

Clinical Research Article

Crinercerfont Lowers Elevated Hormone Markers in Adults With 21-Hydroxylase Deficiency Congenital Adrenal Hyperplasia

Richard J. Auchus,¹ Kyriakie Sarafoglou,² Patricia Y. Fechner,³ Maria G. Vogiatzi,⁴ Erik A. Imel,⁵ Shanlee M. Davis,⁶ Nagdeep Giri,⁷ Julia Sturgeon,⁷ Eiry Roberts,⁷ Jean L. Chan,⁷ and Robert H. Farber⁷

¹Departments of Pharmacology and Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA; ²Department of Pediatrics, Division of Pediatric Endocrinology, University of Minnesota Medical School, Minneapolis, Minnesota 55454, USA; ³Department of Pediatrics, Division of Pediatric Endocrinology, University of Washington School of Medicine, Seattle Children's Hospital, Seattle, Washington 98105, USA; ⁴Division of Endocrinology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104, USA; ⁵Departments of Medicine and Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana 46202, USA; ⁶Department of Pediatrics, Section of Pediatric Endocrinology, University of Colorado School of Medicine, Aurora, Colorado 80045, USA; and ⁷Neurocrine Biosciences Inc, San Diego, California 92130, USA

ORCID numbers: 0000-0001-6815-6181 (R. J. Auchus); 0000-0002-5741-3629 (K. Sarafoglou); 0000-0002-7284-3467 (E. A. Imel).

Abbreviations: 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; ACTH, adrenocorticotropic; CAH, congenital adrenal hyperplasia; CRF1R, corticotropin-releasing factor type 1 receptor; GC, glucocorticoid; TEAE, treatment-emergent adverse event.

Received: 6 July 2021; Editorial Decision: 9 October 2021; First Published Online: 15 October 2021; Corrected and Typeset: 30 October 2021.

Abstract

Context: Classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD) is characterized by impaired cortisol synthesis and excess androgen production. Corticotropin-releasing factor type 1 receptor (CRF1R) antagonism may decrease adrenal androgen production.

Objective: This work aimed to evaluate the safety, tolerability, and efficacy of crinercerfont (NBI-74788), a selective CRF1R antagonist, in 21OHD.

Methods: This open-label, phase 2 study, with sequential cohort design (NCT03525886), took place in 6 centers in the United States. Participants included men and women, aged 18 to 50 years, with 21OHD. Interventions included 4 crinercerfont regimens, each administered orally for 14 consecutive days: 50 or 100 mg once daily at bedtime (cohorts 1 and 2, respectively); 100 mg once daily in the evening (cohort 3); and 100 mg twice daily (cohort 4). Participants could enroll in more than 1 cohort. Main outcomes included

changes from baseline to day 14 in adrenocorticotropin (ACTH), 17-hydroxyprogesterone (17OHP), androstenedione, and testosterone.

Results: Eighteen participants (11 women, 7 men) were enrolled: cohort 1 (n = 8), cohort 2 (n = 7), cohort 3 (n = 8), cohort 4 (n = 8). Mean age was 31 years; 94% were White. Median percent reductions were more than 60% for ACTH (–66%), 17OHP (–64%), and androstenedione (–64%) with crinicerfont 100 mg twice a day. In female participants, 73% (8/11) had a 50% or greater reduction in testosterone levels; male participants had median 26% to 65% decreases in androstenedione/testosterone ratios.

Conclusion: Crinicerfont treatment for 14 days lowered ACTH and afforded clinically meaningful reductions of elevated 17OHP, androstenedione, testosterone (women), or androstenedione/testosterone ratio (men) in adults with 21OHD. Longer-term studies are required to evaluate the effects of crinicerfont on clinical end points of disordered steroidogenesis and glucocorticoid exposure in patients with 21OHD.

Key Words: congenital adrenal hyperplasia, 21-hydroxylase deficiency, 17-hydroxyprogesterone, crinicerfont, NBI-74788

Congenital adrenal hyperplasia (CAH) refers to a group of rare autosomal recessive disorders that result in disordered adrenal steroidogenesis, including impaired cortisol synthesis. Excess adrenal androgen production occurs in patients with CAH due to 21-hydroxylase deficiency (21OHD), a population that accounts for approximately 95% of all CAH cases (1). The cortisol deficiency removes normal negative feedback inhibition on the hypothalamus and the pituitary gland, resulting in increased secretion of corticotropin-releasing factor from the hypothalamus and adrenocorticotropin (ACTH) from the pituitary. Chronic ACTH drive leads to excess production of adrenal androgens (2, 3).

The “classic” form of CAH due to 21OHD, which is associated with more severe enzyme deficiency, occurs in approximately 1:15 000 births (4). The androgen excess during fetal life leads to virilization of 46,XX newborns; 21OHD is the leading cause of atypical genitalia in the female infant. Continued androgen excess during childhood and adolescence causes sexual precocity, virilization, and accelerated somatic growth with advanced bone age, which results ultimately in below-predicted adult height (5, 6). During adulthood, hirsutism and irregular menses are common in women, and men and women with 21OHD suffer from reduced fertility and psychiatric disorders (7). In addition, all patients with 21OHD are at risk for adrenal crises that can result in death if untreated.

Glucocorticoid (GC) therapy, the current standard of care, is used to replace the endogenous cortisol deficiency (8). However, suprathreshold GC doses and nonphysiologic timing (eg, evening dosing) are usually needed to reduce the elevated ACTH secretion and excess androgen production. This chronic exposure to suprathreshold GC doses can lead to serious complications including growth suppression in children, reduced bone mineral density with

increased fracture risk, and metabolic disorders such as obesity, insulin resistance, and hypertension, which can increase cardiovascular risk (1, 2, 8–11). Thus, the challenge of treating 21OHD is to balance adequate control of androgens with the risks of excessive and prolonged GC exposure; undertreatment and overtreatment with GCs both cause side effects and complications.

An antagonist of the corticotropin-releasing factor type 1 receptor (CRF1R) may offer a new approach for treating this disorder. CRF1R antagonism can reduce ACTH secretion, which in 21OHD could decrease the downstream production of androgens and reduce symptoms of hyperandrogenism, while also potentially allowing for GCs to be administered at more physiologic doses. Thus, this approach could help to mitigate the negative consequences of long-term suprathreshold GC treatment. The potential efficacy of CRF1R antagonism was demonstrated in an exploratory study of a CRF1R antagonist (NBI-77860) in 8 female patients with 21OHD (12) that found meaningful reductions in ACTH and 17-hydroxyprogesterone (17OHP) after single-dose administration.

Crinicerfont, an orally administered, nonsteroidal CRF1R antagonist, is currently being studied for the treatment of classic 21OHD. This phase 2 study (NCT03525886) of crinicerfont was conducted to evaluate its safety, tolerability, and effects on ACTH, adrenal androgens, and androgen precursors in adults with classic 21OHD.

Materials and Methods

Study Design and Participants

This phase 2, open-label study used a sequential-cohort design to evaluate the safety, tolerability, and efficacy of 4 different crinicerfont dosing regimens, each dosed for 14 days

in adults with classic 21OHD. The study was performed at 6 centers in the United States and conducted in accordance with Good Clinical Practice guidelines, including International Conference on Harmonization requirements and the United States Code of Federal Regulations for clinical trials; see the supplementary material for details (13). The protocol and patient consent forms were reviewed and approved by the institutional review board at each site. All participants provided written and informed consent prior to any study-related procedures.

