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Supplement Article

Future Directions in the Management of Classic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

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Abstract

Context: The traditional management of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency (21OHD) is difficult and often suboptimal.

Objective: To review improvements in the diagnosis and management of 21OHD.

Design: Literature review, synthesis, and authors' experience.

Setting: United States (2 centers).

Participants: Not applicable.

Interventions: Not applicable.

Main Outcomes: Not applicable.

Results: The 11-oxygenated androgens are abundant in 21OHD, and their measurement might improve diagnosis and medication titration. Several new treatments are under development.

Conclusion: Circadian delivery of hydrocortisone improves disease management of 21OHD compared to conventional glucocorticoids. Glucocorticoid-sparing therapies such as crinecerfont and atumelnant offer the potential for a block-and-replace strategy, with physiologic replacement dosing of hydrocortisone.

Clinical Trial Registration: None.

Key Words: congenital adrenal hyperplasia, 21-hydroxylase deficiency, drug therapy, hydrocortisone, 11-ketotestosterone, crinecerfont

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) has been treated with cortisone or other glucocorticoid therapy (and fludrocortisone acetate when needed) since the pioneering studies of Wilkins and the parallel work of Talbot in the 1950s. Initially, disease control was assessed by measures of steroid metabolites in urine, such as pregnanetriol from 17-hydroxyprogesterone (17OHP) or androsterone and etiocholanolone from both androstenedione (A4) and testosterone (T). Wilkins correctly predicted that the negative feedback effect from exogenous glucocorticoids would reduce ACTH and adrenal androgen production, relieve the suppression of gonadotropins from these androgens, and restore gonadal function. Immunoassays and later liquid chromatography-tandem mass spectrometry assays allowed direct measurements of steroids in serum, and different synthetic glucocorticoids increased the treatment options. Nevertheless, the treatment of 21OHD remains a difficult balance between undertreatment with resultant adrenal-derived androgen excess and overtreatment, with chronic supraphysiologic glucocorticoid exposure. Imperfect laboratory assessments of disease control, limited treatment options and dosage strengths, and the burden of disease to the patient and family all conspire to prevent optimal care. This article will discuss recent advances in monitoring and treatment of 21OHD.

Alternative Steroid Biomarkers

Cytochrome P450 21A2 (steroid 21-hydroxylase), the product of the *CYP21A2* gene, is required for the biosynthesis of aldosterone and cortisol in the adrenal cortex but not for androgens. In the zona fasciculata, where cortisol is produced, the steroid that accumulates immediately above the block at P450 21A2 is 17OHP. Thus, 17OHP has been used to diagnose 21OHD for decades; however, 17OHP can be metabolized via several pathways (1): First, the conventional route,

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albeit with low efficiency, via P450 17A1 (steroid 17-hydroxylase/17,20-lyase) to A4, the immediate precursor of T; second, if 5α-reductase is present, a more efficient alternate pathway from androsterone to androstanediol and then dihydrotestosterone; and third, 11β-hydroxylation by P450 11B1 (steroid 11β-hydroxylase) to 21-deoxycortisol. In addition, A4 is also an excellent substrate for P450 11B1, which produces 11β-hydroxyandrostenedione (11OHA4). Outside the adrenal, 11OHA4 is converted 11-ketotestosterone (11KT), which is only slightly less potent as an androgen than T. Collectively, these 11-oxygenated 19-carbon steroids are referred to as 11-oxygenated androgens or 11-oxyandrogens (11oAs), although only 11KT and its 5α-reduced metabolite have been shown to exhibit significant androgen activity (2, 3). The genetics and steroid production in 21OHD are reviewed in the accompanying papers in this issue.

