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Acute Estradiol and Progesterone Therapy in Hospitalized Adults to Reduce COVID-19 Severity: A Randomized Control Trial

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ABSTRACT

Introduction: As of May 2021, COVID-19, has killed more than 3 million people globally, including over 580,000 in the United States (US). Apart from corticosteroids, most available therapeutic options are at best marginally efficient in reducing disease severity and are extremely expensive. The systematic investigation of clinically approved drugs is a priority to determine what does mitigate disease severity. Estradiol (E2) and progesterone (P4) produce a state of anti-inflammatory immune responses and immune tolerance, and enhanced antibody production. The goal of this trial is to evaluate the efficacy of a short E2 and P4 therapy, in addition to standard of care (SOC), in mitigating disease severity in COVID-19 hospitalized patients.

Methods and analysis: Phase 2, randomized, double blind, placebo-controlled, single center trial. Patients hospitalized for confirmed COVID-19, with scores 3 to 5 on the 9-point World Health Organization (WHO) ordinal scale are randomized between two arms: 1) Estradiol Cypionate (IM) and micronized Progesterone (PO), in addition to SOC, and 2) Placebo, in addition to SOC. The primary outcome is the proportion of patients improving to scores 1 or 2 on the WHO scale through day 28. Secondary outcomes include length of hospital stay, duration of mechanical ventilation, cause of death, readmission rates, change in inflammatory biomarkers between admission and occurrence of primary endpoint, and adverse events. Study sample size will be up to 120 participants. The trial is currently recruiting subjects.

Ethics and dissemination: The sponsor of this study is the Center of Excellence in Sex-Based Biology & Medicine at Tulane University, New Orleans, Louisiana, USA. Ethical approval was obtained from the Tulane institutional review board (IRB) on May 14st 2021. The study was reviewed by the US Food and Drug Administration and granted Investigational New Drug (IND) #152499.Results of the study will be submitted for publication in a peer-reviewed journal.

Clinical Trial Registration: NCT04865029, ClinicalTrials.gov

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. A randomized, double blind, placebo-controlled trial is an efficient way to determine if a short systemic 17β-estradiol (E2) and progesterone (P4) therapy, administered early to hospitalized COVID-19 patients in addition to SOC can reduce the severity of outcomes compared to SOC alone.
2. The trial will include men and women.
3. E2 and P4 produce a state of decreased innate immune cells production of proinflammatory cytokines, enhanced T cells anti-inflammatory responses and immune tolerance, and enhanced B-cell-mediated antibody production.
4. There is depth of knowledge accumulated from decades of clinical and basic studies regarding E2 and P4 efficacy and toxicity.
5. E2 and P4 are widely available in hospitals, inexpensive, manufacturable to scale, and can be prescribed immediately in most countries.

INTRODUCTION

As of May 2021, coronavirus diseases 2019 (COVID-19), has already killed over 3 million people worldwide.[1] Although the vaccination campaign is ramping up in the United States (US), vaccination hesitancy represents up to 25-30% of the population,[2] and hospitalizations and deaths are still high. In Brazil and India, the daily death toll is more than twice as high as it was in 2020.[1] Apart from corticosteroids,[3] most available therapeutic options are at best marginally efficient in reducing disease severity and mortality and are extremely expensive.[4-6] Therefore, the systematic investigation of clinically approved drugs is still a priority in order to determine what compounds could be repurposed to mitigate disease severity, and invest resources to go to full-scale production. Our current understanding of the disease is that COVID-19 deaths result from an inappropriate immune response with outpouring of pro-inflammatory chemokines leading to lung infiltration and hyperactivation of monocytes and macrophages producing pro-inflammatory cytokines (cytokine storm), resulting in lung edema, reduced gas exchange, and ultimately leading to acute respiratory distress syndrome and multiorgan failure.[7-14] Men with COVID-19 have a uniformly more severe outcome than women. In series from China, Europe and the U.S., COVID-19 mortality was consistently 1.5 to 2-fold higher in men than in women, suggesting that female biological sex is protecting women from COVID-19 mortality.[15-20] It is established that women exhibit heightened immune responses to viral infections compared to men,[21] which is at least partially due to the genetic benefit of gene dosage in X-linked immune-response genes. Ovarian steroids, however, also play a protective role. The analysis of electronic health records of over 68,000 COVID-19 patients revealed that estrogen therapy is associated with more than 50% reduction in mortality.[22] The main female steroids, 17β-estradiol (E2) and progesterone (P4) exhibit potent immuno-modulatory and anti-inflammatory actions via estrogen and progesterone receptors expressed in all immune cells, including epithelial cells, macrophages, dendritic cells, CD4+ and CD8+ lymphocytes, and B cells.[21, 23] P4 also acts partially via the glucocorticoid receptor. Together E2 and P4 produce a state of decreased innate immune cells production of proinflammatory cytokines, enhanced T cells anti-inflammatory responses and immune tolerance, and enhanced B-cell-mediated antibody production.[21, 24, 25] The scientific premise of

this trial is that in hospitalized COVID-19 patients, a short and early E2 and P4 therapy, in addition to standard of care (SOC; the National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel recommends the use of dexamethasone for up to 10 days or until hospital Discharge as SOC for the treatment of hospitalized COVID-19 patients), will prevent or mitigate the cytokine storm, and prevent severe outcomes, and without side effects.[25] Therefore, it will provide steroid immunomodulation without immunosuppression. The advantage of repurposing E2 and P4 is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from decades of clinical and basic studies. E2 and P4 are widely available in hospitals, inexpensive, manufacturable to scale, and can be prescribed immediately.

METHODS AND ANALYSIS

Study design, randomization, intervention and study calendar

Phase 2, randomized, double blind, placebo-controlled, single center trial designed to determine to what extent a short systemic E2 and P4 therapy, administered early to hospitalized COVID-19 positive patients of both sexes, in addition to SOC, can reduce the severity of symptoms and outcomes compared to SOC alone.

Hospitalized patients with COVID-19 with scores 3-5 on the 9-point World Health Organization (WHO) ordinal scale[26] are randomized to a 5-day treatment between two arms:

1. Estradiol Cypionate (Depo-Estradiol) 5mg via intramuscular injection on day 1 and micronized progesterone 200mg administered orally, daily for 5 days, in addition to SOC.
2. IM and oral placebos in addition to SOC.

An independent biostatistician will provide a random number table to the unblinded pharmacist for randomization. The random number table is generated based on the total number of subjects to be enrolled in a 1:1 ratio for placebo vs E2/P4. The unblinded pharmacist will use the random number table in sequential order for randomization assignment as subjects are enrolled. The research team will remain blinded to the

randomization assignment throughout the study. Unblinding will only be permissible in case of adverse events.

Drug will be supplied by Tulane Medical Center Research Pharmacy. The supplier of the study drugs is AmerisourceBergan.

Study design and visit schedule are shown in Figure 1.

Study implementation, recruitment, population and eligibility criteria

Participants are recruited at admission by the medical staff of the Department of General Internal Medicine and Geriatrics at Tulane Medical Center (Tertiary care academic hospital), under the leadership of Dr. Bateman (Co-Investigator) who identifies participants meeting study eligibility criteria from their admission list. Dr. Bateman assesses eligibility at screening visit, and notifies the research coordinators of eligible participants. Eligible subjects are enrolled by the research coordinators to partake in a randomized scheme to receive the 5-day active treatment or placebo. The Research Pharmacist at Tulane Medical Center assigns participants to the intervention. We will recruit up to 120 participants hospitalized at Tulane Medical Center with mild to severe COVID-19 (WHO ordinal scale score 3-5, Table 1) confirmed by SARS-CoV-2 PCR test .

Inclusion Criteria:

1. Hospitalization for COVID-19 (WHO Ordinal scale score 3-5, Table 1) confirmed by SARS-CoV-2 PCR at Tulane Medical Center in the Department of General Internal Medicine and Geriatrics.
2. Respiratory symptoms (fever, shortness of breath or cough) or abnormal lung exam or chest imaging characteristic of mild to severe COVID-19 pneumonia.
3. Patient and/or legally authorized representative (LAR) agrees to comply with study procedures and the collection of blood samples per protocol.
4. Patient and/or LAR agrees to be placed on prophylactic dose of anticoagulation for prevention of deep venous thrombosis (DVT) (if necessary).

5. Patient or legally authorized representative has signed informed consent.
6. Women of childbearing age with a negative pregnancy test on admission.

Exclusion Criteria:

1. Patient under 18 years of age
2. Critically-ill COVID-19 (respiratory failure requiring intubation and mechanical ventilation, shock, multi-organ failure).
3. Pregnant women confirmed by pregnancy test.
4. Women within six weeks of postpartum.
5. Patient included in another COVID-19 trial (excluding hydroxychloroquine and dexamethasone).
6. Women already treated by estrogen and or progestogen therapy two weeks prior to admission.
7. Men already treated by testosterone therapy prior to admission.
8. History of breast or endometrial cancer.
9. Abnormal genital bleeding.
10. Active or recent (e.g., within the past year) stroke or myocardial infarction.
11. History of blood clots including deep vein thrombosis related to clotting disease, or pulmonary emboli (prior to hospitalization).
12. History of liver dysfunction or disease.
13. Patients with end-stage renal disease
14. Patients taking inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, and ritonavir.
15. Patients taking St. John's Wort preparations (Hypericum perforatum), phenobarbital, carbamazepine, and rifampin.
16. Patients within 6 weeks of major orthopedic surgery.

Safety measures and monitoring

We will monitor each subject for adverse events/serious adverse events (AEs/SAEs) daily while the subjects are receiving daily therapy (Figure 2). If they develop a grade 3 or 4 AE, we will discontinue the study drug. No dose adjustments will be made. We will also follow-up in person or by telephone for all subjects for any adverse AEs/SAEs and mortality at Day 60.

AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version5 and taken care with the corresponding specialized clinical management of the SAE.

Grade 1 AEs (asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated) or Grade 2 AEs (moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activity of daily living): such as breast tenderness, nausea, vomiting, bloating, stomach cramps, headaches, vaginal itching, abnormal uterine bleeding will not require discontinuation of treatment.

Grade 3 SAEs like DVT and 4 (life-threatening) SAEs such as MI, stroke, anaphylaxis will be managed by treatment discontinuation (except SOC), and appropriate specialized clinical management of the SAE.

The trial is a 5-day intervention during hospitalization and therefore we do not expect problems with adherence. The study team members who are administering the trial drugs will call the PI or the Co-Is in circumstances when subjects have questions or concerns to be addressed in a timely manner to improve adherence.

Study Investigators have formed an independent Data Safety and Monitoring Board (DSMB) to monitor study safety. The DSMB consists of four board members who are clinicians and researchers with the experience necessary to interpret the data and ensure patient safety. Specifically, the board members are knowledgeable about COVID-19 and the treatment involved in this study. The DSMB includes, an infectious disease specialist, an endocrinologist, a hematologist-oncologist, a women's health specialist and a biostatistician. The initial DSMB meeting occurred before the start of the trial to discuss the protocol and guidelines for monitoring the study. At this meeting, guidelines were defined for stopping the study for safety concerns (Study stopping rules) and where relevant, for efficacy based on plans specified in the

protocol. The DSMB will meet on a quarterly basis. After each meeting, the DSMB will provide the PI with a letter describing the results of their review and their recommendations for the continuation of the study.

Outcome Measures

For all randomized patients according to inclusion criteria section above, demographic and clinical variables, including sex, age, race/ethnicity, body mass index (BMI), symptoms, vital signs, blood pressure, respiratory rate, temperature, oxygen saturation, chronic comorbidities through diagnosis codes, medications, clinical course during hospitalization (described in primary outcome below) will be reviewed in the patients' medical charts. Laboratory measurements will include, when available, complete blood count (CBC), blood chemistry, liver function test, creatinine, blood urea nitrogen test (BUN), lactate, troponin, ferritin, C-reactive protein (CRP), procalcitinin, Brain-type natriuretic peptide (BNP), D-dimer, interleukin-6, and glucose-6-phosphate dehydrogenase (G6PD). We will review the participants' medical records for up to 60 days after hospital discharge.