Key inclusion criteria were as follows: male or female, aged 18 to 50 years; medically confirmed classic 21OHD by hormonal and/or molecular testing; body mass index between 18 and 45 kg/m² (inclusive); serum 17OHP greater than or equal to 30.3 nmol/L (≥ 1000 ng/dL), serum cortisol less than 138 nmol/L (< 5 μ g/dL), and plasma ACTH greater than or equal to 4.4 pmol/L (≥ 20 pg/mL) at screening before morning GC dose; receiving a stable GC regimen for at least 30 days before baseline.

Key exclusion criteria were as follows: known or suspected diagnosis of other forms of CAH (eg, 11 β -hydroxylase deficiency); prior or current medical condition requiring daily GC therapy (other than 21OHD); clinically significant unstable medical condition, chronic disease, or malignancy; clinically significant illness within 30 days before screening; clinically relevant laboratory abnormality (eg, hematologic, coagulation, renal, liver enzymes); pregnancy or lactation; corrected QT interval using the Fridericia formula of greater than 450 msec (men) or greater than 470 msec (women); history of significant cardiac abnormality or arrhythmia; recent or current substance abuse or dependence; risk of suicidal or violent behavior; and dexamethasone therapy for 30 days before screening and throughout the study.

The sequential-cohort design comprised 4 open-label crinecerfont dosing regimens, as follows: cohort 1 (50 mg once daily at bedtime); cohort 2 (100 mg once daily at bedtime); cohort 3 (100 mg once daily in the evening); cohort 4 (100 mg twice daily, morning and evening) (Fig. 1). The study medication was taken with 8 ounces (236 mL) of Ensure Plus (16 g protein, 47 g carbohydrate, 11 g fat; Abbott Laboratories) (cohorts 1 and 2) or patients' regular evening meals (cohort 3) or their regular morning and evening meals (cohort 4). Each regimen was administered for 14 consecutive days while continuing their normal daily GC regimen, which was maintained stable over the 14 days. Participants who enrolled in cohort 1 (50 mg once daily at bedtime) or cohort 2 (100 mg once daily at bedtime) were allowed to enter cohort 3 (100 mg once daily in the evening) if no safety concerns were raised. Participants were allowed to enter cohort 4 (100 mg twice a day) from any earlier cohort or de novo.

Study drug (crinecerfont) was provided by Neurocrine Biosciences, Inc. Participants who reenrolled into a new cohort underwent additional screening if their GC regimen changed since their last study visit; otherwise, a new day -7 to -6 baseline sampling could be performed after the day 49 final follow-up visit.

Procedures and Assessments

All participants were admitted to the study center from day -7 to -6 for serial blood sampling to establish baseline ACTH, 17OHP, androstenedione, and testosterone concentration profiles. Serial blood sampling schedules at baseline were time-matched for similar serial sampling on days 1/2 (cohorts 1 and 2) and days 14/15 (all cohorts). On day 1, participants received the first dose of study medication at 22:00 (cohorts 1 and 2) or 19:00 (cohorts 3 and 4) and continued their assigned regimen until day 14. Participants in cohort 4 received the final dose of study medication on the evening of day 14. During treatment in cohort 1 (50 mg once daily at bedtime) and Cohort 2 (100 mg once daily at bedtime), serial sampling started at 15 minutes prior to crinecerfont dosing and continued for 24 hours postdose (at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, and 24 hours) (see Fig. 1). During treatment in cohort 3 (100 mg once daily in the evening) and cohort 4 (100 mg twice a day), serial sampling started at 15 minutes predose and continued for 27 hours postdose (at 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 17, 19, 23, 24, and 27 hours).

During these overnight study admissions, the participants' usual morning GC doses were delayed until after the 10:00 blood sample collection on day 2 and after the 14:00 blood sample collection on day 15. For analysis, the time frame between 06:00 and 10:00 was defined as the "morning window" and included samples obtained at 8, 10, and 12 hours postdose in cohorts 1 and 2 and at 11, 13, and 15 hours postdose in cohorts 3 and 4. Analyses focused on the average (mean) of the 3 morning window time points to evaluate the effect of crinecerfont on the early morning surge of ACTH and the resulting rise in adrenal steroid production, which are difficult to control with physiologic GC regimens (1, 12). Blood samples were sent to a central laboratory for analysis of plasma ACTH; serum 17OHP, androstenedione, testosterone, and cortisol; and crinecerfont pharmacokinetics. See the supplementary material for details (13).

Treatment-emergent adverse events (TEAEs), including serious adverse events and TEAEs leading to discontinuation, were assessed throughout the study. Additional safety assessments included vital signs, 12-lead electrocardiogram, clinical laboratory tests, Brief Psychiatric Rating Scale, and Columbia-Suicide Severity Rating Scale.