In patients with 21OHD, except for well-controlled postpubertal males, the major circulating androgen is 11KT, not T, due to the adrenal origin. Consequently, 11OHA4 and 11KT might be the ideal biomarkers for diagnosis and management of 21OHD. Limited data suggest that 11OHA4 might be used to diagnose nonclassic 21OHD without cosyntropin stimulation testing (4), particularly in conjunction with other steroid biomarkers, at least for women. In children with 21OHD, 11KT was higher in those with poor vs good disease control and was often consistent with clinical assessment of disease control when 17OHP and A4 were discordant (5). In men with testicular adrenal rest tumors (TART), 11OHT showed the highest gradient of all assessed steroids from spermatic vein to peripheral blood samples, consistent with direct production from TART cells (6). The 11OHT in these men with TART appeared to be readily converted to 11KT and/or 11OHA4, because peripheral blood 11OHT was not higher than in men without TART.

Because 11oAs are produced via 11β-hydroxylation of intra-adrenal A4, these steroids do not accumulate significantly in other forms of CAH. Overall, 11oAs, particularly 11OHA4 and 11KT, are relevant biomarkers in 21OHD and show some promise in improving diagnosis and management of 21OHD. For novel nonglucocorticoid treatments of 21OHD, 11oAs decline in parallel with A4 and T with abiraterone acetate therapy (7), yet 11OHA4 is the biomarker that is most difficult to reduce into the normal range for poorly controlled patients. The wider availability of multiplexed liquid chromatography-tandem mass spectrometry assays for 11oAs, which simultaneously measure 17OHP, A4, and T, will allow broader comparison in clinical use.

Alternative Sample Collection Schemes

The current challenge in monitoring hormonal control in response to glucocorticoid therapy is that clinicians must rely on serum measurements of 17OHP and A4 obtained at clinic visits every 3 to 6 months. These measurements only provide assessment of the hypothalamic-pituitary-adrenal (HPA) axis activity and disease control at a single point in time and do not provide information about the state of the HPA axis over the course of the day, during the preceding 3 to 6 months, or even during the preceding few days. Adding to the challenge is that the timing of laboratory assessments vary during clinic visits in relation to the patient's last hydrocortisone dose, which lead to highly variable 17OHP and A4 concentrations that can confound interpretation of the HPA axis control. Figure 1 illustrates that even within a 2-hour time frame after the morning hydrocortisone dose, 17OHP and A4 concentrations can change >60% from 1 to 3 hours post-morning dose. Although it will not provide a complete picture, recording both the dose and the timing of the dose prior to measurement of adrenal steroid concentrations can help with the interpretation of the patient's adrenal pharmacodynamic (PD) response to glucocorticoid therapy, at least within that point in time.

An emerging monitoring paradigm is the development of integrated cortisol pharmacokinetic (PK) and adrenal PD response modeling based on 6-hour serial sampling of cortisol, A4 and 17OHP concentrations performed in an outpatient setting (8, 9), or 24-hour serial sampling of cortisol and 17OHP concentrations performed in an inpatient setting (10). PK/PD modeling provides a fuller picture as it can help predict the effect that changing the amount of dose, time of dose, and frequency of dose will have on an individual's adrenal steroid response over a 24-hour period. Outpatient 6-hour serial sampling after the regular morning hydrocortisone dose can also provide cortisol PK of half-life, clearance, volume of distribution, maximum concentration, time to maximum concentration, minimum concentration, and time to minimum concentration as well as is the PD response to cortisol of 17OHP and A4 (8, 9).

PK/PD modeling has the potential to provide targeted individualized glucocorticoid dose adjustments and monitoring of

