: BE–Appearance (general feelings about appearance), BE–Weight (weight satisfaction), and BE–Attribution (evaluations attributed to others about one's body and appearance).[31]
    - Parental Support Scale – Youth Version – Items designed to assess the level of support that transgender/gender dysphoric youth perceive from their parent(s)

- Social Relationships – Emotional support/friendship/loneliness/perceived hostility/perceived rejection – NIH Toolbox[32]
- Stress/Self-efficacy – Self efficacy (CAT 8-12 / CAT 13-17) – NIH Toolbox[32]
- Harter's Self-Perception Profiles for Adolescents & Children – This is a metric designed to capture an individual's definition of self by assessing self-image and self-esteem using five domains of perceived competence (scholastic competence, social competence, athletic competence, physical appearance, and behavioral conduct).[33]
- Pediatric Quality of Life Inventory (PedsQL) v4.0 (Child Report) – This instrument is used to assess physical, emotional, social, and school functioning in children ages 8 to 12 years old. This measure is designed to be sensitive to children's perceptions of the described domains.[34]
- Physical Activity Questionnaire – A 7-day recall instrument used to assess levels of weight-bearing exercise among school-aged youth[35]

ASEBA Youth Self Report (YSR) – Participants ages 11-17 will complete the YSR. The YSR was designed to assess the emotional and behavioral problems in adolescents in a standardized format. It assesses internalizing (i.e., anxiety, depression, and overcontrolled) and externalizing (i.e., aggressive, hyperactivity, noncompliant, and undercontrolled) behaviors.[52] The YSR will be completed via the online Achenbach System of Empirically Based Assessment (ASEBA), and it will take 0.25 to 1 hour to complete. It will ideally be completed on the same day as the survey.

A structured, clinical diagnostic, face-to-face or teleconference interview will be conducted with the blocker youth participants to assess for the presence of psychiatric diagnoses. The Mini International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. Kid), version 7.0.2, is designed to assess the presence of current DSM-V and ICD-10 psychiatric disorders in children and adolescents aged 6 to 17 years.[36] A portion of the modules of the M.I.N.I. Kid will be completed by blocker cohort participants. The presence of the parent/caregiver during the interview is optional. The interview will ideally be conducted on the same day as the survey. This interview will take approximately 0.5 to 1 hour to complete.

- M.I.N.I. Kid: Major Depressive Episode; Manic and Hypomanic Episodes; Panic Disorder; Agoraphobia; Separation Anxiety Disorder; Social Anxiety Disorder (Social Phobia); Specific Phobia; Obsessive-Compulsive Disorder; Posttraumatic Stress Disorder; Tic Disorders; Attention-Deficit/Hyperactivity Disorder; Conduct Disorder; Oppositional Defiant Disorder; Anorexia Nervosa; Bulimia Nervosa; Binge-Eating Disorder; Generalized Anxiety Disorder; and Adjustment Disorders

6.1.2 Blocker Cohort – Parent/Caregiver Participant

Parent/caregiver study participants will be asked to complete a series of questionnaires via computerized surveys at the baseline visit. The surveys will include demographics, characteristics, and measures to evaluate transgender-specific experiences; mental health; gender dysphoria; the experience of life stressors; parental support; and parent report of social relationships, negative affect, psychological well-being, stress, and self-efficacy; and quality of life.

The measures in the blocker cohort REDCap survey fall into four domains: demographics and characteristics, transgender-specific experiences, mental health and trauma, and additional psychosocial information. The survey will take approximately 1 to 1.5 hours to complete.

- Demographics and characteristics: including assigned sex at birth, gender, age, biological parents' height, race, ethnicity, educational level, socioeconomic status, birth city/country; religiosity and spirituality (Modified Duke University Religion Index)[37]; child's service utilization; relationship to child (adoptive parent, biological parent, foster parent); and child's daily calcium intake.
- Transgender-specific experiences: child's gender identity, child's age of realization of transgender status, child's age of first living in the desired gender role, domains where the child is living in their desired gender role, social transitioning, parental/guardian support, and disclosure of child's transgender status to others.

- Parent Report Gender Identity Questionnaire – An assessment tool that is utilized to identify children with gender dysphoria among gender-referred probands.[38]
- Diagnostic and Statistical Manual of Mental Disorders (DSM-5) – Criteria for gender dysphoria will be assessed by parent report.[39]
- Social Transitioning Scale – Scale assesses domains in which a child's asserted gender identity is affirmed.[40]

- Mental health and trauma assessments: parent reports of child's anxiety, child's experience of trauma and trauma symptoms (including those associated with impending or beginning pubertal development), child's suicide (ideation and attempts), and self-harm.
  - Autism Spectrum Quotient-10 (AQ-10) Child version – A short questionnaire for parents to complete about a child 4-11 years old with suspected autism who does not have a learning disability.[41]
  - Suicidality – Eight parent-proxy items will be used to capture children's suicide ideation and attempts.[29]
  - Self-Harm – Questions about if and where participant has purposefully harmed themselves.
  - COVID-19 – Portions of the COVID-19 Exposure and Family Impact Survey (CEFIS) and additional questions about the effects of COVID-19 on study participants.

- Additional psychosocial and physiologic measures:
  - Parental Support Scale – Parent Version – Questions related to the parent's level of support around their transgender/gender dysphoric child
  - Parenting Stress Index – Short Form (PSI-SF) – A metric designed to capture the stress within a parent-child dyad. This 36-item scale, adapted from the full-length form, is used to assess levels of stress within the following domains: child characteristics, parent characteristics, and situational/demographic life stress[42]
  - Social Relationships – Empathic Behaviors/Peer Rejection/Positive Peer Interactions/Social Withdrawal (Parent Report) – NIH Toolbox[32]
  - Negative Affect – Anger/Fear/Sadness (Parent Report) – NIH Toolbox[32]
  - Psychological Well-Being – General life satisfaction/positive affect (Parent Report) – NIH Toolbox[32]
  - Stress/Self Efficacy – Self-efficacy (Parent Report) – NIH Toolbox[32]
  - Pediatric Quality of Life Inventory (PedsQL) v4.0 (Parent Report) – The parent-proxy report PedsQL questionnaire is used to examine parents' perceptions of their children's physical, emotional, social, and school functioning.[34]

The Child Behavior Checklist (CBCL) will be completed by the parent/caregiver of blocker cohort participants. The CBCL yields scores on internalizing, externalizing, and total problems as well as scores on DSM-IV related scales. [43] The CBCL will be completed online via the Achenbach System of Empirically Based Assessment (ASEBA), and it will take 0.25 to 1 hour to complete. The CBCL will ideally be completed on the same day as the survey.

Parent/Caregiver guardian participation in the Mini International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. Kid), version 7.0.2, conducted with the blocker youth is optional (see section 6.1.1).

6.1.3 Gender-Affirming Hormone Cohort Participants

Study participants in the gender-affirming hormone cohort will be asked to complete a series of questionnaires via computerized surveys at the baseline visit, including measures to evaluate mental health, gender dysphoria, the experience of life stressors, and parental and peer relationships. Participants will also be asked demographic questions, substance use behaviors, and sexual risk questions.

Measures utilized in the REDCap survey fall into five domains: The survey will take approximately 1 to 1.5 hours to complete.

- Demographics and characteristics: including assigned sex at birth, gender, age, race, ethnicity, sexual orientation, educational level, socioeconomic status; birth city/country; religiosity and spirituality (Modified Duke University Religion Index)[37]; and service utilization
- Transgender-specific experiences including gender dysphoria:
    - Transgender Congruence Scale – A construct of congruence to conceptualize the degree to which transgender individuals feel genuine, authentic, and comfortable with their gender identity and external appearance.[45]
    - Diagnostic and Statistical Manual of Mental Disorders (DSM-5) – *see description in section 6.1.1.*
    - History of Blocker Experience – history of a participant's blocker experience
- Mental health and trauma assessments:
    - Autism-Spectrum Quotient -10 (AQ-10) Adult Version – A self-administered questionnaire for adults (16 years and older) with normal intelligence. The AQ-10 is an adaptation of the original 50-item Autism Spectrum Quotient (AQ) questionnaire. Items are used to assess social skills, attention, communication, and imagination.[41]
    - Revised Children's Manifest Anxiety Scale: Second Edition (RCMAS-2 - What I Think and Feel) – A measure used in both clinical and educational settings to determine levels of anxiety in young children and adolescents aged 6 to 19 years old. This measure is designed to capture levels of physiological anxiety, worry, social anxiety, and defensiveness.[27]
    - Beck Depression Inventory II – BDI-II (Adolescent) – A screening tool used to identify symptoms and severity of depression among adolescents and adults with or without self-reported depression.[46]
    - Suicidal Ideation Scale – *see description in section 6.1.1.*
    - Self-Harm – Questions about if and where participant has purposefully harmed themselves.
    - Gender Minority Stress and Resilience (GMSR) Measure – The Gender Minority Stress and Resilience questionnaire is a measure that consists of subscales (i.e., pride, internalized transphobia, non-affirmation, etc.) designed to assess mental health outcomes, such as depression and social anxiety among transgender and gender-nonconforming individuals.[47]
    - Connor-Davidson Resilience Scale (CD-RISC) – *see description in section 6.1.1.*
- Additional psychosocial information including quality of life and relationships with parents and peers
    - Body Esteem – *see description in section 6.1.1.*
    - Body Image Scale – As an assessment tool designed to evaluate requests for sex-reassignment surgery and treatment, the Body Image scale is a 30-item measure that is used to determine body attitudes among transgender men and women, by asking that participants rate their satisfaction with 30 body features on a Likert-type scale[48]
    - Parental Support Scale – Youth Version– *see description in section 6.1.1.*
    - Negative Affect – Anger/Fear/Sadness NIH Toolbox[32]
    - Psychological Well-Being – General life satisfaction/positive affect NIH Toolbox[32]
    - Stress/Self Efficacy – Self-efficacy (CAT 13-17) – NIH Toolbox[32]
    - Health Related Quality of Life – A scale adapted from the HIV-specific, health-related quality of life measure. This measure includes an assessment of perceived burden of being transgender, measured as the degree of satisfaction with domains of life that are potentially affected by being transgender (e.g., social relationships, family burden, medical management), interference with life goals and daily activities, and related worries.[49]
- Behavior risk including alcohol/drug use, sex work and high-risk sexual activities.

- The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) – The revised version of the ASSIST v2.0 consists of eight sections covering use of the following substances: tobacco, alcohol, cannabis, cocaine, amphetamine-type stimulants (including ecstasy), inhalants, sedatives, hallucinogens, opioids, and 'other drugs'.[50]
- Sexual Risk Behavior – A measure modified after the work of Jemmott *et al.*[51] Items are used to assess an individual's sexual activity in the three months prior to completion of the scale to determine the level of HIV risk-associated sexual activity.
- History of Sexually Transmitted Infections (STIs)
- Sexual Attraction and Romantic Partner

- Youth who had previous blocker experience from when they started puberty will also complete the following items via the survey:
  - Physical Activity Questionnaire – A 7-day recall instrument used to assess levels of weight-bearing exercise among school-aged youth[35]
  - Daily calcium intake

The Achenbach System of Empirically Based Assessment (ASEBA) Youth Self Report (YSR) or Adult Self Report (ASR) will be completed by the gender-affirming hormone participants. Determination of the YSR vs. ASR is based on age (11-17 or 18+ respectively) at the study visit. The YSR and ASR were designed to assess the emotional and behavioral problems in adolescents and adults in a standardized format. It assesses internalizing (i.e., anxiety, depression, and overcontrolled) and externalizing (i.e., aggressive, hyperactivity, noncompliant, and undercontrolled) behaviors.[52] The YSR or ASR will be completed via the online ASEBA system, and it will take 0.25 to 1 hour to complete.

A structured, clinical diagnostic, face-to-face in person or teleconference interview will be conducted with the gender-affirming hormone participants to assess for the presence of psychiatric diagnoses. The Mini International Neuropsychiatric Interview for Children and Adolescents (M.I.N.I. Kid) or Mini International Neuropsychiatric Interview (M.I.N.I), version 7.0.2, is designed to assess the presence of current DSM-V and ICD-10 psychiatric disorders.[36] A portion of the modules of the M.I.N.I. Kid or M.I.N.I. will be completed by the gender-affirming hormone participants. The M.I.N.I. Kid will be utilized with participants who are aged 17 and under on the day of the study visit, and the M.I.N.I. will be utilized with participants who are 18 and older on the day of the study visit. The interview will take approximately 0.5 to 1 hour to complete. For modules included in the M.I.N.I. Kid, please see section 6.1.1 above.

M.I.N.I.: Major Depressive Episode; Manic and Hypomanic Episodes; Panic Disorder; Agoraphobia; Separation Anxiety Disorder; Social Anxiety Disorder (Social Phobia); Obsessive-Compulsive Disorder; Posttraumatic Stress Disorder; Anorexia Nervosa; Bulimia Nervosa; Binge-Eating Disorder; Generalized Anxiety Disorder; and Antisocial Personality Disorder

The YSR or ASR and a portion of the modules of the M.I.N.I. Kid or M.I.N.I. will ideally be completed on the same day as the survey.

