Abbreviated Title: Congenital Adrenal Hyperplasia

Protocol number: 96CH0033

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Title: An Open, Randomized, Long-Term Clinical Trial of Flutamide, Testalactone, and Reduced Hydrocortisone Dose vs. Conventional Treatment of Children with Congenital Adrenal Hyperplasia

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Number and type of patients: 62 patients with Congenital Adrenal Hyperplasia
Accrual Ceiling: 62

	Number	Sex	Age Range
Patients	62	male and female	ages 2-18
Volunteers	0	-	-

Multi-site study: No

Précis

To test the hypothesis that the regimen of flutamide (an antiandrogen), testolactone or letrozole (an inhibitor of androgen-to-estrogen conversion), and reduced hydrocortisone dose can normalize the growth and adult stature of children with congenital adrenal hyperplasia, and can avoid the complications of supraphysiologic glucocorticoid dosage, 60 children with this disorder will be randomized to receive either the above regimen or conventional treatment until they have reached age 13 years in a girl or age 14 in a boy. After these ages boys will receive conventional treatment and girls will receive conventional treatment plus flutamide. In girls, flutamide will be continued until 6 months after menarche. All children will be followed until they have attained final adult height. The principal outcome measures will be adult height, body mass index, and bone density.

Introduction

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is an autosomal recessive disorder involving the next-to-last enzyme of cortisol biosynthesis. The deficiency in cortisol results in an elevation of ACTH leading to adrenal hyperplasia and hypersecretion of cortisol precursors. Several of these precursors are enzymatically converted to androgens and estrogens, which cause rapid growth, premature epiphyseal fusion, and adult short stature.

CAH occurs in several clinically distinct variants that reflect differing severity of the underlying enzymatic deficiency. The most severely affected children have an inability to produce enough mineralocorticoid to prevent adrenal crisis; this is termed the salt-wasting form of CAH. The next most severely affected children can produce sufficient mineralocorticoid to avoid adrenal crisis (although they make less than the normal amount), but still produce excessive amounts of adrenal androgens from early fetal life. Girls with this variant usually have ambiguous genitalia at birth. Boys develop precocious pseudopuberty in early childhood. This form is termed simple virilizing CAH. The salt-wasting and simple virilizing forms of CAH are referred to collectively as the "classic" forms of the disorder.

Standard medical therapy for classic CAH (21-hydroxylase deficiency) has involved daily administration of glucocorticoid and mineralocorticoid. Glucocorticoid replacement suppresses ACTH secretion and thereby reduces adrenal stimulation and androgen production. However, it does not affect the intrinsic adrenal abnormality, and thus the adrenal glands continue to secrete an abnormally high ratio of androgen to cortisol at any given level of adrenal activity. Mineralocorticoid replacement suppresses plasma renin activity and angiotensin levels, and corrects the tendency of these patients to lose salt. By a mechanism still not fully understood, this helps to reduce adrenal androgen overproduction. Thus, mineralocorticoid replacement allows a lower dose of hydrocortisone to be used than would be required if glucocorticoid alone were administered.

Despite decades of study, the growth and development of most children with CAH remain abnormal [1, 2] . High levels of sex steroids induce premature epiphyseal closure; while excess glucocorticoid suppresses growth. The median adult height of treated boys was only at the 4th percentile of normal men, and the median adult height of girls, treated from before one year of age, was at the 25th percentile. Maria New and her colleagues reported adult height in all subjects that averaged 1.7 standard deviations below the mean for the normal population, which corresponds to the 9th percentile [3] . These are the results at leading clinics which pioneered hydrocortisone treatment of CAH. Moreover, retrospective studies indicate that the final height of treated patients is relatively independent of the degree of control of adrenal androgen levels, [1, 3-5] suggesting that both hyperandrogenism and hypercortisolism contributed to the observed adult short stature. A meta-analysis of data from 18 centers showed that adult heights in patients with classic CAH averaged 1.4 SD (10 cm) below the population mean [6] . A subsequent meta-analysis of 35 studies of classic CAH found the mean adult height score corrected for parental height of -1.05 standard deviation and mineralocorticoid use was associated with improved height outcome [7]. In addition, many girls encounter problems related to excess androgen despite treatment. Thus, there is need for continuing research to improve the outcome of treatment.

The suboptimal outcome of treatment is related to the theoretical inadequacy of fludrocortisone and hydrocortisone as sole treatment. If hydrocortisone is administered so as to

achieve perfectly physiologic levels, and thus perfectly normal ACTH levels and adrenal size (euplastic adrenals), the adrenals will nonetheless secrete an abnormally high ratio of androgen and estrogen to cortisol because the 21-hydroxylase deficiency shifts an excessive proportion of the steroid intermediates into the androgen and estrogen pathways. This excess androgen and estrogen will, if left unchecked, accelerate growth rate and bone maturation and compromise adult height. If, on the other hand, the hydrocortisone dose is increased above perfectly physiologic levels to control the excess androgen and estrogen production by the 21-hydroxylase-deficient adrenal glands, the resulting iatrogenic hypercortisolism will stunt growth rate, impair adult stature, decrease bone mineral density, alter intermediary metabolism, and cause hypertension. Thus, the existing approach to treatment produces either hypercortisolism, androgen excess, or a combination of these states.

Our hypothesis to improve treatment initially arose from work in the intramural program of NICHD to treat boys with familial male precocious puberty (FMPP). The accelerated growth rate and bone maturation of these boys was controlled effectively with an antiandrogen (spironolactone) and an aromatase inhibitor (testolactone) [8] . We hypothesized that a similar approach would be effective in CAH, and would allow us to avoid the complications of glucocorticoid excess by reducing hydrocortisone dose to strictly physiologic levels. An antiandrogen other than spironolactone will be required in CAH, however, since patients with CAH have a defect of the aldosterone pathway that precludes the use of spironolactone because this drug also has aldosterone antagonist activity. Thus, we have substituted flutamide for spironolactone. The aromatase inhibitor is an essential component of the regimen to prevent the gynecomastia that would otherwise result from use of antiandrogen alone, and to prevent the maturational effects of estrogen upon bone, which are exerted at extremely low levels [9-11].

Flutamide (4^1-nitro-3^1 fluoromethylisobutyranilide) is a nonsteroidal antiandrogen which has been used in the treatment of prostate cancer and benign prostatic hypertrophy [12, 13]. It prevents the action of androgens by blocking the receptor sites in target tissues [14]. It may also produce changes in the metabolism of testosterone as well as estradiol [15, 16]. In comparison with other antiandrogens such as cyproterone acetate and megestrol acetate, flutamide alone has pure antiandrogenic activity, thus making it the most specific androgen antagonist for the treatment of androgen-dependent disorders [17, 18].