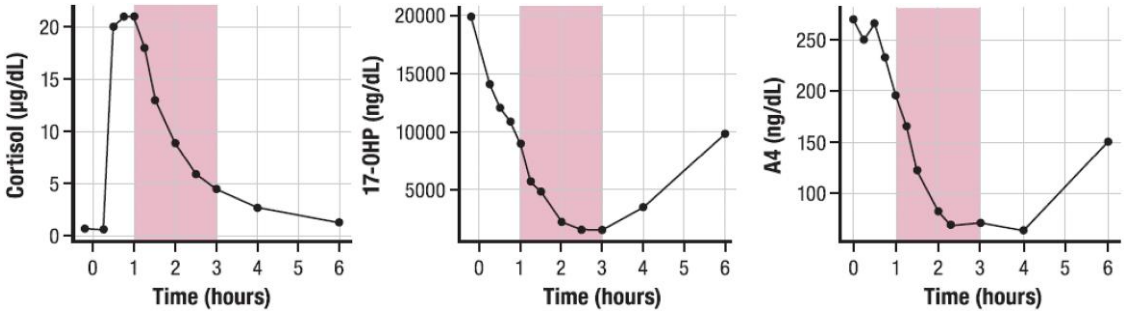

Figure 1. Wide variability of adrenal steroid concentrations within a 2-hour time frame after the morning hydrocortisone dose in a representative child with 21OHD. Reproduced with permission from Sarafoglou 2023 *J Clin Endocrinol Metab*. Observed cortisol, 17OHP, and A4 concentrations over 6 hours. Shaded area depicts a 2-hour time window, 1 to 3 hours post-morning 6 mg hydrocortisone dose. Concentration levels of each adrenal steroid showed a > 60% change within the 2-hour time frame. Child's regular total daily hydrocortisone dose was 12 mg/m^2/day.

Abbreviations: 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; A4, androstenedione.

disease control in children and adults with 21OHD. However, further studies are needed to link the use of PK/PD modeling on long-term outcomes.

An alternative to frequent blood drawing, microdialysis-based tissue sampling has been used to collect interstitial fluid for measurement of free-cortisol profiles (11-14). Upton et al, using a portable microdialysis device (U-RHYTHM), reported the establishment of normal 24-hour patterns of free cortisol, among other hormones, in the interstitial fluid of 214 healthy volunteers during their day-to-day activities including sleep in their own surroundings (13). The U-RHYTHM sampling device collects 20 µL samples of interstitial fluid every 20 minutes using a miniature infusion pump perfusing a microdialysis catheter placed in the abdominal subcutaneous tissue that connects to a portable sample collector. Previously, a good correlation between serum cortisol and interstitial free-cortisol has been reported (11, 12). The sampling device has the potential of assessing 24-hour free-cortisol profiles in response to various cortisol replacement regimens.

For many patients blood tests are invasive, time consuming, and distressing, as accurate monitoring requires frequent measurements (15). As an alternative to blood draws, hormone levels can be measured from saliva, which is an ultrafiltrate of plasma and contains nonprotein-bound steroids. An advantage to saliva over serum or urine is that multiple samples may be collected at home noninvasively, without the stress of phlebotomy or travel to a collection site. Analysis of timed saliva samples throughout the day affords more complete information about disease control and treatment response than a single blood sample, analogous to continuous glucose monitoring for diabetes mellitus, except it is not in "real-time" as glucose monitoring is in diabetes and requires the patient to mail the saliva samples. In 78 children with 21OHD, strong correlations were found between serum and saliva values for 17OHP, A4, T, 11OHA4, and 11KT, although saliva concentrations were lower, especially for T (16). Using multiple saliva samples, the circadian variation of 17OHP and 11oAs, previously observed in serum (17), was replicated in children (18, 19) and adults (20) with 21OHD. The data from serial saliva sampling was used to optimize hydrocortisone dose distribution in children with 21OHD (21).

Steroids also deposit in hair, which then affords a history of hormonal control over several months. A patch of hair is cut at the scalp, bound at the distal end, and cut into pieces, with each 1.2 cm marking 1-month intervals starting from the scalp and proceeding backwards in time to the distal end. This technique has been primarily used to measure cortisol content as a measure of long-term compliance and exposure to hydrocortisone therapy in patients with adrenal insufficiency (22). Although 17OHP and A4 might also be measured in hair samples as a measure of disease control in 21OHD (23, 24), the values are much lower than cortisol, and this approach with low time resolution has not proven to be of utility in treatment titration for patients with 21OHD. Bone age is a more practical way of assessing cumulative exposure to androgen and androgen control for growing children.