***Primary outcome**:*

For all randomized patients with baseline inclusion criteria, WHO 9-point ordinal scale scores 3 to 5, the primary efficacy end point will be the proportion of patients who improve to scores 1 or 2 on the WHO ordinal scale (Table 1) through day 28.

***Secondary outcomes**:*

For all randomized patients, the following secondary outcomes will be assessed at day 14, 28 and 60. Assessment at day 14 and 28 will be performed by electronic medical records review, while assessment at day 60 will be performed via telephone call directly to the study subjects.

1. Length of hospital stay
2. Duration of mechanical ventilation
3. Date and cause of death
4. Readmission

5. Change in biological markers below between admission and occurrence of primary endpoint (2 values at least 2 days apart)
6. Grade 3 and 4 AEs
7. SAEs

Biological markers

Obtained from SOC:

Inflammation: neutrophil:lymphocyte ratio, CRP, ferritin, procalcitonin.

Hypercoagulability: D-dimers, fibrinogen

Tissue injury: troponin, Alanine transaminase (ALT), Aspartate transaminase (AST), Lactate dehydrogenase (LDH)

Metabolome, Proteome, peripheral blood mononuclear cells (PBMCs)

Data collection and management

The collection of routine blood samples and patient monitoring will be conducted as part of the SOC procedures that are utilized at Tulane Medical Center for COVID-19 patients. The collection of blood samples (CBC with differential, blood chemistry, inflammation markers) is to monitor disease severity and is independent from this protocol, but we will use the data in our analysis. Women of childbearing age will have a pregnancy test on admission.

An additional blood sample will be collected at admission and after 5 days of treatment (about 5-25 ml of blood at these two time points). This blood sample will be stored in Dr. Mauvais-Jarvis lab for 1) isolation of PBMC to assess the effect of treatment on immune cells populations by flow cytometry, and PBMC gene expression by RNA-Seq, and 2) metabolomics profiling to determine metabolic and molecular signatures of the efficacy of treatment. De-identified serum and PBMC frozen samples will be transferred to the University of Michigan Metabolomics Core to perform untargeted metabolomic analyses. The serum and PBMC from collected blood samples will be used to perform multi-dimensional

analysis in the Center for Translational Research in Infection and Inflammation at Tulane University School of Medicine. Blood samples will be stored for up to 3 years.

The majority of clinical data, including all blood sample collection and assessment of severity of illness, will occur while the patient is hospitalized, which should minimize patient nonadherence. The only information to be collected after discharge will be a telephone call to assess clinical status at day 60. All available data will be collected and assessed into our analysis. If patients choose to discontinue from the study after day 1 (when they will have already received estradiol if in the treatment group), we will separate the analysis from the treatment group as they will not have completed the progesterone therapy.

All data and research files will be de-identified and stored using the secure web application Research Electronic Data Capture (REDCap). REDCap is protected by a login as well as encryption. All data for this study will be electronic; there will be no paper records. Enrolled subjects will be assigned a sequential study ID (i.e. 001, 002, etc) that is linked to their medical record number. A link between the study ID numbers and medical record number will be kept in a separate file from the de-identified database and stored securely in REDCap. To promote data quality, two different individuals will perform double data entry. Subsequently, comparison of the two records will be done and any non-matching data will be correctly identified and entered. Only the IRB-approved study investigators will have access to the data and link. The PI will store all study research records and data post study closure, as required by NIH and University data retention policies.

Statistical considerations

Sample size

Sample size calculations are based on 80% Power, using a two-sided Pearson's Chi-square Test for Proportion Differences at the 5% significance level. It is estimated that between 60% and 70% of hospitalized patients with mild or severe disease will improve clinically and become ambulatory after standard treatment (WHO ordinal scale score 1-2, Table 1)). Table 2 presents the sample sizes that are required in each treatment group to detect a significant difference in the percent of hospitalized patient

leading to an ambulatory disease (shift from category 3-5 to category 1-2 in 20%, 25%, 30% or 35% of hospitalized patients after treatment with estradiol (E2) and progesterone (P4)). Efforts will be made to recruit 60 patients in each treatment group in order to stratify by disease severity, age and gender.

Interim analysis

An interim analysis will be performed at 50% enrollment to determine to what extent a strong treatment efficacy warrants stopping the trial early. Table 3 presents interim sample sizes and required Z test statistics for comparing two proportions at each of stage of an O'Brien-Fleming alpha-spending sequential design.[27] Based on 80% power and an overall 5% significance level, if the interim analysis for the first 60 enrolled patients results in differences that are more than 2.96 standard deviations from 0, the trial will end, and treatment efficacy will be concluded. Otherwise, the trial will enroll the total 120 patients.

Statistical Analysis

For all subjects who have completed the study, clinical outcomes and demographic characteristics will be summarized and presented separately for the treatment group and the control group, as well as overall for all patients. Categorical variables, such as the primary outcome, readmission, as well as race and sex will be summarized with counts and percentages. Quantitative outcomes, such as length of stay, change in biological markers, age and BMI will be summarized with means, medians, standard deviations, and interquartile ranges. Univariate analyses will be performed as the primary method to assess the unadjusted effect of treatment on the primary outcome and on readmission rate using the Pearson Chi-square test. Differences in average length of stay and in change in biological markers will be assessed with either a two-sample t test or the Mann-Whitney nonparametric test. Multivariate analyses will be performed to compare treatment to control responses while adjusting for various co-factors. Multiple logistic regression will be used as supportive method to test for significant differences in the proportion with the primary outcome, between the active treatment and placebo groups, while adjusting for age, race, sex, BMI and other potential co-factors. For the secondary outcomes, multiple logistic regression will also be used to test for significant differences in the proportion readmits, between the active treatment and

placebo groups, and multiple linear regression will be used to test for significant differences in average length of stay and changes in biological markers, between the treatment and SOC groups, while adjusting for potential influencing co factors.

Patient and public involvement

No patients were involved in the design or implementation of this study.

ETHICS AND DISSEMINATION

Ethics and safety approval

Ethical approval was obtained from the institutional review board (IRB) at Tulane University Clinical Translational Unit on May 14st 2021. Future amendments to the protocol by the study investigators will be submitted to the IRB. Implementation of changes in the study will only be made after the IRB approval is received. Accordingly, trial participants, trial registries and trial staff will be informed in a timely manner. The study has been reviewed by the United States Food and Drug Administration and granted Investigational New Drug (IND) #152499.

Informed consent

The consent process will be administered to the patient (or legally authorized representative (LAR) in the case that the patient is unable to consent) prior to enrollment in the study. Adobe sign feature on iPad or smartphone will be used in order to minimize the safety concerns for study personnel conducting the consent procedures. There is also be a separate section in the consent that allows the subjects to opt in or out of additional blood collection for ancillary studies in the future that are directly related to the current study. The forms and the consenting process have been reviewed and approved by the Tulane IRB.

Dissemination

The data generated in this study will be presented at internal medicine, infections disease and endocrinology national or international conferences and published in a timely fashion. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. Results will be published in open-access journals when possible.

Study protocol and statistical analysis plans will be publically available. Participant-level data set and statistical code access will be considered upon request.

Discussion

The pathogenesis of COVID-19 involves an inappropriate immune response, with outpouring of pro-inflammatory chemokines, leading to lung infiltration and hyperactivation of monocytes and macrophages producing pro-inflammatory cytokines (cytokine storm), resulting in lung edema, reduced gas exchange, and ultimately leading to acute respiratory distress syndrome and multiorgan failure.[28-35] Repurposing approved drugs that have already been tested in humans—and for which detailed information is available on their pharmacology, formulation, dose, and potential toxicity—provides a fast and safe approach for off-label use of potentially life-saving therapeutics. The scientific premise for the use of E2 and P4 in hospitalized patients, and its relevance to COVID-19 pathogenesis, is based on a large body of published literature reviewed recently.[25] In fact, two clinical trials are testing E2 (ClinicalTrials.gov identifier NCT04359329) or P4 (ClinicalTrials.gov identifier NCT04365127) individually in COVID-19 patients. The present study has been reviewed by the US Food and Drug Administration for Investigational New Drug authorization since August 2020, which has delayed its beginning.

The advantage of repurposing E2 and P4 is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from over half a century of clinical and basic research. Hormone therapy using estrogens and progestogens is used by millions of women for contraception or the prevention of menopausal symptoms. There is no acute toxicity. The risk of DVT or pulmonary embolism associated with estradiol and progesterone treatment exists but is minimal. First, the risk of DVT is associated with chronic hormone therapy, over months or years, not following acute hormone therapy of several days. Most importantly, the risk is minimal because of parenteral estradiol administration, which does not alter clotting factors. DVT risk occurs mainly following orally administered estrogens, and synthetic progestins, because of first-pass liver metabolism producing high liver exposures to hormones, increasing hepatic production of clotting and inflammatory factors.[36] Additionally, the DVT risks are usually associated with the use of conjugated equine estrogens alone or associated with a synthetic progestin, like medroxyprogesterone acetate.[36] There is no documented risk associated with natural E2 given

systemically (IM, SC) and no documented risk with the use of natural P4. Finally, the pathogenesis of thrombosis in COVID-19 is a consequence of the immuno-inflammation,[37] and is expected to be ameliorated by the E2 and P4 treatment.

TRIAL STATUS

This trial is currently recruiting subjects.

Competing interests

PI and Co-Is do not have any conflict of interest to disclose.

Authors' Contributions

FMJ- study design, management, analysis, interpretation of data; writing of the report; and decision to submit the report for publication. DL-clinical trial preparatory activities, data management, statistical analysis, interpretation of data and writing of the report. KB- Recruitment of patients, interpretation of data and writing of the report. VF- interpretation of data and writing of the report. JL- statistical analysis, interpretation and writing of the report. MS- visualization and writing of the report.

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REFERENCES

1. John Hopkins Coronavirus Resource Center. Cases by Country. Accessed May 5, 2021. https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6
2. Khubchandani J, Sharma S, Price JH, Wiblishauser MJ, Sharma M, Webb FJ. COVID-19 Vaccination Hesitancy in the United States: A Rapid National Assessment. *J Community Health*. Apr 2021;46(2):270-277. doi:10.1007/s10900-020-00958-x
3. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*. Feb 25 2021;384(8):693-704. doi:10.1056/NEJMoa2021436
4. Kaka AS, MacDonald R, Greer N, et al. Major Update: Remdesivir for Adults With COVID-19 : A Living Systematic Review and Meta-analysis for the American College of Physicians Practice Points. *Ann Intern Med*. Feb 9 2021;doi:10.7326/m20-8148
5. Salvarani C, Dolci G, Massari M, et al. Effect of Tocilizumab vs Standard Care on Clinical Worsening in Patients Hospitalized With COVID-19 Pneumonia: A Randomized Clinical Trial. *JAMA Intern Med*. Jan 1 2021;181(1):24-31. doi:10.1001/jamainternmed.2020.6615
6. Janiaud P, Axfors C, Schmitt AM, et al. Association of Convalescent Plasma Treatment With Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis. *Jama*. Mar 23 2021;325(12):1185-1195. doi:10.1001/jama.2021.2747
7. Mehta SR, Yusuf S, Diaz R, et al. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. *Jama*. Jan 26 2005;293(4):437-46. doi:10.1001/jama.293.4.437
8. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. May 1 2020;130(5):2620-2629. doi:10.1172/jci137244
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. Feb 15 2020;395(10223):497-506. doi:10.1016/s0140-6736(20)30183-5
10. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. May 28 2020;181(5):1036-1045.e9. doi:10.1016/j.cell.2020.04.026
11. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol*. Jul 2017;39(5):517-528. doi:10.1007/s00281-017-0639-8
12. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. Jun 2020;80(6):607-613. doi:10.1016/j.jinf.2020.03.037
13. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. Jul 2016;8(8):959-70. doi:10.2217/imt-2016-0020
14. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev*. Jun 2020;19(6):102537. doi:10.1016/j.autrev.2020.102537
15. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. Apr 30 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032