6.2 Month 6 through Year 2 Psychosocial Assessments

6.2.1 Blocker Cohort – Youth Participants

The blocker youth participant will be asked to complete a series of questionnaires via the computerized surveys at 6, 12, 18, and 24 months from the date the blocker was started +/- 28 days. The measures are described above in section 6.1.1. In addition to the data collected in the baseline survey, the follow-up visit surveys will also collect youth participant's perceptions of the side effects of GnRH agonists. The survey should take about 1 to 1.5 hours to complete. The youth annual surveys will include the Adolescent Life-Change Event Scale (modified to be non-binary) to measure exposure to stress in daily life. A survey to measure the effects of COVID-19 will be added to all timepoints following IRB-approval of version 3 of this protocol.

ASEBA Youth Self Report (YSR) – Participants will complete the YSR or the ASR at the year 1 and year 2 visits and annually thereafter. Determination of the YSR vs. ASR is based on age (11-17 or 18+ respectively) at the study visit. The YSR and ASR were designed to assess the emotional and behavioral problems in adolescents in a standardized format. It assesses internalizing (i.e., anxiety, depression, and overcontrolled) and externalizing (i.e., aggressive, hyperactivity, noncompliant, and undercontrolled) behaviors.[52] The YSR or ASR will be completed via the online Achenbach System of Empirically Based Assessment (ASEBA), and it will take 0.25 to 1 hour to complete.

A portion of the modules of the M.I.N.I. Kid or M.I.N.I. will be completed by blocker youth participants with optional presence of the parent/caretaker at the year 1 and year 2 visits. Determination of the M.I.N.I. Kid vs. M.I.N.I. is based on age (8-17 or 18+) at the study visit. M.I.N.I. components are listed in section 6.1.3. The M.I.N.I. Kid is conducted through a face-to-face in person or teleconference interview. This interview will take 0.5 to 1 hours to complete.

The YSR or ASR and a portion of the modules of the M.I.N.I. Kid or M.I.N.I. will ideally be completed on the same day as the survey.

6.2.2 Blocker Cohort – Parent/Caregiver Participants

Parent participants will be asked to complete a series of questionnaires via the computerized survey at 6, 12, 18, and 24 months from the date the blocker was inserted +/- 28 days. The measures are described above in sections 6.1.2. The 6- and 24-month surveys will collect additional information about parent/caregiver participant's perceptions about barriers to accessing a puberty blocker for their child. The survey should take no more than 1 to 1.5 hours to complete. The parent Month-24 survey will also include questions about causes of delay in accessing a puberty blocker for their child. A survey to measure the effects of COVID-19 will be added to all timepoints following IRB-approval of version 3 of this protocol.

Parents/caregivers of participants will also complete the CBCL at the year 1 and year 2 visits. This will ideally be completed on the same day as the survey. This will take 0.25 to 1 hour to complete.

6.2.3 Gender-Affirming Hormone Cohort Participants

Gender-affirming hormone study participants will be asked to complete online questionnaires at 6, 12, 18, and 24 months from the initiation date of gender-affirming hormones +/- 28 days. In addition to the data collected in the baseline survey (described above in section 6.1.3), the follow-up visit surveys will also collect the following:

- Physical and Emotional Effects of Hormone Use – Questions on the physical and emotional effects of hormone use for the following hormone treatments: testosterone, progesterone, estrogen, spironolactone, and any other hormone blockers as applicable
- For transmasculine participants:
    - Age at menarche;
    - Month and year of last menstrual period;
    - Information regarding use of chest binders;
    - History of male chest reconstruction procedures;
    - Level of interest in other surgeries related to gender identity and presentation; and
    - Chest dysphoria

The annual surveys will also include the following:

- Adolescent Life-Change Event Scale – an instrument to measures exposure to stress in daily life (modified to be non-binary).
- COVID-19 – Portions of the COVID-19 Exposure and Family Impact Survey (CEFIS) and additional questions about the effects of COVID-19 on study participants.

The Month-24 survey will also include the following additional items related to understanding the study participants' perceptions of being in the study:

- Utrecht Gender Dysphoria Scale (UGDS) – a dimensional scale designed specifically to measure gender dysphoria. The adolescent version of the Utrecht Gender Dysphoria scale consists of 12-items to which individuals rate their level of agreement on a 5-point Likert scale.[26]
- Gender Identity/Gender Dysphoria Questionnaire for Adolescents and Adults – A dimensional assessment tool designed to determine the degree of gender dysphoria or gender uncertainty among adolescents and young adults.[44]
- Questions about participants' experience taking part in the Trans Youth Care study, including questions about some of the measures that were used.
- Questions about causes of delay in accessing gender-affirming hormones.

A survey to measure the effects of COVID-19 was added to all timepoints following IRB-approval of version 3 of this protocol.

The REDCap survey should take approximately 1 to 1.5 hours to complete.

The participants will also complete the YSR or ASR at the year 1 and year 2 visits and annually thereafter. These items will take an additional .25 to 1 hour to complete.

A portion of the modules of the M.I.N.I. Kid or M.I.N.I. will be completed by the gender-affirming hormone youth participants at the year 1 and year 2 visits. Determination of the M.I.N.I. Kid vs. M.I.N.I. is based on age (8-17 or 18+) at the study visit. M.I.N.I. components are listed in section 6.1.3. The M.I.N.I. and M.I.N.I. Kid is conducted through a face-to-face in person or teleconference interview. This interview will take 0.5 to 1 hour to complete.

The YSR or ASR and a portion of the modules of the M.I.N.I. Kid or M.I.N.I. will ideally be completed on the same day as the survey.

6.3 Years 3-9 Annual Psychosocial Assessments

Some participants may receive the Year 2 measures for Years 3 and 4 due to the delay in modifying the Year 3-9 study measures.

6.3.1 Blocker Cohort – Youth Participants

Blocker youth participants will continue to complete annual surveys from years 3-9. Revisions to the study measures occur in these years primarily based Baseline through Year 2 data analyses and the aging of the cohort.

Revisions to the survey for these timepoint include removing the following measure:

- Harter's Self-Perception Profiles for Adolescents & Children

Measures that are added for the years 3-9 annual psychosocial assessments include:

- Transgender-specific experiences including gender dysphoria:
    - Steps to Transition Scale by HB Kozee, TL Tylka, and LA Bauerband
    - Transgender Congruence Scale – A construct of congruence to conceptualize the degree to which transgender individuals feel genuine, authentic, and comfortable with their gender identity and external appearance.[45]
    - Gender Development Scale by JF Strang
    - Physical and Emotional Effects of Hormone Use – If participants have started to use gender-affirming hormones, they will also receive questions on the physical and emotional effects of hormone use for the following hormone treatments: testosterone, progesterone, estrogen, spironolactone, and any other hormone blockers as applicable.

- Mental health and trauma assessments:
  - BDI-II for 13+ replacing the BDI-Y for 12 and Under based on participant's age at study visit
  - Adult Manifest Anxiety Scale for 19+ replacing the Revised Children's Manifest Anxiety Scale (RCMAS-2) that is used for participants through age 17 based on participant's age at study visit
  - Child and Adolescent Trauma Screen
  - Adverse Childhood Experiences (ACEs) Questionnaire once participants are age 18 based on participant's age at study visit
  - Autism Spectrum Quotient-10 (AQ-10) Adult Version – Participants will transition to the adult version at age 16 based on participant's age at study visit

- Additional psychosocial information including quality of life and relationships with parents and peers
  - Health Related Quality of Life – A scale adapted from the HIV-specific, health-related quality of life measure. This measure includes an assessment of perceived burden of being transgender, measured as the degree of satisfaction with domains of life that are potentially affected by being transgender (e.g., social relationships, family burden, medical management), interference with life goals and daily activities, and related worries.[49]
  - Body Image Scale – As an assessment tool designed to evaluate requests for sex-reassignment surgery and treatment, the Body Image scale is a 30-item measure that is used to determine body attitudes among transgender men and women, by asking that participants rate their satisfaction with 30 body features on a Likert-type scale[48]
  - NIH Toolbox Emotion Battery – Emotional Support, Instrumental Support, Loneliness, Friendship, Perceived Hostility, Perceived Rejection
  - NIH Toolbox – General Life Satisfaction and Positive Affect with different versions for 13-17-year-old participants vs. 18+ participants based on participant's age at study visit
  - NIH Toolbox Self-Efficacy – the 18+ version will replace the 13-17-year-old version based on the participant's age at study visit
  - Perceived Stress Scale
  - Gender Minority Stress and Resilience (GMSR) Measure for Adolescents – The Gender Minority Stress and Resilience for Adolescents questionnaire is a measure that consists of subscales (i.e., pride, internalized transphobia, non-affirmation, etc.) designed to assess mental health outcomes, such as depression and social anxiety among transgender and gender-nonconforming individuals.[47] For participants 18+ at the time of the study visit, they will complete the Gender Minority Stress and Resilience Scale for Adults.
  - Carver Brief COPE Inventory
  - Deiner Flourishing Scale
  - Hemingway Measure of Adolescent Connectedness
  - Sexual Attraction and Romantic Partner

- Behavior risk including alcohol/drug use, sex work and high-risk sexual activities.
  - Sexual Risk Behavior – A measure modified after the work of Jemmott *et al.*[51] Items are used to assess an individual's sexual activity in the three months prior to completion of the scale to determine the level of HIV risk-associated sexual activity.
  - History of Sexually Transmitted Infections (STIs)
  - The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) – The revised version of the ASSIST v2.0 consists of eight sections covering use of the following substances: tobacco, alcohol, cannabis, cocaine, amphetamine-type stimulants (including ecstasy), inhalants, sedatives, hallucinogens, opioids, and 'other drugs'.[50]

The schedule for the M.I.N.I. or M.I.N.I. Kid reduces from annually to every other year (years 4, 6, and 8) starting at the year 3 visits. These items will take an additional 1 to 2 hours to complete. The portion of the modules of the M.I.N.I. Kid or M.I.N.I. will ideally be completed on the same day as the survey.

6.3.2 Blocker Cohort – Parent/Caregiver Participants

Blocker parent/caregiver participants will continue to complete annual surveys during years 3-9. Revisions to the study measures occur in these years primarily based Baseline through Year 2 data analyses and the aging of the blocker child cohort.

Revisions to the survey for these timepoint include removing the following measure:

- Diagnostic and Statistical Manual of Mental Disorders (DSM-5) – Criteria for gender dysphoria will be assessed by parent report.[39]

Measures that are added for the years 3-9 annual psychosocial assessments include:

- Transgender-specific experiences including gender dysphoria:
  - Steps to Transition Scale by HB Kozee, TL Tylka, and LA Bauerband
  - Gender Development Scale – Parent Report by JF Strang
- Mental health and trauma assessments:
  - PROMIS Measures for Parent Proxy Report – Emotional Distress – Anger, Emotional Distress – Anxiety, Emotional Distress – Depressive Symptoms, and Psychological Stress Experiences for youth participants 6 – 17 years old based on their age at the study visit
  - Autism Spectrum Quotient-10 (AQ-10) Adolescent Version – A short questionnaire for parents to complete about a child 12-15 years old with suspected autism who does not have a learning disability.[41] Parents/caregivers will complete this regardless of whether the youth participant is in the 12-15 age range.

6.3.3 Gender-Affirming Hormone Cohort Participants

Gender-affirming hormone participants will continue to complete annual surveys from years 3-9. Revisions to the study measures occur in these years primarily based Baseline through Year 2 data analyses and the aging of the cohort.

Revisions to the survey for these timepoint include removing the following measure:

- Diagnostic and Statistical Manual of Mental Disorders (DSM-5) – Criteria for gender dysphoria will be assessed by parent report.[39]

Measures that are added for the years 3-9 annual psychosocial assessments include:

- Transgender-specific experiences including gender dysphoria:
  - Steps to Transition Scale by HB Kozee, TL Tylka, and LA Bauerband
  - Gender Development Scale by JF Strang
- Mental health and trauma assessments:
  - Adult Manifest Anxiety Scale for 19+ replacing the Revised Children's Manifest Anxiety Scale (RCMAS-2) that is used for participants through age 17 based on participant's age at study visit
  - Child and Adolescent Trauma Screen
  - Adverse Childhood Experiences (ACEs) Questionnaire once participants are age 18 based on participant's age at study visit
- Additional psychosocial information including quality of life and relationships with parents and peers
  - NIH Toolbox Emotion Battery 18+ will replace the Social Relationships domains for 8-17-year-old participants when the participant turns 18 based on participant's age at study visit. The domains and measures to be use are Social Support – Emotional Support and Instrumental Support; Companionship – Loneliness and Friendship; and Social Distress – Perceived Hostility and Perceived Rejection

- NIH Toolbox Emotion Battery 18+ will replace the Negative Affect domains for 8-17-year-old participants when the participant turns 18 based on participant's age at study visit. The domains and measures to be use are Anger-Affect, Anger-Hostility, Anger-Physical Aggression, Fear-Affect, Fear-Somatic Arousal, and Sadness.
- NIH Toolbox Emotion Battery 18+ will replace the Psychological Well-Being domains for 8-17-year-old participants when the participant turns 18 based on participant's age at study visit. The domains and measures to be use are Positive Affect and General Life Satisfaction.
- NIH Toolbox Self-Efficacy – the 18+ version will replace the 13-17-year-old version based on the participant's age at study visit
- Perceived Stress Scale
- Gender Minority Stress and Resilience (GMSR) Measure for Adults –For participants 18+ at the time of the study visit, the Gender Minority Stress and Resilience Scale for Adults will replace the Gender Minority Stress and Resilience Measure for Adolescents.
- Carver Brief COPE Inventory
- Deiner Flourishing Scale
- Hemingway Measure of Adolescent Connectedness

The schedule for the M.I.N.I. or M.I.N.I. Kid reduces from annually to every other year (years 4, 6, and 8) starting at the year 3 visits. These items will take an additional 1 to 2 hours to complete. The portion of the modules of the M.I.N.I. Kid or M.I.N.I. will ideally be completed on the same day as the survey.