Alternative Hydrocortisone Formulations

Continuous Subcutaneous Hydrocortisone Infusion

In healthy individuals, cortisol levels increase overnight to an early morning peak and then decrease throughout the day to very low or undetectable levels by midnight (25). However, in individuals with cortisol deficiency, conventional oral hydrocortisone treatment cannot replicate this physiologic rhythm, so patients are often under- or overtreated (26). Thus, use of the continuous subcutaneous hydrocortisone infusion (CSHI) has been explored, using insulin pumps programmed with variable infusion rates to replicate physiologic cortisol secretion and potentially improve hormone control (26) (Fig. 2). In proof-of-concept studies in adults with Addison disease and 21OHD, CSHI was shown to decrease

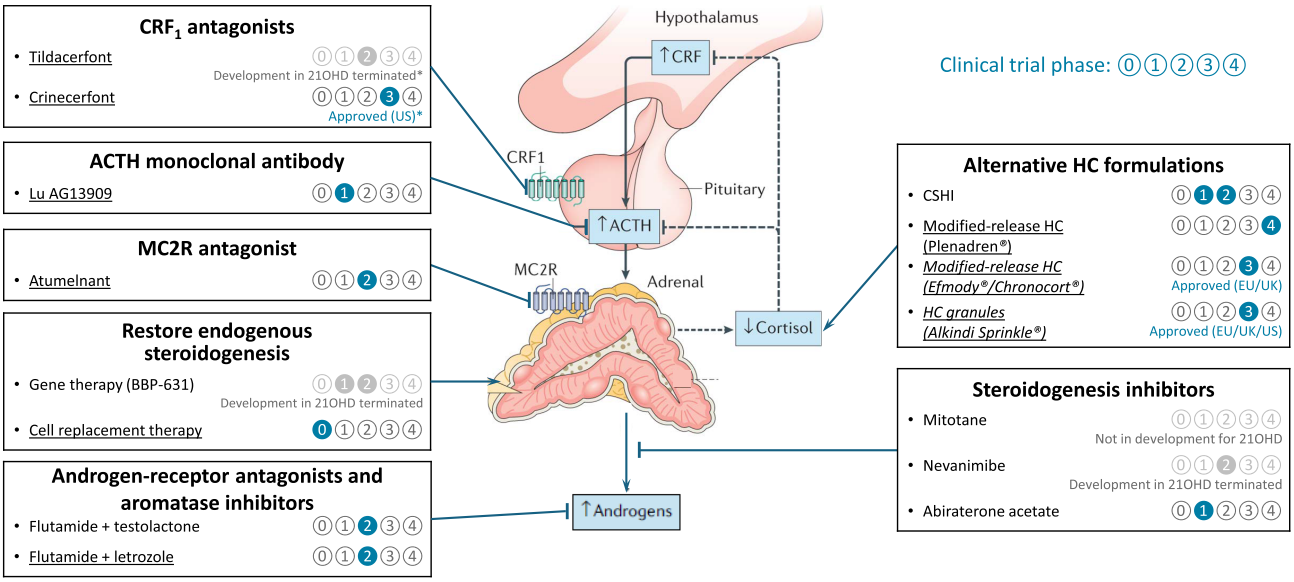

Figure 2. Investigational treatment approaches in development for 21OHD. *As announced in December 2024. Adapted with permission from Mallappa and Merke 2022 *Nat Rev Endocrinol*. Underlined compounds are currently under development for 21OHD. Italicized compounds are approved in ≥1 country for 21OHD.

Abbreviations: 21OHD, 21-hydroxylase deficiency; ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; CRF_1, corticotropin-releasing factor type 1 receptor; HC, hydrocortisone; MC2R, melanocortin receptor type 2.