16. Klein SL, Dhakal S, Ursin RL, Deshpande S, Sandberg K, Mauvais-Jarvis F. Biological sex impacts COVID-19 outcomes. *PLoS Pathog*. Jun 2020;16(6):e1008570. doi:10.1371/journal.ppat.1008570
17. Mauvais-Jarvis F. Aging, Male Sex, Obesity, and Metabolic Inflammation Create the Perfect Storm for COVID-19. *Diabetes*. Sep 2020;69(9):1857-1863. doi:10.2337/dbi19-0023
18. Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet*. Aug 22 2020;396(10250):565-582. doi:10.1016/s0140-6736(20)31561-0
19. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *Jama*. May 26 2020;323(20):2052-2059. doi:10.1001/jama.2020.6775
20. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat Rev Immunol*. Jul 2020;20(7):442-447. doi:10.1038/s41577-020-0348-8
21. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. Oct 2016;16(10):626-38. doi:10.1038/nri.2016.90
22. Seeland U, Coluzzi F, Simmaco M, et al. Evidence for treatment with estradiol for women with SARS-CoV-2 infection. *BMC Med*. Nov 25 2020;18(1):369. doi:10.1186/s12916-020-01851-z
23. Phiel KL, Henderson RA, Adelman SJ, Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol Lett*. Feb 15 2005;97(1):107-13. doi:10.1016/j.imlet.2004.10.007
24. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. Aug 2007;28(5):521-74. doi:10.1210/er.2007-0001
25. Mauvais-Jarvis F, Klein SL, Levin ER. Estradiol, Progesterone, Immunomodulation, and COVID-19 Outcomes. *Endocrinology*. Sep 1 2020;161(9)doi:10.1210/endocr/bqaa127
26. World Health Organization R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis. Accessed April 25, 2021. https://www.who.int/teams/blueprint/covid-19
27. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. Sep 1979;35(3):549-56.
28. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*. 2020/03/28/ 2020;395(10229):1033-1034. doi:https://doi.org/10.1016/S0140-6736(20)30628-0
29. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of clinical investigation*. 04/13/ 2020;130(5)doi:10.1172/JCI137244
30. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
31. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. doi:10.1016/j.cell.2020.04.026
32. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Seminars in Immunopathology*. 2017/07/01 2017;39(5):517-528. doi:10.1007/s00281-017-0639-8
33. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. Apr 10 2020;doi:10.1016/j.jinf.2020.03.037

34. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. 2016;8(8):959-970. doi:10.2217/imt-2016-0020
35. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmunity Reviews*. 2020/04/03/ 2020:102537. doi:https://doi.org/10.1016/j.autrev.2020.102537
36. Mauvais-Jarvis F, Manson JE, Stevenson JC, Fonseca VA. Menopausal hormone therapy and type 2 diabetes prevention: Evidence, mechanisms and clinical implications. *Endocr Rev*. Mar 08 2017;doi:10.1210/er.2016-1146
37. Bonaventura A, Vecchié A, Dagna L, et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat Rev Immunol*. Apr 6 2021:1-11. doi:10.1038/s41577-021-00536-9

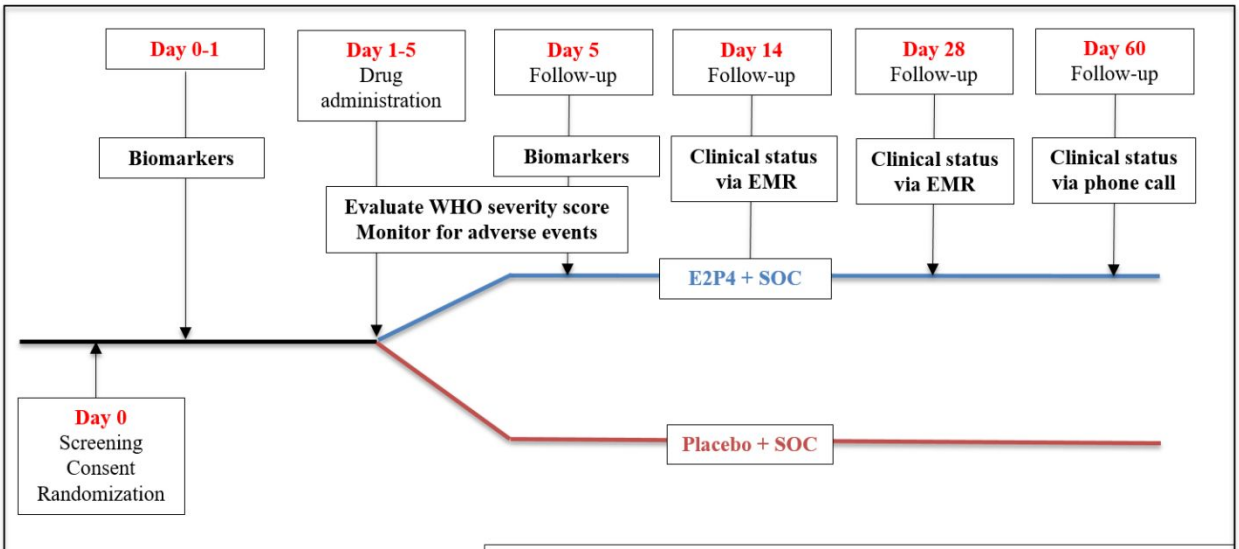

Figure 1. Study Design

E2, Estradiol Cypionate (Intramuscular); P4, micronized Progesterone (Oral); WHO, World health organization; EMR, electronic medical records; SOC, standard of care.

Surveillance	Days following initiation of treatment								
	0	1	2	3	4	5	14	28	60
Pregnancy test*	X								
Injection site		X	X	X	X	X			
E2 side effects		X	X	X	X	X	X		
P4 side effects		X	X	X	X	X	X		
DVT symptoms		X	X	X	X	X	X	X	X
MI symptoms		X	X	X	X	X	X	X	X
Stroke symptoms		X	X	X	X	X	X	X	X
Fluid retention**		X	X	X	X	X	X		

* Women in reproductive age ** Patients with renal impairment

Figure 2. Schedule of safety assessments

Table 1. World Health Organization 9 point Ordinal Scale for Clinical Improvement		
Patient State	**Descriptor**	**Score**
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support- pressors, Renal Replacement Therapy (RRT), Extracorporeal Membrane Oxygenation (ECMO)	7
Dead	Death	8
** Adapted from Ref 26*		

Table 2. Sample size calculations		
Treatment	Percent leading to a scale 1-2	Sample Size
SOC	60%	32
SOC + E2 + P4	90%	32
SOC	60%	22
SOC + E2 + P4	95%	22
SOC	70%	62
SOC + E2 + P4	90%	62
SOC	70%	36
SOC + E2 + P4	95%	36

Table 3. Interim sample size calculations			
	Treatment	Sample Size	Standardized Z test Statistic
Stage 1	SOC	30	2.96
	SOC + E2 + P4	30	
Stage 2	SOC	60	1.96
	SOC + E2 + P4	60	

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Acute Estradiol and Progesterone Therapy in Hospitalized Adults to Reduce COVID-19 Severity: A Randomized Control Trial

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ABSTRACT

Introduction: As of September 2021, COVID-19, has killed more than 4 million people globally, including over 650,000 in the United States (US). Apart from corticosteroids, most available therapeutic options are at best marginally efficient in reducing disease severity and are extremely expensive. The systematic investigation of clinically approved drugs is a priority to determine what does mitigate disease severity. Estradiol (E2) and progesterone (P4) produce a state of anti-inflammatory immune responses and immune tolerance, and enhanced antibody production. The goal of this trial is to evaluate the efficacy of a short E2 and P4 therapy, in addition to standard of care (SOC), in mitigating disease severity in COVID-19 hospitalized patients.

Methods and analysis: Phase 2, randomized, double blind, placebo-controlled, single center trial. Patients hospitalized for confirmed COVID-19, with scores 3 to 5 on the 9-point World Health Organization (WHO) ordinal scale are randomized between two arms: 1) Estradiol Cypionate (IM) and micronized Progesterone (PO), in addition to SOC, and 2) Placebo, in addition to SOC. The primary outcome is the proportion of patients improving to scores 1 or 2 on the WHO scale through day 28. Secondary outcomes include length of hospital stay, duration of mechanical ventilation, cause of death, readmission rates, change in inflammatory biomarkers between admission and occurrence of primary endpoint, and adverse events. Study sample size will be up to 120 participants. The trial is currently recruiting subjects.

Ethics and dissemination: The sponsor of this study is the Center of Excellence in Sex-Based Biology & Medicine at Tulane University, New Orleans, Louisiana, USA. Ethical approval was obtained from the Tulane institutional review board (IRB) on May 14st 2021. The study was reviewed by the US Food and Drug Administration and granted Investigational New Drug (IND) #152499. Results of the study will be submitted for publication in a peer-reviewed journal.

Clinical Trial Registration: NCT04865029, ClinicalTrials.gov

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. The trial will include men and women.
2. E2 and P4 decrease production of proinflammatory cytokines and enhance B-cell-mediated antibody production.
3. The advantage of repurposing E2 and P4 comes from decades of accumulated knowledge regarding their clinical efficacy and toxicity.
4. E2 and P4 are inexpensive and available in hospitals worldwide.
5. A randomized, placebo-controlled trial is an efficient way to determine if a short systemic E2 andP4 therapy, administered early to hospitalized COVID-19 patients in addition to SOC can reduce the severity of outcomes compared to SOC alone.

INTRODUCTION

As of September 2021, coronavirus diseases 2019 (COVID-19), has already killed over 4 million people worldwide.[1] Although the vaccination campaign is ramping up in the United States (US), vaccination hesitancy represents up to 25-30% of the population, [2] and hospitalizations and deaths are still high. In Brazil and India, the daily death toll is more than twice as high as it was in 2020.[1] Apart from corticosteroids,[3] most available therapeutic options are at best marginally efficient in reducing disease severity and mortality and are extremely expensive.[4-6] Therefore, the systematic investigation of clinically approved drugs is still a priority in order to determine what compounds could be repurposed to mitigate disease severity, and invest resources to go to full-scale production. Our current understanding of the disease is that COVID-19 deaths result from an inappropriate immune response with outpouring of pro-inflammatory chemokines leading to lung infiltration and hyperactivation of monocytes and macrophages producing pro-inflammatory cytokines (cytokine storm), resulting in lung edema, reduced gas exchange, and ultimately leading to acute respiratory distress syndrome and multiorgan failure.[7-14] Men with COVID-19 have a uniformly more severe outcome than women. In series from China, Europe and the U.S., COVID-19 mortality was consistently 1.5 to 2-fold higher in men than in women, suggesting that female biological sex is protecting women from COVID-19 mortality.[15-20] It is established that women exhibit heightened immune responses to viral infections compared to men,[21] which is at least partially due to the genetic benefit of gene dosage in X-linked immune-response genes. Ovarian steroids, however, also play a protective role. The analysis of electronic health records of over 68,000 COVID-19 patients revealed that estrogen therapy is associated with more than 50% reduction in mortality.[22] The main female steroids, 17β-estradiol (E2) and progesterone (P4) exhibit potent immuno-modulatory and anti-inflammatory actions via estrogen and progesterone receptors expressed in all immune cells, including epithelial cells, macrophages, dendritic cells, CD4+ and CD8+ lymphocytes, and B cells.[21, 23] P4 also acts partially via the glucocorticoid receptor. Together E2 and P4 produce a state of decreased innate immune cells production of proinflammatory cytokines, enhanced T cells anti-inflammatory responses and immune tolerance, and enhanced B-cell-mediated antibody production.[21, 24, 25] The scientific premise of

this trial is that in hospitalized COVID-19 patients, a short and early E2 and P4 therapy, in addition to standard of care (SOC; the National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel recommends the use of dexamethasone for up to 10 days or until hospital Discharge as SOC for the treatment of hospitalized COVID-19 patients), will prevent or mitigate the cytokine storm, and prevent severe outcomes, and without side effects.[25] Therefore, it will provide steroid immunomodulation without immunosuppression. The advantage of repurposing E2 and P4 is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from decades of clinical and basic studies. E2 and P4 are widely available in hospitals, inexpensive, manufacturable to scale, and can be prescribed immediately.