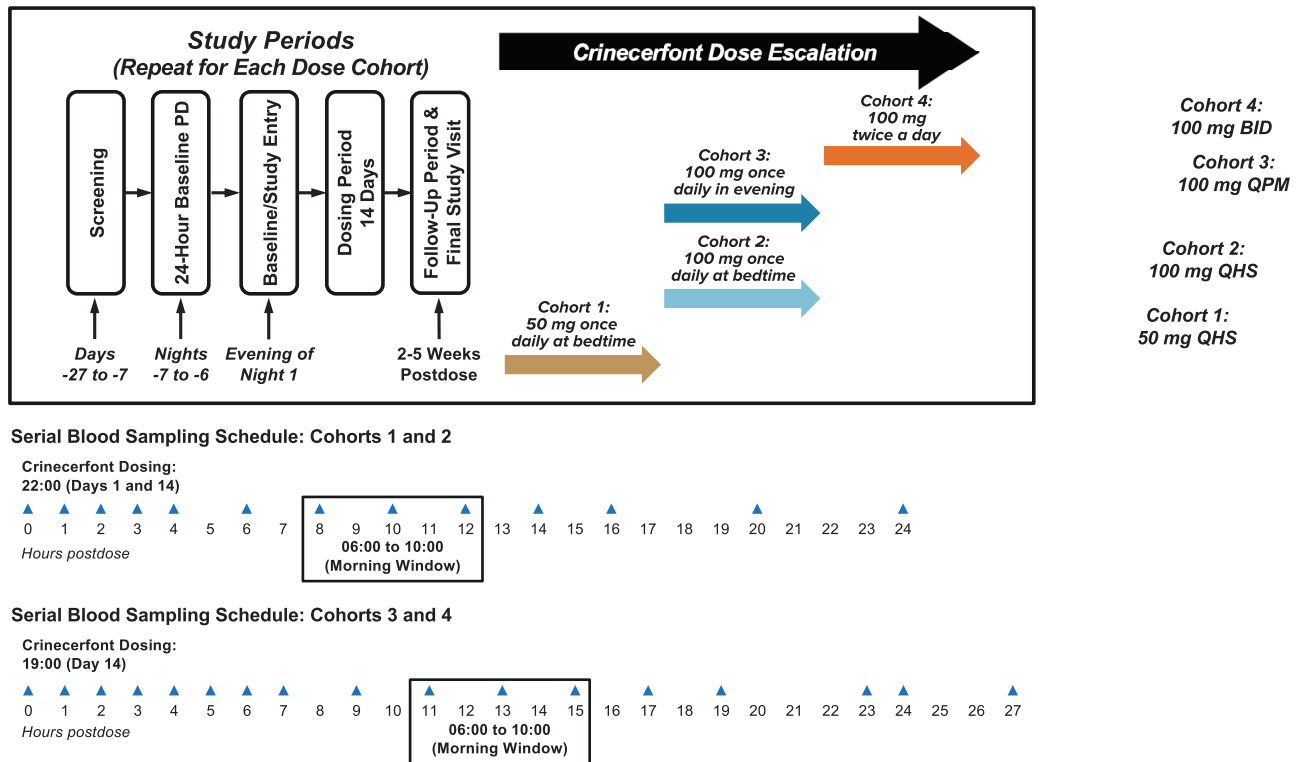


Figure 1. Study design. Blue triangles denote sample collection. In all cohorts, sampling for the 24-hour baseline period (day -7 to -6) matched the sampling schedule during crinecerfont treatment. For the baseline and day 1 visits, participants received their prescribed glucocorticoid dose after sample collection at 10:00 (end of the morning window); for the day 14 visit, glucocorticoid was administered after sample collection at 14:00. GC, glucocorticoid.

Statistical Analyses

Descriptive statistical methods were used to summarize the data. The analysis population included all 18 participants who received at least one dose of study drug. All participants had a baseline and at least one postbaseline pharmacodynamic assessment. Three participants (one each from cohorts 1, 2, and 3) were considered to have a protocol deviation for inadvertently receiving GC dosing before morning window blood sample collection. However, a sensitivity analysis indicated that the inclusion/exclusion of these data did not materially alter the results. Therefore, all 18 participants were included in the pharmacodynamic/efficacy analyses. Sample size was based on practical clinical considerations for a rare disease, without formal statistical power calculations.

Efficacy analyses were based on assessments collected on days -7/-6 (“baseline”), days 1/2 (“day 1”), and days 14/15 (“day 14”); for simplicity, the 24- and 27-hour postdose periods are referred to as “24-hour sampling period.” Key end points for ACTH, 17OHP, androstenedione, and testosterone concentrations were based on available values both in the morning window and the 24-hour sampling period. Analyses based on these values included the following: median change from baseline to day 14 (morning window and 24-hour periods); median

percent change from baseline to day 14 (morning window); and percentage of participants with 50% or greater reduction (ie, “responders”) (12) from baseline to day 14 (morning window). Changes (in men) for androstenedione/testosterone ratios were also analyzed, including achieving a target of less than 0.5 (14). Reduction into the normal range during the morning window was also analyzed in participants whose androstenedione or testosterone (women only) at baseline exceeded 1.2 times the upper limit of normal. Mean values are presented with the standard deviation. Median values are presented with the interquartile range, defined as the absolute difference between the 75th and 25th percentiles (Q3-Q1).

Results

Participants and Crinecerfont Exposure

A total of 18 participants were enrolled in the study. Three participants enrolled in 3 cohorts each; 7 participants enrolled in 2 cohorts each. The median time between cohorts was 183 days (range, 49-343 days). Crinecerfont exposure over 24 hours (area under the curve [AUC]₀₋₂₄) increased with dose across cohorts. In the 100-mg cohorts, mean plasma exposure of crinecerfont was similar irrespective of meal type (liquid dietary supplement vs usual evening

meal) or timing (once daily at bedtime vs once daily in the evening) [AUC_{0-24} : 77.4 $\mu\text{mol} \times \text{h/L}$ vs 72.0 $\mu\text{mol} \times \text{h/L}$]. Crinecerfont dosed at 100 mg twice daily was observed to result in approximately twice the exposure [AUC_{0-24} : 138.5 $\mu\text{mol} \times \text{h/L}$] compared to the exposure observed with 100 mg once daily (once daily at bedtime or once daily in the evening).

Of the 18 enrolled participants, 61% were female and 94% were White; mean age was 31 ± 9.3 years (Table 1). A prior hysterectomy was reported in one female participant who enrolled in cohorts 2 and 4. For GC therapy at baseline, 56% of participants were receiving hydrocortisone alone, and 44% were receiving prednisone (or equivalent) with or without hydrocortisone. Mean baseline total daily GC dose in hydrocortisone equivalents was 26 ± 9.1 mg/day (14 ± 4.8 mg/day/ m^2 when adjusted for body surface area), with 1 mg of prednisolone, methylprednisolone, or prednisone considered equivalent to 4 mg of hydrocortisone. Baseline samples from the morning window indicated that mean and median values for androstenedione, 17OHP, testosterone (in men and women), and androstenedione/testosterone ratios (in men) were above the upper limit of normal (see Tables 1 and 2), indicating inadequate disease control.

Effects of Crinecerfont on Adrenal Androgens and Precursors

At baseline, the 24-hour plasma/serum concentration profile of ACTH, 17OHP, and androstenedione demonstrated the expected early morning increases starting around 04:00 and peaking around 08:00 to 10:00 in the morning (Fig. 2). Treatment with crinecerfont for 14 days led to substantial median reductions for ACTH, 17OHP, and androstenedione relative to baseline, especially during the morning window, across all cohorts (see Fig. 2). The decrease in androstenedione was most pronounced for cohort 4 (100 mg twice a day).

In all cohorts, median ACTH, 17OHP, androstenedione, testosterone (in women), and androstenedione/testosterone ratio (in men) were reduced from baseline to day 14 whether based on samples collected during the morning window (06:00-10:00) or the 24-hour sampling period (see Table 2). For ACTH and 17OHP, median percent decreases from baseline were generally similar across cohorts, ranging from -53% to -66% (Fig. 3). Dose-related decreases in androstenedione were also observed, ranging from a 21% reduction in cohort 1 (50 mg once daily at bedtime) to a 64% reduction in cohort 4 (100 mg twice a day). Median percent reductions for testosterone (in women) ranged from -32% (cohort 1) to -74% (cohort 3) (see Supplementary Fig. S1) (13).

In male participants, testosterone derives from both the adrenals and testes, which complicates the assessment of

testosterone changes in men; however, the androstenedione/testosterone ratio increases as the adrenal contribution to circulating androgens rises (1). While androstenedione declined similarly in male and female participants, testosterone did not fall proportionately in the male participants (see Fig. 3 and Supplementary Fig. S1)(13). Consequently, the median androstenedione/testosterone ratio (in men) during the morning window declined from 26% to 65% in a dose-dependent fashion across the 4 cohorts, and median 24-hour androstenedione/testosterone ratios also declined by 33% (cohort 1) to 59% (cohort 3).

In cohort 4 (100 mg twice a day), a majority of participants had a 50% or greater reduction from baseline to day 14 in ACTH, 17OHP, androstenedione, testosterone (women), and androstenedione/testosterone ratio (men); 2 of 3 male participants achieved an androstenedione/testosterone ratio of less than 0.5 (Table 3). Among female participants, 73% (8/11) had a 50% or greater reduction from baseline in testosterone concentrations. Four of 8 participants in cohort 4 achieved 50% or greater reductions in ACTH, 17OHP, and androstenedione (Fig. 4).