Testolactone has for many years been an approved drug to lower estrogen levels in women with breast cancer [19]. We have **19** years of combined experience using it in children with male-limited precocious puberty and CAH. The rationale of combined treatment of CAH with flutamide and testolactone will be to block the effects of elevated androgen levels with flutamide, and to reduce elevated estrogen levels, which are produced by peripheral conversion from the high androgen levels, with testolactone. Since these drugs act through quite different mechanisms, there is no reason to expect that their combinations will interfere with the efficacy of either drug or cause adverse effects beyond those associated with the use of these drugs individually. In fact, testolactone was found, in our studies of boys with male-limited precocious puberty, to prevent the gynecomastia that accompanied use of antiandrogen alone [8].

Both testolactone and letrozole are aromatase inhibitors. Testolactone was chosen as the drug of choice for protocol 96CH0033 years ago at a time when testolactone was being used in a variety of pediatric endocrine conditions. Over the years, other more potent aromatase inhibitors have become available. Letrozole is given once daily, while testolactone is given thrice daily. Thus,

letrozole is more convenient for the patient. Testolactone occasionally causes gastrointestinal discomfort. This side effect has been observed, but less commonly, with letrozole.

Letrozole is approved by the FDA to lower estrogen levels in adult women with breast and other cancers. Letrozole, like all aromatase inhibitors, has not been formally tested in children and has not been approved by the FDA for use in children. However, other than testolactone, letrozole is the aromatase inhibitor with the most reported pediatric experience and with the most clinical experience by investigators at the Clinical Center. Letrozole was used at the Clinical Center in the treatment of children with McCune Albright syndrome (protocol 00-D-0183). In this protocol, girls with precocious puberty and McCune Albright syndrome experienced an improvement in their puberty without any apparent harmful side-effects[20] . Moreover, pediatric endocrinologists have used letrozole to treat other endocrine conditions in children[21]. Safe and effective use of letrozole in children has been reported for the treatment of idiopathic short stature [22, 23]; and in combination with testosterone for male pubertal delay [24-26].

Continuation of flutamide therapy during puberty has the advantage of possibly protecting the ovaries against the development of polycystic ovarian syndrome (PCOS). Fertility in females with CAH is an ultimate and attainable goal. Delay of the average age of menarche in girls with classic CAH has been reported from various clinics [2, 27]. Menstrual irregularities, anovulation, infertility in females with CAH is not always due to the undertreated state of hyperandrogenism. CAH females have been found to have increased adrenal progesterone secretion [28], as well as elevated estrogen of adrenal origin [27]. A PCO-syndrome type of ovarian dysfunction has been described in women with CAH [29]. Androgens may directly prevent the maturation of follicles and/or affect the hypothalamic-pituitary-gonadal axis, which then induces morphologic and biochemical changes characteristic of polycystic ovarian syndrome. Ovarian dysfunction in females with CAH may be due to an abnormality either at the hypothalamic, pituitary or ovarian level, and inadequate control of excessive adrenal sex steroids (androgens, progestins, estrogens alone or in combination) may contribute to menstrual and reproductive disorders in CAH females. We hypothesize that flutamide treatment during puberty will antagonize intraovarian levels of androgens, normalize the age of onset of menarche and reduce the incidence of PCOS. The use of flutamide will also allow for a lower glucocorticoid dose.

We completed a pilot study which consisted of a 6-month trial of flutamide plus testolactone, added to a modified conventional regimen (modified by restricting hydrocortisone dosage to a physiologic level), compared to conventional treatment with hydrocortisone plus fludrocortisone [30]. The sequence of 6-month periods was chosen randomly with a 3-month "washout" period between each 6-month arm.

Results from this pilot study revealed that the rate of growth, weight gain, and bone maturation normalized with treatment with flutamide, testolactone and low dose hydrocortisone. No significant adverse effects were observed. Because a 6-month treatment period was too short to provide meaningful assessment of the effects of flutamide and testolactone on long-term growth rate, bone age advancement, and predicted adult height, a 2-year trial of flutamide plus testolactone, added to the modified conventional regimen of hydrocortisone and fludrocortisone was undertaken. Children were randomized to receive either conventional treatment or the investigational regimen of flutamide, testolactone, and reduced hydrocortisone dose. An open design was employed because the hydrocortisone dosage adjustments required during conventional treatment would have made it very difficult to carry out a blind study. Results from

this 2-year study show that there is continued normalization of the rate of growth, weight gain, and bone age maturation, without any significant adverse side effects [31]. Based upon these findings, we have decided to conduct a long-term trial to test the hypothesis that this new regimen can normalize the growth and development of children with CAH.

Evaluation of the Project in Relation to Criteria for NICHD Clinical Protocols

(1) The protocol addresses fundamental aspects of human physiology or pathophysiology.

The protocol addresses four fundamental issues related to the pathophysiology of congenital adrenal hyperplasia and its impact on growth and development. First, it tests the hypothesis that some of the undesired outcomes of conventionally treated patients, such as obesity, are due to glucocorticoid excess, and that these undesired outcomes can be avoided by reduction of the glucocorticoid dose to physiologic levels [32, 33]. To our knowledge the current trial is the only long-term randomized study of CAH that involves the restriction of hydrocortisone dose to a strictly physiologic level. Second, the protocol examines the hypothesis that estrogen contributes to the impaired adult height in CAH by using an inhibitor of estrogen synthesis. This hypothesis is in accord with the observation of delayed epiphyseal fusion and tall stature in individuals with a defect in the estrogen receptor [11] or in the estrogen biosynthetic enzyme aromatase [34]. To our knowledge, the current study is the only long-term randomized trial of an aromatase inhibitor in any of the LHRH-independent forms of precocious puberty. Lastly, the protocol addresses the hypotheses that early exposure to androgens influences the developing brain and ovaries and has an effect on cognition and reproductive function in females.

(2) The protocol outcome has a reasonable potential to impact significantly on the public health.

Congenital adrenal hyperplasia occurs in only 1 in 15,000 individuals. Thus, even the complete elimination of the disorder would have at best a minimal impact on the public health. No study of a new treatment regimen for congenital adrenal hyperplasia can be expected to meet the above criterion because the disorder is so rare.

Ancillary studies of the population of children with CAH followed in this protocol have the potential to impact significantly on public health. Extending our studies to the pubertal period in girls with CAH may provide insight into the long-term consequences of early ovarian exposure to androgens. PCO affects 7% of women of reproductive age and is a significant public health issue. The effects of extraovarian sources of androgens (i.e. adrenal) on the development of PCO is not well understood. Moreover, the protocol includes behavioral and cognitive testing which may provide insight into how hormones affect behavior and the developmental consequences of early exposure to androgens. Learning disabilities are a significant public health issue and are much more common in boys than girls. Studies of children with CAH may provide insight into hormonal influences in the development of learning disabilities.

(3) The protocol represents research in the diagnosis or treatment of orphan or rare diseases that is relatively unlikely to be performed outside the Clinical Center.

