Study Protocol Cover page

Title: Effectiveness, safety, and tolerability of different estradiol dosing regimens in transgender females.

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- **Objectives**

The purpose of this open label, pilot, randomized clinical trial is to evaluate the effectiveness, safety and tolerability of estrogen use in transgender female and the degree of testosterone suppression achieved in this population when placed on sublingual 17-beta estradiol in comparison to transdermal 17-beta estradiol for gender affirming pharmacological therapy over the first two-years of treatment.

This study is important because, to our knowledge, this is the first randomized clinical trial evaluating the effectiveness, safety, and tolerability of estrogen in transgender female. There are no previous data comparing the different presentation of estrogen options in transgender women; therefore, this clinical trial will determine the best strategies and methods required to develop a larger randomized clinical trial. This study will also provide information useful to close the medical knowledge gap to provide safe and competent care to transgender female patients.

Aims

Aim 1. In this randomized clinical trial, we will evaluate the degree of testosterone suppression by measuring total testosterone level in transgender female patients undergoing hormonal affirming therapy randomized to sublingual 17 beta estradiol or transdermal 17 beta estradiol, in conjunction with spironolactone (antiandrogen), for a period of 2 years.
Hypothesis 1a. Transgender females undergoing hormonal affirming therapy randomized to sublingual estradiol with multiple daily doses will have higher degree of testosterone suppression. This hypothesis is based on the rationale that higher peak levels obtained with sublingual estradiol will suppress the hypothalamic-pituitary axis to a greater degree than the more stable levels achieved with transdermal delivery. Multiple studies (15) (18) in cisgender women describe the daily biological rhythm of estradiol, and a diurnal cycle of estradiol exhibiting an early morning peak followed by two to four recurrent cycles. However, there is no information on transgender patients regarding best pharmacological intervention (continuous vs pulsatile) to suppress the hypothalamic-pituitary-gonadal axis.

Aim 2. In this randomized clinical trial, we will determine the impact on liver enzymes, metabolic profile, and thrombogenic factors in transgender female patients undergoing hormonal affirming therapy randomized to sublingual 17 beta estradiol or transdermal 17 beta estradiol, in conjunction with spironolactone (an antiandrogen), for a period of 2 years.
Hypothesis 2a. Transgender female patients on sublingual estradiol will have higher levels of estrone, which will lead to an increase on thrombogenic factors and changes in metabolic factors. It has been determined that higher estrone levels in cisgender females, has been linked with a high incidence of venous thromboembolism, breast cancer, and endothelial inflammation. An explanation for the higher thrombotic risk in postmenopausal women includes high production of thrombin due to high levels of estrone (12). Hypothesis 2b. Higher fat mass after FHT will have a negative impact on insulin sensitivity. Estradiol therapy in transgender women appears to be associated with an increase in fat mass and decrease in lean mass, although prospectively controlled data is limited (11). The increase in body fat associated with estrogen therapy raises questions about the effect on insulin sensitivity and cardiovascular risk.

Purpose, Background, and Rationale

- **Background and Significance**

Transgender individuals experience discordance between their self-identified gender and biological birth sex (1). Some transgender individuals experience gender dysphoria which the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) defines as clinically significant distress or impairment in social, occupational, or

other key areas of functioning. Increasing numbers of adolescents seek care at gender identity centers in Western Countries (3).

Studies using short self-reports of gender identity and its variants suggest that 0.17%-1.3% of adolescents and young adults identify as transgender (4). Even using the conservative estimate of 0.3%, the number of people living in the United States who identify as transgender is nearly 1 million. Health care for this population has historically been, and continues to be, overlooked by governmental, health care, and academic establishments (16). This has led to a wide range of health disparities and poor health outcomes compared with their cisgender counterparts (17). Given the widespread acknowledgment of the health care needs of transgender people, priority should be given to those actions that will ensure timely access to appropriate care. Such action includes, first and foremost, coverage of transition care and a requirement to ensure safe, appropriate, and sensitive care in health centers (16) (17).

Transgender women seek feminizing hormone therapy (FHT) to reach feminine physical features consistent with their gender identity. The goal of FHT is to achieve feminine features and maintain estradiol levels within the normal female range while suppressing endogenous testosterone (5). Both the World Professional Association for Transgender Health (WPATH) and the Endocrine Society have created transgender-specific guidelines that serve as a framework for providers caring for gender minority patients. These guidelines are mostly based on clinical experience from experts in the field. Guidelines for estrogen therapy in transgender women are loosely based on treatments used for postmenopausal women (6). Therefore, stronger evidence is needed to guide clinicians on best clinical practices for this population.