Safety

TEAEs are summarized in Table 4. The majority of TEAEs were mild or unrelated to the study drug, with no deaths, severe TEAEs, or discontinuations due to TEAEs. The most common TEAEs (reported in ≥ 2 participants overall) were headache, upper respiratory tract infection, fatigue, contusion (bruising), insomnia, nasopharyngitis, pyrexia, and nausea. One serious TEAE of cholelithiasis, occurring 34 days after the last dose of the study drug, was assessed as unlikely related to the study drug by the investigator. There were no safety concerns with respect to routine clinical laboratory values, vital signs, electrocardiograms, or neuropsychiatric assessments.

Discussion

Since Wilkins et al described life-saving cortisone therapy for 21OHD in the early 1950s (15), GCs have been the mainstay of treatment, both to replace the cortisol deficiency and to reduce the production of adrenal-derived androgens. With routine access to GCs, the introduction of newborn screening, and improved health care delivery, most children with 21OHD survive well into adulthood (1). Supraphysiologic GC dosing, however, is often needed to attenuate the excess adrenal-derived androgen production. This lifelong treatment of excessive GC dosing is associated with a high burden of comorbidities and reduced quality of life in adults with 21OHD (7, 16, 17).

Table 1. Baseline characteristics

	Cohort 1: 50 mg once daily at bedtime (n = 8)	Cohort 2: 100 mg once daily at bedtime (n = 7)	Cohort 3: 100 mg once daily in evening (n = 8)	Cohort 4: 100 mg twice a day (n = 8)	All participants (N = 18)
Demographics					
Women, n (%)	4 (50)	5 (71)	3 (38)	5 (63)	11 (61)
White, n (%) ^a	7 (88)	7 (100)	7 (88)	8 (100)	17 (94)
Age, mean (SD), y	31 (9.4)	33 (9.7)	31 (10.5)	29 (8.2)	31 (9.3)
Body mass index, mean (SD)	29 (5.5)	29 (2.7)	29 (4.7)	31 (2.8)	29 (4.1)
Glucocorticoid treatment, n (%)					
Hydrocortisone	3 (38)	4 (57)	4 (50)	5 (63)	10 (56)
Prednisone or equivalent	4 (50)	3 (43)	3 (38)	2 (25)	7 (39)
Hydrocortisone + prednisone or equivalent	1 (13)	0 (0)	1 (13)	1 (13)	1 (5.6)
Glucocorticoid total daily dose, mean (SD)					
Hydrocortisone equivalent, mg/day	25 (11.1)	26 (6.9)	26 (9.0)	26 (8.0)	26 (9.1)
Hydrocortisone equivalent, mg/m ² /day	14 (6.6)	14 (2.5)	14 (4.9)	13 (3.6)	14 (4.8)
Adrenal androgens and precursors, mean (SD)^b					
ACTH, pmol/L	67 (66)	53 (42)	83 (64)	78 (74)	70 (67)
17OHP, nmol/L	167 (116)	310 (229)	210 (177)	343 (260)	236 (183)
Men	230 (126)	533 (78)	197 (177)	428 (303)	304 (213)
Women	105 (70)	221 (207)	232 (213)	292 (253)	217 (195)
Androstenedione, nmol/L	11 (8.5)	26 (26)	17 (19)	29 (24)	18 (20)
Men	14 (10)	61 (5.7)	18 (23)	40 (27)	28 (24)
Women	7.7 (6.4)	12 (13)	14 (14)	22 (23)	14 (15)
Testosterone-women, nmol/L	1.8 (1.5)	3.1 (1.6)	3.7 (3.7)	3.4 (3.7)	3.0 (2.4)
Testosterone-men, nmol/L	12 (5.8)	12 (0.5)	14 (5.6)	13 (3.8)	13 (4.5)
Androstenedione/testosterone ratio-men	1.2 (1.0)	5.0 (0.3)	1.9 (2.8)	3.5 (2.7)	2.2 (2.1)

Abbreviations: 17OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropic.

^aIncluded one participant who also self-identified as Hispanic or Latino.

^bBased on values from the morning window time points (06:00, 08:00, 10:00). Normal ranges are as follows: ACTH, 2.2 to 13.2 pmol/L (10-60 pg/mL); 17OHP adult men, less than 6.7 nmol/L (< 220 ng/dL); 17OHP follicular women, less than 2.4 nmol/L (< 80 ng/dL); 17OHP luteal women, less than 8.6 nmol/L (< 285 ng/dL); 17OHP postmenopausal women, less than 1.5 nmol/L (< 51 ng/dL); androstenedione adult men, 2.3 to 7.3 nmol/L (65-210 ng/dL); androstenedione adult women, 2.8 to 8.4 nmol/L (80-240 ng/dL); total testosterone women, 0.3 to 2.1 nmol/L (8-60 ng/dL); total testosterone men, 10.4 to 41.6 nmol/L (300-1200 ng/dL). For androstenedione/testosterone men, target ratio was less than 0.5. In cohort 3, results for testosterone women are based on 4 participants who had available baseline morning window values.

These limitations of current treatment regimens illustrate the unmet need for adjunctive therapies that can reduce androgen production without the need for supraphysiologic GC dosing. The major finding of this study is that treatment with crinicerfont (at 50-200 mg/d for 14 days) affords significant, consistent, and dose-dependent reduction of ACTH, 17OHP, and androstenedione in adults with inadequately controlled 21OHD. Crinicerfont also significantly lowered testosterone in most female participants and reduced androstenedione/testosterone ratios in male participants. Moreover, these reductions were achieved in

the early morning hours—the most challenging window to achieve disease control with physiologic GC exposure (1, 18)—despite a protocol-imposed delay of the first morning GC dose to 14:00, which was implemented to observe the isolated effects of crinicerfont in the absence of concurrent GC action. Importantly, the evaluation of multiple dosing regimens permitted a dose-related response to be observed; the greatest effect was seen for the 100-mg twice-daily dose, for which all participants experienced a decrease in ACTH, 17OHP, and androstenedione. Although the treatment duration was limited to 14 days, crinicerfont

Table 2. Effects of crinecerfont on adrenal androgens and precursors^{a,b}

Median (IQR)	Cohort 1: 50 mg once daily at bedtime (n = 8)		Cohort 2: 100 mg once daily at bedtime (n = 7)		Cohort 3: 100 mg once daily in evening (n = 8)		Cohort 4: 100 mg twice a day (n = 8)	
	Morning window ^c	24-h period ^d	Morning window ^c	24-h period ^d	Morning window ^c	24-h period ^d	Morning window ^c	24-h period ^d
ACTH, pmol/L								
At baseline	33 (103)	20 (69)	43 (83)	16 (21)	98 (104)	28 (26)	68 (86)	22 (25)
Change from baseline to day 14	-24 (48)	-7.6 (48)	-34 (42)	-9.2 (16)	-85 (101)	-18 (29)	-45 (57)	-5.8 (15)
17OHP, nmol/L								
At baseline	162 (77)	69 (89)	299 (452)	114 (260)	197 (292)	89 (150)	327 (425)	103 (175)
Change from baseline to day 14	-81 (43)	-20 (43)	-135 (281)	-38 (104)	-102 (208)	-59 (94)	-171 (330)	-41 (74)
Androstenedione, nmol/L								
At baseline	9.4 (12)	7.5 (6.5)	7.8 (51)	7.2 (38)	11 (19)	6.4 (13)	27 (41)	9.9 (27)
Change from baseline to day 14	-3.8 (4.8)	-0.9 (4.2)	-5.8 (12)	-3.5 (8.5)	-8.1 (13)	-4.8 (9.7)	-14 (33)	-4.2 (16)
Testosterone-women, nmol/L^e								
At baseline	1.9 (2.5)	1.4 (1.9)	2.4 (0.3)	1.9 (0.5)	3.0 (7.3)	1.9 (6.2)	2.2 (4.6)	2.0 (2.0)
Change from baseline to day 14	-0.4 (1.2)	-0.2 (1.0)	-1.8 (0.8)	-1.3 (0.6)	-2.6 (5.7)	-1.5 (4.9)	-1.7 (3.0)	-0.6 (1.3)
Testosterone for men, nmol/L^e								
At baseline	12 (8.4)	8.9 (6.3)	12 (0.7)	11 (0.4)	11 (8.7)	8.9 (7.3)	12 (7.0)	9.9 (4.3)
Change from baseline to day 14	2.2 (7.9)	2.7 (5.8)	-0.5 (0.1)	-1.3 (1.7)	0.7 (3.0)	-0.6 (4.4)	0.9 (3.6)	0.8 (1.2)
Androstenedione/testosterone ratio for men^e								
At baseline	0.9 (1.2)	1.0 (1.4)	5.0 (0.4)	4.3 (1.1)	0.6 (1.1)	0.5 (0.9)	3.9 (5.4)	3.2 (3.7)
Change from baseline to day 14	-0.3 (0.8)	-0.3 (0.8)	-1.7 (1.3)	-1.7 (1.7)	-0.5 (0.6)	-0.3 (0.6)	-3.6 (3.7)	-2.4 (2.7)