Congenital adrenal hyperplasia is indeed an orphan or rare disease. During a recent literature review, we failed to find a single study, even a short-term study, in which two or more therapies

had been subjected to a randomized trial. However, there is widespread recognition among the pediatric endocrine community of the need for improved treatments of this disorder. The Clinical Center is uniquely suited to overcome the principal obstacles to a long-term study of this sort, namely, (1) recruitment of patients, (2) successful long-term follow-up, and (3) stable funding over the long duration of the project.

(4) The responsible and principal investigators have clinical training and expertise appropriate for supervising the care of patients on the protocol and the study design is adequate.

The principal investigator, [redacted]

Associate investigator, [redacted]

The study design is similar to that of other NICHD long-term trials (83-CH-199, 87-CH-152, 91-CH-46) in which adult height is a principal outcome measure. The difference in the design of the current study is the absence of a double-blind design. This difference reflects the complexity of CAH treatment, and the need to adjust hydrocortisone dose in the conventional treatment arm but not in the investigational treatment arm. Thus, the decision to choose an open rather than double-blind design reflected our judgement that a double-blind design would be unworkable.

(5) The protocol can only be carried out at the NIH, or can be carried out more effectively and efficiently by intramural than extramural investigators.

As indicated in item 3 above, the intramural program has significant advantages for conducting this protocol due to its ability to recruit an adequate number of subjects with a rare disease, its low drop-out rate in long-term trials, and the availability of stable long-term funding.

(6) The protocol has a favorable cost-benefit ratio.

The ultimate implication of the study for the cost of CAH treatment is difficult to assess for two reasons. First, the effectiveness of aromatase inhibitors for treatment of breast cancer, and of antiandrogens for treatment of prostate cancer, has led to intense pharmaceutical company interest in these drug classes. Several highly promising drugs that cannot now be used in children are under development. By the time the current study can determine whether or not the regimen of antiandrogen and aromatase inhibitor offers long-term benefit, there will probably be alternative drugs available in each class that will have greater potency, fewer side effects, and perhaps lower cost. Second, although the cost of drugs for the investigational regimen always exceeds that for

conventional treatment (because of the two additional drugs), the cost of monitoring will be less. This is due to the reduced need to monitor androgen levels when they are being blocked by an antiandrogen. Since the cost of monitoring multiple steroid levels at 3-6 month intervals throughout childhood can be quite high, the increased drug cost may be offset by reduced monitoring cost. Our prediction is that the cost-benefit ratio for the investigational approach will be favorable if the long-term benefits that we hypothesize are in fact achieved.

Study objectives and endpoints

Success in normalizing adult height is a sensitive measure of control of excess sex steroid action and avoidance of excess glucocorticoid action during childhood.

Primary objective

- To demonstrate the superior efficacy of the combination regimen of an antiandrogen, aromatase inhibitor and reduced hydrocortisone dose compared with standard glucocorticoid replacement therapy in achieving taller adult stature in classic congenital adrenal hyperplasia.

Secondary Objectives

- To assess the safety and tolerability of antiandrogen, aromatase inhibitor and reduced hydrocortisone dose combination therapy.
- To assess the effect of antiandrogen, aromatase inhibitor and reduced hydrocortisone dose combination therapy on height gain, growth velocity and bone maturation prior to puberty.
- To assess the effect of antiandrogen, aromatase inhibitor and reduced hydrocortisone dose combination therapy on height gain and bone maturation during puberty
- To assess the effect of antiandrogen, aromatase inhibitor and reduced hydrocortisone dose combination therapy on development of TART in males and establishment of menstrual regularity in females
- To assess the effect of antiandrogen, aromatase inhibitor and reduced hydrocortisone dose combination therapy on fasting glucose, body mass index, and bone mineral density
- To assess hormonal control based on percent of visits within the normal (or optimal) range for 17OHP, androstenedione, PRA and ACTH
- To assess sex differences in outcomes.

A. Primary Endpoint

The operational definition of attainment of adult height was defined as the study visit at which the incremental growth declined <1.5 cm (0.6 inch) over 12 months.
The primary outcome variable is adult height which will be expressed in SD units relative to the normal population.

B Secondary Endpoints

Secondary outcome variables will be examined between the two study arms at baseline, pubertal onset and adult height (final visit). To examine sex differences, the secondary outcomes variables will be examined by-sex between the two study arms.

'Pubertal Onset' is defined as the visit when patients entered puberty (Tanner 2 breast in females, testicle size > 3mL) or completed therapy with the aromatase inhibitor and or LHRHa.

i. Adult height achieved will also be expressed standard deviation (SD) units and in cm as the difference in height achieved in relation to their target height (i.e., mid-parental height, calculated based on their parental heights)
ii. Height achieved (SD units) from baseline to pubertal onset
iii. Height achieved (SD units) from pubertal onset to adult height
iv. Predicted adult height (using Bayley-Pinneau method) at pubertal onset
v. Predicted adult height (using Bayley-Pinneau method) change from baseline to pubertal onset
vi. Predicted adult height (using Bayley-Pinneau method) change from pubertal onset to final height
vii. Growth velocity (in SD units) from baseline to pubertal onset
viii. Growth velocity (in SD units) from pubertal onset to adult height
ix. Percent of visits with ACTH in normal range from baseline to pubertal onset
x. Percent of visits with ACTH in normal range from pubertal onset to adult height
xi. Percent of visits with androstenedione in normal range from baseline to pubertal onset
xii. Percent of visits with androstenedione in normal range from pubertal onset to adult height
xiii. Percent of visits with 17-hydroxyprogesterone in the optimal range (<1,200 ng/dL) or high (>1,200 ng/dL) from baseline to pubertal onset
xiv. Percent of visits with 17-hydroxyprogesterone in the in the optimal range (<1,200 ng/dL) or high (>1,200 ng/dL) from pubertal onset to adult height
xv. Percent of patients with onset of central precocious puberty (CPP)
xvi. Rate of bone maturation as change in bone age from baseline to pubertal onset / change in chronological age from baseline to pubertal onset; change in bone age from pubertal onset to adult height / change in chronological age from pubertal onset to adult height; change in bone age from baseline to adult height / change in chronological age from baseline to adult height. Bone age assessment will be performed manually using Greulich and Pyle atlas method and by automated software (BoneXpert) in a subset of patients visit who have availability of digital bone age images.
xvii. Number of years bone age remain unchanged from baseline to pubertal onset
xviii. Dose of hydrocortisone at baseline, pubertal onset and adult height, baseline to pubertal and pubertal to adult height
xix. Cumulative glucocorticoid exposure over time

C. Exploratory Endpoints

i. Insulin resistance based on HOMA-IR-at the final visit
ii. BMI (as SD units) change from baseline to 'pubertal onset' visit
iii. BMI (as SD units) change from 'pubertal onset' to adult height
iv. BMI (as SD units) at adult height

v. BMD (Z-score) at adult height
vi. Age at menarche
vii. Percent of patients (females only) with normal menstrual cyclicity at adult height

