

Title: Phase IIa Clinical Trial to Evaluate Pharmacokinetics and Safety of Slow Release DHEA

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Phase IIa Clinical Trial to Evaluate Pharmacokinetics and Safety of Slow Release DHEA

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1 BACKGROUND AND RATIONALE

Severe asthma results in significant mortality and morbidity and in annual healthcare expenditures >\$10 billion (1). Glucocorticoids (GCs) are the mainstay of treatment but are only incompletely effective in this condition. There is a critical need for novel, targeted and corticosteroid-sparing therapies for patients with severe asthma. Since asthma is sexually dimorphic with women being more affected than men (2,3), since GC treatment suppresses endogenous dehydroepiandrosterone (DHEA) production (4), and since our preliminary data demonstrate a variety of protective DHEA effects on automatic exposure control (AEC) function and inflammatory responses, there is a strong biological rationale to test whether DHEA-based treatment strategies are beneficial in this condition. Our data suggest that patients with low dehydroepiandrosterone-sulfate (DHEA-S) levels and HSD3B1 AA or AC genotypes represent a subgroup that will derive particular benefit from DHEA treatment, suggesting an exciting new potential for personalized medicine strategies in severe asthma. Our experience with studying DHEA treatment in asthma (Marozkina (5)), HSD3B1 genotype-phenotype correlations in asthma (Zein (4)) and sex hormone signaling in lung disease (Lahm (6)), make us uniquely equipped to perform the proposed study. Our overall goal is to 1) determine pharmacokinetics (PK) and evaluate the safety of DHEA treatment in asthma and 2) perform a randomized, placebo-controlled pilot study to evaluate DHEA effects on FEV1 and FeNO. This protocol outlines goal #1. A separate protocol will be developed for goal #2. This study will allow us to gather critical baseline data for designing larger trials in the future. Ultimately, this will allow for developing novel and personalized therapies for severe asthma.

PK studies of DHEA in asthma in general, and severe asthma in particular, have never been done. In many diseases, PK in subjects with disease differs from that of control subjects and those with other conditions. For example, cystic fibrosis is a pulmonary condition in which PK is unique to the disease population (7). Therefore, we are investigating if PK levels to slow release DHEA are different in subjects with severe asthma who have the HSD3B1 AA or AC phenotypes. Previous work has shown that the HSD3B1 genotype does not classically affect circulating DHEA-S levels, but rather affects tissue androgen levels (4, 8, 9). Based on our preliminary data and published reports (5), we anticipate that the maximal effect of DHEA will be observed in patients with the responsive genotype (4) and low DHEA-S levels (4) (**Table 1**). Because the homozygous HSD3B1(1245) AA genotype and heterozygous AC genotype in severe asthma populations are similar in lung function (4), and because the CC genotype is uncommon (12%; **Table 2**), we will focus our PK studies on the “optimal response” cohort (AA/AC genotype & low DHEA-S levels). If we are successful, additional studies will be

Table 1. Predicted biomarker response (based on previously published work from our group)(4,5).

	Serum DHEA-S levels (µg/dL)	
	<90 in males or <45 in females	≥90 in males or ≥45 in females
HSD3B1(1245) genotype		
CC	Intermediate Response	Poor Response
AA or AC	Optimal Response	Intermediate Response

Table 2: HSD3B1(1245) genotype distribution in SARP I, II & III

Genotype	American Indian/ Alaskan Native	Asian	African American	White/ Caucasian	Hispanic	Other
AA - n(%)	4 (80)	30 (83)	375 (77)	411 (46)	22 (52)	27 (63)
AC - n(%)	1 (20)	5 (14)	105 (22)	386 (43)	18 (43)	13 (30)
CC - n(%)	0 (0)	1 (3)	6 (1)	100 (12)	2 (5)	3 (7)

performed in subsequent, larger trials. We suspect that the dose used for our pilot study in non-severe asthma (100 mg twice/day (5)) may be higher than what is needed for severe, susceptible

asthma. Indeed, steady state levels in the pilot study were $\sim 725 \mu\text{g/dL}$ (5). Therefore, we will study 50 mg & 100 mg of DHEA (both within commonly used dosing ranges (10,11)).

2 CLINICAL SIGNIFICANCE

Severe asthma only accounts for $\sim 10\%$ of all asthma in the United States, but it accounts for nearly half of the annual mortality and cost of the disease (12-16). Estimates are that severe asthma takes over 1,000 lives per year, and that the annual financial burden of severe asthma may be several billion dollars in the United States alone. Important improvements in asthma outcomes have been realized with antibody-based therapies (for example, 17-19). However, these therapies are primarily effective for specific subpopulations of the Th-lymphocyte Type 2-high (“T2-high”) asthma population, are normally not indicated in the “T2 low” population (17-21) and are expensive. Here, we propose to investigate potentially less expensive, less invasive, personalized approaches to managing certain severe patients, particularly including those who are not candidates for, or have failed, conventional severe asthma therapies and biological approaches.

3 STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVE:

- Determine dose of slow release DHEA in patients with asthma and AA or AC genotype in conjunction with low serum DHEA-S.

3.2 SECONDARY OBJECTIVE:

- Evaluate the safety and tolerability of DHEA in asthma.
 - Safety outcomes include changes in:
 - Vital signs
 - Pulse oximetry
 - Physical examination
 - Symptoms
 - Adverse events (AEs)
 - Serious adverse events (SAEs)
 - Blood tests (CBC with differential, comprehensive chemistry)
 - Pulmonary function (FEV1, FVC, FEV1/FVC, FEF25-75)
 - An asthma exacerbation (physician decision to treat) will be considered an event of particular interest.

4 STUDY DESIGN

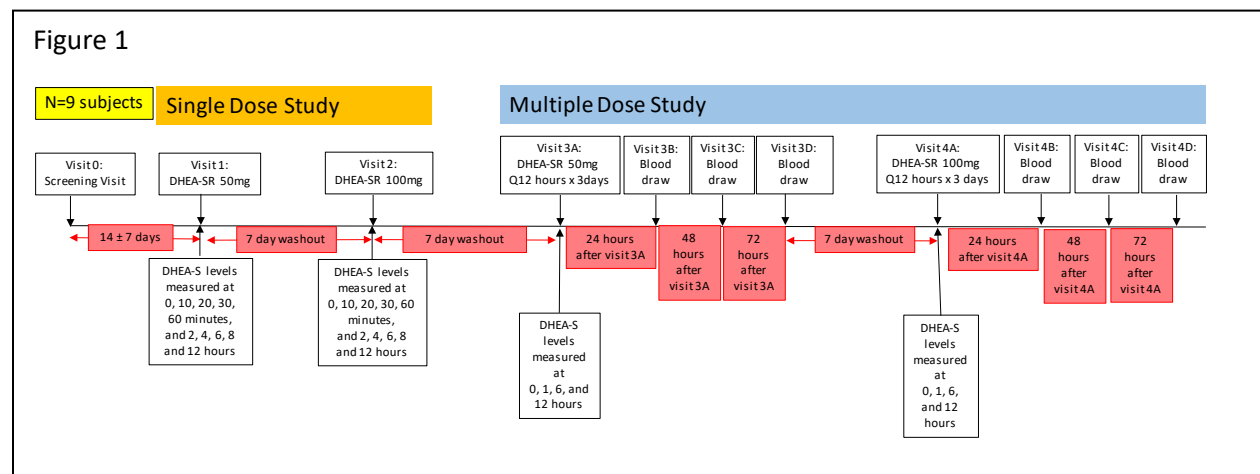
This is an unblinded pharmacokinetic (PK) study to determine optimal dosing for future studies. This study will start with a one-time dose and progress to twice daily dosing for 3 days as described below.