6.4 Medical Record Abstraction

Project staff at each site will record data through medical chart abstraction or asking participants at baseline, 6 months, 12 months, 18 months, 24 months, and annually thereafter. Data recorded will be from baseline or the previous visit date through the Month 6, 12, 18, and Year 2-9 target dates based on the date the blocker was inserted or gender-affirming hormones were started. Data to be collected are:

- Ongoing prescription medications (may be asked directly of the participant and/or parent and/or abstracted from the medical record)
- Clinically significant diagnoses (may be asked directly of the participant and/or parent and/or abstracted from the medical record)
- Anthropometric measures – most recent results of height, weight, blood pressure, and Tanner Stage, and for those who have started gender-affirming hormones: breast hemi-circumference and/or Modified Ferriman-Gallwey scale (to be completed by participant).
- Physiologic parameters:
  - Hormone levels (ultrasensitive luteinizing hormone, ultrasensitive follicle stimulating hormone, ultrasensitive serum estradiol or ultrasensitive serum testosterone, based on assigned sex at birth)
  - White blood cell count, hemoglobin, hematocrit, platelets, glycosylated hemoglobin, sodium, potassium, chloride, CO2 Total, BUN, creatinine, fasting and non-fasting glucose, AST, ALT, and fasting or non-fasting lipids (triglycerides, cholesterol, cholesterol HDL, cholesterol LDL, and cholesterol VLDL)
  - Bone health (25-hydroxy vitamin D, calcium, serum phosphorus, and alkaline phosphatase)
- Additional measures to be collected at annual visits from the date the blocker was started for participants who have/had a blocker are:
  - Bone mineral density measured via a DXA or QCT
  - Bone age
- Start date for the GnRH agonist. This information will be entered into the database after the baseline survey visit, once the GnRH agonist has been started.

- Date of initiation of gender-affirming hormones.
- Insurance status.

7.0 DATA COLLECTION AND SITE MONITORING

7.1 Data Records

Participants will be assigned a unique participant identification (PID) number. No personal identifying information will be used on the paper CRFs or electronic REDCap, CBCL, YSR, M.I.N.I., or M.I.N.I. Kid data records. The paper M.I.N.I. or M.I.N.I. Kid instruments may be identified by the PID. Alternatively, the paper M.I.N.I. or M.I.N.I. Kid instruments may be identified by the participant's medical record number, name, and/or date of birth and be maintained with other source documentation under double lock and key when not in use and with restricted access during work hours and/or when unattended. Consent forms will be filed and stored separate from the raw data in a secured location under double lock when not in use and with restricted access during work hours and/or when unattended. A key file that matches the ID number to the participant and organization will be maintained in a secure data repository within the project offices at each of the four sites. Data will be kept strictly confidential, except as required by law, and stored on a secure network, with password protection such that only authorized users will have access to the file server.

Data will remain on the CHLA server, the online Achenbach System of Empirically Based Assessment, or in REDCap during data collection, verification, cleaning, and analysis. At the closure of the study, electronic and hardcopy data will be maintained for a minimum of six years from the closure of the study per CHLA IRB policy. The local site data will be retained at the local site for the length of time as defined by the local site's IRB policy. Project binders at CHLA containing archival information will be stored a minimum of six years from the closure of the study and will then be eligible to be destroyed.

7.2 Data Collection and Submission

7.2.1 Case Report Forms

Anthropometric measures, physiologic parameters, hormone levels, measures related to bone health, diagnoses, concomitant medications, and other protocol-related data elements will be collected on case report forms (CRFs) created by CHLA or study coordinators may enter the data elements directly into the Access database based upon source documentation. All CRFs (see Appendix V) will be entered into password-protected Access databases located on the CHLA network. A password will be used to access survey data and chart abstraction data files and will be made accessible only to the Principal Investigators and study staff. These data will be archived in a password-protected database on the CHLA network daily. If hard copies of CRFs are used, they will be securely filed at each respective study site. Study-wide data management procedures, including integration and verification of multi-site data, will take place at CHLA. All data reported on CRFs or in the Access database must have corresponding source documentation at the clinical site to substantiate all submitted data.

7.2.2 Data Collection Methods

All data collected will be through software installed on a portable laptop computer or through web-based software. For the baseline and follow-up surveys, each participant will be assigned a PID number and their baseline and follow-up surveys will be linked via their PID number. A master list of the PID numbers assigned to participants will be kept in a secure, password protected file or in a secured location at each site under double lock when not in use and with restricted access during work hours and/or when unattended. ACASI data collected prior to the implementation of REDCap will be collected on the laptop computer at the collaborating site and encrypted and transferred to the CHLA network via VPN, Microsoft OneDrive, Microsoft SharePoint, or encrypted email. All data collected via the USC HIPAA-compliant Research Electronic Data Capture (REDCap) administration will be accessed through a unique password for each

user. All data collected from the administration of the CBCL and YSR will be accessed through password protected Achenbach System of Empirically Based Assessment (ASEBA)-Web Software. All data obtained from ASEBA-Web software will be securely exported to CHLA. M.I.N.I. and M.I.N.I. Kid data will be entered into the secure REDCap online database. CRF data will be entered into an Access database located on a secure CHLA network drive via VPN or VDI software. All data transferred to CHLA will be stored on CHLA's secured network (with firewall protection), which cannot be accessed by anyone outside of CHLA.

7.2.2.1 Data Security

Only participants with an individual link will be able to open the surveys, and if a participant needs to return to a survey, a password is required. All in-person diagnostic interviews will be conducted in a private office to ensure privacy and confidentiality of participant responses. If the participant is completing the diagnostic interview via teleconference, they will be advised prior to the interview that they should be in a private space during the interview. If the participant completing either the surveys or the diagnostic interview requires a short break, it is possible to stop and return later to complete it. The surveys must be completed within 7 days from when they are initiated and within the study visit window.

All data collected using ASEBA-Web or REDCap software is electronically stored, under policies compliant with the Health Information Portability and Accountability Act (HIPAA) and the Health Information Technology for Economic and Clinical Health Act.

All computers used for in-person visits will be located in locked facilities or securely stored during Safer-At-Home orders, and consent forms will be filed and stored separate from the raw data in a secured location under double-lock when not in use and with restricted access during work hours and/or when unattended. Any temporary data files kept on removable storage devices, as well as printouts derived from data analysis, will be stored in a locked compartment when not in use.

7.3 Data Quality Assurance

Investigators receiving federal funding must adhere to the Code of Federal Regulations to protect research participants and produce reliable study information. The data manager at each study site will work with site staff to facilitate the receipt of data, provide technical assistance as necessary, and meet regularly via telephone with the staff person responsible for site-specific quality assurance. Data edits through range checks and field inconsistencies will be built into the database to enable real time correction of key entries and CRF completion errors.

7.4 Data Management

Data must be cleaned and stored uniformly in order to perform useful analyses and generate meaningful conclusions. Data coordination staff at CHLA will conduct trainings of data collection personnel at each site concerning appropriate procedures for recruitment, enrollment, and use of computer-assisted data collection. Additionally, CHLA staff will be responsible for verifying that data from all sites are comparable. The CHLA data manager will be responsible for ensuring that site data are safely transferred via secure protocols to CHLA for cleaning and merging. The CHLA data manager will generate tracking reports regarding the accrual of participants at each site, as well as specific characteristics of the emerging cross-site data file (such as balance of male and female transgender respondents, previous blocker use for gender-affirming hormone cohort, age categories, etc.).

7.5 Study Site Monitoring and Record Availability

Each of the participating study sites will review a selected portion of the individual subject records, including assent/consent forms, CRFs and supporting source documentation to ensure the protection of study participants, compliance with the protocol, and accuracy and completeness of records. Regulatory files, as required, will also be inspected to ensure that regulatory requirements are being followed.

The primary investigators at each site will make study documents (e.g., consent forms, case report forms) and pertinent hospital or clinic records readily available for inspection by the local IRB, CHLA staff, the NICHD, the Office of Human Research Protection, or the sponsor's designee for confirmation of the study data.

8.0 PARTICIPANT MANAGEMENT

8.1 Tracking Participants/Follow-up

All participants will be contacted before each follow-up study visit (i.e., 6, 12, 18, and 24 months after the initiation date of gender-affirming hormones for the gender-affirming hormone cohort or after blocker insertion date for the blocker cohort). Multiple contact methods will be used for participants who are difficult to reach (e.g., phone, email, text message). Participants will be asked whether messages can be left for each of the phone numbers that they provide. They will be informed that messages will not contain any information regarding the nature of the project.

8.2 Study Visit Management

All study visits are to be conducted according to the Schedule of Evaluations in Appendix I. The preferred timeframe for all follow-up visits including dates of physiologic and anthropomorphic data is within 28 days prior to or after the target study visit date. If the participant is unable to attend a visit within this timeframe, the site staff should work with the participant to identify a day closest to the scheduled visit to perform the visit. Extension of the visit window must be communicated to and approved by the CHLA coordinating center via TransYouthCare@chla.usc.edu. Scheduling of study visits will not be recalibrated based on the actual date that a visit was made. All follow-up study visits must be made based on the elapsed time from the date of enrollment.

8.2.1 Completing the Survey, M.I.N.I., M.I.N.I. Kid, YSR, or CBCL

Participants should be in a quiet, private area to complete the survey or interview. Prior to starting the survey or interview, the participant should be reminded of their right to discontinue at any time with no penalty and the right to leave any questions unanswered that make them feel uncomfortable. Project staff at each study site will assist with unbiased, survey tutorials, if required. If the participant requires breaks, project staff at each site will instruct the participant in how to make sure the computer program is exited and re-entered properly, so that the participant's confidentiality is maintained. Participants must be given adequate breaks in between surveys, as needed. If participants are not able to complete all study activities on one day, they may return within 7 days to complete the study activities. If a participant needs more than 7 days to complete all elements of the study visit, the study coordinator should notify the CHLA coordinating center via TransYouthCare@chla.usc.edu.

8.2.2 Debriefing and Referral Procedures for Participants

Because responding to questions in the surveys or interviews may be distressing to participants, a short debriefing of all participants will be conducted upon completion of the survey and/or interview to ensure that study staff has an opportunity to assess the participant's reaction to the survey or interview, whether each item was wholly or partially completed. Study staff will ask participants if there was any part of the survey and/or interview that was upsetting to them or that they would like to discuss. Further, if participants indicate current suicidal thoughts on the REDCap survey, the program will flag those responses via an email to the study coordinator at the completion of the survey. The study staff will then talk with the participant to determine whether the participant currently presents a danger to self or others. If the participant's response to the debriefing interview or suicide assessment indicates a potential risk to the participant's safety and an urgent need of mental health assistance, site staff should follow their individual site procedures for acute mental health referrals. Ideally, site staff should contact a supervisor immediately and stay with the study participant in person or remotely until a parent/caregiver, licensed clinician, mental health professional, or emergency services, if needed, arrives. Results of the debriefing interview are kept

at the sites; however, sites are instructed to inform the CHLA team when the site PI determines that the event may be attributable to participation in or the conduct of the study and complete and submit the Monitoring Untoward Event Form.

If a participant wholly or partially completes the survey, becomes distraught, and leaves the clinic without the debriefing interview being conducted, the site staff will report the protocol deviation to their local IRB and the CHLA team. In addition, they will complete a Monitoring Untoward Event Form and submit it to the CHLA team.

Participants who do not appear distressed and do not want to discuss anything about the survey or interview will be informed that they can contact study personnel for referrals in the event that issues or concerns arise later.

8.3 Compensation

The decisions around compensation will be determined separately by each site and approved by each site's IRB.

8.4 Intervening on "Social Harms"

The research staff and protocol will be certified through the local IRB at each of the four sites to conduct research on human participants, particularly as related to children. This observational study is 46.404, research not involving greater than minimal risk, and 46.408 requirements for permission by parents or guardians and for assent by children will be followed. All sites have specific policies governing the treatment of human participants. These policies specify that medical and psychological assistance will be available in the immediate environment in the event a participant should experience any adverse reactions resulting from study procedures.