Abbreviations: 17OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropic; GC, glucocorticoid; IQR, interquartile range (Q3-Q1).

^aNormal ranges are as follows: ACTH, 2.2-13.2 pmol/L (10-60 pg/mL); 17OHP adult men, less than 6.7 nmol/L (< 220 ng/dL); 17OHP follicular women, less than 2.4 nmol/L (< 80 ng/dL); 17OHP luteal women, less than 8.6 nmol/L (< 285 ng/dL); 17OHP postmenopausal women, less than 1.5 nmol/L (< 51 ng/dL); androstenedione adult men, 2.3 to 7.3 nmol/L (65-210 ng/dL); androstenedione adult women, 2.8 to 8.4 nmol/L (80-240 ng/dL); total testosterone women, 0.3 to 2.1 nmol/L (8-60 ng/dL); total testosterone men, 10.4 to 41.6 nmol/L (300-1200 ng/dL). For androstenedione/testosterone men, target ratio was less than 0.5.

^bAside from GC increases found in 3 participants with a protocol deviation (received GC dosing before blood sample collection in cohorts 1, 2, and 3 [each n = 1]), no clinically meaningful changes in cortisol levels were found.

^cBased on values from the morning window time points (06:00, 08:00, 10:00).

^dBased on values from all time points in serial blood sampling period: cohorts 1 and 2 (from 23:00 to 22:00 [following day]); cohorts 3 and 4 (from 20:00 to 22:00 [following day]).

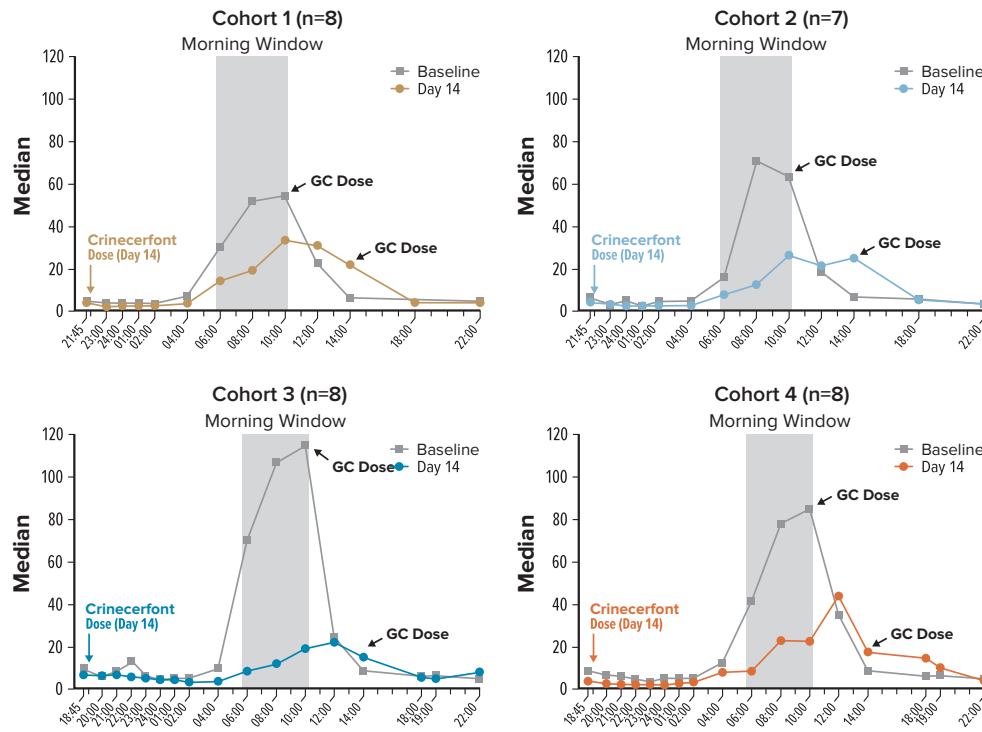
^en values for testosterone and androstenedione/testosterone were as follows: females (cohort 1, n = 4; cohort 2, n = 5; cohort 3, n = 3; cohort 4, n = 4); males (cohort 1, n = 4; cohort 2, n = 2; cohort 3, n = 5; cohort 4, n = 3).

was well tolerated, with the majority of adverse events assessed as mild and/or unrelated and without evidence of relationship to dose. Thus, these data demonstrate that crinecerfont shows promise as adjunctive treatment to control androgen excess in 21OHD. If such improvements are sustained during chronic treatment, GC dosing might be reduced to physiologic replacement schedules, which in turn could mitigate the long-term adverse health outcomes observed in adults with 21OHD (19).

One prior published study evaluated a single bedtime dose of the CRF1R antagonist NBI-77860 in 8 adult women with 21OHD at doses of 300 mg and 600 mg (12). A majority of

participants (50%-75%) experienced a substantial 50% or greater reduction in ACTH and/or 17OHP during the early-morning window as described in this study. A significant pharmacokinetic-pharmacodynamic correlation was observed, as most participants with peak drug concentrations of greater than 500 ng/mL showed a good response to treatment. In a second trial, 6 days of treatment with abiraterone acetate, a 17-hydroxylase/17,20-lyase (CYP17A1) inhibitor, normalized androstenedione on day 7 in all 6 adult women with 21OHD at a dose of 250 mg/d (20). A third study of nevanimibe, a cholesterol acyltransferase enzyme (SOAT1 or ACAT1) inhibitor, found reductions in 17OHP but not androstenedione