Table 1. Observational and nonregistration trials of agents not under further development for 21OHD indication

Agent	Phase/NCT (Ref)	Primary endpoint(s)	Key results
Continuous subcutaneous hydrocortisone infusion	Phase 1-2/ NCT01859312 (30, 31)	• Morning 17OHP ≤1200 ng/dL at 6 months	• No change from BL in percent of participants with morning 17OHP ≤1200 ng/dL • Reductions in morning and 24-hour ACTH, 17OHP, and A4 • Cortisol pharmacokinetic profile approximated physiologic secretion • Improvements in HRQoL, fatigue, osteocalcin, and c-telopeptide; no changes in GC dose or in disease- and GC-related comorbidities • At month 18, HC dose was reduced from month 6; reductions in biomarkers and improvements in HRQoL and fatigue were maintained • No serious AEs, but 2 of 8 participants found pump incompatible with their lifestyle and switched to oral therapy
Mitotane	Retrospective study (32)	• Effect on TARTs at 5 years[a]	• 40% (2/5) of participants had complete disappearance of TARTs; 40% (2/5) had stabilization of TARTs; 20% (1/5) had 50% decrease in TART volume • 40% (2/5) of participants had improved sperm count
Nevanimibe	Phase 2/ NCT02804178 (33)	• Morning 17OHP ≤2× ULN at 14 days	• 20% (2/10) of participants achieved morning 17OHP ≤2× ULN • Decreased levels of 17OHP and A4 • No dose-response up to 1000 mg BID
Flutamide + testolactone + reduced HC dose	Pilot study (34)	• Changes in linear growth, weight gain, and bone maturation at 6 months[a]	• Greater reductions in linear growth rate, weight gain, and bone maturation compared to participants receiving conventional treatment • Increased 17OHP and ACTH but no change in A4; reduced urinary cortisol
	Pilot Study (35)	• Changes in linear growth, bone maturation, predicted height, and weight gain at 2 years[a]	• Greater reductions in linear growth and bone maturation, with increased predicted height compared to participants receiving conventional treatment; no difference in weight velocity or body mass • Increased 17OHP, A4, T, and ACTH; reduced urinary cortisol
Abiraterone acetate	Phase 1/ NCT01495910 (7, 36)	• A4 ≤ULN at 6 days	• 50% (3/6) and 83% (5/6) of participants receiving 100 and 250 mg/day, respectively, had A4 ≤ ULN • Mean A4 declined to 66 and 38 ng/dL with 100 and 250 mg/day, respectively; urinary androgen metabolites declined similarly • 56%-77% reductions in serum 11oAs
BBP-631	Phase 1-2/ NCT04783181 (37)	• Safety and dose-finding	• 1 SAE (redness at the infusion site) reported • Increased 11-deoxycortisol at higher doses

Abbreviations: 11oA, 11-oxygenated androgen; 17OHP, 17-hydroxyprogesterone; 21OHD, 21-hydroxylase deficiency; A4, androstenedione; ACTH, adrenocorticotropic hormone; AE, adverse event; AUC, area under the curve; BID, twice daily; BL, baseline; GC, glucocorticoid; HC, hydrocortisone; HRQoL, health-related quality of life; TART, testicular adrenal rest tumor; ULN, upper limit of normal.
[a]For studies without a primary endpoint, the study objective is provided.

morning ACTH, 17OHP, and/or cortisol levels, with 24-hour salivary cortisol curves that resembled normal circadian variation (27-29). In addition, mean glucocorticoid doses were decreased from baseline, and there were improvements in subjective health scores.