While participants will be informed that they may refuse to answer any question at any time, responses or reactions to certain questions may indicate distress on the part of the participants. If at any time during the study a participant divulges being at risk for harm, including but not limited to being abused or experiencing violence; if harm is suspected or likely; or if the participant reports suicidal/homicidal intentions, measures will be taken to ensure the participant's safety per each site's IRB requirements and safety protocol. Reporting will be done as appropriate to the specific situation and the local legal statues, including reporting to child protection agencies, or other appropriate agencies and referrals will be provided to appropriate support, counseling, or treatment resources. In addition, social harms will be reported to the CHLA Team as part of study conduct.

8.5 Premature Discontinuation from the REDCap Survey, CBCL, YSR, or M.I.N.I./M.I.N.I. Kid Interview

If a participant appears to be distressed while completing a survey or participating in an interview, research staff will ask the participant if there is anything upsetting them and if they would like to stop the survey or interview. If they choose to stop the survey or interview, the research staff will follow their local site procedures for assessing and intervening. Medical and psychological professionals on the project team will be available to provide support where necessary. Participants will continue on study, unless the participant requests to end study participation as discussed in the Premature Study Discontinuation section.

8.6 Premature Study Discontinuation

Participants will be prematurely discontinued from the study if any of the following occurs:

- The participant withdraws consent (or assent and/or parent/legal guardian withdraws permission, if applicable);
- Participant is diagnosed with osteoporosis at baseline via bone densitometry;
- The participant is lost to follow-up;
- The participant experiences an untoward event that warrants discontinuation form the study;

- The participant develops a health problem and needs treatment that would affect the results of this study;
- The study is cancelled by *The Eunice Kennedy Shriver* NICHD;
- The study is cancelled for other administrative reasons; or
- Death of the participant.

If a youth participant in the Blocker Cohort is prematurely discontinued from participating in the study, the parent or caregiver participant will also be prematurely discontinued from the study.

Study coordinators should complete the Off Study Form when the decision is made to permanently discontinue the participant from the study and no further data collection will occur. Data through the time that the participant is taken off study will still be used for study purposes.

9.0 MONITORING UNTOWARD EVENTS

Site staff must first follow their own IRB's procedure for reporting and managing untoward events. Study staff will record any untoward event experienced by the participant. Reporting is required for occurrences including social harms, psychological distress and serious life threatening events such as suicide attempts. These may be immediately apparent to the study staff, such as the participant's emotional upset requiring referral for counseling; or they may be delayed and reported later to study staff, such as physical harm to an individual for having participated in the study. Study staff will notify CHLA of these untoward events as soon as possible, but no later than 48 hours after awareness of the event. In addition, study staff will complete the Untoward Event Form and enter it into the study database within three working days after awareness of the event. Study staff will be briefed during the training on the scope of possible untoward events and instructed to report them.

10.0 STATISTICAL/ANALYTIC CONSIDERATIONS

10.1 Introduction

This is a longitudinal observational multi-site study to study the impact of medical treatments for gender dysphoria in youth who are initiating puberty suppression *or* pursuing a phenotypic gender change with gender-affirming hormones. Participants will be studied prospectively up to a 9-year period from the initiation of a GnRH agonist or gender-affirming hormones.

10.1.1 Population for Analysis

Gender dysphoric youth ages 8 through 20 at enrollment who are seeking care at one of the participating sites. All participants with available endpoint data will be included in the analysis.

10.1.2 Study Hypotheses

Effects of Hormonal Interventions on Mental Health and Psychological Well-Being:

- Hypothesis 1a: Patients treated with GnRH agonists will exhibit decreased symptoms of gender dysphoria, depression, anxiety, trauma symptoms, self-injury, and suicidality and increased body esteem and quality of life over time
- Hypothesis 2a: Patients treated with gender-affirming hormones will exhibit decreased symptoms of anxiety and depression, gender dysphoria, self-injury, trauma symptoms, and suicidality and increase body esteem and quality of life over time

Safety of Hormonal Interventions:

- Hypothesis 1b: GnRH agonists are tolerable and safe for transgender youth in Tanner stage 2 or 3 of sexual development, i.e., lipids, glucose, liver enzymes, electrolytes, and HgbA1c will not increase above clinically safe ranges

- Hypothesis 2b: Gender-affirming hormones are tolerable and safe to use with transgender youth initiating phenotypic transition, i.e., will not increase lipids, glucose, liver enzymes, electrolytes, hemoglobin A1c and hemoglobin above clinically safe ranges

Bone Density in Blocker Cohort:

- Hypothesis 1c: Raw bone density scores will remain stable for youth receiving GnRH agonists; however, age-matched z-scores may decrease

10.1.3 Exploratory Aim

Risk Behavior in Gender-affirming Hormone Youth:

- Based on evidence of high rates of substance use and HIV infection in some transgender adolescents, we will measure substance use and sexual risk behaviors over time

10.2 Study Endpoints

The primary study endpoint for each participant will be reached at the end of the observational period – two years after the initiation of treatment. However, it is the intention of the PIs to extend this study at a future time point. At that time, IRB approval of the extension will be obtained.

10.3 Sample Size and Power Analysis

10.3.1 Blocker Cohort – Power Analysis

Unique to the blocker cohort is the need to assess the effect of GnRH agonists on bone health (Hypothesis 1c). Although we hypothesize that there is no net change in raw bone density over time (precluding power analysis), it is important to assess nontrivial lags in development compared to age-matched peers. Using G*Power version 3.1.3 to conduct an a priori power analysis in a repeated measures MANOVA framework with effect size f=.20 (a moderate effect size equivalent to Cohen's d=.40), alpha=.05, adequate statistical power=.80, and 4 measurement time points, a sample of 73 participants would be sufficient to detect significant decrease in age-matched z-scores over time. Thus, we will recruit a minimum sample of 80 evaluable participants, across all study sites, in the blocker cohort, which will yield comparable power to detect moderate changes in mental health outcomes over time (Hypothesis 1a) and good (.89) power to detect significant differences in metabolic and physiologic lab values of one-third of a standard deviation from clinical cutoffs (Hypothesis 1b). Additional participants may be recruited to provide more diversity in ethnicity, race, or gender.

10.3.2 Gender-Affirming Hormone Cohort – Power Analysis

In the absence of available longitudinal metabolic and physiological data, the study is powered to assess changes in mental health and psychological well-being (Hypothesis 2a) based on evidence from our preliminary data. Using G*Power version 3.1.3 and conducting an a priori power analysis in a repeated measures MANOVA framework with effect size f=.11 (a small effect size equivalent to Cohen's d=.22), alpha=.05, adequate statistical power=.80, and 4 measurement time points, with a small natural correlation among repeated measures of r=.15, a total sample of 196 participants is needed for adequate power to detect multivariate significance. This sample will generate adequate (.80) power to detect effects as small as d=.17, or less than a fifth of a standard deviation from clinical cutoffs, in the safety analyses of Hypothesis 2b. Therefore, we will recruit a minimum total sample of 200 evaluable participants, across all study sites, in the gender-affirming hormone cohort to ensure adequate statistical power to test the two hypotheses of Aim 2 and to conduct the exploratory analysis. Additional participants may be recruited to provide more diversity in ethnicity, race, or gender.

10.4 Randomization Procedure

This is an observational study and participants will not be randomly assigned to treatment.

10.5 Statistical Analysis

10.5.1 Primary Objective: *Effects of Hormonal Interventions on Mental Health and Psychological Well-Being:*

Hypotheses under the primary objective will be tested in each cohort, respectively, using repeated measures multivariate analysis of variance (MANOVA) to assess the trajectories of continuous mental health outcomes and psychological well-being over time within each cohort. The MANOVA approach will preserve statistical power to detect significant effects among this set of related continuous outcomes without the inflated Type I error rates associated with a series of individual ANOVA or regression analyses. The MANOVA analyses will investigate the changes over time in gender dysphoria, depression, anxiety, trauma symptoms, self-injury, suicidality, body esteem, and quality of life. The model will incorporate time (i.e., measurement time point: baseline, 6-month, 12-month, 18-month or 24-month survey) as a within-participants factor. Asserted gender, age, ethnicity, and other socio-demographic variables may additionally be entered as possible covariates (i.e., ANCOVA) to improve statistical power to detect significant time effects. However, we do not propose any a priori hypotheses about demographic effects on these outcomes, and any demographic variables that do not contribute significantly to the model will be removed from the analysis in order to preserve power and increase model parsimony.

In keeping with conventional practice, analysis will first proceed with a review of Box's test for the equality of covariance matrices. Violations of this assumption would require the use of Pillai's trace, as opposed to Wilks' Lambda, to determine multivariate statistical significance. If, as hypothesized, the within-participants time variable demonstrates significant multivariate effects, the follow-up univariate results will be inspected as appropriate. The assumption of sphericity via Mauchly's test will be checked for each measured outcome; if sphericity is violated, the Huyhn-Feldt correction for degrees of freedom will be applied to that outcome. Finally, for outcomes showing significant time effects, linear and quadratic contrasts will be checked for significance and marginal means will be computed and plotted to create a visual display of significant trajectories. An a priori p-value of 0.05 will be applied as the criterion for statistical significance in all analyses.

10.5.2 Secondary Objectives

Safety of Hormonal Interventions:

Unlike the mental health and psychological well-being measures, the question of interest for these metabolic and physiological parameters is not whether they show significant fluctuation over time (which may or may not be meaningful), but rather whether development after initiation of hormonal interventions pushes any physiological indicator above the clinically safe range for that indicator, i.e., above predetermined safety cutoff values based on previous literature and clinical guidelines. Safety will be assessed cross-sectionally with one-sided one-sample t-tests comparing cohort mean scores to the cutoff value. We hypothesize that the cohort means will be significantly lower than the cutoff score. We will use the Benjamini-Hochberg procedure to account for inflated family-wise alpha due to multiple comparisons at each time point.

Additionally, ranges of raw scores from all patient labs will be computed at each time point as part of the preliminary data cleaning and descriptive analysis phase. This will provide an immediate assessment whether the indicator value for any individual patient has crossed the safety threshold for that indicator as data are collected at each time point. In the event any patient experiences an individual increase in laboratory values above the threshold, medication adjustments will be made to protect the well-being of the patient according to the discretion of the medical provider at the site where they are receiving care regardless of the whole-cohort significance test results for that time point.

Bone Density in Blocker Cohort and Gender-Affirming Hormone Cohort with Previous GnRH Agonist Experience at Puberty:

We will use repeated measures ANOVA to estimate trajectories of raw and age-matched bone density scores over time in blocker cohort youth and the gender-affirming cohort who utilized GnRH agonists to

prevent puberty. As before, asserted gender and socio-demographic variables may be entered as possible covariates, linear and quadratic contrasts will be assessed, and marginal means will be computed and plotted to create a visual display of trajectories for both outcomes. We hypothesize that for raw scores, the linear term will not differ significantly from zero, indicating net stability in bone density over time. However, for age-matched z-scores, the linear term may be negative as gender non-conforming youth receiving GnRH agonists fail to add bone density at a rate comparable to their age-matched peers.

10.5.3 Exploratory Objective: *Risk Behavior in Youth Initiating Gender-Affirming Hormones*

We will conduct an exploratory assessment of sexual risk and substance use behavior in the gender-affirming hormone cohort, using repeated measures MANOVA to model trajectories of these risk outcomes over time. As before, asserted gender and socio-demographic variables may be entered as possible covariates. Given that sexual risk and substance use behaviors increase during adolescence in normative samples, we do not specify a priori hypotheses regarding the impact of hormone treatment on these risk outcomes in our transgender population. However, linear and quadratic contrasts will be assessed. Significant positive terms (indicating increased risk over time) would be indicative of a typical adolescent risk trajectory, whereas significant negative terms (indicating decreasing engagement in risky behaviors) or non-significant time effects (suggesting no net change in risk) would instead support a "treatment-as-prevention" explanation. Again, Box's test will be reviewed for equality of covariance matrices and multivariate test statistic determined accordingly, and sphericity will be assessed via Mauchly's test with the Huyhn-Feldt correction applied as needed.

10.5.4 Additional Analytic Considerations: Site Clustering Effects

Although the observational study will take place at four sites nationwide, we do not anticipate substantial site effects. To verify this, a group identifier for each participant is included in the merged analytic dataset, and the intra-class correlation (ICC) for each outcome will be calculated prior to conducting multivariate analyses. If, as anticipated, no significant variance is carried at the group level, we will reduce the model to a traditional one-level model. If significant group-level variance does emerge, dummy codes to control site-specific variance will be used to enhance statistical power.

10.6 Missing Data

CHLA will conduct missing data analyses in order to differentiate between data that are missing at random (MAR) and data that are missing related to gender or aspects of the treatment plan (e.g., hormone dosing). If missing data can be regarded as MAR, multiple imputations may be used. If the MAR assumption is not plausible, sensitivity analyses will be conducted to evaluate the impact of MAR violations on analyses by specifying models for non-ignorable missing data mechanisms.

11.0 HUMAN SUBJECTS

This study is being conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice guidelines, and 45 Code of Federal Regulations (CFR) §46.