METHODS AND ANALYSIS

Study design, randomization, intervention and study calendar

Phase 2, randomized, double blind, placebo-controlled, single center trial designed to determine to what extent a short systemic E2 and P4 therapy, administered early to hospitalized COVID-19 positive patients of both sexes, in addition to SOC, can reduce the severity of symptoms and outcomes compared to SOC alone.

Hospitalized patients with COVID-19 with scores 3-5 on the 9-point World Health Organization (WHO) ordinal scale[26] are randomized to a 5-day treatment between two arms:

1. Estradiol Cypionate (Depo-Estradiol) 5mg via intramuscular injection on day 1 and micronized progesterone 200mg administered orally, daily for 5 days, in addition to SOC.
2. IM and oral placebos in addition to SOC.

We chose E2 administration via IM injection to avoid first-pass liver metabolism and change in coagulation markers while we chose P4 oral preparation due to ease of administration and low side effect profile. Drug will be supplied by Tulane Medical Center Research Pharmacy. The manufacturers of the study drugs are: Pfizer for E2 (NDC: 0009-0271-01) and VIRTUS for P4 (NDC: 69543-375-10).

An independent biostatistician will provide a random number table to the unblinded pharmacist for randomization. The random number table is generated based on the total number of subjects to be enrolled in a 1:1 ratio for placebo vs E2/P4. The unblinded pharmacist will use the random number table in sequential order for randomization assignment as subjects are enrolled. The research team will remain blinded to the randomization assignment throughout the study. Unblinding will only be permissible in case of adverse events.

Study design and visit schedule are shown in Figure 1.

Study implementation, recruitment, population and eligibility criteria

Participants are recruited at admission by the medical staff of the Department of General Internal Medicine and Geriatrics at Tulane Medical Center (Tertiary care academic hospital), under the leadership of Dr. Bateman (Co-Investigator) who identifies participants meeting study eligibility criteria from their admission list. Dr. Bateman assesses eligibility at screening visit, and notifies the research coordinators of eligible participants. Eligible subjects are enrolled by the research coordinators to partake in a randomized scheme to receive the 5-day active treatment or placebo. The Research Pharmacist at Tulane Medical Center assigns participants to the intervention. We will recruit up to 120 participants hospitalized at Tulane Medical Center with mild to severe COVID-19 (WHO ordinal scale score 3-5, Table 1) confirmed by SARS-CoV-2 PCR test .

Inclusion Criteria:

1. Hospitalization for COVID-19 (WHO Ordinal scale score 3-5, Table 1) confirmed by SARS-CoV-2 PCR at Tulane Medical Center in the Department of General Internal Medicine and Geriatrics.
2. Respiratory symptoms (fever, shortness of breath or cough) or abnormal lung exam or chest imaging characteristic of mild to severe COVID-19 pneumonia.

3. Patient and/or legally authorized representative (LAR) agrees to comply with study procedures and the collection of blood samples per protocol.
4. Patient and/or LAR agrees to be placed on prophylactic dose of anticoagulation for prevention of deep venous thrombosis (DVT) (if necessary).
5. Patient or legally authorized representative has signed informed consent.
6. Women of childbearing age with a negative pregnancy test on admission.

Exclusion Criteria:

1. Patient under 18 years of age
2. Critically-ill COVID-19 (respiratory failure requiring intubation and mechanical ventilation, shock, multi-organ failure).
3. Pregnant women confirmed by pregnancy test.
4. Women within six weeks of postpartum.
5. Patient included in another COVID-19 trial (excluding hydroxychloroquine and dexamethasone).
6. Women already treated by estrogen and or progestogen therapy two weeks prior to admission.
7. Men already treated by testosterone therapy prior to admission.
8. History of breast or endometrial cancer.
9. Abnormal genital bleeding.
10. Active or recent (e.g., within the past year) stroke or myocardial infarction.
11. History of blood clots including deep vein thrombosis related to clotting disease, or pulmonary emboli (prior to hospitalization).
12. History of liver dysfunction or disease.
13. Patients with end-stage renal disease
14. Patients taking inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, and ritonavir.

15. Patients taking St. John's Wort preparations (Hypericum perforatum), phenobarbital, carbamazepine, and rifampin.
16. Patients within 6 weeks of major orthopedic surgery.

Safety measures and monitoring

We will monitor each subject for adverse events/serious adverse events (AEs/SAEs) daily while the subjects are receiving daily therapy (Figure 2). If they develop a grade 3 or 4 AE, we will discontinue the study drug. No dose adjustments will be made. We will also follow-up in person or by telephone for all subjects for any adverse AEs/SAEs and mortality at Day 60.

AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version5 and taken care with the corresponding specialized clinical management of the SAE.

Grade 1 AEs (asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated) or Grade 2 AEs (moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activity of daily living): such as breast tenderness, nausea, vomiting, bloating, stomach cramps, headaches, vaginal itching, abnormal uterine bleeding will not require discontinuation of treatment.

Grade 3 SAEs like DVT and 4 (life-threatening) SAEs such as MI, stroke, anaphylaxis will be managed by treatment discontinuation (except SOC), and appropriate specialized clinical management of the SAE.

The trial is a 5-day intervention during hospitalization and therefore we do not expect problems with adherence. The study team members who are administering the trial drugs will call the PI or the Co-Is in circumstances when subjects have questions or concerns to be addressed in a timely manner to improve adherence.

Study Investigators have formed an independent Data Safety and Monitoring Board (DSMB) to monitor study safety. The DSMB consists of four board members who are clinicians and researchers with the experience necessary to interpret the data and ensure patient safety. Specifically, the board members are knowledgeable about COVID-19 and the treatment involved in this study. The DSMB includes, an

infectious disease specialist, an endocrinologist, a hematologist-oncologist, a women's health specialist and a biostatistician. The initial DSMB meeting occurred before the start of the trial to discuss the protocol and guidelines for monitoring the study. At this meeting, guidelines were defined for stopping the study for safety concerns (Study stopping rules) and where relevant, for efficacy based on plans specified in the protocol. The DSMB will meet on a quarterly basis. After each meeting, the DSMB will provide the PI with a letter describing the results of their review and their recommendations for the continuation of the study.

Outcome Measures

For all randomized patients according to inclusion criteria section above, demographic and clinical variables, including sex, age, race/ethnicity, body mass index (BMI), symptoms, vital signs, blood pressure, respiratory rate, temperature, oxygen saturation, chronic comorbidities through diagnosis codes, medications, clinical course during hospitalization (described in primary outcome below) will be reviewed in the patients' medical charts. Laboratory measurements will include, when available, complete blood count (CBC), blood chemistry, liver function test, creatinine, blood urea nitrogen test (BUN), lactate, troponin, ferritin, C-reactive protein (CRP), procalcitinin, Brain-type natriuretic peptide (BNP), D-dimer, and interleukin-6.We will review the participants' medical records for up to 60 days after hospital discharge.

***Primary outcome**:*

For all randomized patients with baseline inclusion criteria, WHO 9-point ordinal scale scores 3 to 5, the primary efficacy end point will be the proportion of patients who improve to scores 1 or 2 on the WHO ordinal scale (Table 1) through day 28.

***Secondary outcomes**:*

For all randomized patients, the following secondary outcomes will be assessed at day 14, 28 and 60. Assessment at day 14 and 28 will be performed by electronic medical records review, while assessment at day 60 will be performed via telephone call directly to the study subjects.

1. Length of hospital stay
2. Duration of mechanical ventilation

3. Date and cause of death
4. Readmission
5. Change in biological markers below between admission and occurrence of primary endpoint (2 values at least 2 days apart)
6. Grade 3 and 4 AEs
7. SAEs

Biological markers

Obtained from SOC:

Inflammation: neutrophil:lymphocyte ratio, CRP, ferritin, procalcitonin.

Hypercoagulability: D-dimers, fibrinogen

Tissue injury: troponin, Alanine transaminase (ALT), Aspartate transaminase (AST), Lactate dehydrogenase (LDH)

Metabolome, Proteome, peripheral blood mononuclear cells (PBMCs)

Data collection and management

The collection of routine blood samples and patient monitoring will be conducted as part of the SOC procedures that are utilized at Tulane Medical Center for COVID-19 patients. The collection of blood samples (CBC with differential, blood chemistry, inflammation markers) is to monitor disease severity and is independent from this protocol, but we will use the data in our analysis. Women of childbearing age will have a pregnancy test on admission.

An additional blood sample will be collected at admission and after 5 days of treatment (about 5-25 ml of blood at these two time points). This blood sample will be stored in Dr. Mauvais-Jarvis lab for 1) isolation of PBMC to assess the effect of treatment on immune cells populations by flow cytometry, and PBMC gene expression by RNA-Seq, and 2) metabolomics profiling to determine metabolic and molecular signatures of the efficacy of treatment. De-identified serum and PBMC frozen samples will be transferred to the University of Michigan Metabolomics Core to perform untargeted metabolomic

analyses. The serum and PBMC from collected blood samples will be used to perform multi-dimensional analysis in the Center for Translational Research in Infection and Inflammation at Tulane University School of Medicine. Blood samples will be stored for up to 3 years.

The majority of clinical data, including all blood sample collection and assessment of severity of illness, will occur while the patient is hospitalized, which should minimize patient nonadherence. The only information to be collected after discharge will be a telephone call to assess clinical status at day 60. All available data will be collected and assessed into our analysis. If patients choose to discontinue from the study after day 1 (when they will have already received estradiol if in the treatment group), we will separate the analysis from the treatment group as they will not have completed the progesterone therapy.

All data and research files will be de-identified and stored using the secure web application Research Electronic Data Capture (REDCap). REDCap is protected by a login as well as encryption. All data for this study will be electronic; there will be no paper records. Enrolled subjects will be assigned a sequential study ID (i.e. 001, 002, etc) that is linked to their medical record number. A link between the study ID numbers and medical record number will be kept in a separate file from the de-identified database and stored securely in REDCap. To promote data quality, two different individuals will perform double data entry. Subsequently, comparison of the two records will be done and any non-matching data will be correctly identified and entered. Only the IRB-approved study investigators will have access to the data and link. The PI will store all study research records and data post study closure, as required by NIH and University data retention policies.

Statistical considerations

Sample size

Sample size calculations are based on 80% Power, using a two-sided Pearson's Chi-square Test for Proportion Differences at the 5% significance level. It is estimated that between 60% and 70% of hospitalized patients with mild or severe disease will improve clinically and become ambulatory after standard treatment (WHO ordinal scale score 1-2, Table 1)). Table 2 presents the sample sizes that are

required in each treatment group to detect a significant difference in the percent of hospitalized patient leading to an ambulatory disease (shift from category 3-5 to category 1-2 in 20%, 25%, 30% or 35% of hospitalized patients after treatment with estradiol (E2) and progesterone (P4)). Efforts will be made to recruit 60 patients in each treatment group in order to stratify by disease severity, age and gender.

Interim analysis

One and only interim analysis will be performed at 50% study completion (after 60th subject end of study visit) to determine to what extent a strong treatment efficacy warrants stopping the trial early. Table 3 presents interim sample sizes and required Z test statistics for comparing two proportions at each of stage of an O'Brien-Fleming alpha-spending sequential design.[27] Based on 80% power and an overall 5% significance level, if the interim analysis for the first 60 enrolled patients results in differences that are more than 2.96 standard deviations from 0, the trial will end, and treatment efficacy will be concluded. Otherwise, the trial will enroll the total 120 patients.