The current approach to FHT is not uniform or FDA approved and depends on the health care system, cost considerations, and differences in the regional availability of estrogens and antiandrogens. A typical regimen includes estrogen to provide feminizing effects in conjunction with therapy to block testosterone (antiandrogens or gonadotropin-releasing hormone [GnRH] analogs). Estrogen also inhibits testosterone secretion (9)(13). *Ethinyl estradiol* was previously the mainstay of most estrogen-directed therapies; this is no longer the case due to its increased risk of cardiovascular death and increased incidence of deep venous thrombosis (7). 17-beta estradiol, which can be provided in tablet, patch, and injection, is currently the preferred formulation (8).

Androgen blockade is usually established in all transgender females undergoing feminizing therapy and is achieved by either using gonadotropin blockade or antiandrogens. The latter, defined as medications other than estradiol which are used to decrease the synthesis of or actions of androgens. In the United States, spironolactone is commonly prescribed. Cyproterone acetate (CPA) is not licensed for use in the United States; however, CPA appears to be favored in many European countries and forms standard care as part of the European Network for the Investigation of Gender Incongruence (ENIGI) treatment protocol (10).

- **Innovation**

Transgender female patients suffer from poor health outcomes compared with their cisgender peers, mostly because feminizing therapy with estrogen has been based on data from postmenopausal women and expert opinion. This represents a critical gap in knowledge that prevents the delivery of safe and appropriate care for the transgender population. This proposal is innovative because the project is aimed to study the tolerability, safety, and differences in testosterone suppression of the most common 17-beta estradiol presentation while using the same antiandrogen (spironolactone). Any differences detected between the groups will be attributed to the estrogen effect based on their route of administration. Moreover, this randomized clinical trial will allow the collection of valuable preliminary data to determine the best strategies and methods required to develop a larger randomized clinical trial. This knowledge will set the stage for future feasible and sustainable interventions for transgender youth to improve health related outcomes.

- **Methods, Research Plan, and Design**
- **Study design**

This study will be a prospective 2-year randomized, open label, clinical trial, using daily dose sublingual versus twice daily dose sublingual versus transdermal 17-beta estradiol in transgender female patients. Primary outcome is degree of testosterone suppression. Secondary outcome to measure safety and tolerability will

include liver enzymes, thrombogenic, and metabolic factors. Patients will be evaluated every 6 months during the length of the study

b. Subjects

Inclusion criteria: Female transgender patients between the ages of 18 to 45 years of age who are seen at the Washington University Transgender Center. Patients must have met the eligibility and readiness criteria for gender-affirming hormone therapy.

Exclusion criteria: Use of GnRH agonist for the last 12 months, history of liver disease, dyslipidemia requiring treatment, drug use, alcohol use (>2drinks per day), cigarette smoking, history of blood clot (venous thromboembolism, pulmonary embolism), history of hyperkalemia, history of Addison's disease, concomitant use of eplerenone.

Patients who have been referred to the Washington University Transgender Center for feminizing hormonal therapy and meet the inclusion criteria, will undergo consent process before initial evaluation. Methods for recruitment includes phone calls, emails, letters, and EPIC slicer dicer. For consent, physical or electronic consent form will be provided.

If consent is obtained, patients will be assigned to either daily sublingual vs twice daily sublingual vs transdermal 17-beta estradiol. Assignment will be done through block randomization.. Using the inclusion criteria, our center provides care to 150-200 new transgender female patients in a year for initiation of female hormone therapy.

We aim to enroll a total of 70 patients. This is a pilot study, which results will guide the feasibility, design, and implementation of a larger clinical trial. To account for 15% of potential dropout, we have planned to enroll a total of 80 patients.

- **Research Plan**

The proposed clinical trial involves 2 research aims to be conducted over two years (Table 1). Subjects will be recruited from the Washington University Transgender Center. The Center is projected to see more than 1,000 patients every year, of which 150-200 are new transgender female patients for initiation of FHT.

Patients who agree to participate in the study and meet inclusion criteria will undergo the evaluation described in Table 2. Data of interest will be collected on all patients enrolled and will be saved on a database created in Research Electronic Data Capture (REDCap). Information to be collected will include:

1. Demographic and anthropometric variables: Parameters will be routinely collected through chart review.

2. Laboratory testing: During each visit, we will obtain hormone levels, coagulation factors, metabolic factors, electrolytes level. A hematologist will review the lab results and communicate any recommended interventions (including stopping medication) to the participant's treatment team.