11.1 Participants' Confidentiality

Participants will be assigned a unique identification number code (PID). A key file that matches the PID number to the participant and organization will be maintained in a secure data repository within the project offices at each of the four sites on in a password-protected file on an institutional server. Data will be kept strictly confidential, except as required by law, and stored on a secure network, with password protection such that only authorized users will have access to the file server. All computers will be located in locked facilities or in a secure location during Safer-At-Home. Consent forms will be filed and stored separate from the raw data in a secured online location, on a secure institutional network, or at a physical location that's under double lock when not in use and with restricted access during work hours and/or when unattended. Any temporary data files kept on removable storage devices, as well as printouts derived from data analysis, will be stored in a locked compartment when not in use.

11.2 Certificate of Confidentiality

To further protect the privacy of the study participants, a Certificate of Confidentiality has been obtained from the U.S. Department of Health and Human Services. With this Certificate in place, the researchers at the study sites cannot be forced to turn over identifying information about a study participant in any Federal, State, or local criminal, administrative, legislative, or other proceedings. This Certificate does not prevent a study participant from volunteering to turn over their research information nor does it prevent researchers from providing research-related information to others when requested by the study participant, or when required by law such as in cases of suspected or actual harm to or by the study participant.

11.3 Risks and Benefits

11.3.1 Risks

The Principal Investigators have determined that this study does not involve greater than minimal risk (45 CFR §46.404 and 21 CFR §50.51). Participation in this study poses no more harms or discomforts to participants than they may experience in normal daily life or during routine psychological examinations or tests.

Due to the personal nature of the information being collected in this study, there is some risk of emotional discomfort or distress. Participants will be informed that they are free to decline to answer any questions. Furthermore, participants will be informed that at any point they may stop if they do not wish to continue the questionnaire/interview. In the event of discomfort or upset, there are counselors at study sites with whom participants can talk and who can provide ongoing support as needed. Every effort will be made to keep the participant's participation in the study and personal information private and confidential, but absolute confidentiality cannot be guaranteed.

As this is an observational study, there are no alternative treatments or procedures.

11.3.2 Benefits

There may be no direct benefit to the participants for their participation in this study, but information learned from this study may benefit other youth now or in the future. This research provides the opportunity to obtain a better understanding of transgender youth, improve their care, and share information on a local and national level about how to provide care and hormone therapy for gender dysphoric children and adolescents. The information that is learned from this project will support innovative approaches to identifying, understanding, and providing optimal care for multi-ethnic transgender youth.

11.4 Institutional Review Board Review and Informed Consent

The Institutional Review Boards requires that all research participants review and sign an informed consent/permission/assent form. The informed consent/permission/assent form covers information about the overall purpose of the study, what the study entails, potential risks, potential benefits to participating individuals and society, the confidentiality of data, and contact information for the Principal Investigator and the IRB. Once informed consent/permission/assent has been obtained, the research staff will have the form reviewed by a fellow research team member, who will confirm that it is fully completed before it is filed in a secure location under double-lock when not in use and with restricted access during work hours and/or when unattended.

For participants aged 7 to 13 years old, the participant will sign an age-appropriate assent form, and the parent/legal guardian will sign a permission form. For participants aged 14 to 17 years old, the participant and the parent/legal guardian will sign a combined permission/assent form. For participants aged 18 years or older (including parent/caregiver participants), the participant will sign a consent form.

11.5 Requirement for Consenting Participants Enrolled as Minors Who Reach Age of Majority While on Study

Pursuant to guidance requested from OHRP, when a minor participant is enrolled with parental permission into the study, and the study will extend beyond the participant's age of legal majority, research staff must establish a mechanism to track the participant to obtain a legally effective consent when the participant reaches majority to remain on study.

11.6 Prisoner Participation

The Principal Investigators and NICHD have concluded that this protocol does not meet Federal requirements governing prisoner participation in human participant research and should not be considered by local IRBs for the recruitment of prisoners. Participants may not engage in study activities if they become incarcerated or are placed in detention. In addition, research staff will not collect study-related data during the time that the participant is incarcerated or placed in detention.

11.7 45 CFR Parts 160 and 164 Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule" Pursuant to the Health Insurance Portability and Accountability Act - HIPAA)

Each site is responsible for adherence to their individual institution's HIPAA policies and procedures.

11.8 Study Discontinuation

This study may be discontinued at any time by *The Eunice Kennedy Shriver* NICHD.

11.9 Community Participation

This study seeks contributions from community experts and advisory board members who identify as transgender or gender diverse. Community input is valuable for maintaining and creating robust research questions, improving perspective about measurement tools; assessing participants' experiences in the study; analyses of study data; dissemination of study results; and developing future research inquiries. This study will not collect individually identifiable data from or about community partners.

12.0 PUBLICATION OF RESEARCH FINDINGS

Data will be made available to other NIH investigators under the data-sharing agreement with NICHD after a reasonable time period that includes enough opportunity to prepare and have submitted for publication four manuscripts presenting the basic outcomes of the project. Beginning in Year 3, peer-reviewed publications will be developed pertaining to cross-sectional hypotheses and research questions found in primary and secondary objectives, though the bulk of publications are longitudinal in nature and will be developed in Year 5. Dissemination of findings to State and County officials, policy makers, and organizations will begin in Year 3, when preliminary data become available.

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APPENDIX I: SCHEDULE OF EVALUATIONS

	Pre-screening Worksheet[1]	Participant Consent/ Assent[2]	Locator Form[3]	Eligibility Confirmation[4]	Computerized Survey & Debriefing[5]	CBCL/ YSR/ ASR[6]	M.I.N.I. or M.I.N.I. Kid[6]	Chart Abstraction
Pre-Screening	X							
Baseline	X	X	X	X	X	X	X	X[7]
6 Months			X		X[8]			X[9]
12 Months			X		X[8]	X	X	X[9]
18 Months			X		X[8]			X[9]
Years 2-9 Annual Visits			X		X[8]	X	X (years 2, 4, 6, & 8)	X[9]
Premature Discontinuation			X		X	X		X[9]

1 Pre-Screening Worksheet must be completed by an IRB-approved medical provider.

2 Participant consent/assent must be obtained within 30 days prior to enrollment (baseline survey). Consent/assent must be reaffirmed prior to enrollment if > 30 days has elapsed.

3 The Locator Form data should be confirmed to be correct at every visit.

4 If eligibility is confirmed prior to the baseline visit date, eligibility must be reconfirmed at the baseline visit before administering the survey.

5 Participation in the Baseline survey = Enrollment. Ideally, the survey should be completed in a single day. If extenuating circumstances prevent completion in a single day, the subject may return within the next 7 days to retake it from the beginning again. If retaken, the subject's first survey will be deleted.

6 The M.I.N.I., M.I.N.I. Kid, CBCL, YSR, and/or ASR will ideally be completed on the same day as the survey.

7 The Baseline Chart Abstraction is for data from the most recent previous medical visit and labs.

8 For the Blocker Cohort, the timeline for the follow-up surveys is based on the date of the insertion of the GnRH agonist. For the Gender-Affirming Hormone Cohort, the timeline for the follow-up surveys is based on the date of initiation of gender-affirming hormones.

9 The follow-up Chart Abstractions should collect data from the previous the visit through the date of the survey.

Trans Youth Care Letter of Amendment

DATE: August 9, 2016

TO: TYC Site Principal Investigators and Study Coordinators

FROM: TYC Coordinating Center

SUBJECT: TYC, v. 2.2, dated 7/19/16: Letter of Amendment #1: TYC Revisions to ACASI Script

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The following information impacts the TYC study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your TYC regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the ATN DOC Regulatory Office.

This serves as Letter of Amendment (LoA) #1 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued because of a change in the ACASI script for the blocker parent, blocker youth, and cross-sex hormone cohorts.

SUMMARY OF CHANGES

Revised the ACASI scripts:

1) To make the responses clearer for the measures that could be edited because they are not validated/published;
2) By adding instructions to clarify what was meant by same and opposite sex for the validated measures that could not be revised;
3) To correct some skip patterns;
4) To correct a missing "not"; and
5) To correct minor grammar issues.

GUIDANCE FOR SITES

Submit the following version of the ACASI scripts to your IRB within 30 days:

- Blocker Parent Cohort: Blocker Parent Cohort rev8.5.16 EN Qx
- Blocker Youth Cohort: Blocker Cohort Youth_rev8.4.16 En Qx
- Cross-Sex Hormone Cohort: CSH Cohort_rev8.4.16 EN Qx

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: September 16, 2016

TO: TYC Site Principal Investigators and Study Coordinators

FROM: TYC Coordinating Center

SUBJECT: TYC, v. 2.2, dated 7/19/16: Letter of Amendment #2: Pre-Screener & CRF Revisions

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The following information impacts the TYC study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your TYC regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #2 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued because of a change in the Pre-Screening Worksheets and CRFs.

SUMMARY OF CHANGES

1. Revised the Pre-Screening Worksheets to change the wording on question 18/Blocker or question 15/Cross-Sex Hormone and delete "not eligible" and added a response option to 18a/15a indicating that the subject lives too far from the study site.
2. Updated the CSH Laboratory CRF and the QCT/DXA CRF to indicate that bone-related measures should **only be collected for those youth who had a blocker to prevent puberty**.
3. Updated the Blocker Laboratory CRF to correct a unit of measurement (ng vs. mg).
4. Updated the Data Collection Forms Schedule to reflect the above revision dates.

GUIDANCE FOR SITES

Submit the following version of the Pre-Screeners and CRFs to your IRB within 30 days if required by your IRB:

- Data Collection Forms Schedule R6 08-25-16.pdf
- F1 Blocker Cohort Pre-Screening Worksheet R2 08-30-16.pdf
- F2 Cross-Sex Hormone Cohort Pre-Screening Worksheet R3 08-30-16.pdf
- F6 QCT and DXA CRF R1 8-25-16.pdf
- F7 Blocker Lab CRF R3 08-25-16.pdf
- F8 Cross-Sex Hormones Lab CRF R5 08-25-16.pdf

The revised Pre-Screening Worksheets are available on the Google Drive under TYC CRFs & Source Docs\TYC Source Docs\Version 2.2. The revised CRFs are available at TYC CRFs & Source Docs\TYC CRFs\Version 2.2.

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: October 12, 2016

TO: TYC Site Principal Investigators and Study Coordinators

FROM: TYC Coordinating Center

SUBJECT: TYC, v. 2.2, dated 7/19/16: Letter of Amendment #3: Removal of the DISC

The following information impacts the TYC study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your TYC regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #3 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the removal of the DISC from the TYC protocol.

SUMMARY OF CHANGES

1. We are removing the use of the Parent-Informant Diagnostic interview Schedule for Children for the parents of the child participants in the Blocker Cohort and the Youth-Informant Diagnostic Interview Schedule for Children (DISC-Y) for the adolescent participants in the Cross-Sex Cohort.
2. The length of time it took to complete the DISC interview was found to be longer than originally expected due to the large number of positive answers to the diagnostic questions, especially for those youth who had many potential diagnoses.
3. In addition, it was felt that the use of the DISC interview questions further pathologized gender identity, which has the potential to increase harm to subjects.
4. As this is a minimal risk study, although completion of fewer surveys may produce even less risk, the risk/benefit ratio has not changed.

GUIDANCE FOR SITES

Submit this amendment to your IRB within 30 days:

- Discontinue use of the DISC upon approval by your IRB, or sooner if allowed by your IRB due to the coordinating center's prior approval of the DISC-related protocol deviation.
- If protocol deviations are required to be submitted to your IRB, forward the completed protocol deviation to the coordinating center.
- Site's informed consent/assent/permission forms may need to be revised.

The CHLA IRB approval letter for this amendment can be found on the Google Drive in the CHLA IRB Approval Letters folder.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: December 1, 2016

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: TYC, v. 2.2, dated 7/19/16: Letter of Amendment #4: MINI, Addition of Breast Measurement & Ferriman-Gallwey Scale, Additional Bone Health Measures, & CRF Revisions

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The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #4 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued because of the addition of the MINI and MINI KID, addition of the collection of breast measurement and the Ferriman-Gallwey scale, expansion of the data collected on bone health, and CRF revisions.

SUMMARY OF CHANGES

1. Addition of the MINI 7.0.1 and MINI KID 7.0.1 to the protocol. Participants 16 and younger at baseline will complete the MINI KID 7.0.1 throughout their study participation. Participants 17 and older at baseline will complete the MINI 7.0.1 throughout their study participation.
2. Addition of CRF F13, Breast & Modified Ferriman-Gallwey Measurements Form. Report breast hemi-circumference measurements for transfeminine participants at all study visits. Complete the Modified Ferriman-Gallwey Scale for transmasculine participants at all study visits. These items may be completed by the provider, or if reported in the medical record, data may be abstracted by the study coordinator. If being used for source documentation, ensure that CRF is signed and dated by the staff person completing the form.
3. Revised CRF F6 QCT and DXA Results Form to collect bone age and additional DXA measurements and Z-scores.
4. Revised CRF F4 Visit Report Form to replace reference to DISC in question 1 with MINI.

5. Updated the Data Collection Forms Schedule to reflect the above items.

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- MINI 7.0.1 – communicate to IRB that participant name will not be provided to coordinating center
- MINI KID 7.0.1 – communicate to IRB that participant name will not be provided to coordinating center
- F4 Visit Report Form R3 11-17-16.pdf
- F6 QCT and DXA CRF R2 11-17-16.pdf
- F13 Breast & Ferriman-Gallwey Measurements CRF 11-18-16.pdf
- Data Collection Forms Schedule R7 11-18-16.pdf

The MINI 7.0.1 and MINI KID 7.0.1 are available at Trans Youth Care\Measures\Version 2.2. The revised CRFs and Data Collection Forms Schedule are available at Trans Youth Care\TYC CRFs & Source Docs\TYC CRFs\Version 2.2.