Statistical Analysis

For all subjects who have completed the study, clinical outcomes and demographic characteristics will be summarized and presented separately for the treatment group and the control group, as well as overall for all patients. Categorical variables, such as the primary outcome, readmission, as well as race and sex will be summarized with counts and percentages. Quantitative outcomes, such as length of stay, change in biological markers, age and BMI will be summarized with means, medians, standard deviations, and interquartile ranges. Univariate analyses will be performed as the primary method to assess the unadjusted effect of treatment on the primary outcome and on readmission rate using the Pearson Chi-square test. Differences in average length of stay and in change in biological markers will be assessed with either a two-sample t test or the Mann-Whitney nonparametric test. Multivariate analyses will be performed to compare treatment to control responses while adjusting for various co-factors. Multiple logistic regression will be used as supportive method to test for significant differences in the proportion with the primary outcome, between the active treatment and placebo groups, while adjusting for age, race, sex, BMI and other potential co-factors. For the secondary outcomes, multiple logistic regression will also

be used to test for significant differences in the proportion readmits, between the active treatment and placebo groups, and multiple linear regression will be used to test for significant differences in average length of stay and changes in biological markers, between the treatment and SOC groups, while adjusting for potential influencing co factors.

Patient and public involvement

No patients were involved in the design or implementation of this study.

ETHICS AND DISSEMINATION

Ethics and safety approval

Ethical approval was obtained from the institutional review board (IRB) at Tulane University Clinical Translational Unit on May 14st 2021. Future amendments to the protocol by the study investigators will be submitted to the IRB. Implementation of changes in the study will only be made after the IRB approval is received. Accordingly, trial participants, trial registries and trial staff will be informed in a timely manner. The study has been reviewed by the United States Food and Drug Administration and granted Investigational New Drug (IND) #152499.

Informed consent

The consent process will be administered to the patient (or legally authorized representative (LAR) in the case that the patient is unable to consent) prior to enrollment in the study. Adobe sign feature on iPad or smartphone will be used in order to minimize the safety concerns for study personnel conducting the consent procedures. There is also be a separate section in the consent that allows the subjects to opt in or out of additional blood collection for ancillary studies in the future that are directly related to the current study. The forms and the consenting process have been reviewed and approved by the Tulane IRB.

Dissemination

The data generated in this study will be presented at internal medicine, infections disease and endocrinology national or international conferences and published in a timely fashion. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. Results will be published in open-access journals when possible.

Study protocol and statistical analysis plans will be publically available. Participant-level data will not be shared

Discussion

The pathogenesis of COVID-19 involves an inappropriate immune response, with outpouring of pro-inflammatory chemokines, leading to lung infiltration and hyperactivation of monocytes and macrophages producing pro-inflammatory cytokines (cytokine storm), resulting in lung edema, reduced gas exchange, and ultimately leading to acute respiratory distress syndrome and multiorgan failure.[28-35] Repurposing approved drugs that have already been tested in humans—and for which detailed information is available on their pharmacology, formulation, dose, and potential toxicity—provides a fast and safe approach for off-label use of potentially life-saving therapeutics. The scientific premise for the use of E2 and P4 in hospitalized patients, and its relevance to COVID-19 pathogenesis, is based on a large body of published literature reviewed recently.[25] In fact, two clinical trials are testing E2 (ClinicalTrials.gov identifier NCT04359329) or P4 (ClinicalTrials.gov identifier NCT04365127) individually in COVID-19 patients. While this protocol was in review, the trial with P4 reported that in men hospitalized with moderate to severe COVID-19, a 5 day progesterone treatment resulted in three fewer days of supplemental oxygen need and 2.5 fewer days of hospital stay as compared with control subjects.[36] The present study has been reviewed by the US Food and Drug Administration for Investigational New Drug authorization since August 2020, which has delayed its beginning.

The advantage of repurposing E2 and P4 is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from over half a century of clinical and basic research. Hormone therapy using estrogens and progestogens is used by millions of women for contraception or the prevention of menopausal symptoms. There is no acute toxicity. The risk of DVT or pulmonary embolism associated with estradiol and progesterone treatment exists but is minimal. First, the risk of DVT is associated with chronic hormone therapy, over months or years, not following acute hormone therapy of several days. Most importantly, the risk is minimal because of parenteral estradiol administration, which does not alter clotting factors. DVT risk occurs mainly following orally administered estrogens, and synthetic progestins, because of first-pass liver metabolism producing high liver exposures to hormones, increasing

hepatic production of clotting and inflammatory factors.[37] Additionally, the DVT risks are usually associated with the use of conjugated equine estrogens alone or associated with a synthetic progestin, like medroxyprogesterone acetate.[37] There is no documented risk associated with natural E2 given systemically (IM, SC) and no documented risk with the use of natural P4. Finally, the pathogenesis of thrombosis in COVID-19 is a consequence of the immuno-inflammation,[38] and is expected to be ameliorated by the E2 and P4 treatment.

TRIAL STATUS

This trial is currently recruiting subjects.

Competing interests

PI and Co-Is do not have any conflict of interest to disclose.

Authors' Contributions

FMJ- study design, management, analysis, interpretation of data; writing of the report; and decision to submit the report for publication. DL-clinical trial preparatory activities, data management, statistical analysis, interpretation of data and writing of the report. KB- Recruitment of patients, interpretation of data and writing of the report. VF- interpretation of data and writing of the report. JL- statistical analysis, interpretation and writing of the report. MS- visualization and writing of the report.

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REFERENCES

1. John Hopkins Coronavirus Resource Center. Cases by Country. Accessed Sept 8, 2021. https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6
2. Khubchandani J, Sharma S, Price JH, Wiblishauser MJ, Sharma M, Webb FJ. COVID-19 Vaccination Hesitancy in the United States: A Rapid National Assessment. *J Community Health*. Apr 2021;46(2):270-277. doi:10.1007/s10900-020-00958-x
3. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*. Feb 25 2021;384(8):693-704. doi:10.1056/NEJMoa2021436
4. Kaka AS, MacDonald R, Greer N, et al. Major Update: Remdesivir for Adults With COVID-19 : A Living Systematic Review and Meta-analysis for the American College of Physicians Practice Points. *Ann Intern Med*. Feb 9 2021;doi:10.7326/m20-8148
5. Salvarani C, Dolci G, Massari M, et al. Effect of Tocilizumab vs Standard Care on Clinical Worsening in Patients Hospitalized With COVID-19 Pneumonia: A Randomized Clinical Trial. *JAMA Intern Med*. Jan 1 2021;181(1):24-31. doi:10.1001/jamainternmed.2020.6615
6. Janiaud P, Axfors C, Schmitt AM, et al. Association of Convalescent Plasma Treatment With Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis. *Jama*. Mar 23 2021;325(12):1185-1195. doi:10.1001/jama.2021.2747
7. Mehta SR, Yusuf S, Diaz R, et al. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. *Jama*. Jan 26 2005;293(4):437-46. doi:10.1001/jama.293.4.437
8. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. May 1 2020;130(5):2620-2629. doi:10.1172/jci137244
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. Feb 15 2020;395(10223):497-506. doi:10.1016/s0140-6736(20)30183-5
10. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. May 28 2020;181(5):1036-1045.e9. doi:10.1016/j.cell.2020.04.026
11. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol*. Jul 2017;39(5):517-528. doi:10.1007/s00281-017-0639-8
12. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. Jun 2020;80(6):607-613. doi:10.1016/j.jinf.2020.03.037
13. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. Jul 2016;8(8):959-70. doi:10.2217/imt-2016-0020
14. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev*. Jun 2020;19(6):102537. doi:10.1016/j.autrev.2020.102537
15. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. Apr 30 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032
16. Klein SL, Dhakal S, Ursin RL, Deshpande S, Sandberg K, Mauvais-Jarvis F. Biological sex impacts COVID-19 outcomes. *PLoS Pathog*. Jun 2020;16(6):e1008570. doi:10.1371/journal.ppat.1008570
17. Mauvais-Jarvis F. Aging, Male Sex, Obesity, and Metabolic Inflammation Create the Perfect Storm for COVID-19. *Diabetes*. Sep 2020;69(9):1857-1863. doi:10.2337/dbi19-0023
18. Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet*. Aug 22 2020;396(10250):565-582. doi:10.1016/s0140-6736(20)31561-0
19. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *Jama*. May 26 2020;323(20):2052-2059. doi:10.1001/jama.2020.6775

20. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat Rev Immunol*. Jul 2020;20(7):442-447. doi:10.1038/s41577-020-0348-8
21. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. Oct 2016;16(10):626-38. doi:10.1038/nri.2016.90
22. Seeland U, Coluzzi F, Simmaco M, et al. Evidence for treatment with estradiol for women with SARS-CoV-2 infection. *BMC Med*. Nov 25 2020;18(1):369. doi:10.1186/s12916-020-01851-z
23. Phiel KL, Henderson RA, Adelman SJ, Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol Lett*. Feb 15 2005;97(1):107-13. doi:10.1016/j.imlet.2004.10.007
24. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. Aug 2007;28(5):521-74. doi:10.1210/er.2007-0001
25. Mauvais-Jarvis F, Klein SL, Levin ER. Estradiol, Progesterone, Immunomodulation, and COVID-19 Outcomes. *Endocrinology*. Sep 1 2020;161(9)doi:10.1210/endocr/bqaa127
26. World Health Organization R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis. Accessed April 25, 2021. https://www.who.int/teams/blueprint/covid-19
27. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. Sep 1979;35(3):549-56.
28. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*. 2020/03/28/ 2020;395(10229):1033-1034. doi:https://doi.org/10.1016/S0140-6736(20)30628-0
29. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of clinical investigation*. 04/13/ 2020;130(5)doi:10.1172/JCI137244
30. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
31. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. doi:10.1016/j.cell.2020.04.026
32. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Seminars in Immunopathology*. 2017/07/01 2017;39(5):517-528. doi:10.1007/s00281-017-0639-8
33. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. Apr 10 2020;doi:10.1016/j.jinf.2020.03.037
34. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. 2016;8(8):959-970. doi:10.2217/imt-2016-0020
35. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmunity Reviews*. 2020/04/03/ 2020:102537. doi:https://doi.org/10.1016/j.autrev.2020.102537
36. Ghandehari S, Matusov Y, Pepkowitz S, et al. Progesterone in Addition to Standard of Care vs Standard of Care Alone in the Treatment of Men Hospitalized With Moderate to Severe COVID-19: A Randomized, Controlled Pilot Trial. *Chest*. Jul 2021;160(1):74-84. doi:10.1016/j.chest.2021.02.024
37. Mauvais-Jarvis F, Manson JE, Stevenson JC, Fonseca VA. Menopausal hormone therapy and type 2 diabetes prevention: Evidence, mechanisms and clinical implications. *Endocr Rev*. Mar 08 2017;doi:10.1210/er.2016-1146
38. Bonaventura A, Vecchié A, Dagna L, et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat Rev Immunol*. Apr 6 2021:1-11. doi:10.1038/s41577-021-00536-9

Table 1. World Health Organization 9 point Ordinal Scale for Clinical Improvement		
Patient State	**Descriptor**	**Score**
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support- pressors, Renal Replacement Therapy (RRT), Extracorporeal Membrane Oxygenation (ECMO)	7
Dead	Death	8
** Adapted from Ref 26*		