D. Safety Endpoints

i. Adverse events (AEs) – particularly GI related adverse events, abnormal liver function tests.
ii. Percent of patients (males only) with development of Testicular adrenal rest tumors (TART) at pubertal onset and adult height

Study Design and Methods

Patient Selection

Subjects will be boys with bone ages 2 to 13 years and girls with bone ages 2 to 11 years with classic 21-hydroxylase. Subjects must either not yet have undergone pubertal activation of the hypothalamic-pituitary-gonadal axis, or, if pubertal activation has occurred, must be receiving an LHRH agonist to suppress their secondary central precocious puberty. This requirement is to ensure that growth during the study will not be compromised by secondary central precocious puberty. Children with a bone age of 1 to 2 years may enroll in the protocol for optimization of conventional therapy as described below, but will not be randomized to a study arm until the bone age reaches 2. Monitoring of such children will be identical to older children randomized to the conventional arm.

Exclusion criteria include children who have concurrent illnesses requiring glucocorticoid treatment (such as severe asthma), or requiring drugs that markedly alter hydrocortisone metabolism (such as anticonvulsants), and children who cannot be brought into reasonable control with conventional treatment (an unusual occurrence).

Protocol

Before entry into the study, adequacy of the patient's CAH management will be documented. Treatment will be modified as needed to achieve optimal control, according to state-of-the-art concepts of CAH management, as outlined below.

Secondary central precocious puberty will be defined by meeting one or more of the following criteria:
1) Peak Leuprolide-stimulated LH/FSH ratio consistent with central puberty.

3) Baseline LH-ICMA of greater than 0.3 [35].

4) Clinical evidence of central precocious puberty (breast development in girls and testicular enlargement in boys).

Patients who are in secondary central precocious puberty will be offered treatment with a commercially available LHRH agonist, Lupron Depot(administered via monthly intramuscular injection, TAP) or an investigational LHRH agonist, Deslorelin (administered via daily subcutaneous injection). Since 1979, Deslorelin has been used at the NIH for the treatment of

central precocious puberty and has been shown to be safe and effective [35]. Other commercially available products include Histrelin (Roberts) and Lupron Injection, both administered via daily subcutaneous injection. We have chosen to continue to use Deslorelin because it is readily available through the NIH Pharmacy (there was a recent shortage of Histrelin), it is less expensive than the commercially available subcutaneous products, and we have extensive personal experience in its use.

Choice of medication will be based on patient and family preferences. Families will be counseled on the advantages and disadvantages of these two therapies. Both medications are known to effectively suppress the hypothalamic-pituitary-gonadal axis. Known side effects include rash, and local injection site reactions (milder with Deslorelin). Lupron has the advantage of monthly administration and the disadvantage of an increase risk of local injection site abscess formation (Deslorelin may cause redness at the injection site but has not been reported to result in abscess formation). Lupron also may require more dose adjustments in the beginning of therapy. Anaphylactoid reactions are an extremely rare risk for both medications. Children will return to the NIH day hospital 3 months after starting LHRH analog therapy for evaluation of hypothalamic-pituitary-gonadal suppression. Those patients who are currently being treated with Deslorelin (previously under protocol 79-CH-112) will be continued on Deslorelin or switched to Lupron, depending on patient preference.

Once degree of CAH control has been judged optimal, children will be randomized to receive either the regimen of flutamide, testolactone or letrozole, and reduced hydrocortisone dose or conventional treatment (with hydrocortisone and fludrocortisone) until the age of 13 in the girls and 14 in the boys. At that time the regimen of flutamide, testolactone, and reduced hydrocortisone dose will be discontinued in boys so that the subjects can progress through puberty. At age 13 years, girls who are receiving flutamide and testolactone or letrozole therapy will discontinue the aromatase inhibitor therapy (to allow normal progression of puberty) and continue flutamide (to continue androgen blockade). Flutamide therapy will continue until 2 years after menarche or when final height is reached, whichever occurs first.

Children who are also receiving concurrent LHRH analog to suppress central puberty will also discontinue this agent at age 13 in the girls or age 14 in the boys. The operational definition of age 13 or 14 will be the NIH study visit that is closest to their thirteenth or fourteenth birthday. The operational definition of attainment of final height, as in our other long-term trials, will be the study visit at which the incremental growth over the previous 12 months declines to less than 1.5 cm (0.6 inch).

During flutamide and aromatase inhibitor treatment hydrocortisone dose will be decreased in 4 equal weekly steps to approximately 8 mg/m^2/day. During this month close contact will be maintained with the family by telephone and/or outpatient visits (for local patients), or referring physician visits (for distant patients), to check for symptoms or signs of adrenal insufficiency. If the hydrocortisone dosage of approximately 8 m^2/day produces symptoms or signs of adrenal insufficiency in any subject, the dosage will be adjusted on an individual basis to a dosage that will not cause symptoms or signs of adrenal insufficiency.

Patients will initially be placed on Testolactone 20 mg/kg/day of testolactone. The dose will then be increased at weekly intervals to 30 and then 40 mg/kg/day, which will be the maximum dose for the study (approximately equivalent to the usual maximal adult dose of 2 grams per day).

Patients placed on letrozole will receive a dose comparable to the dose used to suppress estrogens in adult women (1.5 mg per square meter of body surface area). This is the same dose of letrozole used for the treatment of girls with McCune Albright Syndrome.

Flutamide will also be administered in increasing doses. Patients will initially be placed on 5 mg/kg/day of flutamide. The dose will then be increased at weekly intervals to 7.5 and then 10 mg/kg/day, which will be the maximum dose for the study. This is approximately the same dose used to treat men with prostate cancer (750 mg/day, or about 10 mg/kg/day).

Subjects will be evaluated as outpatients, day-hospital patients, or inpatients on the pediatric endocrine ward at 6-month intervals until final adult height is attained. The following evaluations will be made:

(1) Ten standing height measurements by stadiometer.
(2) Ten sitting height measurements.
(3) Weight.
(4) Bone age.
(5) Two 24-hour urine collections for cortisol.
(6) A leuprolide stimulation test, to assess pubertal status of the hypothalamic-pituitary-gonadal axis will be performed at the first visit and all subsequent visits after the child has reached a bone age of 10 in boys or 9 in girls, or if there is clinical suspicion of hypothalamic-pituitary-gonadal activation (i.e. testicular enlargement in boys, breast tissue in girls). Children who are started on an LHRH analog for central precocious puberty and those who switch LHRH analog medication type will have leuprolide stimulation test performed 3 months after beginning treatment to confirm efficacy of this treatment. Additional leuprolide stimulation testing while receiving an LHRH analog will only be done if clinically indicated. This testing will resume once the patient is taken off LHRH analog therapy. Measurement of gonadotropins by third-generation immunochemiluminometric assays (ICMA) will be done. Blood withdrawal: 12 mL.
(7) Measurement of plasma 17-hydroxyprogesterone, DHEA-S, androstenedione, estradiol, testosterone, cortisol, and ACTH, estradiol starting as near 0800 h as feasible. The morning hydrocortisone dose will be held until after these samples have been obtained. Blood withdrawal: 18 mL.
(8) Blood to be frozen for possible future hormone measurements: 17 mL.
(9) Upright and supine plasma renin activity. Blood withdrawal: 14 mL.
(10) Routine CBC, thyroid panel and SMAC. Blood withdrawal: 8 mL.
(11) One-hour cortrosyn (ACTH) test to assess levels of steroid intermediates in response to ACTH stimulation. Cortisol, 17-hydroxyprogesterone, androstenedione, and DHEA will be measured at time 0 and 60 minutes after ACTH bolus infusion. Blood withdrawal: 24 mL. This test will be performed at the beginning and end of the study, and, if indicated, at some other visits.
(12) Fasting glucose, insulin and leptin levels. Blood withdrawal: 5 mL.
(13) Lipid profile. Blood withdrawal: 2.5 mL.
(14) Plasma and urinary catecholamines and metanephrines Blood withdrawal: 20 mL.
(15) Bone densitometry (DEXA): to be performed at start of treatment, annually thereafter (e.g., every other NIH visit), and at attainment of final adult height, to measure bone density of the forearm, femoral neck, and spine (AP and lateral).
(16) MRI abdomen: to be performed after 2-3 years on the protocol and after the patient's 6th birthday.
(17) Boys will have a testicular ultrasound and girls will have a pelvic ultrasound done

each visit.

(18) Photographs will be taken with the patient wearing a bathing suit or underwear. This will be done at the beginning of the study and then yearly thereafter.

(19) If clinically indicated, an adrenal ultrasound and/or an additional MRI of the abdomen and adrenals may be done at follow-up visits.

(20) An apparent cortisol metabolic clearance rate determination will be performed in all patients. In patients in the flutamide-testolactone treatment group, this test will be performed twice, once off flutamide and testolactone and once on flutamide and testolactone.

Determining the apparent cortisol metabolic clearance rate in our CAH patients will enable us to determine if a patient's dose of hydrocortisone is related to their metabolic clearance of cortisol and if flutamide and/or testolactone interfere with the metabolic clearance of hydrocortisone. Cortisol metabolic clearance rate will be determined by infusing hydrocortisone overnight, starting at 6:00 p.m. and ending the hydrocortisone infusion at 2:00 a.m., at an infusion rate of 0.6 mg/m2/hour. Oral hydrocortisone will be withheld on the day of testing. This testing will be done at the randomization visit for all new patients. Patients already on flutamide and testolactone will have one of their visits extended by 3 days in order to perform this testing on and off flutamide and testolactone.

(21) Psychological assessment will be performed at baseline and completion of the study. The following standard tests will be performed: Wechsler Intelligence Scale to determine IQ, California Verbal Learning Test to evaluate verbal-memory skills, Woodcock Johnson Achievement Tests to identify learning disabilities, Bender-Gestalt Test to evaluate visual-motor skills and Continuous Performance Test to evaluate attention. Additional testing may be performed if clinically indicated. If desired, a report will be provided to the parents for potential use by the child’s school. Patients will be invited to participate in an NIMH study involving magnetic resonance imaging of the brain to examine the effects of androgen exposure on brain morphology. The proposed psychological assessment will augment this data. We plan to collect data prior to the onset of puberty and after puberty.

Many children with CAH enrolled in 96CH003 are in special education classes or have documented language or perceptual delays by parental report. Learning disabilities are 6 times more likely to occur in males than females. The role of androgens in this phenomenon is unknown. Sex differences in patterns of brain maturation in children have been found [36]. This sexual dimorphism is of interest in light of the differences in prevalence, age of onset, and symptomatology noted in nearly every neuropsychiatric disorder of childhood onset. Sexually distinct patterns of normal brain development may be due to the X or Y chromosomes, effects of hormones, or environmental factors. Several studies on CAH children have suggested that CAH females are similar to males in both their behavior and/or cognition. For example, a study of CAH control females with equivalent general intelligence found CAH patients to have inferior verbal vs. visual and arithmetic scores typical of male vs. female differences [37]. CAH females have been shown to have improved spatial skills, typical of males [38]. Studies of gender-related behavior have shown that CAH females have more masculine type orientation than their unaffected sisters [39] and have more masculinized play preferences [40]. These type of data have been interpreted as a consequence of early in utero androgen exposure since testosterone is known to promote development of the right hemisphere and delays maturation of the left hemisphere. We hypothesize that in CAH females, the organization of the brain is more like males than females, while CAH boys are more like unaffected males.

(22) At the time of menarche, girls will be asked to keep a calendar documenting their menstrual history.

Due to the known hepatotoxicity of flutamide in some patients (see Risks below), patients who randomize to the flutamide and aromatase inhibitor arm will have liver function tests drawn by their local physician every 2 weeks for the first 3 months, then once a month for the next 9 months, then every 3 months for the duration of the study. Parents will also be carefully instructed about the signs and symptoms of liver toxicity and the procedure to follow if such symptoms occur (see consent). We have not, however, observed flutamide-associated liver toxicity in any child receiving flutamide during our initial 6-month pilot study or the ongoing current study.

If a patient develops premature pubertal activation of the hypothalamic-pituitary-gonadal axis during the study, he or she will receive treatment with an LHRH analog. Since we cannot predict with certainty who may develop secondary central precocious puberty during the study, all patients and their families will receive an explanation of the use of LHRH analogs and available treatment regimens. If a child develops premature pubertal activation of the hypothalamic-pituitary-gonadal axis, the NIH will supply a commercially available LHRH agonist, Lupron (monthly intramuscular injection) or an investigational LHRH agonist, Deslorelin (daily subcutaneous injection) Choice of medication will be based on family preference and demonstrated efficacy in each patient.

Because the degree of control of CAH often varies over time for unexplained reasons, adjustments in total hydrocortisone dose will be permitted during the conventional treatment arm throughout the study, as would be the case in clinical practice. These dose adjustments will be guided by both clinical and biochemical criteria as outlined in the following discussion of the principles that are currently used in managing these patients.

Hydrocortisone Dose Adjustment During Conventional Treatment

We will attempt to optimize the child's management during a baseline period. While administering hydrocortisone (preferably in a dose of 10-15 $mg/m^2/day$, and not exceeding 25 $mg/m^2/day$), the mineralocorticoid dose will be adjusted to maintain a normal plasma renin activity in children with 21-hydroxylase deficiency. With plasma renin activity in the normal range, hydrocortisone dose will generally be increased if:

(1) the growth rate is 2 standard deviation (SD) units or more above the median normal value for age;
(2) the rate of bone age advancement exceeds 2 years per chronologic year; or
(3) plasma 17-hydroxyprogesterone (17-OHP) levels are excessively elevated in association with growth rate and rate of bone maturation that are above the median normal values.