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: December 27, 2016

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: TYC, v. 2.2, dated 7/19/16: Letter of Amendment #5: Reduced Version of the MINI & Back-Up Method to Transfer Data via Encrypted Email

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #5 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued because in LOA #4, the CHLA Coordinating Center mistakenly submitted full versions of the MINI and MINI KID rather than the reduced versions. In addition, **encrypted** email is now approved to be used as a back-up method for data transmission.

SUMMARY OF CHANGES

1. LOA #4 provided approval for the full MINI and MINI KID to be used. This was an error.
2. This LOA removes the following sections of the MINI: Suicidality, Alcohol Use Disorder, Substance Use Disorder, Psychotic Disorders, Optional Assessment Measures to Track Changes Over Time, and information re: the MINI Plus.
3. This LOA removes the following sections of the MINI KID: Suicidality, Alcohol Use Disorder, Substance Use Disorder, and Psychotic Disorders.
4. If data transmission via the VPN or the Virtual Desktop is not an option due to technical difficulties, data can be submitted to the CHLA Coordinating Center via an **encrypted** email.

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- Reduced version of the MINI 7.0.1 – communicate to IRB that participant name will not

be provided to coordinating center

- Reduced version of the MINI KID 7.0.1 – communicate to IRB that participant name will not be provided to coordinating center

The MINI 7.0.1 and MINI KID 7.0.1 are available at Trans Youth Care\Measures\Version 2.2.

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: February 22, 2017

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: TYC, v. 2.2, dated 7/19/16: Letter of Amendment #6: Version 7.02 for the MINI & MINI Kid; Addition of 6-month Symptom Questions to ACASI; Data Submission via OneDrive & Survey Monkey; Minor Revision to Medication CRF Instructions

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The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #6 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to an update to version 7.0.2 of the MINI and MINI Kid and the approval of the revised youth participant ACASIs, which include symptom questions. Minor revisions have been made to the Medication CRF instructions, and two additional methods for data transmission have been approved (Microsoft OneDrive and Survey Monkey).

SUMMARY OF CHANGES

1. This LOA provides approval for version 7.0.2 of the MINI and MINI Kids to be used.
2. This LOA provides approval of revised ACASI surveys for blocker youth and cross-sex hormone youth cohorts. Starting at the 6 Month study visit, the ACASIs include symptom questions that are not in the baseline ACASI. (No changes were made to the blocker parent cohort ACASI survey.)
3. This LOA made a minor revision to the F10 Medications Form. It provided more applicable examples and removed the mistaken reference to RDC. The Data Collections Form Schedule was also respectively updated.
4. This LOA provides for the use of Microsoft OneDrive for data transmission as a back-up method. It also provides for the use of Survey Monkey for data transmission of the data from the MINI and MINI Kid interviews.

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- Blocker Youth ACASI;
- Cross-Sex Hormone Youth ACASI;
- Abbreviated version of the MINI 7.0.2 – communicate to IRB that participant name will not be provided to coordinating center;
- Abbreviated version of the MINI KID 7.0.2 – communicate to IRB that participant name will not be provided to coordinating center; and
- Revised version of the F10 Medications Form and Data Collection Form Schedule (both dated 02-01-17), if required by your IRB.

The MINI 7.0.2, MINI KID 7.0.2, Blocker Cohort Youth 1.27.2017 EN Qx.pdf, and CSH Cohort 1.27.2017 EN Qx.pdf are available at Trans Youth Care\Measures\Version 2.2.

The revised F10 and Data Collection Schedule are available at Trans Youth Care\TYC CRFs & Source Docs\TYC CRFs\Version 2.2.

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: June 13, 2017

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #7: Trans Youth Care Protocol Version 2.3, dated 5/12/17

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #7 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the distribution of version 2.3 of the protocol with a date of 5/12/17. Version 2.3 incorporates all previously distributed amendments. It also includes a revised version of the computerized script to include measures related to chest dysphoria and chest surgery; it expands the eligibility criteria for the blocker cohort to include Tanner 4 youth; it changes the mechanism for collecting MINI data to REDCap; and it removed Scott Leibowitz as Co-Investigator.

SUMMARY OF CHANGES

1. This LOA provides approval for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth, Version 2.3, dated 5/12/17.
2. Version 2.3 of the protocol incorporates Letters of Amendments 1 – 6.
3. Version 2.3 of the protocol removed Scott Leibowitz as Co-Investigator at Lurie Children's Hospital as he is no longer with the institution.
4. Version 2.3 of the protocol added Tanner stage 4 youth as eligible for the blocker cohort to expand the cohort to include all youth going onto a blocker the opportunity to participate in the study.
5. Version 2.3 of the protocol added the use of Research Electronic Data Capture (REDCap), an online database, for the electronic transmission of the M.I.N.I. and M.I.N.I. Kid data.
6. Version 2.3 of the protocol updated the blocker youth parent ACASI script to include the collection of race and ethnicity data from the parents of the blocker

youth. This is required for NIH reporting purposes as the parents are research subjects. The programming of the ACASI script was revised to allow age responses greater than 20 due to aging of the youth subject population.

7. Version 2.3 of the protocol updated the cross-sex hormone youth ACASI script to include surgery and chest dysphoria items. The programming of the ACASI script was revised to allow age responses greater than 20 due to aging of the youth subject population.
8. Version 2.3 of the protocol made a minor revision to the F8 Cross-Sex Hormones Lab CRF, revision 6, dated 5/8/17. It provided clarification that a laboratory test was not done or not needed. The Data Collections Form Schedule was also respectively updated (revision 9, dated 5/8/17).

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- Version 2.3, dated 5/12/17, of the protocol;
- Blocker Parent Cohort ACASI: Blocker Parent Cohort Baseline_6MO v2_w.race 2017 - agelimit revised 6.7.2017 En Qx.pdf
- CSH Youth Cohort ACASI: CSH Cohort 6-MONTH Follow Up v2 revised 3.22.2017 - agelimit revised 6.5.2017 En Qx.pdf
- If required by your IRB, revised versions of the:
    - F8 Cross-Sex Hormones Lab CRF, revision 6, dated 5/8/17, and
    - Data Collections Form Schedule, revision 9, dated 5/8/17.

Version 2.3 of the protocol is available at Trans Youth Care\TYC Protocol\Version 2.3.

The ACASI scripts are available at Trans Youth Care\Measures\Version 2.3.

The revised F8 and Data Collection Schedule are available at Trans Youth Care\TYC CRFs & Source Docs\TYC CRFs\Version 2.3.

Please note: The Blocker Youth Cohort ACASI script has not changed since Amendment #6. It may be titled: Blocker Cohort Youth 6-MONTH Follow Up_1.27.2017 EN Qx.pdf or Blocker Cohort Youth 1.27.2017 EN Qx.pdf. Either file is correct; they just have different file names.

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: September 15, 2017

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: TYC, v. 2.3, dated 6/13/17: Letter of Amendment #8: Tanner Stage 4 on Pre-Screener

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #7 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued to update the Blocker Cohort Pre-Screening Form to include Tanner Stage 4 as eligible.

SUMMARY OF CHANGES

1. This LOA provides approval for a revised version of F1 Blocker Cohort Pre-Screening Worksheet R3 09-12-17 to include Tanner Stage 4 youth as eligible per protocol version 2.3.
2. The Data Collections Form Schedule was also respectively updated.

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- Revised version of the F1 Blocker Cohort Pre-Screening Worksheet R3 09-12-17 and the updated Data Collection Forms Schedule R10 09-15-17, if required by your IRB.

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: November 8, 2017

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: TYC, v. 2.3, dated 6/13/17: Letter of Amendment #9: Addition of Life Events Scale and MINI/MINI Kid Revision

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The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #9 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued to include the Life Event Scale in the Blocker Youth and Cross-Sex Hormone ACASIs and to update the M.I.N.I. and M.I.N.I. Kid to add Refuse to Answer and Can't Determine options. In addition, the page numbering in the M.I.N.I. and M.I.N.I. Kid were corrected.

SUMMARY OF CHANGES

1. The Life Event Scale has been added to the ACASIs for the Blocker Youth and the Cross-Sex Hormone Youth. The Blocker Parents will not complete the scale. The scale will be completed at the 12 and 24 month data collection points.
2. The M.I.N.I. and the M.I.N.I. Kid were updated to instruct the study coordinators to document on the instrument when the participant refuses to answer a question and to circle Can't Determine when the study coordinator is unable to determine if a skip pattern should be followed or if a diagnosis should be indicated because the participant refused to answer previous questions.
3. The page numbering in the M.I.N.I. and M.I.N.I. Kid were revised to be sequential. Previously the numbers skipped based on the numbers of pages that had been removed from the documents.

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- Blocker Cohort Youth Revised 10.27.17 EN Qx
- CSH Cohort Revised 10.27.17 En Qx
- MINI 7.0.2 Standard – Abbreviated 10-19-17
- MINI Kid 7.0.2 Standard – Abbreviated 10-20-17

This LoA does not have any effect on subject risk and does not require a change to the informed consent document.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

May 19, 2018, 04:10pm

To: Johanna Olson-Kennedy, M.D.
ADOLESCENT MEDICINE - CHLA
Julie McAvoy-Banerjea, M.P.H.
ADOLESCENT MEDICINE - CHLA

From: Moore Rhys, C.I.P.
Chair Designee, Children's Hospital Los Angeles Institutional Review Board

Re: CHLA-16-00108 Johanna Olson-Kennedy, M.D.

The Impact of Early Medical Treatment in Transgender Youth

NOTICE OF IRB APPROVAL OF AMENDMENT
(Reference: TYC - Development of Questions re: Participant Experience - CHLA-16-00108-AM017)

Valid from: 5/19/2018 Expires: 3/12/2019

Document(s) Reviewed: • iStar study application and amendment AM017 (dated 5/9/18)

The amendment was reviewed by a member of the CHLA IRB. The amendment is now approved per 45 CFR 46.110[b][2].

This is an auto-generated email. Please do not respond directly to this message using the "reply" address. A response sent in this manner cannot be answered. If you have further questions, please contact iStar Support at (323) 276-2238 or istar@usc.edu.

USC

Date: Friday, August 12, 2022 1:59:33 PM Print Close

iStar ID: APP-16-00076 Application Version Date:5/9/2018 **APP-18-02674** AM15 - Other
Version:18.0

Study: Trans Youth Care **(APP-18-02674)**

AM15. Other

This screen is required if you indicated this amendment involves other changes not included on the previous screens (Question AM1.2.)

AM15. Please describe each of the changes you wish to make to the previously approved study and provide a rationale for the change:
Under the guidance of the principal investigators and co-investigators, three study coordinators (Boston Children's Hospital, CHLA, and UCSF) are creating a sub-study to investigate participants' perceptions of their involvement in this research study. Utilizing an analysis of which questions are most often refused to be answered by participants in addition to validated research involvement measures, they are creating additional questions that will be incorporated into the 24-month survey to obtain feedback from participants. Once the questions have been finalized, the revised survey will be submitted to all IRBs for review and approval. With this additional data, the study coordinators will be able to disseminate information to researchers about the experience of youth who identify as transgender and gender nonconforming of participating in a research study focused on their gender identity.

As part of the process of developing these additional sub-study questions, the study coordinators would like to obtain input from the transgender and gender nonconforming community regarding the questions and the response choices. This will ensure that the sub-study fully incorporates community participation. They will approach community advisory board participants and transgender and gender nonconforming experts to review the questions and answer choices and provide feedback on the survey.

You must make your changes in the body of the main study application.

Trans Youth Care Letter of Amendment

DATE: July 10, 2018

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: TYC, v. 2.3, dated 6/13/17: Letter of Amendment #11: Increase in Study Sample Size, Revised ACASI Survey, & Revised CSH Lab CRF

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #11 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued to increase the study sample size, revise the ACASI survey script, and revise the F8 Cross-Sex Hormones Lab CRF.