4.1 SINGLE DOSE STUDY

Study cohort will be 9 subjects with asthma. DHEA dose will be 50 mg via slow release (SR) capsules. Endpoints will be serum DHEA and DHEA-S levels at 10, 20, 30, 60 min & 2, 4, 6, 8, 12h after administration. After a one-week washout period, the protocol will be repeated using 100 mg of SR-DHEA.

4.2 MULTIPLE DOSE STUDY

The same subject cohort will be studied after a one-week washout, first with 50 mg at dosing intervals, based on the single-dose PK. Then, after a one-week washout, we will employ 100 mg at intervals. Drug will be given for 3 days; DHEA and DHEA-S concentrations will be determined at 1, 6, 12, 24, 48, and 72 hours (**Figure 1**).



4.3 STUDY LOCATION

Indiana University School of Medicine & IU Health
 Riley Hospital for Children/Children's Clinical Research Center
 705 Riley Hospital Drive, Rm 2606
 Indianapolis, IN 46202

Indiana University School of Medicine & IU Health
 University Hospital/Clinical Research Center
 550 N. University Blvd., 5th Floor, Rm 5595
 Indianapolis, IN 46202

4.4 PROJECTED STUDY ENROLLMENT

9 adults

5 STUDY SUBJECT ELIGIBILITY

5.1 INCLUSION CRITERIA

1. Adult male or female age ≥ 18 and ≤ 50 years at the time of enrollment
2. Evidence of asthma demonstrated by reversibility at visit 0 or by historical methacholine or bronchodilator reversibility if testing was performed under either the 2017 ERS technical standard (22) or the 1999 ATS Guidelines (23) or outside studies, provided that full sets of flow volume loops have been reviewed and approved by the PI. These criteria are defined as one of the following:
 - a. For bronchodilator reversibility: An increase in FEV1 $\geq 10\%$ (24) compared to the baseline (and 200 ml) after up to 8 puffs of albuterol

- b. For historical methacholine responsiveness: Positive methacholine defined as PC20 $\leq$ 16 mg/ml, or PD20 $\leq$ 400 mcg
- 3. Physician diagnosis of asthma according to NHLBI guidelines
- 4. Consistent use of an ICS inhaler for the prior 2 months
- 5. Non-smoker
- 6. Females must not be pregnant or lactating (breastfeeding)
- 7. Absence of non-allergic comorbidities
- 8. Genotype testing positive for either HSD3B1 AA or AC specific variant

5.2 EXCLUSION CRITERIA

- 1. Pregnant or actively trying to become pregnant; breast feeding
- 2. Positive urine pregnancy test
- 3. Known lung disease other than asthma
- 4. Acute (non asthma-related) dyspnea, viral respiratory illness or asthma exacerbation within 4 weeks of screening
- 5. Systemic glucocorticoid dosing for maintenance >10 mg/day of prednisone or equivalent
- 6. Patients with significant non-allergic comorbidities (e.g. cerebral palsy, heart disease, kidney disease, liver disease, etc.)
- 7. Patients with any known central or peripheral endocrine abnormality such as precocious puberty or diabetes
- 8. Patients with any known previous adverse reaction to DHEA
- 9. Current smoker or pack year history > 5 years (includes vaping/nicotine inhalation devices)
- 10. Positive urine cotinine test (> 100 mg/mL)
- 11. Use of prednisone or antibiotics in the last 4 weeks
- 12. Use of any performance-enhancing drugs in the last 2 weeks
- 13. Use of DHEA in the last 2 weeks
- 14. Androgen use for any reason
- 15. HSD3B1 CC phenotype
- 16. Any other condition or finding that would compromise the safety of the subject or the quality of the study data, or otherwise interfere with achieving the study objectives, as determined by the PI
- 17. Menopausal amenorrhea by history
- 18. Positive PSA (>4 ng/ml) (Prostate Specific Antigen)
- 19. Prior diagnosis of vocal cord dysfunction, bronchopulmonary dysplasia, cystic fibrosis, chronic obstructive pulmonary disorder, or other lung disease
- 20. Systolic blood pressure > 150 mm Hg and/or diastolic blood pressure >90 mm Hg
- 21. Heart rates outside the range of 50 to 120 beats per minutes or with a pathologic irregularity
- 22. Patients afflicted with any additional acute or chronic pathology that in the opinion of the screening physician makes them unsuitable for study or increases the risks associated with the study.

6 SCHEDULED EVENTS

	Visit 0	Visit 1	Visit 2	Visit 3A	Visit 3B	Visit 3C	Visit 3D	Visit 4A	Visit 4B	Visit 4C	Visit 4D
Assessments and Procedures	Screening Day 0	Day 14 $\pm$ 7 days	Day 28 $\pm$ 7 days	Day 42 $\pm$ 7 days	1 day (24 hrs $\pm$ 1 hr) after Visit 3A	2 days (48 hrs $\pm$ 1 hr) after Visit 3A	3 days (72 hrs $\pm$ 1 hr) after Visit 3A	Day 56 $\pm$ 7 days	1 day (24 hrs $\pm$ 1 hr) after Visit 4A	2 days (48 hrs $\pm$ 1 hr) after Visit 4A	3 days (72 hrs $\pm$ 1 hr) after Visit 4A
Informed consent	X										
Confirm ongoing consent		X	X	X				X			
Medical history and demographics	X										
Concomitant medications	X	X	X	X				X			
Review inclusion/exclusion criteria	X	X	X	X				X			
Asthma Control Test (ACT) & Asthma Control Questionnaire (ACQ)	X	X	X	X				X			X
Height	X										
Weight, Pulse oximetry, Vital signs	X	X	X	X				X			
Urine pregnancy test (when applicable)	X	X	X	X				X			
Urine cotinine test	X										
Prostate specific antigen test (when applicable)	X										
Spirometry with bronchodilator response (maximum bronchodilator)	X						X				X
Spirometry. If $\geq$ 10% decrease in FEV1 from baseline, give bronchodilator and measure response		X	X	X				X			
Abbreviated physical exam	X	X	X	X				X			
Blood draw for: -CBC with differential -comprehensive metabolic panel -dehydroepiandrosterone sulfate (DHEA-S) level -HSD3B1 genotyping	X										
Blood draw for: -baseline DHEA-S level -cytokine studies/ peripheral blood		X									