A. Plasma ACTH, pmol/L



B. Serum 17OHP, nmol/L

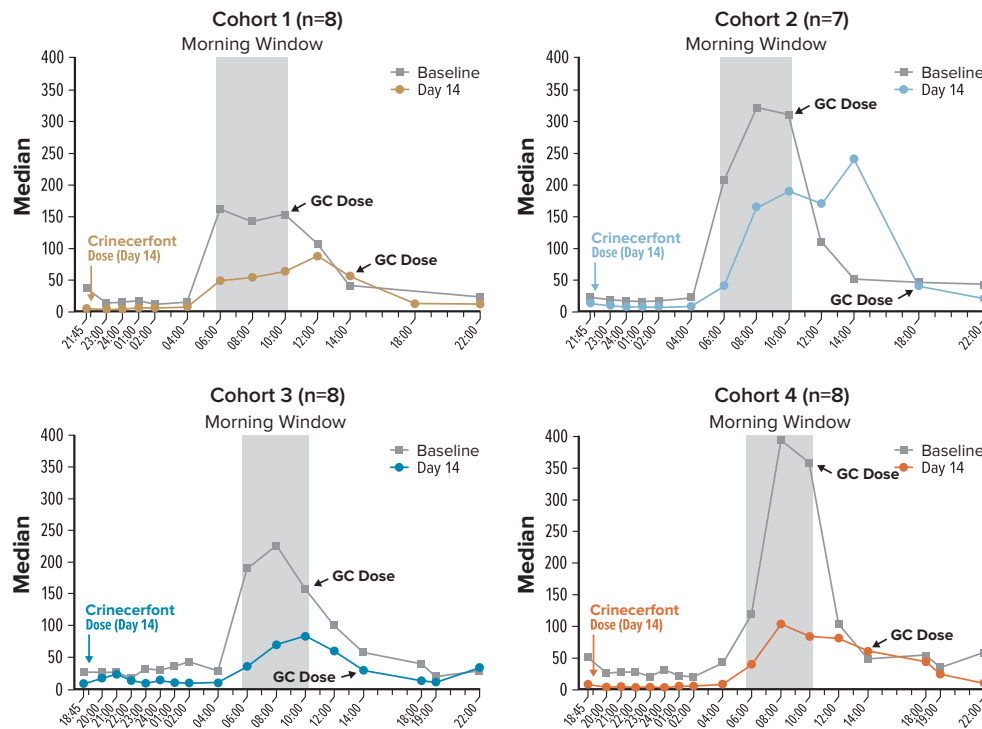


Figure 2. Twenty-four-hour profiles. For cohorts 1 and 2, crinercerfont dosing was at 22:00 on day 14; predose sampling was at 21:45. For cohorts 3 and 4, crinercerfont dosing was at 19:00 on day 14; predose sampling was at 18:45. 17OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropic; GC, glucocorticoid.

compared to placebo without a dose-response relationship (21). Studies of slow-release hydrocortisone (22, 23) and continuous subcutaneous hydrocortisone infusion (24) have demonstrated improved biomarker control, but GC exposure

remained above physiologic levels (> 20 mg/d) in these studies. In contrast, we found that crinercerfont lowered ACTH and consequently afforded meaningful reductions in 17OHP and androstenedione in adults with 21OHD. Additionally,

C. Serum androstenedione, nmol/L

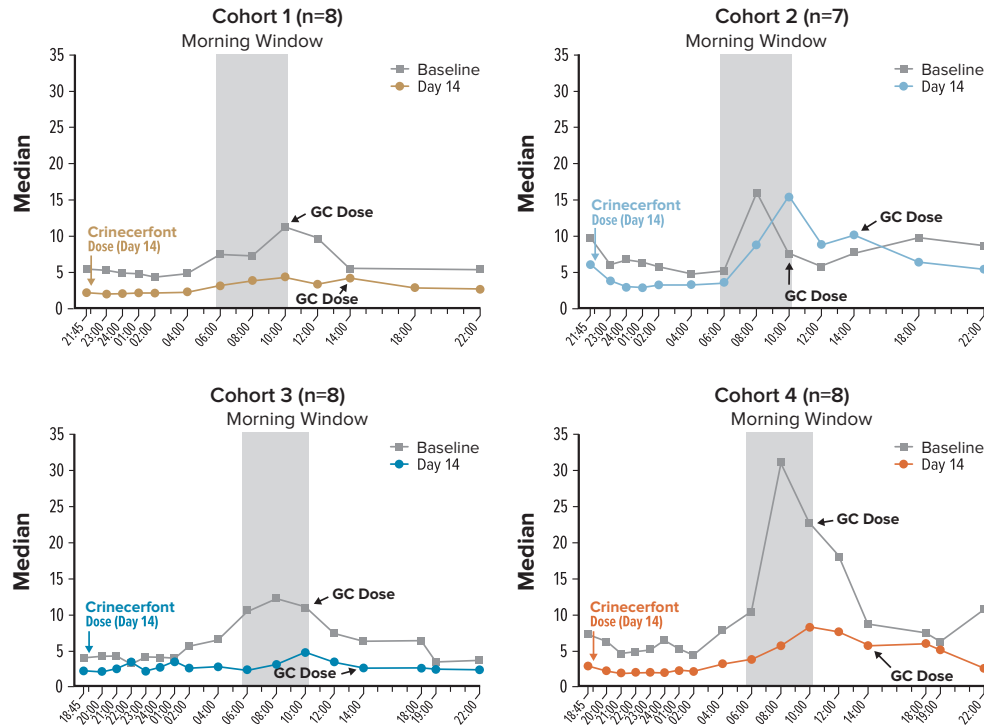


Figure 2. Continued.

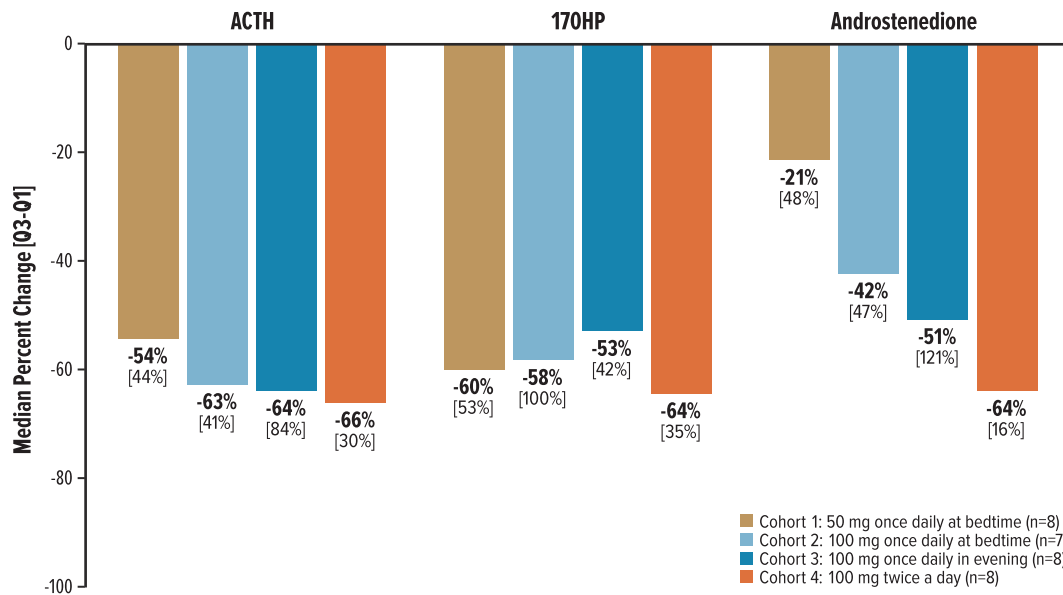


Figure 3. Median percent reductions from baseline to day 14 based on morning window values. Based on each participant's values from the morning window time points (06:00, 08:00, 10:00). The interquartile ranges (absolute value of Q3-Q1) for median percent reductions are shown in brackets. 17OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropicin.

crinicerfont is the first non-GC therapy to show reductions in testosterone (in women) and androstenedione/testosterone ratios (in men) over 14 days of treatment.