SUMMARY OF CHANGES

1. The sample size has been increased as follows to increase the representation of ethnic and racial minority youth.
    a. Blocker cohort youth: from 88 to 110 participants
    b. Blocker cohort parents/LARs: from 88 to 110 participants
    c. Cross-sex hormone cohort: from 300 to 350 participants
    d. Total sample size: from 476 to 570 participants
2. ACASI scripts revised to change bi-gendered to bi-gender for all cohorts and the following cohort specific changes:
    a. Blocker youth & cross-sex hormone participant surveys - moved the question asking the participant's sex assigned at birth to the beginning of the survey so that it would be completed by the study coordinator when they are answering the survey set-up questions. We did this due to feedback from participants that asking that question in the survey was upsetting.
    b. Blocker parent & cross-sex hormone participant surveys - modified the race question to check all that apply; this was an error in the initial programming of the survey & participants may identify as more than one race
    c. Blocker parent survey - added a question asking the parent's gender; this provides demographic data re: the study parent/caregiver participants

3. Revised the CSH Lab CRF to add an extra digit field for the white blood cell count.

GUIDANCE FOR SITES

Submit the following to your local IRB within 30 days:

- This Letter of Amendment #11
- Blocker Cohort Youth_Revised 7.09.2018 En Script
- Blocker Parent Cohort Revised 7.09.2018 En Script
- CSH Cohort_Revised 7.09.2018 En Script
- F8 Cross-Sex Hormones Lab CRF R7 07-05-2018

This LoA does not have any effect on subject risk; however, your IRB may require a change to your local informed assent, consent, or permission document to indicate the increase in study participants.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: January 29, 2019

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #12: Trans Youth Care Protocol Version 2.4, dated 1/8/19

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #12 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the distribution of version 2.4 of the protocol with a date of 1/8/19. Version 2.4 incorporates all previously distributed amendments. It also includes adding questions to assess GAH participants' perceptions of participating in the study and some gender specific measures; adding questions to obtain information about barriers encountered in accessing a blocker or GAHs; revising language from cross-sex hormone to gender-affirming hormone; lifting the co-enrollment restriction; expanding the visit window from +/- 14 days +/- 28 days; adding a Community Participation section to the protocol; and other smaller and editorial changes.

SUMMARY OF CHANGES

1. This LOA provides approval for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth, Version 2.4, dated 1/8/19.
2. Version 2.4 of the protocol incorporates Letters of Amendments 7 – 11.
3. Version 2.4 renames the Cross-Sex Hormone Cohort as the Gender-Affirming Hormone Cohort, and all language referencing cross-sex hormones was changed to gender-affirming hormones, including CRFs.
4. Version 2.4 corrects the contact information for Marco Hidalgo and updates the NICHD Health Science Administrator information.
5. Version 2.4 increases the length of the study recruitment period from 2 to 3 years.
6. Version 2.4 changes the administration of the MINI vs. the MINI Kid to be based

on the age of the participant at the visit rather than the participant's age at baseline. This also expands the use of the MINI to the Blocker Cohort, if the age requirement is met. The ages are 17 and younger for the MINI Kid and 18 and older for the MINI.

7. Version 2.4, per LOA #11, increases sample sized from 88 blocker youth and 88 parents/caregivers/legal guardians to 110 of each, and 300 gender-affirming hormone participants to 350 participants. Total sample size increases from 460 youth and 110 parents/caregivers/legal guardians. The protocol revision provides the opportunity to recruit additional participants to increase diversity in ethnicity, race, or gender.
8. Version 2.4 eliminates any co-enrollment prohibitions.
9. Version 2.4 clarifies that it is ideal that the same parent/caregiver/legal guardian is the adult participant for the blocker cohort through the study, but it is no longer required.
10. Version 2.4 removes the UGDS from the blocker youth assessments.
11. Version 2.4 removes the descriptors "(for those youth 8-11 years)" for the BDI-Y and the "(8-10.5)" for the Body Esteem Scale for the blocker youth assessments.
12. Version 2.4 lists the inadvertently missing "Self-Harm" measure to the blocker youth, parent/guardian, and GAH assessments.
13. Version 2.4 adds gender and clarifies that it's the biological parents' height being requested in the demographics section of the parent/guardian assessments.
14. Version 2.4 adds gender to the demographics section of the GAH assessments.
15. Version 2.4 removes the GIGDQAA and UGDS from the GAH assessments, except at the month 24 visit.
16. Version 2.4 expands the visit window from +/- 14 days to +/- 28 days.
17. Version 2.4 adds questions about parents'/guardians' perceptions about barriers to accessing a puberty blocker for their child at the month 6 and 24 assessments.
18. Version 2.4 adds the modified Adolescent Life-Change Event Scale to the month 12 and 24 assessments for parents/guardians and GAH participants.
19. Version 2.4 adds questions about GAH participants' experience taking part in the study, including questions about some of the measures that were used.
20. Version 2.4 adds questions about causes of delay for GAH participants in accessing gender-affirming hormones.
21. Version 2.4 provides clarification re: when to complete Monitoring Untoward Event Forms.
22. Version 2.4 adds a section 11.9 re: Community Participation.
23. Version 2.4 updates Appendix II: Measures per the revisions to the measures.
24. Version 2.4 editorial changes for ease of reading or grammar purposes.

GUIDANCE FOR SITES

Submit the following to your IRB within 30 days:

- Version 2.4, dated 1/8/19, of the protocol;
- Blocker Parent Cohort ACASI: Blocker Parent Cohort Revised 12.11.2018 Codebook.pdf
- Blocker Youth Cohort ACASI: GAH Cohort Revised 12.28.2018 Codebook.pdf
- CSH Youth Cohort ACASI: GAH Cohort Revised 12.28.2018 Codebook.pdf

- If required by your IRB, revised versions of the:
    - Data Collections Form Schedule, revision 11, dated 1/22/19,
    - F2 Gender-Affirming Hormone Cohort Pre-Screening Worksheet R4 01-22-19,
    - F8 Gender-Affirming Hormone Lab CRF R8 1-22-19, and
    - F13 Breast & Ferriman-Gallwey Measurements CRF R1 1-22-19.

Version 2.4 of the protocol is available at Trans Youth Care\TYC Protocol\Version 2.4.

The ACASI scripts are available at Trans Youth Care\Measures\Version 2.4.

The revised Data Collection Schedule, F8, and F13 are available at Trans Youth Care\TYC CRFs & Source Docs\TYC CRFs\Version 2.4.

The revised F2 worksheet is available at Trans Youth Care\TYC CRFs & Source Docs\TYC Source Docs\Version 2.4.

The CHLA IRB determined that the following consent/assent/permission forms be revised:

- Blocker consent/permission/assent,
- Consent addendum for subjects turning 18,
- Gender-affirming assent,
- Gender affirming consent/permission/assent, and
- Gender-affirming hormone cohort participant perceptions addendum (new required form).

Samples are available at Trans Youth Care\Sample Informed Consents-Assents-Permission Forms.

Once approved by your local IRB, please send documentation of the approval to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: May 21, 2019

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #13: Trans Youth Care Protocol Version 2.5, dated 5/16/19

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #13 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the distribution of version 2.5 of the protocol with a date of 5/16/19. Version 2.5 revises sections 4.8 and 5.1 of the protocol. The CHLA Coordinating Center IRB is NOT requiring that the parent/caregiver be re-consented for their own participation in the study.

SUMMARY OF CHANGES

1. This LOA provides approval for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth, Version 2.5, dated 5/16/19.
2. Version 2.5 of the protocol includes revised language in sections 4.8 and 5.1 to more clearly indicate that the parents/caregivers of blocker participants are enrolling in the study as participants themselves.
3. Version 2.5 updates Appendices VI through IX and adds Appendix X (sample informed consent/assent/permission forms) to include a parent/caregiver consent form for their own study participation. In addition, the parent/LAR permission form no longer serves as a parent/caregiver consent form; it only serves as a parent/LAR permission form for the child to participate and a child assent form if the child is 14 years or older.
4. Version 2.5 provides guidance that when a minor study participant turns 18, they must sign as an adult a consent form or consent addendum at their first study visit after they turn 18.
5. Version 2.5 includes editorial changes to the Table of Contents to update page numbering.

6. Version 2.5 includes an editorial change to Appendix II, Measures due to a missing “for”.

GUIDANCE FOR SITES

Submit Version 2.5, dated 5/16/19, of the protocol to your IRB within 30 days.

If your site does not have a separate consent form for the parent/caregiver of the blocker participant, submit the following to your IRB:

- A consent form for the parent/caregiver participant;
- A parent/LAR permission form for the parent/LAR to sign to provide permission the blocker child to participate.
- The parent/caregiver consent form may no longer be combined with the assent/parent permission form for the blocker child.

Once approved, these approved forms **MUST** be submitted to the CHLA Coordinating Center.

Version 2.5 of the protocol is available at Trans Youth Care\TYC Protocol\Version 2.5.

Once approved by your local IRB, please send documentation of the approval and the approved consent and assent/parent permission forms to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: June 5, 2020

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #14: Trans Youth Care Protocol Version 3.0, dated 5/13/20

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #14 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the distribution of version 3.0 of the protocol with a date of 5/13/20. Version 2.5 revises sections 4.8 and 5.1 of the protocol. The CHLA Coordinating Center IRB is NOT requiring that the parent/caregiver be re-consented for their own participation in the study.

SUMMARY OF CHANGES

1. This LOA provides approval for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth, Version 3.0, dated 5/13/20.
2. Version 3.0 includes numerous revisions:
    a. Copy edits for readability and understanding
    b. Editorial changes to the Table of Contents to update page numbering
    c. Updates to the Protocol Team Roster
    d. Removal of references to ACASI and addition of REDCap for surveys
    e. Inclusion of the option for remote visits
    f. Extension of the study period to 9 years at annual timepoints following year 2
    g. Extension of enrollment period to permit reenrollment of previous participants, and that the PID will remain the same
    h. Addition of the adult version of the MINI for the GnRHa cohort participants who are 18 or older.
    i. Inclusion of HIPAA-compliant teleconferences

j. Inclusion of remote consent conferences through HIPAA-compliant teleconferences and REDCap & guidance around privacy requirements
k. Clarify that enrollment may be restricted within a cohort due to underrepresentation of a specific gender.
l. Added "approximately" to the number of parent/caregiver participants due to the need for a replacement parent/caregiver to enroll in the study
m. Clarification that a new consent conference needs to occur when a replacement parent/caregiver enrolls in the study (updating protocol to recognize process already taking place)
n. Added COVID-19 survey that will occur with all study participants at a single point in time as soon as possible after site IRB approval and ongoing in all future timepoint surveys
o. Collapsed the annual visit into one visit, although participants can always terminate a visit and complete it on another day within 7 days
p. Added history of blocker experience to GAH cohort surveys
q. Clarification of where electronic survey and CRF data will be stored
r. Update re: the process for the flag for SI from the REDCap survey
s. Update re: SI response process for remote visits
t. Clarification on reporting of untoward events
u. Updates to Appendix I: Schedule of Evaluations
v. Updates to Appendix II: Measures

3. Version 3.0 updates Appendix IV: Locator Form
4. Version 3.0 updates Appendix V: Case Report Forms
5. Version 3.0 updates Appendices VI through IX and adds Appendix X (sample informed consent/assent/permission forms) to include new and revised information.

GUIDANCE FOR SITES

Submit Version 3.0, dated 5/13/20, of the protocol to your IRB within 30 days.

Update your site-specific consent, assent, and permission forms.

Once approved, these approved forms **MUST** be submitted to the CHLA Coordinating Center. They will be used for creating your REDCap electronic consent, assent, and permission forms.

Trans Youth Care Google Drive:

1. Clean and tracked changes of version 3.0 of the protocol are available at Trans Youth Care\TYC Protocol\Version 3.0.
2. CHLA IRB Approval Letter for version 3.0 is available at Trans Youth Care\CHLA IRB Approval Letters
3. IRB-approved measures are available at Trans Youth Care\Measures\Version 3.0
4. Sample informed consent, assent, and permission forms are available at Trans Youth Care\Sample Informed Consents-Assents-Permission Forms

5. IRB-approved CRFs and the Data Collection Forms Schedule are available at Trans Youth Care\TYD CRFs & Source Docs\TYC CRFs\Version 3.0
6. IRB-approved source docs are available at Trans Youth Care\TYD CRFs & Source Docs\TYC Source Docs\Version 3.0

Once approved by your local IRB, please send documentation of the approval and the approved consent and assent/parent permission forms to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: July 2, 2020

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #15: Trans Youth Care Protocol Version 3.0, dated 5/13/20

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #15 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the revision of the COVID-19 questions to make them more applicable to an adolescent and young adult population.

SUMMARY OF CHANGES

1. This LOA provides approval for the revision of the COVID-19 questions to make them more applicable to the adolescent and young adult population.
2. The measures documents that changed were:
    a. Impact of COVID19 Youth Point in Time Survey 6-5-20
    b. Impact of COVID19 for Parents Point in Time Survey 6-5-20
    c. TYC Blocker Parent Survey 6-5-20
    d. TYC Blocker Youth Survey 6-5-20
    e. TYC GAH Survey 6-5-20

GUIDANCE FOR SITES

Submit the revised versions of the survey measures to your IRB within 30 days.

Trans Youth Care Google Drive – the IRB-approved measures are available at Trans Youth Care\Measures\Version 3.0

Once approved by your local IRB, please send documentation of the approval of the revised COVID-19 measures to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: December 10, 2020

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #16: Trans Youth Care Protocol Version 3.0, dated 5/13/20

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #16 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the removal of the one-off episode of the COVID-19 questions & the related compensation.

SUMMARY OF CHANGES

1. This LOA provides for the removal of the one-time episode of COVID-19 questions. They will remain in the regularly scheduled visits.
2. Remove the $10 compensation for the one-time episode of COVID-19 questions since the survey is being removed.
3. For CHLA (may not be applicable to other sites): corrected the CHLA consent, assent, and permission forms to align with the revised CHLA templates/language; and state that the participant feedback survey is at the year 2 visit, not the last visit. In addition, IRB-approval was obtained to use DocuSign for documenting consent, assent, and permission.