Assessments and Procedures	Visit 0	Visit 1	Visit 2	Visit 3A	Visit 3B	Visit 3C	Visit 3D	Visit 4A	Visit 4B	Visit 4C	Visit 4D
	Screening Day 0	Day 14 ± 7 days	Day 28 ± 7 days	Day 42 ± 7 days	1 day (24 hrs ± 1 hr) after Visit 3A	2 days (48 hrs ± 1 hr) after Visit 3A	3 days (72 hrs ± 1 hr) after Visit 3A	Day 56 ± 7 days	1 day (24 hrs ± 1 hr) after Visit 4A	2 days (48 hrs ± 1 hr) after Visit 4A	3 days (72 hrs ± 1 hr) after Visit 4A
mononuclear cells (PBMCs) -endocrine analysis -genomics											
Blood draw pre-DHEA administration for: -baseline DHEA-S			X	X				X			
Blood draw after DHEA administration at: 10, 20, 30, 60 minutes, and 2, 4, 6, 8 and 12 hours for DHEA-S		X	X								
Blood draw after DHEA administration at: 0, 1, 6 and 12 hours for DHEA-S				X				X			
Blood draw after DHEA administration at 24 hours for DHEA-S					X				X		
Blood draw after DHEA administration at 48 hours for DHEA-S, cytokine studies and genomics						X				X	
Blood draw after DHEA administration and cytokine studies at 72 hours for: -cytokine studies/PBMCs- -endocrine analysis -DHEA-S -genomics							X				
Blood draw after DHEA administration at 72 hours for: -CBC with differential -comprehensive metabolic panel -cytokine studies/PBMCs -endocrine analysis -DHEA-S -genomics											X
DHEA administration		X	X	X				X			
Collect any unused DHEA capsules from subject							X				X

	Visit 0	Visit 1	Visit 2	Visit 3A	Visit 3B	Visit 3C	Visit 3D	Visit 4A	Visit 4B	Visit 4C	Visit 4D
Assessments and Procedures	Screening Day 0	Day 14 $\pm$ 7 days	Day 28 $\pm$ 7 days	Day 42 $\pm$ 7 days	1 day (24 hrs $\pm$ 1 hr) after Visit 3A	2 days (48 hrs $\pm$ 1 hr) after Visit 3A	3 days (72 hrs $\pm$ 1 hr) after Visit 3A	Day 56 $\pm$ 7 days	1 day (24 hrs $\pm$ 1 hr) after Visit 4A	2 days (48 hrs $\pm$ 1 hr) after Visit 4A	3 days (72 hrs $\pm$ 1 hr) after Visit 4A
Adverse events	X	X	X	X	X	X	X	X	X	X	X
Give subject Dosing & Symptom Diary				X				X			
Collect Dosing & Symptom Diary							X				X

7 DESCRIPTION OF EVENTS

Medical history and demographics, height, weight and vital signs

Relevant medical history, including history of current disease, spirometry results, other pertinent respiratory history, and information regarding underlying diseases will be recorded. Demographic and baseline clinical characteristics including age, gender, and race will be recorded. Pulse oximetry, height and weight will be measured, as well as vital signs (blood pressure, temperature, heart rate and respiration rate).

Concomitant medications

All concomitant medications and concurrent therapies will be documented at visits 0-4A. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured for the 30 days prior to screening and between each visit.

Asthma control questionnaire

Subjects will be asked to complete two questionnaires: the Asthma Control Test (ACT) and the Asthma Control Questionnaire (ACQ).

- The ACT is a validated asthma assessment tool used to assess asthma symptoms over a 4-week period. Each item on the ACT has a possible score ranging from 0 to 5, and the total score is used to determine asthma control.
- The ACQ includes six questions on symptoms, activity limitation and beta2-agonist use, with an optional assessment on airway caliber over a 7-day period. Each question is scored from 0 (well controlled) to 6 (extremely poorly controlled) with the total score being the average of the questions.

Urine pregnancy test

A urine pregnancy test will be performed at Visits 0-4A if the subject is a female of child bearing potential.

Urine cotinine test

A urine cotinine test will be performed at Visit 0 for all subjects to measure cotinine levels.

Prostate specific antigen test

Five mL of blood will be obtained from male subjects for PSA because administration of supplemental DHEA in people with prostate cancer theoretically could result in a flare-up of their prostate cancer.

Spirometry with maximum bronchodilator response

Pre-bronchodilator FEV1 and post-bronchodilator FEV1 will be measured using spirometry. The FEV1 is the volume of air which can be forcibly exhaled from the lungs in the first second, measured in liters. Spirometry will be done according to American Thoracic Society/European Respiratory Society procedural guidelines (25). Current reference equations (26) will be used.

Airway reversibility will be demonstrated by measuring the change in FEV1 before and after inhalation of albuterol; reversibility testing should be attempted after withholding long-acting beta-agonist (LABA) therapy for at least 12 hours and short-acting beta agonist (SABA) therapy for at least 6 hours. Up to 8 puffs of albuterol should be used for reversibility testing, as tolerated. If a subject's FEV1 improves at least 10% between the 2 tests, then he/she will be deemed as having airway reversibility.

Abbreviated physical exam

An abbreviated physical exam will consist of HEENT, cardiovascular system, chest/lung, abdomen, extremities, skin, and any other areas as appropriate. This exam will be performed by a physician.

Blood collection

Blood will be drawn at each visit. A CBC with differential and comprehensive metabolic panel will be performed at baseline and at the conclusion of the study. A prostate specific antigen (PSA) level will be measured in male participants at the screening visit. DHEA-S levels will be measured at the screening visit, before the initial dose of DHEA, and at designated timepoints after DHEA is administered. Blood will be obtained at Visit 1, 3D, and 4D for cytokine studies, PBMCs, genotyping and for endocrine analysis to measure hormone levels.

The following amounts of blood will be drawn during this study and used as specified in the **Description of Visits** section.

Visit	# mL	Visit	# mL
0	20	3D	35
1	101	4A	56
2	80	4B	14
3A	56	4C	14
3B	14	4D	45
3C	14		

Blood will be collected at various intervals throughout the study. Each interval will include a window of time before and after to account for unplanned mishaps, equipment failure, or human error. This also applies to study drug administration, both in clinic, and when the subject is at home. Due to the number of intervals, adding this $\pm$ timeframe to each occurrence creates excessively busy text and an

opportunity for errors and inconsistencies. Therefore, we are listing these timeframes here and they apply to all study activities.

- All minute intervals (10, 20, 30, and 60) will include a window of ± 2 minutes
- All hour intervals (1, 2, 4, 6, 8, 12) will include a window of ± 10 minutes
- Study drug is taken every 12 hours, ± 1 hour

Genotyping

Single nucleotide polymorphism testing for HSD3B1 genotype will be performed from blood drawn at the screening visit and results must be available before the first dose of study drug. If a genotype test has been performed previously and is documented in the subject's medical record, the subject's eligibility must be approved by the PI. If a historic genotype result is not available at screening or if the historic genotype result is not approved by the medical monitor, subjects will be tested for genotype at screening and the results must be reviewed before the first dose of study drug. Subjects will not receive the study drug if genotype is not confirmed by both an approved historic genotype and approval of medical monitor result OR by genotype testing at screening. Study subjects with HSD3B1 AA or AC specific variants will be enrolled in the study. No further genetic studies will be performed on the subject's DNA. The subject will be fully informed of the genotype results relevant to this study.