Because spot measurements of plasma 17-OHP correlate rather poorly with growth rate and bone maturation, plasma 17-OHP will be used as an aid to judgement rather than as a treatment goal in itself. At the present time, 0800-0900 plasma 17-OHP levels of 500 to 1300 ng/dL are considered appropriate, levels of 1300 to 3000 ng/dL prompt consideration of a hydrocortisone dose increase (assuming that plasma renin activity is normal), levels of 3000 to 6000 ng/dL prompt strong consideration, and levels >6000 ng/dL will ordinarily lead to a dosage increase.

Hydrocortisone dose will generally be decreased if:

(1) the growth rate is 2 SD units or more below the median normal value for age, if the bone age is prepubertal, or the growth rate seems inappropriately suppressed relative to age and bone age, if the bone age is in the pubertal age range;

(2) the body mass index exceeds 2 SD units above the median value for age, or if signs of Cushing's syndrome are appearing;

(3) there is no detectable bone maturation over a period of more than 6 months; or

(4) plasma 17-OHP appears excessively suppressed in association with a subnormal growth rate or rate of bone maturation. Plasma 17-OHP levels below 500 ng/dL will ordinarily raise consideration of a dosage decrease; levels below

200 ng/dL will raise strong consideration.

We have chosen a thrice-daily hydrocortisone dose schedule for this study. There are no controlled studies that compare a twice-daily and thrice-daily approach; however the thrice-daily hydrocortisone dose schedule is the most commonly used conventional regimen.

Hydrocortisone Dose Adjustment During Investigational Treatment

Congenital adrenal hyperplasia occurs in several clinically distinct variants that reflect differing severity of the underlying enzymatic deficiency, even among the classic forms. For example, the salt-wasting and simple-virilizing forms of 21-hydroxylase deficiency have different clinical courses: salt-wasters experience many more episodes of adrenal crisis and may require more glucocorticoid for adequate control. Even within the salt-waster and simple-virilizer subgroups of CAH, clinical heterogeneity is seen. This may be due to varying genotypes or other unknown factors.

A hydrocortisone dose of 8 mg/m2/day was chosen in our initial pilot study based on estimates of normal production rate; however, children may vary in their glucocorticoid daily requirements. Our hypothesis that the regimen of flutamide, an aromatase inhibitor and a reduced hydrocortisone dose can normalize the growth of children with, CAH while avoiding the complications of supraphysiologic glucocorticoid, does not require identical glucocorticoid doses in all children on the new regimen. Regimens will be more closely tailored to the individual differences in glucocorticoid requirement. The hydrocortisone dose will be allowed to vary in the flutamide-aromatase inhibitor treatment arm.

With plasma renin activity in the normal range, hydrocortisone dose will generally be increased if 2 or more of the following occur:

1) the rate of bone age advancement exceeds 2 years per chronologic year and there is no evidence of central precocious puberty as the cause;

2) plasma 17-hydroxyprogesterone levels exceed 10,000 ng/dL;

3) plasma androstenedione levels exceed 400 ng/dL;

4) testosterone levels exceed 100 ng/dL in girls or prepubertal boys;

5) signs of virilization (clitoral growth, voice deepening, acne) on physical examination;

6) presence of adrenal rest tissue on testicular ultrasound in boys (see below);

7) evidence of macronodular disease on adrenal ultrasound or MRI (see below).

With plasma renin activity in the normal range, hydrocortisone dose may be decreased if 2 or more of the following occur:

1) plasma 17-hydroxyprogesterone levels are below 500 ng/dL;

2) plasma androstenedione levels are below 50 ng/dL;

3) the rate of bone age advancement is less than 1 year per chronologic year;

4) weight percentile is greater than or equal to height percentile.

Treatment of Testicular Adrenal Rest Tissue or Macronodular Disease.

Testicular ultrasound is a sensitive method for detecting testicular adrenal rest tissue; however, testicular ultrasounds are not routinely done on CAH patients and the incidence of adrenal rest tissue with this sensitive method is unknown. Typically, testicular adrenal rests are thought to be evidence of poor control, but this may only be the case if significant growth occurs. If a small amount of adrenal rest tissue is present, and there is no growth over time, a change in treatment may not be necessary. Our studies of male patients with CAH support this approach [41]. If interval growth occurs between visits (i.e. over 6 months), glucocorticoid dose will be increased 20%, or to a minimum of 12 mg/m2/day in the flutamide-aromatase inhibitor group. If interval growth occurs on a hydrocortisone dose of at least 12 mg/m2/day in the flutamide-aromatase inhibitor treatment group, or 18 mg/m2/day in the conventional treatment group, then a 10-day course of dexamethasone (15 mcg/kg/day given qhs) will be instituted. Flutamide and aromatase inhibitor therapy will be continued.

If the rare occurrence of adrenal macronodular disease develops, a 10 day course of dexamethasone (15 mcg/kg/day given qhs) will be instituted. Subsequently, glucocorticoid dose will be increased to a minimum of 12 mg/m2/day in the flutamide-aromatase inhibitor treatment group, and flutamide and aromatase inhibitor therapy will be continued. Glucocorticoid dose will be increased to a minimum of 15 mg/m2/day in the conventional treatment arm.

The boy with significant testicular adrenal rest tissue and the patient who is developing macronodular enlargement represent special cases in which it may not be appropriate to restrict hydrocortisone dosage to the physiologic range. Fortunately, this is a small proportion of patients with CAH. We recognize that the final analysis of the data will need to take account of these subgroups.

Monitoring and Criteria for Withdrawal of Subjects from Study

Patients will be monitored every 3 months, with visits alternating between their local physician and NIH (every 6 month visit to NIH). At the visit to the local physician, a single blood sample

will be obtained and sent to NIH for analysis of the same hormonal measures and blood chemistries that are performed during NIH visits (see protocol section above). The other monitoring during NIH visits has been described in detail above.

In addition to this monitoring, patients who are randomized to flutamide will receive intensive monitoring of liver function as a precaution against flutamide-associated liver toxicity (see Risks below). This monitoring will consist of a blood sample to determine liver function every 2 weeks for the initial 3 months, every month for the next 9 months, and every 3 months thereafter.

Patients who experience flutamide-associated liver toxicity (the estimated incidence is 1 in 273 patients), or who do not tolerate the flutamide and aromatase inhibitor regimen, will be withdrawn from this treatment arm and followed on conventional treatment so that their outcome will be available for an intention-to-treat analysis. We will also ask patients who elect to withdraw from the study to continue to make semiannual height measurements at home and to return to NIH for one final height and weight measurement once they attain the final height criteria.