Initial evaluation will include patient history, physical examination, and baseline labs. During the initial visit, patients will be started on spironolactone and 17-beta estradiol transdermal or or daily sublingual or twice daily sublingual based on their treatment group assignment. Our team will check hormonal levels every 4 weeks (+/- 2 weeks) until estradiol level is within goal (see dosing guidelines) as established by standard of care. Once patients are started on estradiol, they will be asked to attend 6 more visits for the 2 years duration of the study as described in table 2.

Dosing guidelines:

Per the current standard of treatment, all study medications are initiated at the lowest available clinical dose and titrated to effectiveness at the study clinician's discretion by 6 months of study participation, using plasma total testosterone level in the cisgender female range (<50ng/dL) with serum estradiol level that should not excess the peak physiologic range 100-200 pg/mL. In some cases, medication doses will be titrated at monthly study follow up visits to optimize treatment response.

Spironolactone:
Starting dose will be 50 mg daily
Plan to increase 50 mg monthly to standard dose of 200 mg daily

For patients randomized to transdermal (patch) estradiol:

Starting dose will be 100 mcg/24hrs
Plan to increase by 100 mcg/24hrs every month
Max dose 400 mcg/24hrs
Goal is to achieve a serum estradiol level between 100-200 pg/mL and to suppress testosterone to cisgender female levels
Estradiol levels will be measured in the morning (fasting sample)

For patients randomized to daily sublingual estradiol:

Starting dose will be 2 mg daily
Plan to increase every month by 2 mg daily
Goal is to achieve serum estradiol level between 100-200 pg/mL mL and to suppress testosterone to cisgender female levels.
Estradiol levels will be measured in the morning (fasting sample).

For patients randomized to twice daily dose sublingual estradiol

Starting dose will be 1 mg twice daily
Plan to increase every month by 2 mg daily
Goal is to achieve serum estradiol level between 100-200 pg/mL mL and to suppress testosterone to cisgender female levels
Estradiol levels will be measured in the morning (fasting sample).

Table 1. Planned Research Timeline

Task	Year 1				Year 2				Year 3			
	1	2	3	4	1	2	3	4	1	2	3	4
Obtain IRB approval			•									
Database creation (REDCap)			•									
Recruitment			•	•	•							
Collection of blood samples			•	•	•	•	•	•	•	•	•	
Data entry			•	•	•	•	•	•	•	•	•	

Data cleaning and analysis									•	•	•	•
Grant application					•	•	•	•				
Final results manuscript										•	•	•
Presentation preparation										•	•	•

Table 2. Description of the evaluation and laboratory testing in each visit during the clinical trial

Study visit	Baseline	6 months +/- 2 months	12 months +/- 2 months	18 months +/- 2 months	24 months +/- 2 months
Anthropometric measurement	•	•	•	•	•
Metabolic factors	•	•	•	•	•
Hormone levels	•	•	•	•	•
Electrolytes	•	•	•	•	•
Liver function test	•	•	•	•	•
Coagulation factors	•	•	•	•	•
Lipid Panel	•		•		•

- **Benefit/Risk assessment**

i. Benefits

Direct benefits from this study to participants are anticipated. During the research study, their tolerability and side effects will be monitored closely so any changes can be detected earlier. Also, participants may feel gratified by contributing to the field. Patients will have the opportunity to help us improve services and treatment options for them and future generations.

ii. Risks

Potential risks with this study are no different than the risks that may occur while receiving gender affirming therapy. This may include changes in their lipoprotein profile and triglycerides level (14) and increase risk of venous thromboembolism, diabetes, high blood pressure, coronary heart disease, hepatotoxicity. While the patient is receiving gender affirming therapy, the investigators will provide careful attention to laboratory results.

Laboratory testing will be obtained every 3 months for the first year, and every 6 months on the second year. For this purpose, placement of an intravenous (IV) access may be required. IV access or venipuncture may be associated with possible minor discomfort or pain when inserted.

- **Statistical methods and Data Analysis**

Baseline characteristics and laboratory results will be reported as mean± SD, median (interquartile range), or percentages. Independent T-test will be used to analyze the difference between treatment groups. We may use Wilcoxon rank sum test in case of non-normal distribution.

g. Limitations and contingent plans

A potential limitation to this study is the difficulty of recruitment since the PI is a First Year Clinical Fellow with a heavy clinical rotation. Our project is also supported by the director of the Washington University Transgender Center which will improve the recruitment of patients. Also, the co-directors of the Washington University Transgender Center at St Louis Children's Hospital are supporting the project, therefore, we could increase our enrollment numbers by recruitment patients seen in their clinic. Another limitation is the cost of the entire panel of thrombogenic factors. We could address this by just testing the most valuable test (thrombin) which has been used in previous research to assess the thrombotic risk in postmenopausal women on hormone replacement therapy.