DHEA

DHEA is available as an over-the-counter supplement that is available on the market without prescription. The DHEA used in this study will be formulated for slow release and will be provided by Arena District Pharmacy of the Central Ohio Compounding Pharmacy in Columbus, OH 43215. They produce 50mg and 100mg capsules that will be used for this study. Arena District will provide capsules by prescription only. The prescribed DHEA capsules will be received, stored and distributed by the Gaston Pediatric Translational Research Lab Group in the Herman B Wells Center for Pediatric Research. Accountability records will be maintained by the study coordinator.

Subjects will be provided DHEA capsules as outlined below:

1. One 50mg capsules at visit 1 to take at the onset of the 12 hour PK study.
2. One 100mg capsule at visit 2 to take at the onset of the 12 hour PK study.
3. Six 50mg capsules at visit 3. They will take their first capsule at the onset of the 72 hour PK study and continue to self-administer one 50mg capsule every 12 hours until all 6 capsules have been consumed. Time of dosing will be entered into their Dosing and Symptom Diary.
4. Six 100mg capsules at visit 4. They will take their first capsule at the onset of the 72 hour PK study and continue to self-administer one 100mg capsule every 12 hours until all 6 capsules have been consumed. Time of dosing will be entered into their Dosing and Symptom Diary.

At visits 3 and 4, subjects should continue taking the DHEA capsules every 12 hours with the last dose being taken 60 hours after taking the initial dose. The supply of DHEA given to the subject will be sufficient to cover dosing for 60 hours. As part of visits 3 and 4, subjects will be asked to present for a blood draw at 24, 48 and 72 hours after initial administration. Subjects will be asked to bring all unused DHEA capsules and packaging back to the clinic at visits 3D and 4D.

Dosing and Symptom diary

Subjects will complete a dosing and symptom diary at home starting after visit 3A until the end of the study.

Adverse events (AEs)

Information regarding occurrence of AEs will be captured throughout the study from Visit 1 until the final visit. Subjects will be given a phone number to notify the study coordinator regarding an asthma exacerbation or other adverse event.

8 DESCRIPTION OF VISITS

In general, the order of the items listed below will be the chronological order in which they will be performed at each study visit. However, this order may change once the logistics are finalized for each visit prior to the start of the clinical trial.

In addition to the following scheduled visits, we are also including an unscheduled visit that may be used for any reason when a subject needs to return to the study site to complete study activities.

Visit 0 (Screening Visit)

1. Review the study with the subject and obtain written informed consent and HIPAA authorization
2. Assign the subject a unique screening number
3. Complete asthma control test and asthma control questionnaire
4. Record demographics and other baseline data
5. Record medical and surgical history, including a history of asthma, diagnosis date, and asthma signs and symptoms
6. Record concomitant medications
7. Review inclusion/exclusion criteria
8. Measure and record height and weight without shoes
9. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
10. Perform and record oximetry
11. Perform abbreviated physical exam
12. Obtain urine to perform urine pregnancy test (when applicable) and urine cotinine test
13. Obtain blood for prostate specific antigen test (when applicable)
14. Obtain spirometry with maximum bronchodilator response
15. Obtain blood draw for baseline CBC with differential, comprehensive metabolic panel, prostate specific antigen level (PSA), dehydroepiandrosterone sulfate (DHEA-S) level and HSD3B1 genotyping

Visit 1 (Day 14 ± 7)

1. Complete asthma control test and asthma control questionnaire
2. Review and record any changes to concomitant medications
3. Record any adverse events (AEs) that may have occurred since last visit
4. Review inclusion/exclusion criteria
5. Measure and record weight without shoes

6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical exam
9. Obtain urine to perform urine pregnancy test (when applicable)
10. Perform spirometry
11. Place IV and obtain blood for DHEA-S level (time 0), cytokines, PBMCs, endocrine analysis, and genomics.
12. Administer 50mg of DHEA PO
13. For PK blood draws at 10, 20, 30, 60 minutes, and 2, 4, 6, 8 and 12 hours:
 - a. Draw back 1-2 mL of blood in a syringe or discard tube
 - b. Discard the syringe or tube
 - c. Draw blood with appropriate tube or syringe. Flush IV saline lock with 2-3 ml of normal saline
 - d. If IV stops working, another IV can be placed OR venous blood draw(s) can be performed in place of IV blood draw
14. Schedule visit 2

Visit 2 (Day 28 $\pm$ 7) (Must be at least 7 days after completing Visit 1)

1. Complete asthma control test and asthma control questionnaire
2. Review and record any changes to concomitant medications
3. Record any adverse events (AEs) that may have occurred since last visit
4. Review inclusion/exclusion criteria
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical exam
9. Obtain urine to perform urine pregnancy test (when applicable)
10. Perform spirometry
11. Place IV and obtain blood for DHEA-S level (time 0)
12. Administer 100mg of DHEA PO
13. For PK blood draws at 10, 20, 30, 60 minutes, and 2, 4, 6, 8 and 12 hours:
 - a. Draw back 1-2 mL of blood in a syringe or discard tube
 - b. Discard the syringe or tube
 - c. Draw blood with appropriate tube or syringe. Flush IV saline lock with 2-3 ml of normal saline
 - d. If IV stops working, another IV can be placed OR venous blood draw(s) can be performed in place of IV blood draw
14. Schedule visit 3

Visit 3A (Day 42 $\pm$ 7) (Must be at least 7 days after completing Visit 2)

1. Complete asthma control test and asthma control questionnaire
2. Review and record any changes to concomitant medications
3. Record any adverse events (AEs) that may have occurred since last visit

4. Review inclusion/exclusion criteria
5. Measure and record weight without shoes
6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical exam
9. Obtain urine to perform urine pregnancy test (when applicable)
10. Perform spirometry
11. Give subject a dosing and symptom diary
12. Place IV and obtain blood for DHEA-S level (time 0)
13. Administer 50mg of DHEA PO
14. For PK blood draws at 1, 6, and 12 hours:
 - a. Draw back 1-2 mL of blood in a syringe or discard tube
 - b. Discard the syringe or tube
 - c. Draw blood with appropriate tube or syringe. Flush IV saline lock with 2-3 ml of normal saline
 - d. If IV stops working, another IV can be placed OR venous blood draw(s) can be performed in place of IV blood draw
15. Remove PIV after 12-hour blood draw
16. 12 hours after time 0 and after 12-hour blood draw, administer 50mg DHEA
17. Send subject home with four DHEA 50mg tablets to take at 24, 36, 48 and 60 hours after time 0
18. Schedule venous blood draw at: 24, 48 and 72 hours (visits 3B, 3C and 3D)

Visit 3B (24 hours (± 1 hr) post-administration of DHEA at time 0 in visit 3A)

1. Obtain blood for DHEA-S levels
2. Record any adverse events (AEs) that may have occurred since last visit

Visit 3C (48 hours (± 1 hr) post-administration of DHEA at time 0 in visit 3A)

1. Obtain blood for DHEA-S levels, cytokine studies and genomics
2. Record any adverse events (AEs) that may have occurred since last visit