Plasma exposure of crinicerfont was similar when dosed with a liquid dietary supplement or with regular meals. A 100-mg twice-daily dose resulted in twice the exposure

observed with a 100-mg daily dose over a 24-hour period. Crinicerfont plasma exposures following a 100-mg twice-daily dose of crinicerfont are expected to result in an effective and sustained degree of CRF1R antagonism throughout the day, and this regimen was observed to result in the greatest reduction of androstenedione.

Table 3. Proportion of participants having 50% or greater reduction in morning window hormone values from baseline to day 14 and proportion achieving normal values

Participants, n/N (%)	Cohort 1: 50 mg once daily at bedtime	Cohort 2: 100 mg once daily at bedtime	Cohort 3: 100 mg once daily in evening	Cohort 4: 100 mg twice a day
With $\geq 50\%$ reduction from baseline				
ACTH	4/8 (50)	5/7 (71)	5/8 (63)	6/8 (75)
17OHP	4/8 (50)	4/7 (57)	5/8 (63)	6/8 (75)
Androstenedione	2/8 (25)	3/7 (43)	4/8 (50)	6/8 (75)
Testosterone for women	2/4 (50)	3/5 (60)	2/3 (67)	3/4 (75)
Androstenedione/testosterone ratio for men	1/4 (25)	0/2 (0)	4/5 (80)	2/3 (67)
With return to normal values ^a				
Androstenedione	2/5 (40)	1/4 (25)	2/5 (40)	2/6 (33)
Testosterone for women	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)
Androstenedione/testosterone ratio for men	1/3 (33)	0/2 (0)	2/4 (50)	2/3 (67)

Abbreviations: 17OHP, 17-hydroxyprogesterone; ACTH, adrenocorticotropin.

^aIn the subset of participants who had baseline androstenedione or (in women) testosterone values that were greater than $1.2 \times$ upper limit of normal (age- and sex-matched) or androstenedione/testosterone ratio (in men) greater than or equal to 0.5.

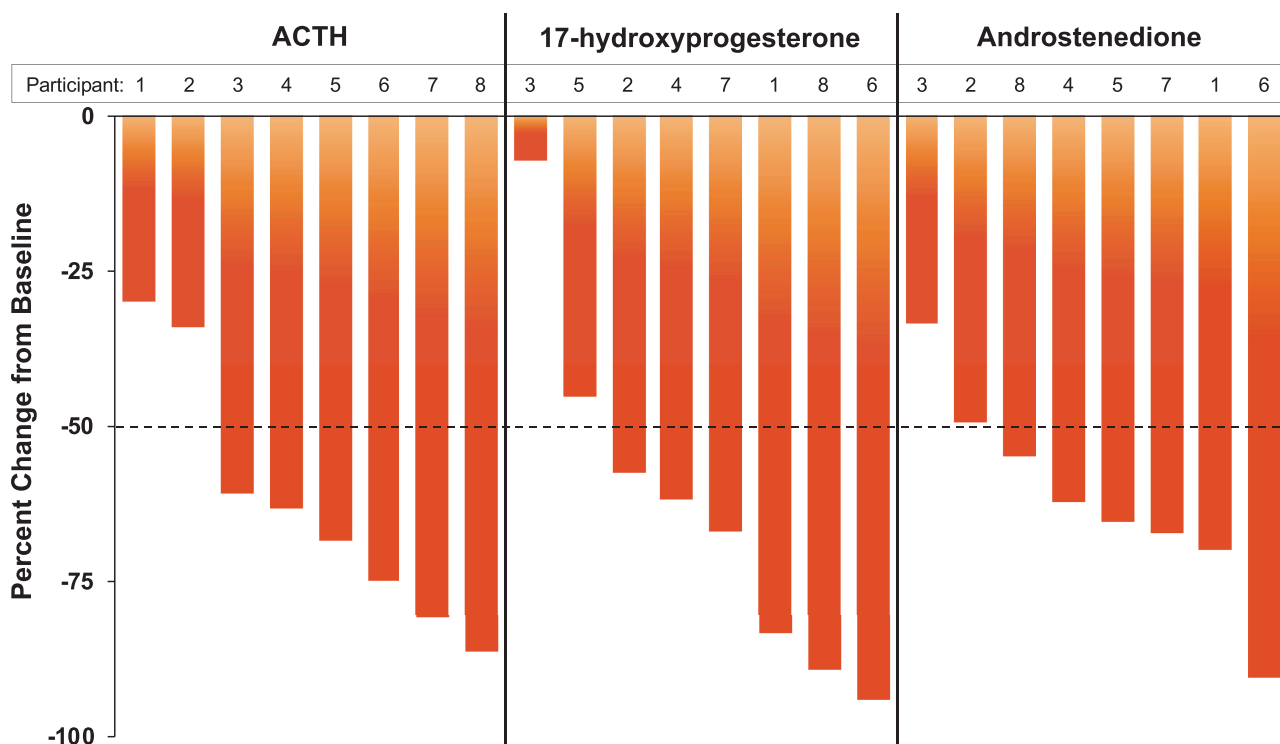


Figure 4. Percent change from baseline to day 14 in participants with crinecerfont 100 mg twice a day (cohort 4). Based on each participant's average values from the morning window time points (06:00, 08:00, 10:00) on day 14 compared to the average of their morning window values at baseline. ACTH, adrenocorticotropin.

The limitations to this study are primarily related to the small number of participants in each cohort and the wide range of adrenal steroid levels at baseline despite various conventional GC regimens. A study of this size was not powered to demonstrate statistical significance of a treatment effect or between-cohort differences, and data analyses were restricted to descriptive

statistics. Nevertheless, the consistent and dose-dependent changes of ACTH, 17OHP, androstenedione, testosterone (in women), and androstenedione/testosterone ratio (in men) with decreases ranging from 20% to 75% provide convincing evidence that crinecerfont can be added to conventional GC regimens to treat the androgen excess due to 21OHD. The data from

Table 4. Treatment-emergent adverse events

	Cohort 1: 50 mg once daily at bedtime (n = 8)	Cohort 2: 100 mg once daily at bedtime (n = 7)	Cohort 3: 100 mg once daily in evening (n = 8)	Cohort 4: 100 mg twice a day (n = 8)
Adverse event summary, n (%)				
Any TEAE	7 (88)	5 (71)	5 (63)	5 (63)
Any SAE	0 (0)	1 (14) ^a	0 (0)	0 (0)
Any TEAE leading to discontinuation	0 (0)	0 (0)	0 (0)	0 (0)
Any TEAE resulting in death	0 (0)	0 (0)	0 (0)	0 (0)
TEAEs by MedDRA-preferred term, n (%)				
Headache	3 (38)	1 (14)	0 (0)	1 (13)
Upper respiratory tract infection	3 (38)	0 (0)	1 (13)	0 (0)
Fatigue	1 (13)	0 (0)	1 (13)	1 (13)
Contusion	2 (25)	0 (0)	0 (0)	0 (0)
Insomnia	0 (0)	1 (14)	0 (0)	1 (13)
Nasopharyngitis	0 (0)	0 (0)	0 (0)	2 (25)
Nausea	1 (13)	1 (14)	0 (0)	0 (0)
Pyrexia	0 (0)	0 (0)	1 (13)	1 (13)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; SAE, serious adverse event; TEAE, treatment emergent adverse event.