- **References**

- Unger CA. Hormone therapy for transgender patients. *Transl Androl Urol*. 2016;5(6):877-884. doi:10.21037/tau.2016.09.04
- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Washington, DC: American Psychiatric Press; 2013
- Kaltiala-Heino R, Bergman H, Työläjärvi M, Frisén L. Gender dysphoria in adolescence: current perspectives. *Adolesc Health Med Ther*. 2018;9:31-41. Published 2018 Mar 2. doi:10.2147/AHMT.S135432
- Connolly MD, Zervos MJ, Barone CJ 2nd, Johnson CC, Joseph CL. The Mental Health of Transgender Youth: Advances in Understanding. *J Adolesc Health*. 2016;59(5):489-495. doi:10.1016/j.jadohealth.2016.06.012
- Hamidi O, Davidge-Pitts CJ. Transfeminine Hormone Therapy. *Endocrinol Metab Clin North Am*. 2019;48(2):341-355. doi:10.1016/j.ecl.2019.02.001
- Unger CA. Hormone therapy for transgender patients. *Transl Androl Urol*. 2016;5(6):877-884. doi:10.21037/tau.2016.09.04
- Asscheman H, Giltay EJ, Megens JA, de Ronde WP, van Trotsenburg MA, Gooren LJ. A long-term follow-up study of mortality in transsexuals receiving treatment with cross-sex hormones. *Eur J Endocrinol*. 2011;164(4):635-642. doi:10.1530/EJE-10-1038
- Unger CA. Hormone therapy for transgender patients. *Transl Androl Urol*. 2016;5(6):877-884. doi:10.21037/tau.2016.09.04
- Hamidi O, Davidge-Pitts CJ. Transfeminine Hormone Therapy. *Endocrinol Metab Clin North Am*. 2019;48(2):341-355. doi:10.1016/j.ecl.2019.02.001
- Angus LM, Nolan BJ, Zajac JD, Cheung AS. A systematic review of antiandrogens and feminization in transgender women [published online ahead of print, 2020 Sep 14]. *Clin Endocrinol (Oxf)*. 2020;10.1111/cen.14329. doi:10.1111/cen.14329
- Spanos C, Bretherton I, Zajac JD, Cheung AS. Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: A systematic review. *World J Diabetes*. 2020;11(3):66-77. doi:10.4239/wjd.v11.i3.66
- Bagot CN, Marsh MS, Whitehead M, et al. The effect of estrone on thrombin generation may explain the different thrombotic risk between oral and transdermal hormone replacement therapy. *J Thromb Haemost*. 2010;8(8):1736-1744. doi:10.1111/j.1538-7836.2010.03953.x
- Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons: An Endocrine Society Clinical Practice Guideline [published correction appears in J Clin Endocrinol Metab. 2018 Feb 1;103(2):699] [published correction appears in J Clin Endocrinol Metab. 2018 Jul 1;103(7):2758-2759]. *J Clin Endocrinol Metab*. 2017;102(11):3869-3903. doi:10.1210/jc.2017-01658
- Bao AM, Liu RY, van Someren EJ, Hofman MA, Cao YX, Zhou JN. Diurnal rhythm of free estradiol

during the menstrual cycle. *Eur J Endocrinol*. 2003;148(2):227-232. doi:10.1530/eje.0.1480227

- Dutra E, Lee J, Torbati T, Garcia M, Merz CNB, Shufelt C. Cardiovascular implications of gender-affirming hormone treatment in the transgender population. *Maturitas*. 2019;129:45-49. doi:10.1016/j.maturitas.2019.08.010
- Neyman A, Fuqua JS, Eugster EA. Bicalutamide as an Androgen Blocker With Secondary Effect of Promoting Feminization in Male-to-Female Transgender Adolescents. *J Adolesc Health*. 2019;64(4):544-546. doi:10.1016/j.jadohealth.2018.10.296
- Stroumsa D. The state of transgender health care: policy, law, and medical frameworks. *Am J Public Health*. 2014;104(3):e31-e38. doi:10.2105/AJPH.2013.301789
- Butler KG. Relationship Between the Cortisol-Estradiol Phase Difference and Affect in Women. *J Circadian Rhythms*. 2018;16:3. Published 2018 Feb 21. doi:10.5334/jcr.154