Visit 3D (72 hours (± 1 hr) post-administration of DHEA at time 0 in visit 3A)

1. Obtain blood for DHEA-S levels, cytokines, PBMCs, endocrine analysis, and genomics
2. Obtain spirometry with maximum bronchodilator response
3. Record any adverse events (AEs) that may have occurred since last visit
4. Collect any unused DHEA capsules from visit 3
5. Collect dosing and symptom diary

Visit 4A (Day 56 ± 7) (Must be at least 7 days after completing Visit 3)

1. Complete asthma control test and asthma control questionnaire
2. Review and record any changes to concomitant medications
3. Record any adverse events (AEs) that may have occurred since last visit
4. Review inclusion/exclusion criteria
5. Measure and record weight without shoes

6. Perform and record vital signs, including a blood pressure after the subject has been seated for 5 minutes
7. Perform and record oximetry
8. Perform abbreviated physical exam
9. Obtain urine to perform urine pregnancy test (when applicable)
10. Perform spirometry
11. Give subject a dosing and symptom diary
12. Place IV and obtain blood for DHEA-S level (time 0)
13. Administer 100mg of DHEA PO
14. For PK blood draws at 1, 6 and 12 hours:
 - a. Draw back 1-2 mL of blood in a syringe or discard tube
 - b. Discard the syringe or tube
 - c. Draw DHEA-S levels with appropriate tube or syringe. Flush IV saline lock with 2-3 ml of normal saline
 - d. If IV stops working, another IV can be placed OR venous blood draw(s) can be performed in place of IV blood draw
15. 12 hours after time 0 and after 12-hour blood draw, (± 10 min) administer 100mg DHEA
16. Send subject home with four DHEA 100mg tablets to take at 24, 36, 48 and 60 hours after time 0
17. Schedule venous blood draws at: 24, 48 and 72 hours (visits 4B, 4C and 4D)

Visit 4B (24 hours (± 1 hr) post-administration of DHEA at time 0 in visit 4A)

1. Obtain blood for DHEA-S levels
2. Record any adverse events (AEs) that may have occurred since last visit

Visit 4C (48 hours (± 1 hr) post-administration of DHEA at time 0 in visit 4A)

1. Obtain blood for DHEA-S levels, cytokine studies, and genomics
2. Record any adverse events (AEs) that may have occurred since last visit

Visit 4D (72 hours (± 1 hr) post-administration of DHEA at time 0 in visit 4A)

1. Obtain blood for DHEA-S levels, CBC with differential, comprehensive metabolic panel, and cytokines, PBMCs, endocrine analysis, and genomics
2. Obtain spirometry with maximum bronchodilator response
3. Complete asthma control test and asthma control questionnaire
4. Record any adverse events (AEs) that may have occurred since last visit
5. Collect any unused DHEA capsules from subject
6. Collect dosing and symptom diary

9 STUDY RISKS

DHEA: DHEA is commercially available without a prescription. The DHEA capsules used in this study are formulated for slow release. Most of the known data pertaining to risks come from studies in adults with adrenal insufficiency who were treated with long courses of DHEA (e.g. 3-12 months). Many of these trials were included in a recent meta-analysis (27). This paper included 10 trials where 264 women treated with 20-50mg (most were 50mg) daily for 3-12 months (most were 4-6 months). None of the trials reported any serious adverse effects from prolonged therapy with DHEA. Minor side effects reported include greasy skin, hirsutism, acne, scalp itching and increased sweating. In two trials

including 36 men, reported side effects included diarrhea (n=1), mild acne (n=1) and increased facial hair (n=1) (28, 29). In a study of 23 adolescent girls and young women (ages 13-25) treated with 25mg of DHEA for 1 year, adverse events were similar between DHEA and placebo (30). One girl developed mild hirsutism and no subjects developed acne. We are aware of a single case report of a 55-year-old patient developing palpitations after 2 weeks of DHEA therapy (50mg/day) that were found to represent premature atrial and ventricular contractions (PACs and PVCs) that resolved with an oral beta-blocker therapy (31). Finally, we previously performed a pilot study of DHEA repletion in female asthma patients with DHEA-S levels <200 µg/dl. The short-term (2 week) regimen was associated with a significant improvement in post-bronchodilator FEV1 and no major side effects were observed in the pilot study (5). These results support feasibility of the proposed trial and suggest that asthma patients with low DHEA-S plasma levels will tolerate DHEA treatment.

Blood collection and genotyping: When blood samples are taken from a vein, subjects may have discomfort or pain where the blood was taken. Sometimes a person may become dizzy or faint when blood is taken. There is also a risk of infection (rare), bleeding, redness or bruising at the skin puncture. Bleeding and bruising can usually be reduced by putting pressure on the place where the blood was taken. The chance of infection is lowered by using standard skin cleaning and sterile needles.

Genotype data is obtained from blood analysis. The only risk specifically associated with genotyping is breach of confidentiality. Every effort will be made to maintain confidentiality and all study data will be treated as confidential information. No identifiable information released or shared with individuals outside the study team.

Spirometry: There is a small risk of lightheadedness, wheezing, shortness of breath or increased cough when performing spirometry. These symptoms usually resolve quickly without the need for treatment. Withholding short-acting/long-acting beta-agonists prior to study visits: There is a risk of shortness of breath or breathing difficulty. Subjects will be advised that if they experience any worsening symptoms during the pre-visit withholding period, they should use their usual medication as needed and call the study team to cancel or reschedule the study visit.

Urine Collection: Urine will be collected by clean-catch. There are no foreseeable risks to its collection. However, there is the risk of emotional distress if a subject discovers that she is pregnant. Our study coordinators are highly trained and experienced regarding advising women who discover that they are pregnant as part of a research protocol.

10 STUDY BENEFITS

There are no expected direct benefits to individual subjects participating in this research. The information obtained from this research study may prove helpful for future asthma patients and other pulmonary research projects.

11 WITHDRAWAL FROM STUDY PARTICIPATION

An individual subject will be withdrawn from the trial if the subject withdraws consent (the subject is not required to justify this decision). All withdrawals will be documented and the reason (if provided) for withdrawal will be recorded. There is no need to follow subjects after withdrawal. If a subject wishes to

withdraw consent, the PI will have his/her stored specimens discarded. Specimens already tested or used by another researcher will not be destroyed. Information already learned from the testing of the sample will not be destroyed. If a subject starts taking the DHEA and then calls in prior to completion of the PK lab testing to say that they want to stop taking it/withdraw from the study, we will ask the subject to come back for their next lab draw and if they are taking the twice daily doses, to return their unused DHEA capsules.