Since CAH is a chronic disorder requiring lifelong treatment, and since the final height of all subjects is important for the performance of an intention-to-treat analysis, we do not foresee circumstances that would lead us to withdraw a subject from study. If an unrelated illness, such as chronic active hepatitis, were to preclude use of the investigational regimen, we would nonetheless wish to follow the subject on the conventional treatment.

Adverse Event Reporting and Data Monitoring

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801. [redacted] the primary investigator is responsible for monitoring the protocol.

[redacted] is responsible for all aspects of the study. [redacted] are responsible for coordinating data collection and will review the data for accuracy and completeness within 1 month of each subject visit, including review of patient consent documents. Review of literature and results of related studies will be assessed throughout the study for any impact on patient safety or ethical questions.

[redacted] is responsible for maintaining IRB documentation, including records of all reviews of the study and submissions to the IRB.

Missed blood draws or other testing (i.e. radiological studies) due to scheduling or patient related issues commonly encountered in clinical care will not be reported as protocol deviations.

[redacted] will maintain subjects' records for at least three years after completion of the study.

Waiver of consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions

with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.
If reconsent is not feasible, we request waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.
Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.

    a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.
(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
(5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

Research Use, Storage and Disposition of Human Subjects' Samples and Data

Samples will be stored according to NICHD and NIH policy. Samples may be used in the future, with identifiers, for studies not described here, based on the subject's consent. Blood samples may be shared with collaborators, without personal identifiers.

All of the stored study research specimens are labeled by a numeric, alpha-numeric identification code that only the study team can link to the subject. Specimens are stored in locked lab freezers and in the NICHD biorepository, which is accessed via a locked door. All stored research specimens are logged into a specimen tracking database. Variables included in the database are ID, specimen type, aliquot, date of collection, and location. All usage and/or disbursement of the specimens is documented and tracked in the database. Only investigators or their designees will have access to the specimens and/or data.

Subject confidentiality will be maintained to the greatest extent possible. Research results will be

labeled using a unique code. Publications will not include subject names nor will they contain personal identifying information.

Any loss or unanticipated destruction of specimens (e.g. due to freezer malfunction) or data (e.g. misplacing a print-out of data with identifiers) that would affect the scientific integrity of the study will be reported to the IRB as an unanticipated problem by the PI.

This protocol will be kept open as long as there is a possibility of future research use of the specimens and/or data. When there is no longer a need, the specimens will be destroyed or, after IRB approval, transferred to another repository. Data will be archived by the investigator in compliance with requirements for retention of research records, or after IRB approval, destroyed or transferred to another repository.

Analysis of Study (please also see attached SAP Appendix A)

- The primary outcome (adult height expressed in SD units) will be compared between groups at the conclusion of the study using the unpaired two-tailed Student's t-test, or nonparametric Wilcoxon rank sum test, as appropriate

- Secondary outcome measures during the study include hormone levels (percent of visits in ranged defined above) and treatment doses, BMI (as SD units), final BMD (Z-score), growth velocity (in SD units), rate of bone maturation, and predicted adult height (Bayley-Pinneau method, automated software in a subset of patients) at baseline, pubertal onset, and final visit. These outcomes will be compared between the study arms by multivariable mixed models for repeated measures. These models will allow for within-subject comparisons between timepoints, as well as account for their correlations across time, adjust for potential confounders, and specify the appropriate outcome distribution (e.g., normal, binomial, or Poisson distribution for type of data). They are a robust method for accommodating missing data, especially in measures that are repeated and collected longitudinally. Post-hoc comparisons will identify the driving factors and group effect influencing any observed differences, and will be corrected for multiple comparisons as applicable.

We have chosen a group size of 30, giving this study the same statistical power as the study of Deslorelin-induced pubertal delay (83-CH-199). With an expected standard deviation of 6 cm for the final height measurements (approximately that of the normal population), the group size required to give a 80% power to detect a 5-cm difference (about 2 inches) at a $p<0.05$ is 24. The group size needed to detect a 6-cm difference is 17. This is regarded by the pediatric endocrine community as a reasonable power for a growth study - for example, the Genentech Collaborative Study of Turner Syndrome had a group size of 17. Thus, the study is designed to detect at least a 5 cm difference in final height, a clinically meaningful difference, and allows for a 25% drop out rate over the course of the study. This is regarded by the pediatric endocrine community as a reasonable power for a growth study - for example, the Genentech Collaborative Study of Turner Syndrome had a group size of 17. Thus, the study is designed to detect at least a 5 cm difference in final height, a clinically meaningful difference, and allows for a 25% drop out rate over the

course of the study.

Please find attached study analysis plan.

Human Subject Protections

Subject Selection

Exclusion criteria include children who have concurrent illnesses requiring glucocorticoid treatment (such as severe asthma), or requiring drugs that markedly alter hydrocortisone metabolism (such as anticonvulsants), and children who cannot be brought into reasonable control with conventional treatment (an unusual occurrence).

The incidence of classical CAH appears to be nearly the same in all ethnic groups, and lower in African Americans (ref: Therrell BL, Jr., Berenbaum SA, Manter-Kapanke V, et al. Results of screening 1.9 million Texas newborns for 21-hydroxylase-deficient congenital adrenal hyperplasia. *Pediatrics* 1998; **101**(4 Pt 1)**:** 583-90. Based upon our previous studies, the anticipated gender and ethnic distribution of this study will reflect the gender and ethnic distribution of the United States population.

The study includes a vulnerable population (children) because treatments designed to address the growth abnormalities in CAH can only be evaluated in subjects who are growing. However, the procedures involved in this study are essentially the same as would be required in children receiving conventional treatment for CAH outside of a study. Additionally, the expertise provided by the study team, may reduce the psychological stress of evaluation compared to some other settings. Moreover, the expertise of the physician team may mitigate some of the physical consequences of the disorder (such as iatrogenic Cushing syndrome), even for those subjects who are in the conventional treatment arm.

Evaluation of Benefits and Risks/Discomforts

Data from this study will be evaluated annually by the NICHD Data Safety Monitoring Committee.

The potential benefits include a 50% chance of receiving state-of-the-art monitoring and drug adjustment, if one is randomized to conventional treatment, and a 50% chance of receiving the hypothesized benefits of the new investigational regimen, if one is randomized to that arm and if the hypothesized benefits are in fact realized. The psychological assessment has the benefit of identifying an unknown learning disability.

The risks and discomforts of participation in this study include:

(1) pain associated with having an intravenous needle inserted into a hand or arm vein, and the possibility of a bruise;

(2) the inconvenience of urine collections;
(3) blood withdrawal: 120 mL per 6 months for the conventional arm. If the leuprolide

stimulation test and ACTH test are omitted, blood withdrawal is 84 mL. For those receiving flutamide, additional blood withdrawal will be necessary for liver function tests: 12 mL in the first 6 months, 6 mL for the subsequent 6 months and 2 mL thereafter. These amounts are within the National Institutes of Health guidelines of 7 mL per kg body weight per 6 weeks. If a child meets this blood limit, certain tests will be omitted.