GUIDANCE FOR SITES

Clean and tracked changes sample copies of the CHLA consent, assent, and permission forms are available in the Google Drive (Trans Youth Care > Sample Informed Consents-Assents-Permission Forms).

If sites would like to use the CHLA DocuSign system, please provide PDF, stamped versions of your site's consent, assent, and permission forms to Julie. Please note that you may want to

revise your consent, assent, and permission forms to collect participant identifiers (e.g., name & DOB) and the required signature lines. Please see the CHLA forms as examples.

Once approved by your local IRB, please send documentation of the approval of the revised COVID-19 measures to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-361-5023.

Trans Youth Care Letter of Amendment

DATE: April 2, 2021

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #17: Trans Youth Care Protocol Version 4.0, dated 03/03/21

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #17 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the approval of version 4.0 of the TYC protocol, dated 03/03/21.

SUMMARY OF CHANGES

1. Informed Consent Templates were revised as follows. Please remember that individual site templates may differ from the CHLA template.
    a. Revised key information section per updated CHLA institutional templates
    b. Revised language re: transgender or gender non-conforming children to state "transgender or gender diverse" to reflect updated language
    c. Updated Number of Participants sections to reflect increase in expected enrollment numbers
    d. Added additional items that we will be collecting per the new version of the survey
    e. Revised the MINI/MINI Kid interview from annual to every 2 years
    f. Revised Costs to You for Being in this Study section and Compensation sections to reflect that we will no longer be providing reimbursement for travel and parking costs
    g. Revised the Compensation section to pay only $60 for the annual visits that do not have a MINI being conducted
    h. Corrected the response to the first question assessing understanding of the study by deleting the word "being"

i. Changed tick boxes to lines to avoid formatting errors when iStar converts the Word documents to PDFs so that it won't create small circles for checking

2. Number of Participants Across All Sites
    a. 400 gender-affirming hormone youth
    b. 120 puberty blocker youth
    c. 135 puberty blocker parents/caregivers
3. Procedures and/or Protocol
    a. Updated the Protocol Team Roster
    b. Updated the number of participants throughout the protocol
    c. Revised the protocol aims per the new grant funding
    d. Clarified electronic storage of documents due to safer at home & no longer require the completion of paper CRFs
    e. Created a PID numbering system for replacement parent/caregiver participants
    f. Added Year 3-9 measure sections for each cohort (please see bullets below)
    g. Changed the frequency of conducting the MINI or MINI Kid to once every 2 years instead of annually
    h. Expanded data abstraction so that is similar for blocker cohort participants and gender-affirming hormone participants because many of the blocker participants are starting gender-affirming hormones
    i. Added Microsoft SharePoint as a data transfer or storage location
    j. Deleted Table of Measures in Appendix due to multiple changes over time occurring; table will be stored locally rather than in the protocol
    k. Because the youth participants are aging, we needed to revise the Year 3-9 surveys so that they would be better suited for the age of the participants. In addition, we removed some measures that either were not found to be of use during analysis or because a better measure was found. We also added measures (some of which we modified for transgender or gender-diverse youth) to replace less favorable measures. The Baseline – Year 2 surveys remain the same.
        i. Puberty Blocker Youth:
            1. Added:
                a. Steps to Transition Scale
                b. Transgender Congruence Scale
                c. Strang Gender Development Scale
                d. Child and Adolescent Trauma Screen
                e. Adverse Childhood Experiences Questionnaire (18+)
                f. Health-Related Quality of Life Scale
                g. Body Image Scale
                h. Perceived Stress Scale
                i. Gender Minority Stress & Resilience Scale for Adolescents (12-18) or Adults (18+)
                j. Deiner Flourishing Scale
                k. Carver Brief Cope Inventory
                l. Hemingway Measure of Adolescent Connectedness

m. Sexual Attraction and Romantic Partner
n. Sexual Risk Behavior Questions
o. STI Questions
p. Alcohol, Smoking and Substance Involvement Screening Test
q. Questions re: surgery
r. Hormone Use and Side Effects
s. ASEBA Youth Self-Report

2. Updated:
    a. Self-Harm Questions
3. Revisions based on age:
    a. BDI-II
    b. Adult Manifest Anxiety Scale (18+)
    c. NIH Toolbox Emotion Battery (18+)
    d. NIH Toolbox General Life Satisfaction/Positive Affect (13-17 or 18+)
    e. NIH Toolbox Self-Efficacy (18+)
    f. Autism-Spectrum Quotient (16+)
4. Deletions:
    a. UGDS
    b. GIGD
    c. Harter's Self-Perception Profiles for Adolescents & Children

ii. Puberty Blocker Parent/Caregiver
1. Additions:
    a. Steps to Transition Scale
    b. Strang Gender Development Scale
    c. PROMIS Parent Proxy Report Social Health (Peer Relationships/Family Relationships); Mental Health (Emotional Distress-Anger; Emotional Distress-Anxiety; Emotional Distress-Depressive Symptoms; Psychological Stress Experiences)
2. Updated:
    a. Self-Harm Questions
3. Revisions based on age:
    a. Autism Spectrum Quotient - adolescent version
4. Deleted:
    a. STS Social Transition
    b. DSM 5 questions re: gender dysphoria dx

iii. Gender-Affirming Youth
1. Added:
    a. Steps to Transition Scale
    b. Strang Gender Development Scale
    c. Child and Adolescent Trauma Screen

d. Adverse Childhood Experiences Questionnaire (18+)
e. Perceived Stress Scale
f. Deiner Flourishing Scale
g. Carver Brief COPE Inventory
h. Hemingway Measure of Adolescent Connectedness
i. Adolescent Life-Change Event Scale
j. Questions re: surgery

2. Revisions based on age:
    a. Adult Manifest Anxiety Scale (18+)
    b. NIH Toolbox Emotion Battery (18+) (Emotional Support, Instrumental Support, Loneliness, Friendship, Perceived Hostility, Perceived Rejection; Anger-Affect; Anger-Hostility; Anger-Physical Aggression; Fear-Affect; Fear-Somatic Arousal; Sadness)
    c. NIH Toolbox Positive Affect/General Life Satisfaction (18+)
    d. Gender Minority Stress & Resilience Scale for Adolescents Adults (18+)
    e. NIH Toolbox Self-Efficacy (18+)
    f. ASEBA Adult Self-Report
3. Updated:
    a. Self-Harm Questions
4. Deleted:
    a. DSM 5 questions re: gender dysphoria dx
    b. NIH Toolbox - Social Relationships

l. CRFs
    i. Revisions
        1. Data Collection Forms Schedule
        2. F13 Breast & Ferriman-Gallwey Measurements CRF R3 2-24-21
    ii. Added
        1. F16 Re-Enrollment Screening Log 2-23-21 – ***this is a new Excel tracking sheet that study coordinators should record all participants contacted to continue or re-enroll in the study after the Year 2 visit***
        2. F18 LAB CRF 2-23-21 – ***this is a single lab CRF that is to be used for both the blocker and GAH cohort participants***
    iii. Deleted
        1. F7 Blocker Lab CRF R4 05-04-20
        2. F8 Gender-Affirming Hormone Lab CRF R9 05-07-20

GUIDANCE FOR SITES

Clean and tracked changes sample copies of the CHLA consent, assent, and permission forms are available in the Google Drive (Trans Youth Care > Sample Informed Consents-Assents-Permission Forms). It is suggested that you edit your informed consent, assent, and permission forms so that they work smoothly in DocuSign. This may mean adding extra blank lines where

information such as names and signatures need to be added and having lines for X marks to select a Yes or No option with initialing being separate (rather than simply initialing next to the Yes or No option). Please see the CHLA forms as examples.

Participant reimbursement and compensation is determined at the individual site level. At CHLA, we removed compensation for travel or parking reimbursement due to reduced NICHD funding and because visits may now be completed from home. We also reduced compensation for year 2, 4, 6, and 8 visits to $60 due to removal of MINI or MINI Kid from study activities. The total compensation amount was reduced to $900.

Participant reimbursement and compensation is decided on an individual site level. At CHLA we Removed compensation for travel or parking reimbursement due to reduced NICHD funding and because visits may now be completed from home. Reduced compensation for year 2, 4, 6, and 8 visits to $60 due to removal of MINI or MINI Kid from study activities. Reduced total compensation amount to $900.

Once approved by your local IRB, please send documentation of the approval of the revised COVID-19 measures to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-709-9920 (cell) or 323-361-5023 (office).

Trans Youth Care Letter of Amendment

DATE: June 29, 2021

TO: TYC Site Principal Investigators and Study Coordinators

FROM: Trans Youth Care Coordinating Center

SUBJECT: Letter of Amendment #18: Trans Youth Care Protocol Version 5.0, dated 05/11/21

The following information impacts the Trans Youth Care study and must be submitted to your local Institutional Review Board (IRB) within the next 30 calendar days.

Maintain this letter and other related IRB correspondence in your Trans Youth Care regulatory files.

A copy of your IRB's approval of this letter of amendment must be submitted to the CHLA Coordinating Center.

This serves as Letter of Amendment (LoA) #18 for Trans Youth Care: The Impact of Early Medical Treatment in Transgender Youth. It is being issued due to the approval of version 5.0 of the TYC protocol, dated 05/11/21.

SUMMARY OF CHANGES

1. Editorial - Minor editorial changes to clarify study procedures; Table of Contents updated
2. Funding - Uploaded the NICHD Notice of Award to continue the study through 1/31/26
3. Informed Consent Templates were revised as follows. Please remember that individual site templates may differ from the CHLA template.
    a. Blocker assent
        i. added "how you act with your friends and family," and "how you behave with other people or in different situations" to the procedures to clarify that we're also asking about behaviors.
    b. Blocker Consent for Parent-Caregiver
        i. added information about obtaining information re: the impact of gender-affirming hormones on child participants as they get older to make it clear that we would like participants to continue in the study even if they are no longer on a blocker.
        ii. changed MINI/MINI Kid so it will also be conducted at the year 1 visit and every other year starting with year 2 in the text and the schedule of study procedures

c. Blocker Consent Permission Assent
    i. added "how you behave in different situations or with different people" to the procedures to clarify that we're also asking about behaviors
    ii. made "survey" into "surveys" since there is more than one survey
    iii. added "Answer a survey about your behaviors and physical and mental health at the baseline and every year. This will take about 30 minutes." to separate the REDCap survey from the ASEBA survey
    iv. changed MINI/MINI Kid so it will also be conducted at the year 1 visit and every other year starting with year 2 in the text, compensation, and the schedule of study procedures
    v. changed the compensation to $940 since the MINI will be done at the year 1 visit, which is an additional $40
d. Gender-Affirming Hormone Assent
    i. changed the length of time to 2-3 hours because this bullet also includes the YSR survey time (it's not split out separately)
    ii. included "your behaviors and your physical and mental health" to broaden the items covered by the surveys
e. Gender-Affirming Hormone Consent/Permission/Assent
    i. added, "We will do this by asking you complete surveys and interviews" to the Purpose section to make the connection between the surveys/interviews and the purpose clearer.
    ii. removed "additional" as it's not needed
    iii. changed MINI/MINI Kid so it will also be conducted at the year 1 visit and every other year starting with year 2 in the text, compensation, and the schedule of study procedures
    iv. changed the compensation to $940 since the MINI will be done at the year 1 visit, which is an additional $40
f. All ICFs - changed consent comprehension question #4 from "come in for" to "do" a study visit to make it clear that participants do not need to come to the clinic to complete a study visit.

4. Procedures and/or Protocol
    a. Created version 5 of the protocol, with a date of 5/11/2021
    b. Updated the Protocol Team information.
    c. There was an oversight in the previous amendment. While the Gender Development Scale was listed in the protocol as an additional measure, it was not included in the Blocker Youth and GAH surveys that were uploaded to item 21 in iStar. The individual Gender Development Scale can be provided as a separate document upon request.
    d. We have revised the questions about starting, stopping, and length of time using gender-affirming hormones in blocker youth and GAH youth/young adult surveys so that we can better collect data on GAH use and the reasons behind its use or discontinuation.
    e. We have revised the timeline for the MINI/MINI Kid. Participants will also complete the MINI or MINI Kid at the Year 1 visit.

f. Clarified the protocol so that the Blocker Participants could complete the ASR as of the Year 2 visit if they are 18 years old.
g. Removed any ordering requirements for the REDCap survey, ASEBA surveys, and MINI or MINI Kid.

5. Subject Reimbursement/Compensation
    i. Revised the youth/young adult subject compensation to a total of $940 because the Year 1 visit will be compensated at $100 due to completion of the MINI or MINI Kid.
6. Other
    a. Updated the CoC to 1/31/26, the end date for this new funding period.

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The CHLA IRB determined that the CHLA site will re-consent all participants prior to their next study visit.

Once approved by your local IRB, please send documentation of the approval of the revised COVID-19 measures to transyouthcare@chla.usc.edu.

For questions regarding this LoA, please contact Julie McAvoy-Banerjea, Clinical Research Manager at jmcavoy@chla.usc.edu or 323-709-9920 (cell) or 323-361-5023 (office).