In addition, a subject may be discontinued from the study at any time if the PI feels that it is not in the subject's best interest to continue. Possible reasons for study discontinuation include but are not limited to:

- Subject withdrawal of consent
- Subject is not compliant with or tolerating study procedures
- Subject is not adherent to study drug schedule
- Adverse event that, in the opinion of the PI, places the subject at increased risk
- Lost to follow-up
- Physician request for early termination of study
- Subject becomes pregnant

Being a part of this clinical trial while pregnant may expose the unborn child to significant risks. Therefore, pregnant women will be excluded from the study. A pregnancy test will be done at the beginning at every study visit for any women of childbearing potential, and it must be negative before they can enter and continue in this study. All subjects must agree to use appropriate contraceptive measures during the study. Medically acceptable contraceptives include: (1) surgical sterilization, (2) approved hormonal contraceptives such as birth control pills, (3) barrier methods (such as a condom or diaphragm) used with a spermicide, (4) abstinence, or (5) an intrauterine device (IUD). If they become pregnant during this study, they must inform a member of the research team immediately. In this scenario, study drug will be discontinued immediately, and the participant will be monitored throughout the pregnancy and for 1 year after study drug was discontinued. The baby will be monitored for adverse events for 1 year after birth.

12 DATA AND SAFETY MONITORING

This study will have a Data and Safety Monitoring Board (DSMB). The DSMB will meet regularly to review reports of all adverse events. The DSMB may review all procedures, protocol changes, ICFs and annual reports. Notification of all Serious AEs will be sent to DSMB, the principal investigator and the study's Steering Committee (comprised of research project leaders). The DSMB Chair also will function as an independent medical monitor and AE adjudicator. AEs will be reviewed and categorized as serious, non-serious, drug related, or procedure related. Based on the safety data, the DSMB has the authority to request a modification to the study design. This overview is only meant to be used as a guideline by the DSMB to review the study and to make a recommendation to continue or modify the protocol, not necessarily a mandate to the board for study termination. The members will not be involved in the planned study but will have the requisite clinical expertise to oversee subject safety. The DSMB will meet at least once per year, will review a written report prepared annually by the study team, and in response, will prepare a written report of the results of these meetings. The PI will alert the DSMB, along with the IRB, the study's Steering Committee, and the NHLBI Program Officer of any serious AEs. Procedures and requirements for prompt reporting of AEs and unanticipated problems involving risk to subjects or others have been established by our IRB and will be adhered to for all aspects of this project.

Any member of the study team will report any unexpected event or problem and any AE, either serious or non-serious, to the PI immediately upon discovery. The PI will then proceed to report the event within the specified timeframes mandated by the DSMB Charter, the IRB and the NHLBI. Any non-serious AE, either study or not study related, can be reported by the research team at the date of the next continuing review. Current regulations require that any AE that is (1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the study-related documents, and (b) the characteristics of the subject population being studied, (2) related or possibly related to study participation, AND (3) suggesting that the research places subjects or others at greater risk of harm than was previously known must be reported to the IRB within 5 business days. A SAE that is non-study related can be reported at the next continuing review.

13 SAFETY ASSESSMENTS

The monitoring in place for assessing the subject's responses will ensure that any untoward change in physiologic status is immediately known.

This includes the following:

1. Measuring pulmonary function at the beginning of each visit and measuring bronchodilator response if there is a drop $\geq 10\%$ from baseline.
2. Obtaining ACT scores at each visit to assess subjects' asthma control.
3. Performing an abbreviated physical exam at visits 0, 1, 2, 3A, and 4A.
4. Checking vital signs, including pulse oximetry, to ensure the subject is at their baseline.
5. Asking about interim history to ensure the subject has not had an asthma exacerbation since the previous visit.

As a result of the study design, subjects will be monitored on a case-by-case basis by the study coordinator. The experimental design has each subject extensively monitored; any manner of medical intervention is immediately available, and the appearance of any suspected untoward effect will result in cessation of protocol procedures if decided by the principal investigator.

Any applicable safety concerns will be reported as described above.

14 ADVERSE EVENTS AND SEVERITY GRADING

The IU IRB and the FDA have well-established policies (and means) for prompt reporting of AEs and unanticipated problems involving risk to subjects and these policies will be strictly followed.

Adverse and serious adverse events (AE and SAE, respectively) will be defined according to FDA and OHRP guidelines as per IU IRB and NIH requirements.

14.1 ADVERSE EVENT ASSESSMENT

All adverse events will be reported to the Indiana University IRB and FDA as required per their adverse event reporting policies. At the time of the annual review of the study, adverse events will be reported as per the Indiana University HRPP Reportable Events Policy and Guidance. An Adverse Reaction Reporting Form will be completed for any serious and unexpected adverse reaction associated with the

study, and the Indiana University IRB will be notified per IU HRPP Reportable Events Policy and Guidance.

An IND safety report will be submitted to the FDA for an event that is a (1) suspected adverse reaction, (2) serious, and (3) unexpected, per FDA guidance.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event.

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the protocol.

Any unexpected fatal or life-threatening suspected adverse reaction will be submitted as an IND safety report to the FDA within seven (7) calendar days after initial receipt of information. Any potential serious risks (serious and unexpected suspected adverse reactions, findings from other studies, findings from animal or in vitro testing, or increased rate of occurrence of serious suspected adverse reactions) will be submitted as an IND safety report to the FDA within fifteen (15) calendar days after initial receipt of the information.

All adverse events will be classified and recorded according to the NCI Toxicity Grading Scale. All study related toxicities will be graded (Grades 1, 2, 3 or 4) according to the Toxicity Grading Scale and will be employed along with potential study drug specific toxicities in the individual subject stopping rules. Subject and study stopping rules will be determined by the DSMB. If the DSMB recommends that the study change or be closed early for patient safety reasons or for slow accrual, the PI will act to implement the change as expeditiously as possible. If a recommendation is made to change the study for other than patient safety reasons or slow accrual, the DSMB will provide an adequate rationale for its decision.

Adverse events will be graded as follows:

Grade 0 – No adverse effects

Grade 1 - Mild adverse events

Grade 2 – Moderate adverse events

Grade 3 – Severe adverse events

Grade 4 – Life threatening or disabling adverse events

Grade 5 – Death related to adverse events

Adverse events will be assessed using the NCI Common Toxicity Criteria guidelines listed in (<http://ctep.cancer.gov/forms/CTCAEv3.pdf>) along with potential study drug specific toxicities in the individual subject stopping rules.

Deaths will be reported to the Indiana University IRB per their institutional guidelines if they occur within 30 days of study intervention. The principal investigator will be responsible for reporting adverse effects to the IRB and other relevant bodies.

14.2 TRACKING OF EVENTS

The following AE's are discussed in the protocol, are discussed with patients as part of the informed consent process, and will be tracked as part of the protocol:

- Complications of venipuncture, blood draw, IV placement, or IV infusion
- Complications related to study drug

14.3 ADVERSE EVENT REPORTING

- Every event that is reported to either the PI or the designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented.
- An AE report will be generated for each event and each report will include: (1) description of the event; (2) when it was reported; (3) severity; and (4) a determination of attribution.
- All SAE's will be reported once the event has been verified and determination of attribution has been completed in accordance with IU IRB reporting requirements and DSMB requirements. All other AEs will be reported according to the IU IRB reporting requirements.

DSMB requirements are that SAE's be reported to the board within 24 hours of learning of the confirmed event. DSMB will proceed to review, discuss and convey recommendations.

14.4 FOLLOW UP FOR ADVERSE EVENTS

Subject follow-up will be on a case-by-case basis as directed by the PI and/or the IU IRB and the DSMB as appropriate.