(4) inconvenience and discomfort of being upright for 2 hours for an upright renin test.

(5) Aromatase inhibitor. Over twenty years of testolactone use, and our own experience with protocols 82-CH-165 and 85-CH-16, have revealed only minor dose-related gastrointestinal side effects and no serious toxicity. This side effect has been observed, but less commonly, with letrozole.
Other than testolactone, letrozole is the aromatase inhibitor with the most reported pediatric experience and with the most clinical experience by investigators at the Clinical Center. No harmful side-effects have been reported.

(6) Flutamide. Aside from infrequent nausea, diarrhea, and dizziness, the most common side-effect when flutamide was used in prostate cancer has been gynecomastia and breast tenderness (34% of patients). We have found, however, that combined treatment with aromatase inhibitor prevents development of gynecomastia, as was the case during combined spironolactone plus testolactone treatment [8]. Infrequent adverse events reported in the literature include thromboembolic complications [15] and neutropenia [42]. Should the latter develop, the drug will be discontinued immediately.

The most serious adverse effect of flutamide is hepatotoxicity. This risk was not detected in animal or pre-marketing clinical studies. It became apparent through the spontaneous reporting of 15 deaths believed to be due to flutamide-related hepatotoxicity from among the nearly 700,000 flutamide prescriptions for prostate cancer between 1989 and 1991 [43]. The median duration of flutamide treatment among the fatal cases was 2 months, with the longest duration being 7 months. In each fatal case flutamide administration was continued despite evidence of deteriorating liver function. In other cases in which flutamide was discontinued after liver toxicity was recognized, the abnormality of liver function was reversible.

In a study of 1,091 consecutive patients with prostate cancer treated with flutamide, 4 (or 1 in 273) developed significant liver dysfunction during the first month of treatment, which resolved completely with discontinuation of the drug. Thus, liver toxicity due to flutamide usually develops early in the course of treatment. This is the rationale for the schedule of hepatic monitoring in the flutamide-treated patients in this study: every 2 weeks for the initial 3 months of treatment, every month for the next 9 months, and every 3 months thereafter. In addition to the monitoring, each parent will be instructed in the signs and symptoms of liver toxicity and what to do if they should occur (see consent) .

If an elevation in liver function tests occurs, the following monitoring will be done. If the liver enzyme values are greater than 3 times the upper limit of normal, flutamide will be discontinued immediately. If the liver enzyme values are elevated but less than a 3-fold increase, liver function tests will be repeated immediately but the drug will not be discontinued. If the elevation is confirmed by repeat testing, the child will have weekly liver function tests for 3 weeks. If the

values are increasing by more than 10%, flutamide will be discontinued. If values are remaining the same (or within 10%), flutamide will be continued and the previous regimen of liver function test monitoring will be resumed with a minimum of monthly evaluations for the first three months. The above monitoring program was developed after consulting both the Food and Drug Administration and an internationally recognized expert on drug-induced hepatic injury. With these precautions, it is believed that the risk of serious hepatic injury, such as occurred in adults before this risk of the drug was recognized [43], can be eliminated. During the 8 years that we have used flutamide, involving approximately 30 children, no hepatotoxicity has been observed.

The use of flutamide during pregnancy may result in ambiguous genitalia in a male fetus. Other potential teratogenic effects are unknown. Ovulation and pregnancy either prior to menarche or in the first 6 months following menarche in girls with classic CAH is extremely unlikely. All female subjects have undergone genital surgery for correction of ambiguous genitalia and many require a second surgery in adolescence before sexual intercourse will be possible. These structural abnormalities further reduce the unlikely possibility of pregnancy. Patients and their parents will be counseled together and separately as to the importance of not becoming pregnant while on flutamide. We will perform a pregnancy test each visit for pubertal girls on flutamide.

(6) Since hydrocortisone dosage will be reduced below that usually employed, some patients may develop symptoms or signs of adrenal insufficiency. We consider that this is unlikely to be a serious risk because; (1) patients will continue to receive an optimal fludrocortisone dose, (2) CAH patients remain capable of producing some endogenous hydrocortisone, and (3) the restricted dosage of approximately 8 mg/m^2/day is equivalent to the most recent estimates of normal production rate and, in concert with the first 2 factors, should prevent any subjects from experiencing adrenal crisis. If, however, symptoms or signs of adrenal insufficiency do occur at this dosage, the hydrocortisone dose will be increased on an individual basis to a dose that does not cause such signs or symptoms.

(7) Efficacy of the new treatment approach. Since the new approach to be tested in this study has only been employed previously in short-term studies, there is the theoretical possibility that its long-term effectiveness could be less than that of conventional therapy. In this event participants randomized to the investigational regimen would miss the full benefit of conventional treatment.

(8) Leuprolide stimulation test. This is a standard test of pubertal onset. It has no known risk.
(9) ACTH test. This is a standard test of adrenal function. It has no known risk.

(10) Bone density of the forearm, femur, and lumbar spine will be determined annually by DEXA. (Bone age x-rays of the wrist will also be performed every six months). The maximal tissue dose is 90 mrem to skin of lower back. The total effective dose is less than 1 mrem. Effective dose is the uniform whole-body dose, which is used to relate the dose received by each organ to a single value. The effective dose is well within the RSC guidelines for children of 500 mrem.

(11) Magnetic Resonance Imaging (MRI). This procedure is without known risk and involves no radiation exposure. Approximately 1% of subjects are too claustrophobic to be studied. During the MRI scan, the patient hears rhythmic tapping sounds due to switching on and off of the gradient coils. Subjects are warned of this prior to scanning and most do not find it objectional. Ear plugs are provided. Metal objects are exclusionary criteria and patients will be carefully screened for these exclusionary criteria. There are no known adverse effects of magnetic resonance imaging

but guidelines for use have been established by the FDA. For purposes of this protocol the NIH s MRI units operate well within those guidelines and their use has been previously approved in an IRB protocol for child age volunteers (CC#87-CC-91).

(12) There are possible discomforts associated with talking about topics such as behavior problems, or mental health problems.

Recruitment, Consent, and Assent Processes

Subjects will be recruited through announcements mailed to pediatricians and family practitioners (see sample at end of this section).

Consent will be obtained by one of the members of the research team. Consent will be documented in writing and witnessed on the consent document (see below). A copy of the consent document will be provided to the parent for reference about the study details. Parents will be encouraged to ask any questions that they may have, before or after the consent process.

Children will generally be present and have the opportunity to ask questions during explanation of the study and the consent process with the parents. Whenever it is appropriate for the childs age and level of understanding, the child will be given a chance to read (or have read to them) and sign the study assent document. The assent will be obtained by one of the members of the research team.

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