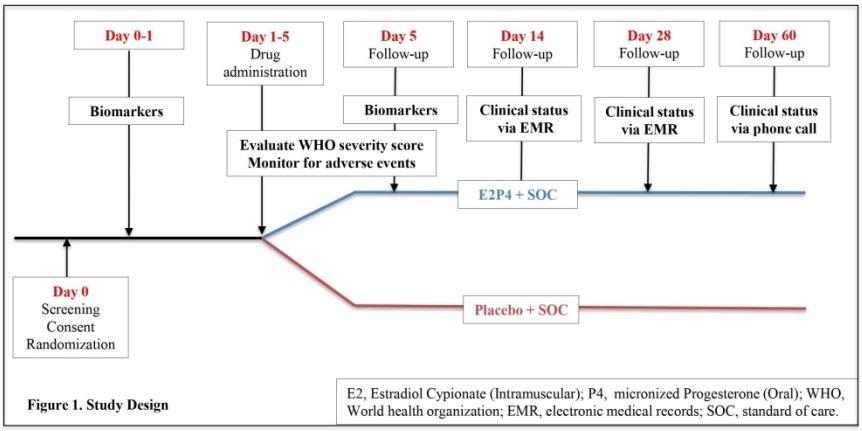
Table 2. Sample size calculations		
Treatment	Percent leading to a scale 1-2	Sample Size
SOC	60%	32
SOC + E2 + P4	90%	32
SOC	60%	22
SOC + E2 + P4	95%	22
SOC	70%	62
SOC + E2 + P4	90%	62
SOC	70%	36
SOC + E2 + P4	95%	36

Table 3. Interim analysis sample size calculation for a binary endpoint		
Stage	1	2
Information rate	50%	100%
Efficacy boundary (z-value scale)	2.963	1.969
Overall power	0.1641	0.8000
Number of subjects	61.8	123.7
Cumulative alpha spent	0.0031	0.0500
Two-sided local significance level	0.0031	0.0490
Lower efficacy boundary (t)	-0.377	-0.172
Upper efficacy boundary (t)	0.277	0.148
Sequential analysis with a maximum of 2 looks (group sequential design). The sample size was calculated for a two-sample test for rates (two-sided), H0: pi(1) - pi(2) = 0, H1; treatment rate pi(1) = 0.9, control rate pi(2) = 0.7. (t): approximate treatment effect scale		

FIGURE LEGENDS

Figure 1. Study design.
E2 5mg IM injection will be administered once on day 1 and P4 200mg will be administered daily PO day 1-5. Clinical status follow up via electronic medical records (EMR) review will be performed day 14th and day 28th. Clinical status for the end of study visit will be on day 60 via telephone and EMR review.

Figure 2. Schedule of safety assessments.
Study subjects will be monitored for symptoms daily while receiving the study drugs day 1-5. Relevant safety assessments otherwise will be performed via chart review on day 14 and 28th. End of study visit safety assessment will be on day 60 via telephone and EMR review.



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254x190mm (300 x 300 DPI)

Surveillance	Days following initiation of treatment								
	0	1	2	3	4	5	14	28	60
Pregnancy test*	X								
Injection site		X	X	X	X	X			
E2 side effects		X	X	X	X	X	X		
P4 side effects		X	X	X	X	X	X		
DVT symptoms		X	X	X	X	X	X	X	X
MI symptoms		X	X	X	X	X	X	X	X
Stroke symptoms		X	X	X	X	X	X	X	X
Fluid retention**		X	X	X	X	X	X		

* Women in reproductive age ** Patients with renal impairment

Figure 2. Schedule of safety assessments

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Study subjects will be monitored for symptoms daily while receiving the study drugs day 1-5. Relevant safety assessments otherwise will be performed via chart review on day 14 and 28th. End of study visit safety assessment will be on day 60 via telephone and EMR review.

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Acute Estradiol and Progesterone Therapy in Hospitalized Adults to Reduce COVID-19 Severity: A Randomized Control Trial

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Acute Estradiol and Progesterone Therapy in Hospitalized Adults to Reduce COVID-19 Severity: A Randomized Control Trial

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ABSTRACT

Introduction: As of October 2021, COVID-19, has killed more than 4 million people globally, including over 720,000 in the United States (US). Apart from corticosteroids, most available therapeutic options are at best marginally efficient in reducing disease severity and are extremely expensive. The systematic investigation of clinically approved drugs is a priority to determine what does mitigate disease severity. Estradiol (E2) and progesterone (P4) produce a state of anti-inflammatory immune responses and immune tolerance, and enhanced antibody production. The goal of this trial is to evaluate the efficacy of a short E2 and P4 therapy, in addition to standard of care (SOC), in mitigating disease severity in COVID-19 hospitalized patients.

Methods and analysis: Phase 2, randomized, double blind, placebo-controlled, single center trial. Patients hospitalized for confirmed COVID-19, with scores 3 to 5 on the 9-point World Health Organization (WHO) ordinal scale are randomized between two arms: 1) Estradiol Cypionate (IM) and micronized Progesterone (PO), in addition to SOC, and 2) Placebo, in addition to SOC. The primary outcome is the proportion of patients improving to scores 1 or 2 on the WHO scale through day 28. Secondary outcomes include length of hospital stay, duration of mechanical ventilation, cause of death, readmission rates, change in inflammatory biomarkers between admission and occurrence of primary endpoint, and adverse events. Study sample size will be up to 120 participants. The trial is currently recruiting subjects.

Ethics and dissemination: The sponsor of this study is the Center of Excellence in Sex-Based Biology & Medicine at Tulane University, New Orleans, Louisiana, USA. Ethical approval was obtained from the Tulane institutional review board (IRB) on May 14st 2021. The study was reviewed by the US Food and Drug Administration and granted Investigational New Drug (IND) #152499. Results of the study will be submitted for publication in a peer-reviewed journal.

Clinical Trial Registration: NCT04865029, ClinicalTrials.gov

STRENGTHS AND LIMITATIONS OF THIS STUDY

1. The trial will include men and women.
2. E2 and P4 decrease production of proinflammatory cytokines and enhance B-cell-mediated antibody production.
3. The advantage of repurposing E2 and P4 comes from decades of accumulated knowledge regarding their clinical efficacy and toxicity.
4. E2 and P4 are inexpensive and available in hospitals worldwide.
5. A randomized, placebo-controlled trial is an efficient way to determine if a short systemic E2 andP4 therapy, administered early to hospitalized COVID-19 patients in addition to SOC can reduce the severity of outcomes compared to SOC alone.

INTRODUCTION

As of October 2021, coronavirus diseases 2019 (COVID-19), has already killed over 4 million people worldwide.[1] Although the vaccination campaign is ramping up in the United States (US), vaccination hesitancy represents up to 25-30% of the population, [2] and hospitalizations and deaths are still high. In Brazil and India, the daily death toll is more than twice as high as it was in 2020.[1] Apart from corticosteroids,[3] most available therapeutic options are at best marginally efficient in reducing disease severity and mortality and are extremely expensive.[4-6] Therefore, the systematic investigation of clinically approved drugs is still a priority in order to determine what compounds could be repurposed to mitigate disease severity, and invest resources to go to full-scale production. Our current understanding of the disease is that COVID-19 deaths result from an inappropriate immune response with outpouring of pro-inflammatory chemokines leading to lung infiltration and hyperactivation of monocytes and macrophages producing pro-inflammatory cytokines (cytokine storm), resulting in lung edema, reduced gas exchange, and ultimately leading to acute respiratory distress syndrome and multiorgan failure.[7-14] Men with COVID-19 have a uniformly more severe outcome than women. In series from China, Europe and the U.S., COVID-19 mortality was consistently 1.5 to 2-fold higher in men than in women, suggesting that female biological sex is protecting women from COVID-19 mortality.[15-20] It is established that women exhibit heightened immune responses to viral infections compared to men,[21] which is at least partially due to the genetic benefit of gene dosage in X-linked immune-response genes. Ovarian steroids, however, also play a protective role. The analysis of electronic health records of over 68,000 COVID-19 patients revealed that estrogen therapy is associated with more than 50% reduction in mortality.[22] The main female steroids, 17β-estradiol (E2) and progesterone (P4) exhibit potent immuno-modulatory and anti-inflammatory actions via estrogen and progesterone receptors expressed in all immune cells, including epithelial cells, macrophages, dendritic cells, CD4+ and CD8+ lymphocytes, and B cells.[21, 23] P4 also acts partially via the glucocorticoid receptor. Together E2 and P4 produce a state of decreased innate immune cells production of proinflammatory cytokines, enhanced T cells anti-inflammatory responses and immune tolerance, and enhanced B-cell-mediated antibody production.[21, 24, 25] The scientific premise of

this trial is that in hospitalized COVID-19 patients, a short and early E2 and P4 therapy, in addition to standard of care (SOC; the National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel recommends the use of dexamethasone for up to 10 days or until hospital Discharge as SOC for the treatment of hospitalized COVID-19 patients), will prevent or mitigate the cytokine storm, and prevent severe outcomes, and without side effects.[25] Therefore, it will provide steroid immunomodulation without immunosuppression. The advantage of repurposing E2 and P4 is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from decades of clinical and basic studies. E2 and P4 are widely available in hospitals, inexpensive, manufacturable to scale, and can be prescribed immediately.

METHODS AND ANALYSIS

Study design, randomization, intervention and study calendar

Phase 2, randomized, double blind, placebo-controlled, single center trial designed to determine to what extent a short systemic E2 and P4 therapy, administered early to hospitalized COVID-19 positive patients of both sexes, in addition to SOC, can reduce the severity of symptoms and outcomes compared to SOC alone.

Hospitalized patients with COVID-19 with scores 3-5 on the 9-point World Health Organization (WHO) ordinal scale[26] are randomized to a 5-day treatment between two arms:

1. Estradiol Cypionate (Depo-Estradiol) 5mg via intramuscular injection on day 1 and micronized progesterone 200mg administered orally, daily for 5 days, in addition to SOC.
2. IM and oral placebos in addition to SOC.

We chose E2 administration via IM injection to avoid first-pass liver metabolism and change in coagulation markers while we chose P4 oral preparation due to ease of administration and low side effect profile. Drug will be supplied by Tulane Medical Center Research Pharmacy. The manufacturers of the study drugs are: Pfizer for E2 (NDC: 0009-0271-01) and VIRTUS for P4 (NDC: 69543-375-10).

An independent biostatistician will provide a random number table to the unblinded pharmacist for randomization. The random number table is generated based on the total number of subjects to be enrolled in a 1:1 ratio for placebo vs E2/P4. The unblinded pharmacist will use the random number table in sequential order for randomization assignment as subjects are enrolled. The research team will remain blinded to the randomization assignment throughout the study. Unblinding will only be permissible in case of adverse events.

Study design and visit schedule are shown in Figure 1.

Study implementation, recruitment, population and eligibility criteria

Participants are recruited at admission by the medical staff of the Department of General Internal Medicine and Geriatrics at Tulane Medical Center (Tertiary care academic hospital), under the leadership of Dr. Bateman (Co-Investigator) who identifies participants meeting study eligibility criteria from their admission list. Dr. Bateman assesses eligibility at screening visit, and notifies the research coordinators of eligible participants. Eligible subjects are enrolled by the research coordinators to partake in a randomized scheme to receive the 5-day active treatment or placebo. The Research Pharmacist at Tulane Medical Center assigns participants to the intervention. We will recruit up to 120 participants hospitalized at Tulane Medical Center with mild to severe COVID-19 (WHO ordinal scale score 3-5, Table 1) confirmed by SARS-CoV-2 PCR test .

Inclusion Criteria:

1. Hospitalization for COVID-19 (WHO Ordinal scale score 3-5, Table 1) confirmed by SARS-CoV-2 PCR at Tulane Medical Center in the Department of General Internal Medicine and Geriatrics.
2. Respiratory symptoms (fever, shortness of breath or cough) or abnormal lung exam or chest imaging characteristic of mild to severe COVID-19 pneumonia.

3. Patient and/or legally authorized representative (LAR) agrees to comply with study procedures and the collection of blood samples per protocol.
4. Patient and/or LAR agrees to be placed on prophylactic dose of anticoagulation for prevention of deep venous thrombosis (DVT) (if necessary).
5. Patient or legally authorized representative has signed informed consent.
6. Women of childbearing age with a negative pregnancy test on admission.