15 STUDY MONITORING

This is a single-site investigator initiated study, taking place at University Hospital on the IUPUI campus. This site will be monitored by representatives from the Indiana Clinical and Translational Sciences Institute. The Investigator grants permission to the monitoring office, and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

16 SUBJECT RIGHTS AND CONFIDENTIALITY

16.1 ALTERNATIVES TO STUDY PARTICIPATION

This is a voluntary study. The alternative is to choose to not participate. A subject's decision will in no

way influence any care a subject might receive at the respective hospital nor will it affect interactions with any of the study personnel.

16.2 FINANCIAL INFORMATION

Subjects will be paid for their participation in this study. Details are provided in the informed consent form.

16.3 RESEARCH RELATED INJURY

If injury occurs as a result of their involvement in this research, medical treatment is available from the respective hospital or another medical facility but the subject or their medical insurance will be responsible for the cost of this treatment. A research injury is an injury that happens as a result of taking part in this research study. There are no plans for payment of medical expenses or other payments, including lost wages, for any research related injury.

16.4 USE OF SPECIMENS

Test results will not be used to guide clinical care, nor will they be used for purposes outside the scope of this research study.

16.5 CONFIDENTIALITY

All study data will be treated as confidential information. Similarly, the subject's medical history will also be treated as confidential with no identifiable information released or shared with individuals outside the study team. If the study results are published, subject names will not be used. Once all the results are collected any identifiers will be removed and the information assigned a code. The data will be identified by a study number and not by name or identifying information. The code assignment key will be maintained by the study team on a password-secured computer kept in a locked office. Access to the code key will be limited to a need-only basis.

16.6 PRIVACY

Study team members are committed to protecting the privacy of research subjects. Recruitment discussions will be conducted with as much privacy as possible. The consent process will take place in a private room. Subjects will be assured that all study staff working on the study will make every effort to protect their privacy.

16.7 DATA/SAMPLE CONFIDENTIALITY

The study team will make an ongoing effort to keep personal information of the subjects confidential. Personal information will be disclosed only if required by law. Subjects will be informed that they are assigned unique study identification numbers to protect their identity. Any paper and electronic files containing study data, as well as any specimens, will be coded with the study identification number and not with the subject's name.

Study information, which does not identify the subjects, may be given to regulatory agencies and the Institutional Review Board (IRB). Staff members from these agencies have the right to review each subject's medical and study records only as needed for safety purposes, to verify the data, assess compliance with the protocol and regulation, or to review the results of the study. By signing the study

consent, subjects agree to let these organizations see their medical and study records. The records will not be used for any other purposes or disclosed to any other party without subject permission. Some of the records reviewed will contain identifying information; however, the study team are required to keep this information confidential. Efforts will be made to keep personal information in the subject's research record private and confidential, but absolute confidentiality cannot be guaranteed.

16.8 DATA/SAMPLE SECURITY

Health information/specimens will be labeled with subject ID number and any other pertinent information (e.g., study name, visit number, sample date). Subject records will be stored by the study coordinator. Specimens will be stored in the Pediatric Translational Research Lab in the Herman B. Wells Center for Pediatric Research, Room 132. Only study personnel for this study designated by the PI will have access to the data/specimens. Electronic files will be stored on password-protected IU systems.

16.9 DATA ENTRY & STORAGE

The study team will adhere to 21 CFR Part 11 guidelines and requirements for data entry and storage practices. This study will use a secured, institutional REDCap database for data entry and storage, with original hard copy, paper documents maintained for cross reference. The institutional REDCap will be designed for this study.

17 STATISTICAL ANALYSIS

Descriptive statistics of the study cohort will be summarized and used to determine safety. If an AE occurs, the maximum tolerable dose (MTD) will be determined following a design with inpatient dose escalation. Assuming a one-week washout period is sufficient, the difference in DHEA and DHEA-S levels under different dose levels will be first calculated for each subject. Mixed-effects models for repeated measures will be considered to test the significance of the difference at various measurement time points. Based on simulation, with nine subjects, using a mixed-effects model, the power of identifying a difference with an estimated effect size of 1.03 is 88% at the significance level of 0.05 and assuming the correlation between measurements is 0.5.

18 REFERENCES

1. Sadatsafavi M, Lynd L, Marra C, Carleton B, Tan WC, Sullivan S and Fitzgerald JM. Direct health care costs associated with asthma in British Columbia. *Can Respir J*. 2010;17:74-80. PMID: 20422063 PMCID: PMC2866215
2. Han MK, Arteaga-Solis E, Blenis J, Bourjeily G, Clegg DJ, DeMeo D, Duffy J, Gaston B, Heller NM, Hemnes A, Henske EP, Jain R, Lahm T, Lancaster LH, Lee J, Legato MJ, McKee S, Mehra R, Morris A, Prakash YS, Stampfli MR, Gopal-Srivastava R, Laposky AD, Punturieri A, Reineck L, Tigno X and Clayton J. Female Sex and Gender in Lung/Sleep Health and Disease. Increased Understanding of Basic Biological, Pathophysiological, and Behavioral Mechanisms Leading to Better Health for Female Patients with Lung Disease. *Am J Resp Crit Care Med*. 2018;198:850-858. PMID 29746147 PMCID PMC6173069
3. Zein JG and Erzurum SC. Asthma is Different in Women. *Curr Allergy Asthma Rep*. 2015;15:28. PMID 26141573 PMCID PMC4572514