^aSingle event of cholelithiasis, assessed by the investigator as moderate in intensity and unrelated to treatment. The participant underwent a cholecystectomy with intraoperative cholangiogram, followed by appropriate medical treatment. The cholelithiasis was resolved and the participant remained in the study.

this 14-day, open-label study strongly support the continued development of crinicerfont for the treatment of 21OHD. Long-term studies of crinicerfont in children and adults with 21OHD are currently underway to determine if these GC dose reductions, combined with androgen control, translate to improved quality of life and clinical outcomes (eg, weight, growth, development) with a favorable benefit-risk profile.

Acknowledgments

The authors thank the clinical staff and patients who participated in the study, along with the following coinvestigators and study coordinators: Adina F. Turcu and Tobias Else (University of Michigan Medical School, Ann Arbor, Michigan); Elizabeth Ramey, Paige Hill, Kristin Boxwell, and Jensina Ericksen (University of Minnesota Medical School, Minneapolis, Minnesota); Susan Kearns (Seattle Children's Hospital, Seattle, Washington); Marian Hart (Indiana University School of Medicine, Indianapolis, Indiana); and Natalie Nokoff (Children's Hospital Colorado, Aurora, Colorado). Medical writing assistance was provided by Mildred Bahn (Prescott Medical Communications Group, Chicago, Illinois) with support from the study sponsor.

Financial Support: This work was supported by Neurocrine Biosciences, Inc. The sponsor contributed to the study design, data analysis, interpretation of results, and content development of this report, in addition to financial support for writing and editorial assistance. All authors had full access to the data, provided critical reviews of all manuscript drafts, and approved the final draft to be submitted for publication.

Clinical Trial Information: Registration number NCT03525886 (registered May 14, 2018).

Additional Information

Correspondence: Richard Auchus, MD, PhD, Departments of Pharmacology and Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan Medical School, 5560A MSRB II, 1150 W Medical Center Dr, Ann Arbor, MI 48109-5632, USA. Email: rauchus@med.umich.edu.

Disclosures: R.J.A.: research funding from and previously served as a consultant to Neurocrine Biosciences, Inc; research funding from Spruce Biosciences; consulting fees from OMass Therapeutics, Crinetics Pharmaceuticals, and Adrenas Therapeutics. K.S.: research funding from Neurocrine Biosciences, Inc, Spruce Biosciences, and Adrenas Therapeutics; advisory board for Eton Pharmaceuticals. P.Y.F.: research funding from and previously served as a consultant to Neurocrine Biosciences, Inc.; research funding from Spruce Biosciences; consulting fees from Eton Pharmaceuticals. M.G.V.: research funding from Neurocrine Biosciences, Inc and Spruce Biosciences; consulting for Adrenas Therapeutics and Eton Pharmaceuticals. E.A.I.: research funding from Neurocrine Biosciences, Inc. S.D.: previously served as a consultant to Antares Pharma. N.G., J.S., E.R., J.L.C., and R.H.F.: Employees of Neurocrine Biosciences, Inc.

Data Availability: Some or all data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

References

- Speiser PW, Arlt W, Auchus RJ, et al. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103(11):4043-4088.

2. Whittle E, Falhammar H. Glucocorticoid regimens in the treatment of congenital adrenal hyperplasia: a systematic review and meta-analysis. *J Endocr Soc*. 2019;3(6):1227-1245.
3. Speiser PW. Emerging medical therapies for congenital adrenal hyperplasia. *F1000Res*. 2019;8:363.
4. Merke DP, Bornstein SR. Congenital adrenal hyperplasia. *Lancet*. 2005;365(9477):2125-2136.
5. Turcu AF, Auchus RJ. Adrenal steroidogenesis and congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am*. 2015;44(2):275-296.
6. Pignatelli D, Pereira SS, Pasquali R. Androgens in congenital adrenal hyperplasia. *Front Horm Res*. 2019;53:65-76.
7. Reisch N. Review of health problems in adult patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Exp Clin Endocrinol Diabetes*. 2019;127(2-03):171-177.
8. Turcu AF, Auchus RJ. The next 150 years of congenital adrenal hyperplasia. *J Steroid Biochem Mol Biol*. 2015;153:63-71.
9. Maccabee-Ryaboy N, Thomas W, Kylo J, et al. Hypertension in children with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)*. 2016;85(4):528-534.
10. Sarafoglou K, Yaw Addo O, Turcotte L, et al. Impact of hydrocortisone on adult height in congenital adrenal hyperplasia—the Minnesota cohort. *J Pediatr*. 2014;164(5):1141-1146.e1141.
11. Sarafoglou K, Forlenza GP, Yaw Addo O, et al. Obesity in children with congenital adrenal hyperplasia in the Minnesota cohort: importance of adjusting body mass index for height-age. *Clin Endocrinol (Oxf)*. 2017;86(5):708-716.
12. Turcu AF, Spencer-Segal JL, Farber RH, et al. Single-dose study of a corticotropin-releasing factor receptor-1 antagonist in women with 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2016;101(3):1174-1180.
13. Auchus RJ, Sarafoglou K, Fechner PY, et al. Supplementary data for “Crinicerfont lowers elevated hormone markers in adults with 21-hydroxylase deficiency congenital adrenal hyperplasia.” *Mendeley Data*. Uploaded July 2, 2021. <https://data.mendeley.com/datasets/bj6nn9343p/1>
14. Auchus RJ, Arlt W. Approach to the patient: the adult with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2013;98(7):2645-2655.
15. Wilkins L, Lewis RA, Klein R, Rosemberg E. The suppression of androgen secretion by cortisone in a case of congenital adrenal hyperplasia. *Bull Johns Hopkins Hosp*. 1950;86(4):249-252.
16. Arlt W, Willis DS, Wild SH, et al; United Kingdom Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE). Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab*. 2010;95(11):5110-5121.
17. Finkelstain GP, Chen W, Mehta SP, et al. Comprehensive genetic analysis of 182 unrelated families with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2011;96(1):E161-E172.
18. Al-Kofahi M, Ahmed MA, Jaber MM, et al. An integrated PK-PD model for cortisol and the 17-hydroxyprogesterone and androstenedione biomarkers in children with congenital adrenal hyperplasia. *Br J Clin Pharmacol*. 2021;87(3):1098-1110.
19. Merke DP, Auchus RJ. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *N Engl J Med*. 2020;383(13):1248-1261.
20. Auchus RJ, Buschur EO, Chang AY, et al. Abiraterone acetate to lower androgens in women with classic 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2014;99(8):2763-2770.
21. El-Maouche D, Merke DP, Vogiatzi MG, et al. A phase 2, multicenter study of nevanimibe for the treatment of congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2020;105(8):2771-2778.
22. Mallappa A, Sinaii N, Kumar P, et al. A phase 2 study of Chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2015;100(3):1137-1145.
23. Merke DP, Mallappa A, Arlt W, et al. Modified-release hydrocortisone in congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2021;106(5):e2063-e2077.
24. Nella AA, Mallappa A, Perritt AF, et al. A phase 2 study of continuous subcutaneous hydrocortisone infusion in adults with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2016;101(12):4690-4698.