Exclusion Criteria:

1. Patient under 18 years of age
2. Critically-ill COVID-19 (respiratory failure requiring intubation and mechanical ventilation, shock, multi-organ failure).
3. Pregnant women confirmed by pregnancy test.
4. Women within six weeks of postpartum.
5. Patient included in another COVID-19 trial (excluding hydroxychloroquine and dexamethasone).
6. Women already treated by estrogen and or progestogen therapy two weeks prior to admission.
7. Men already treated by testosterone therapy prior to admission.
8. History of breast or endometrial cancer.
9. Abnormal genital bleeding.
10. Active or recent (e.g., within the past year) stroke or myocardial infarction.
11. History of blood clots including deep vein thrombosis related to clotting disease, or pulmonary emboli (prior to hospitalization).
12. History of liver dysfunction or disease.
13. Patients with end-stage renal disease
14. Patients taking inhibitors of CYP3A4 such as erythromycin, clarithromycin, ketoconazole, itraconazole, and ritonavir.

15. Patients taking St. John's Wort preparations (Hypericum perforatum), phenobarbital, carbamazepine, and rifampin.
16. Patients within 6 weeks of major orthopedic surgery.

Safety measures and monitoring

We will monitor each subject for adverse events/serious adverse events (AEs/SAEs) daily while the subjects are receiving daily therapy (Figure 2). If they develop a grade 3 or 4 AE, we will discontinue the study drug. No dose adjustments will be made. We will also follow-up in person or by telephone for all subjects for any adverse AEs/SAEs and mortality at Day 60.

AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version5 and taken care with the corresponding specialized clinical management of the SAE.

Grade 1 AEs (asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated) or Grade 2 AEs (moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activity of daily living): such as breast tenderness, nausea, vomiting, bloating, stomach cramps, headaches, vaginal itching, abnormal uterine bleeding will not require discontinuation of treatment.

Grade 3 SAEs like DVT and 4 (life-threatening) SAEs such as MI, stroke, anaphylaxis will be managed by treatment discontinuation (except SOC), and appropriate specialized clinical management of the SAE.

The trial is a 5-day intervention during hospitalization and therefore we do not expect problems with adherence. The study team members who are administering the trial drugs will call the PI or the Co-Is in circumstances when subjects have questions or concerns to be addressed in a timely manner to improve adherence.

Study Investigators have formed an independent Data Safety and Monitoring Board (DSMB) to monitor study safety. The DSMB consists of four board members who are clinicians and researchers with the experience necessary to interpret the data and ensure patient safety. Specifically, the board members are knowledgeable about COVID-19 and the treatment involved in this study. The DSMB includes, an

infectious disease specialist, an endocrinologist, a hematologist-oncologist, a women's health specialist and a biostatistician. The initial DSMB meeting occurred before the start of the trial to discuss the protocol and guidelines for monitoring the study. At this meeting, guidelines were defined for stopping the study for safety concerns (Study stopping rules) and where relevant, for efficacy based on plans specified in the protocol. The DSMB will meet on a quarterly basis. After each meeting, the DSMB will provide the PI with a letter describing the results of their review and their recommendations for the continuation of the study.

Outcome Measures

For all randomized patients according to inclusion criteria section above, demographic and clinical variables, including sex, age, race/ethnicity, body mass index (BMI), symptoms, vital signs, blood pressure, respiratory rate, temperature, oxygen saturation, chronic comorbidities through diagnosis codes, medications, clinical course during hospitalization (described in primary outcome below) will be reviewed in the patients' medical charts. Laboratory measurements will include, when available, complete blood count (CBC), blood chemistry, liver function test, creatinine, blood urea nitrogen test (BUN), lactate, troponin, ferritin, C-reactive protein (CRP), procalcitinin, Brain-type natriuretic peptide (BNP), D-dimer, and interleukin-6.We will review the participants' medical records for up to 60 days after hospital discharge.

***Primary outcome**:*

For all randomized patients with baseline inclusion criteria, WHO 9-point ordinal scale scores 3 to 5, the primary efficacy end point will be the proportion of patients who improve to scores 1 or 2 on the WHO ordinal scale (Table 1) through day 28.

***Secondary outcomes**:*

For all randomized patients, the following secondary outcomes will be assessed at day 14, 28 and 60. Assessment at day 14 and 28 will be performed by electronic medical records review, while assessment at day 60 will be performed via telephone call directly to the study subjects.

1. Length of hospital stay
2. Duration of mechanical ventilation

3. Date and cause of death
4. Readmission
5. Change in biological markers below between admission and occurrence of primary endpoint (2 values at least 2 days apart)
6. Grade 3 and 4 AEs
7. SAEs

Biological markers

Obtained from SOC:

Inflammation: neutrophil:lymphocyte ratio, CRP, ferritin, procalcitonin.

Hypercoagulability: D-dimers, fibrinogen

Tissue injury: troponin, Alanine transaminase (ALT), Aspartate transaminase (AST), Lactate dehydrogenase (LDH)

Metabolome, Proteome, peripheral blood mononuclear cells (PBMCs)

Data collection and management

The collection of routine blood samples and patient monitoring will be conducted as part of the SOC procedures that are utilized at Tulane Medical Center for COVID-19 patients. The collection of blood samples (CBC with differential, blood chemistry, inflammation markers) is to monitor disease severity and is independent from this protocol, but we will use the data in our analysis. Women of childbearing age will have a pregnancy test on admission.

An additional blood sample will be collected at admission and after 5 days of treatment (about 5-25 ml of blood at these two time points). This blood sample will be stored in Dr. Mauvais-Jarvis lab for 1) isolation of PBMC to assess the effect of treatment on immune cells populations by flow cytometry, and PBMC gene expression by RNA-Seq, and 2) metabolomics profiling to determine metabolic and molecular signatures of the efficacy of treatment. De-identified serum and PBMC frozen samples will be transferred to the University of Michigan Metabolomics Core to perform untargeted metabolomic

analyses. The serum and PBMC from collected blood samples will be used to perform multi-dimensional analysis in the Center for Translational Research in Infection and Inflammation at Tulane University School of Medicine. Blood samples will be stored for up to 3 years.

The majority of clinical data, including all blood sample collection and assessment of severity of illness, will occur while the patient is hospitalized, which should minimize patient nonadherence. The only information to be collected after discharge will be a telephone call to assess clinical status at day 60. All available data will be collected and assessed into our analysis. If patients choose to discontinue from the study after day 1 (when they will have already received estradiol if in the treatment group), we will separate the analysis from the treatment group as they will not have completed the progesterone therapy.

All data and research files will be de-identified and stored using the secure web application Research Electronic Data Capture (REDCap). REDCap is protected by a login as well as encryption. All data for this study will be electronic; there will be no paper records. Enrolled subjects will be assigned a sequential study ID (i.e. 001, 002, etc) that is linked to their medical record number. A link between the study ID numbers and medical record number will be kept in a separate file from the de-identified database and stored securely in REDCap. To promote data quality, two different individuals will perform double data entry. Subsequently, comparison of the two records will be done and any non-matching data will be correctly identified and entered. Only the IRB-approved study investigators will have access to the data and link. The PI will store all study research records and data post study closure, as required by NIH and University data retention policies.

Statistical considerations

Sample size

Sample size calculations are based on 80% Power, using a two-sided Pearson's Chi-square Test for Proportion Differences at the 5% significance level. It is estimated that between 60% and 70% of hospitalized patients with mild or severe disease will improve clinically and become ambulatory after standard treatment (WHO ordinal scale score 1-2, Table 1)). Table 2 presents the sample sizes that are

required in each treatment group to detect a significant difference in the percent of hospitalized patient leading to an ambulatory disease (shift from category 3-5 to category 1-2 in 20%, 25%, 30% or 35% of hospitalized patients after treatment with estradiol (E2) and progesterone (P4)). Efforts will be made to recruit 60 patients in each treatment group in order to stratify by disease severity, age and gender.

Interim analysis

One and only interim analysis will be performed at 50% study completion (after 60th subject end of study visit) to determine to what extent a strong treatment efficacy warrants stopping the trial early. Table 3 presents interim sample sizes and required Z test statistics for comparing two proportions at each of stage of an O'Brien-Fleming alpha-spending sequential design.[27] Based on 80% power and an overall 5% significance level, if the interim analysis for the first 60 enrolled patients results in differences that are more than 2.96 standard deviations from 0, the trial will end, and treatment efficacy will be concluded. Otherwise, the trial will enroll the total 120 patients.

Statistical Analysis

For all subjects who have completed the study, clinical outcomes and demographic characteristics will be summarized and presented separately for the treatment group and the control group, as well as overall for all patients. Categorical variables, such as the primary outcome, readmission, as well as race and sex will be summarized with counts and percentages. Quantitative outcomes, such as length of stay, change in biological markers, age and BMI will be summarized with means, medians, standard deviations, and interquartile ranges. Univariate analyses will be performed as the primary method to assess the unadjusted effect of treatment on the primary outcome and on readmission rate using the Pearson Chi-square test. Differences in average length of stay and in change in biological markers will be assessed with either a two-sample t test or the Mann-Whitney nonparametric test. Multivariate analyses will be performed to compare treatment to control responses while adjusting for various co-factors. Multiple logistic regression will be used as supportive method to test for significant differences in the proportion with the primary outcome, between the active treatment and placebo groups, while adjusting for age, race, sex, BMI and other potential co-factors. For the secondary outcomes, multiple logistic regression will also

be used to test for significant differences in the proportion readmits, between the active treatment and placebo groups, and multiple linear regression will be used to test for significant differences in average length of stay and changes in biological markers, between the treatment and SOC groups, while adjusting for potential influencing co factors.

Patient and public involvement

No patients were involved in the design or implementation of this study.

ETHICS AND DISSEMINATION

Ethics and safety approval

Ethical approval was obtained from the institutional review board (IRB) at Tulane University Clinical Translational Unit on May 14st 2021. Future amendments to the protocol by the study investigators will be submitted to the IRB. Implementation of changes in the study will only be made after the IRB approval is received. Accordingly, trial participants, trial registries and trial staff will be informed in a timely manner. The study has been reviewed by the United States Food and Drug Administration and granted Investigational New Drug (IND) #152499.

Informed consent

The consent process will be administered to the patient (or legally authorized representative (LAR) in the case that the patient is unable to consent) prior to enrollment in the study. Adobe sign feature on iPad or smartphone will be used in order to minimize the safety concerns for study personnel conducting the consent procedures. There is also be a separate section in the consent that allows the subjects to opt in or out of additional blood collection for ancillary studies in the future that are directly related to the current study. The forms and the consenting process have been reviewed and approved by the Tulane IRB.

Dissemination

The data generated in this study will be presented at internal medicine, infections disease and endocrinology national or international conferences and published in a timely fashion. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. Results will be published in open-access journals when possible.

Study protocol and statistical analysis plans will be publically available. Participant-level data will not be shared

Discussion

The pathogenesis of COVID-19 involves an inappropriate immune response, with outpouring of pro-inflammatory chemokines, leading to lung infiltration and hyperactivation of monocytes and macrophages producing pro-inflammatory cytokines (cytokine storm), resulting in lung edema, reduced gas exchange, and ultimately leading to acute respiratory distress syndrome and multiorgan failure.[28-35] Repurposing approved drugs that have already been tested in humans—and for which detailed information is available on their pharmacology, formulation, dose, and potential toxicity—provides a fast and safe approach for off-label use of potentially life-saving therapeutics. The scientific premise for the use of E2 and P4 in hospitalized patients, and its relevance to COVID-19 pathogenesis, is based on a large body of published literature reviewed recently.[25] In fact, two clinical trials are testing E2 (ClinicalTrials.gov identifier NCT04359329) or P4 (ClinicalTrials.gov identifier NCT04365127) individually in COVID-19 patients. While this protocol was in review, the trial with P4 reported that in men hospitalized with moderate to severe COVID-19, a 5 day progesterone treatment resulted in three fewer days of supplemental oxygen need and 2.5 fewer days of hospital stay as compared with control subjects.[36] The present study has been reviewed by the US Food and Drug Administration for Investigational New Drug authorization since August 2020, which has delayed its beginning.