4. Zein J, Gaston B, Bazeley P, DeBoer MD, Igo RP, Jr., Bleecker ER, Meyers D, Comhair S, Marozkina NV, Cotton C, Patel M, Alyamani M, Xu W, Busse WW, Calhoun WJ, Ortega V, Hawkins GA, Castro M, Chung KF, Fahy JV, Fitzpatrick AM, Israel E, Jarjour NN, Levy B, Mauger DT, Moore WC, Noel P, Peters SP, Teague WG, Wenzel SE, Erzurum SC and Sharifi N. HSD3B1 genotype identifies glucocorticoid responsiveness in severe asthma. *Proc Natl Acad Sci USA*. 2020;117:2187-2193. PMID 31932420 PMCID PMC6995013
5. Marozkina N, Zein J, DeBoer MD, Logan L, Veri L, Ross K and Gaston B. Dehydroepiandrosterone Supplementation May Benefit Women with Asthma Who Have Low Androgen Levels: A Pilot Study. *Pulm Ther*. 2019;5:213-220. PMID 32026412 PMCID PMC6967310
6. Lahm T, Albrecht M, Fisher AJ, Selej M, Patel NG, Brown JA, Justice MJ, Brown MB, Van Demark M, Trulock KM, Dieudonne D, Reddy JG, Presson RG and Petrache I. 17beta-Estradiol attenuates hypoxic pulmonary hypertension via estrogen receptor-mediated effects. *Am J Resp Crit Care Med*. 2012;185:965-80. PMID: 22383500 PMCID:: 3359941
7. Bentley JK and Hershenson MB. Airway smooth muscle growth in asthma: proliferation, hypertrophy, and migration. *Proc Am Thorac Soc*. 2008;5:89-96. PMID: 18094090 PMCID: PMC2645305
8. Chang KH, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, Vessella R, Nelson PS, Kapur P, Guo X, Mirzaei H, Auchus RJ and Sharifi N. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. *Cell*. 2013;154:1074-1084. PMID 23993097 PMCID PMC3931012
9. Hall SL, Baker T, Lajoie S, Richgels PK, Yang Y, McAlees JW, van Lier A, Wills-Karp M, Sivaprasad U, Acciani TH, LeCras TD, Myers JB, Kovacic MB and Lewkowich IP. IL-17A enhances IL-13 activity by enhancing IL-13-induced signal transducer and activator of transcription 6 activation. *J Allergy Clin Immunol*. 2017;139:462-471 e14. PMID: 27417023 PMCID: PMC5149451
10. Bulitta JB, Jiao Y, Drescher SK, Oliver A, Louie A, Moya B, Tao X, Wittau M, Tsuji BT, Zavascki AP, Shin BS, Drusano GL, Sorgel F and Landersdorfer CB. Four Decades of beta-Lactam Antibiotic Pharmacokinetics in Cystic Fibrosis. *Clin Pharmacokinet*. 2019;58:143-156. PMID: 29936678
11. Hettel D, Zhang A, Alyamani M, Berk M and Sharifi N. AR Signaling in Prostate Cancer Regulates a FeedForward Mechanism of Androgen Synthesis by Way of HSD3B1 Upregulation. *Endocrinology*. 2018;159:2884-2890. PMID: 29850791 PMCID: PMC6456955
12. Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnelle D, Mullins ME, Sugarbaker DJ, Chee C, Singel DJ, Loscalzo J and Stamler JS. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci USA*. 1993;90:10957-61. PMID 8248198 PMCID PMC479800
13. Gaston B, Sears S, Woods J, Hunt J, Ponaman M, McMahon T and Stamler JS. Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure. *Lancet*. 1998;351:1317-9. PMID 9643794
14. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA and Gaston B. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Resp Crit Care Med*. 2000;161:694-9. PMID 10712309
15. Carraro S, Doherty J, Zaman K, Gainov I, Turner R, Vaughan J, Hunt JF, Marquez J and Gaston B. Snitrosothiols regulate cell-surface pH buffering by airway epithelial cells during the human immune response to rhinovirus. *Am J Physiol Lung Cell Mol Physiol*. 2006;290:L827-32. PMID 16603595
16. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP, Brightling C, Chanez P, Dahlen SE, Djukanovic R, Frey U, Gaga M, Gibson

- P, Hamid Q, Jajour NN, Mauad T, Sorkness RL and Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Resp J*. 2014;43:343-73. PMID: 24337046
17. GINA (2019). Retrieved from: <https://ginasthma.org/wp-content/uploads/2019/06/GINA-2019-main-reportJune-2019-wms.pdf>. Accessed November 26, 2019.
 18. Watson AC, Rosenfield RL and Fang VS. Recovery from glucocorticoid inhibition of the responses to corticotrophin-releasing hormone. *Clin Endocrin*. 1988;28:471-7. PMID: 2850875
 19. Jollin L, Thomasson R, Le Panse B, Baillot A, Vibarel-Rebot N, Lecoq AM, Amiot V, De Ceaurriz J and Collomp K. Saliva DHEA and cortisol responses following short-term corticosteroid intake. *Eur J Clin Invest*. 2010;40:183-6. PMID: 19874391
 20. Al-Tarrah K, Moiemmen N and Lord JM. The influence of sex steroid hormones on the response to trauma and burn injury. *Burns Trauma*. 2017;5:29. PMID: 28920065 PMCID: PMC5597997
 21. Phipatanakul W, Mauger DT, Sorkness RL, Gaffin JM, Holguin F, Woodruff PG, Ly NP, Bacharier LB, Bhakta NR, Moore WC, Bleecker ER, Hastie AT, Meyers DA, Castro M, Fahy JV, Fitzpatrick AM, Gaston BM, Jarjour NN, Levy BD, Peters SP, Teague WG, Fajt M, Wenzel SE, Erzurum SC, Israel E and Severe Asthma Research P. Effects of Age and Disease Severity on Systemic Corticosteroid Responses in Asthma. *Am J Resp Crit Care Med*. 2017;195:1439-1448. PMID: 27967215 PMCID: PMC5470749
 22. Coates AL, Wanger J, Cockcroft DW, Culver BH; Bronchoprovocation Testing Task Force: Kai-Håkon Carlsen, Diamant Z, Gauvreau G, Hall GL, Hallstrand TS, Horvath I, de Jongh FHC, Joos G, Kaminsky DA, Laube BL, Leuppi JD, Sterk PJ. ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J*. 2017 May 1;49(5):1601526. doi: 10.1183/13993003.01526-2016. PMID: 28461290.
 23. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med*. 2000 Jan;161(1):309-29. doi: 10.1164/ajrccm.161.1.ats11-99. PMID: 10619836.
 24. Stanojevic S, Kaminsky DA, Miller MR, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. *Eur Respir J* 2022 Jul 13; 60(1): 2101499 [DOI: 10.1183/13993003.01499-2021].
 25. Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, McCarthy K, McCormack MC, Oropez CE, Rosenfeld M, Stanojevic S, Swanney MP, Thompson BR. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med*. 2019 Oct 15;200(8):e70-e88. doi: 10.1164/rccm.201908-1590ST. PMID: 31613151; PMCID: PMC6794117.
 26. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999 Jan;159(1):179-87. doi: 10.1164/ajrccm.159.1.9712108. PMID: 9872837. Alkatib AA, *J Clin Endocrin Metab*, October 2009, 94(10):3676-3681
 27. Hunt PJ, *J Clin Endo & Metab*, 2000 85(12):4650-4656
 28. Brooke AM, *J Clin Endo & Metab*, 2006;91:3773-3779
 29. Binder G, *J Clin Endo & Metab*, 2009;94(4):1182-1190

30. Sahelian R, Ann Intern Med. 1998;129(7):588
31. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): Guidelines for the Diagnosis and Management of Asthma-Summary Report 2007. J Allergy Clin Immunol. 2007 Nov;120(5 Suppl):S94-138. doi: 10.1016/j.jaci.2007.09.043. Erratum in: J Allergy Clin Immunol. 2008 Jun;121(6):1330. PMID: 17983880.

19 PROTOCOL SIGNATURE PAGE

Protocol Title:	Phase IIa Clinical Trial to Evaluate Pharmacokinetics and Safety of Slow Release DHEA
IRB #:	15038
IND:	162875
Protocol Version/Date:	24Sep2024 (A015)

The signature below constitutes approval of this study in full accordance with the provisions of this protocol and the attachments. I agree to conduct this study in compliance with the protocol, in-country and local regulatory requirements, applicable United States (US) Code of Federal Regulations (CFR) and ICH Good Clinical Practices (E6).

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining Sponsor and IRB approval, except when necessary to protect the safety, rights, or welfare of participants.

Printed Name of the Principal Investigator

Date

Signature of the Principal Investigator