The advantage of repurposing E2 and P4 is the depth of knowledge regarding their clinical efficacy and toxicity that has accumulated from over half a century of clinical and basic research. Hormone therapy using estrogens and progestogens is used by millions of women for contraception or the prevention of menopausal symptoms. There is no acute toxicity. The risk of DVT or pulmonary embolism associated with estradiol and progesterone treatment exists but is minimal. First, the risk of DVT is associated with chronic hormone therapy, over months or years, not following acute hormone therapy of several days. Most importantly, the risk is minimal because of parenteral estradiol administration, which does not alter clotting factors. DVT risk occurs mainly following orally administered estrogens, and synthetic progestins, because of first-pass liver metabolism producing high liver exposures to hormones, increasing

hepatic production of clotting and inflammatory factors.[37] Additionally, the DVT risks are usually associated with the use of conjugated equine estrogens alone or associated with a synthetic progestin, like medroxyprogesterone acetate.[37] There is no documented risk associated with natural E2 given systemically (IM, SC) and no documented risk with the use of natural P4. Finally, the pathogenesis of thrombosis in COVID-19 is a consequence of the immuno-inflammation,[38] and is expected to be ameliorated by the E2 and P4 treatment.

TRIAL STATUS

This trial is currently recruiting subjects.

Competing interests

PI and Co-Is do not have any conflict of interest to disclose.

Authors' Contributions

FMJ- study design, management, analysis, interpretation of data; writing of the report; and decision to submit the report for publication. DL-clinical trial preparatory activities, data management, statistical analysis, interpretation of data and writing of the report. KB- Recruitment of patients, interpretation of data and writing of the report. VF- interpretation of data and writing of the report. JL- statistical analysis, interpretation and writing of the report. MS- visualization and writing of the report.

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REFERENCES

1. John Hopkins Coronavirus Resource Center. Cases by Country. Accessed Sept 8, 2021. https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6
2. Khubchandani J, Sharma S, Price JH, Wiblishauser MJ, Sharma M, Webb FJ. COVID-19 Vaccination Hesitancy in the United States: A Rapid National Assessment. *J Community Health*. Apr 2021;46(2):270-277. doi:10.1007/s10900-020-00958-x
3. Horby P, Lim WS, Emberson JR, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*. Feb 25 2021;384(8):693-704. doi:10.1056/NEJMoa2021436
4. Kaka AS, MacDonald R, Greer N, et al. Major Update: Remdesivir for Adults With COVID-19 : A Living Systematic Review and Meta-analysis for the American College of Physicians Practice Points. *Ann Intern Med*. Feb 9 2021;doi:10.7326/m20-8148
5. Salvarani C, Dolci G, Massari M, et al. Effect of Tocilizumab vs Standard Care on Clinical Worsening in Patients Hospitalized With COVID-19 Pneumonia: A Randomized Clinical Trial. *JAMA Intern Med*. Jan 1 2021;181(1):24-31. doi:10.1001/jamainternmed.2020.6615
6. Janiaud P, Axfors C, Schmitt AM, et al. Association of Convalescent Plasma Treatment With Clinical Outcomes in Patients With COVID-19: A Systematic Review and Meta-analysis. *Jama*. Mar 23 2021;325(12):1185-1195. doi:10.1001/jama.2021.2747
7. Mehta SR, Yusuf S, Diaz R, et al. Effect of glucose-insulin-potassium infusion on mortality in patients with acute ST-segment elevation myocardial infarction: the CREATE-ECLA randomized controlled trial. *Jama*. Jan 26 2005;293(4):437-46. doi:10.1001/jama.293.4.437
8. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. May 1 2020;130(5):2620-2629. doi:10.1172/jci137244
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. Feb 15 2020;395(10223):497-506. doi:10.1016/s0140-6736(20)30183-5
10. Blanco-Melo D, Nilsson-Payant BE, Liu WC, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. May 28 2020;181(5):1036-1045.e9. doi:10.1016/j.cell.2020.04.026
11. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol*. Jul 2017;39(5):517-528. doi:10.1007/s00281-017-0639-8
12. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. Jun 2020;80(6):607-613. doi:10.1016/j.jinf.2020.03.037
13. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. Jul 2016;8(8):959-70. doi:10.2217/imt-2016-0020
14. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev*. Jun 2020;19(6):102537. doi:10.1016/j.autrev.2020.102537
15. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. Apr 30 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032
16. Klein SL, Dhakal S, Ursin RL, Deshpande S, Sandberg K, Mauvais-Jarvis F. Biological sex impacts COVID-19 outcomes. *PLoS Pathog*. Jun 2020;16(6):e1008570. doi:10.1371/journal.ppat.1008570
17. Mauvais-Jarvis F. Aging, Male Sex, Obesity, and Metabolic Inflammation Create the Perfect Storm for COVID-19. *Diabetes*. Sep 2020;69(9):1857-1863. doi:10.2337/dbi19-0023
18. Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, et al. Sex and gender: modifiers of health, disease, and medicine. *Lancet*. Aug 22 2020;396(10250):565-582. doi:10.1016/s0140-6736(20)31561-0
19. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *Jama*. May 26 2020;323(20):2052-2059. doi:10.1001/jama.2020.6775

20. Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. *Nat Rev Immunol*. Jul 2020;20(7):442-447. doi:10.1038/s41577-020-0348-8
21. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. Oct 2016;16(10):626-38. doi:10.1038/nri.2016.90
22. Seeland U, Coluzzi F, Simmaco M, et al. Evidence for treatment with estradiol for women with SARS-CoV-2 infection. *BMC Med*. Nov 25 2020;18(1):369. doi:10.1186/s12916-020-01851-z
23. Phiel KL, Henderson RA, Adelman SJ, Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunol Lett*. Feb 15 2005;97(1):107-13. doi:10.1016/j.imlet.2004.10.007
24. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. Aug 2007;28(5):521-74. doi:10.1210/er.2007-0001
25. Mauvais-Jarvis F, Klein SL, Levin ER. Estradiol, Progesterone, Immunomodulation, and COVID-19 Outcomes. *Endocrinology*. Sep 1 2020;161(9)doi:10.1210/endocr/bqaa127
26. World Health Organization R&D Blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis. Accessed April 25, 2021. https://www.who.int/teams/blueprint/covid-19
27. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. Sep 1979;35(3):549-56.
28. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*. 2020/03/28/ 2020;395(10229):1033-1034. doi:https://doi.org/10.1016/S0140-6736(20)30628-0
29. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of clinical investigation*. 04/13/ 2020;130(5)doi:10.1172/JCI137244
30. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet*. 2020;395(10223):497-506. doi:10.1016/S0140-6736(20)30183-5
31. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell*. doi:10.1016/j.cell.2020.04.026
32. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Seminars in Immunopathology*. 2017/07/01 2017;39(5):517-528. doi:10.1007/s00281-017-0639-8
33. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *J Infect*. Apr 10 2020;doi:10.1016/j.jinf.2020.03.037
34. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy*. 2016;8(8):959-970. doi:10.2217/imt-2016-0020
35. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmunity Reviews*. 2020/04/03/ 2020:102537. doi:https://doi.org/10.1016/j.autrev.2020.102537
36. Ghandehari S, Matusov Y, Pepkowitz S, et al. Progesterone in Addition to Standard of Care vs Standard of Care Alone in the Treatment of Men Hospitalized With Moderate to Severe COVID-19: A Randomized, Controlled Pilot Trial. *Chest*. Jul 2021;160(1):74-84. doi:10.1016/j.chest.2021.02.024
37. Mauvais-Jarvis F, Manson JE, Stevenson JC, Fonseca VA. Menopausal hormone therapy and type 2 diabetes prevention: Evidence, mechanisms and clinical implications. *Endocr Rev*. Mar 08 2017;doi:10.1210/er.2016-1146
38. Bonaventura A, Vecchié A, Dagna L, et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. *Nat Rev Immunol*. Apr 6 2021:1-11. doi:10.1038/s41577-021-00536-9

Table 1. World Health Organization 9 point Ordinal Scale for Clinical Improvement		
Patient State	**Descriptor**	**Score**
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized mild disease	Hospitalized, no oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized Severe Disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Ventilation + additional organ support- pressors, Renal Replacement Therapy (RRT), Extracorporeal Membrane Oxygenation (ECMO)	7
Dead	Death	8
** Adapted from Ref 26*		

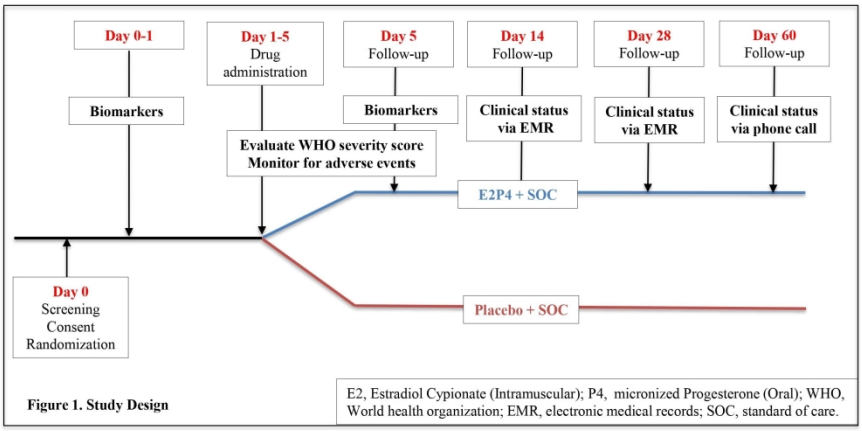
Table 2. Sample size calculations		
Treatment	Percent leading to a scale 1-2	Sample Size
SOC	60%	32
SOC + E2 + P4	90%	32
SOC	60%	22
SOC + E2 + P4	95%	22
SOC	70%	62
SOC + E2 + P4	90%	62
SOC	70%	36
SOC + E2 + P4	95%	36

Table 3. Interim analysis sample size calculation for a binary endpoint		
Stage	1	2
Information rate	50%	100%
Efficacy boundary (z-value scale)	2.963	1.969
Overall power	0.1641	0.8000
Number of subjects	61.8	123.7
Cumulative alpha spent	0.0031	0.0500
Two-sided local significance level	0.0031	0.0490
Lower efficacy boundary (t)	-0.377	-0.172
Upper efficacy boundary (t)	0.277	0.148
Sequential analysis with a maximum of 2 looks (group sequential design). The sample size was calculated for a two-sample test for rates (two-sided), H0: pi(1) - pi(2) = 0, H1; treatment rate pi(1) = 0.9, control rate pi(2) = 0.7. (t): approximate treatment effect scale		

FIGURE LEGENDS

Figure 1. Study design.
E2 5mg IM injection will be administered once on day 1 and P4 200mg will be administered daily PO day 1-5. Clinical status follow up via electronic medical records (EMR) review will be performed day 14th and day 28th. Clinical status for the end of study visit will be on day 60 via telephone and EMR review.

Figure 2. Schedule of safety assessments.
Study subjects will be monitored for symptoms daily while receiving the study drugs day 1-5. Relevant safety assessments otherwise will be performed via chart review on day 14 and 28th. End of study visit safety assessment will be on day 60 via telephone and EMR review.



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254x190mm (300 x 300 DPI)

Surveillance	Days following initiation of treatment								
	0	1	2	3	4	5	14	28	60
Pregnancy test*	X								
Injection site		X	X	X	X	X			
E2 side effects		X	X	X	X	X	X		
P4 side effects		X	X	X	X	X	X		
DVT symptoms		X	X	X	X	X	X	X	X
MI symptoms		X	X	X	X	X	X	X	X
Stroke symptoms		X	X	X	X	X	X	X	X
Fluid retention**		X	X	X	X	X	X		

* Women in reproductive age ** Patients with renal impairment

Figure 2. Schedule of safety assessments

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