In a phase 2 study in 8 adults with 21OHD who had elevated adrenal steroids (17OHP >1200 ng/dL and/or A4 above normal range) and ≥1 disease- or glucocorticoid-related comorbidities, treatment with CSHI was shown to achieve a cortisol profile similar to physiologic cortisol secretion (Table 1) (30). Compared with baseline, 6 months of CSHI resulted in decreased early morning and 24-hour levels of 17OHP, ACTH, A4, and progesterone, as well as marked improvements in health-related quality of life, and fatigue. In addition, CSHI treatment increased bone turnover markers and lean mass; however, other longstanding disease- and glucocorticoid-related comorbidities (eg, fatty liver, insulin resistance, adrenal hypertrophy, etc.) did not change, and weight gain occurred in all but 1 patient. Although there were no serious adverse events related to mechanical failure, CSHI therapy is a more time-intensive therapy compared to oral glucocorticoid replacement regimens, and 2 of 8 patients found the pump incompatible with their lifestyle and opted to switch to oral therapy. In the 6 patients who continued CSHI therapy, the improvements in androgen control, health-related quality of life, lean mass, and bone turnover markers observed at 6 months were maintained at 18 months, and the average hydrocortisone dose decreased from 38.3 mg/d at 6 months to 33.6 mg/d at 18 months (31). Weight, insulin resistance, fat mass, and liver enzymes (aspartate aminotransferase, alanine aminotransferase) remained stable, but there were some improvements in liver fat. An additional phase 1 study in children

was recently completed, but the results have not yet been published (NCT03718234).

Modified-release Hydrocortisone

The poor PK of oral hydrocortisone is a major impediment to therapy of 21OHD. Although the drug is nearly 100% bioavailable, some first-pass metabolism variably occurs, and serum cortisol concentrations peak at 60 to 90 minutes with a 60- to 90-minute elimination half-life. PK/PD studies have shown that 17OHP begins to rise when serum cortisol concentrations drop to <5 μg/dL (140 nmol/L), and a serum cortisol of ~1.7 μg/dL (50 nmol/L) inhibits 17OHP production by 50% (10). Doubling the dose of hydrocortisone will roughly double the peak concentration but will also double the initial rate of elimination. Consequently, a 10 mg dose for an adult will yield a peak cortisol of ~20 μg/dL ~1 hour after dosing, which will fall to ~5 μg/dL in 2 to 3 hours, but serum cortisol following a 20 mg dose will yield the same ~5 μg/dL concentration in 3 to 4 hours, only 1 hour longer, not twice the time. Therefore, for hydrocortisone to be effective as the sole glucocorticoid therapy for 21OHD, 3 to 4 divided doses are needed. This demanding schedule is very difficult for patients and family to adhere day after day and results in alternating periods of suppression and escape throughout the day.

To simplify the regimen and to improve the PK of hydrocortisone, modified-release preparations have been developed (Fig. 2). Plenadren® (Takeda) has a slow-release core with an immediate-release outer shell, which provides sustained hydrocortisone exposure from a single morning dose of 30 mg for over 16 hours. Thus, a single morning dose of Plenadren provides adequate if not excessive cortisol replacement for the waking hours, but for the management of 21OHD, it creates a cortisol-free period in the early morning hours when ACTH and thus adrenal-derived androgens rise maximally under the circadian rhythm. A second dose of Plenadren late in the day would be needed to prevent this rise in unwanted adrenal steroids for patients with 21OHD. Although a study of Plenadren in 21OHD has been presented in abstract form (NCT03760835), no publications on the topic have appeared (Table 2). Plenadren is available in some European countries.

A second strategy to improve hydrocortisone PK is to add an enteric coating to hydrocortisone particles, which can be encapsulated at various dose sizes. This formulation, called Efmody® (development name Chronocort®; Neurocrine UK, Ltd [formerly Diurnal LTD]), delays the initial cortisol exposure 1 to 4 hours after administration with a gradual increase and broad peak ~10 hours later, followed by a more gradual decline over the following 6 to 12 hours. Therefore, the larger "morning" dose is taken at bedtime to provide exposure both prior to and during the morning rise of ACTH, to conveniently attenuate the production of adrenal-derived androgens before the patient awakens. A second smaller dose is recommended upon arising to guarantee cortisol exposure for a full 24 hours. Compared to 3 doses of immediate-release hydrocortisone, 2 doses of Chronocort achieved superior reduction of 17OHP and A4 in the early morning hours at a total daily dose of ~30 mg/day (38) (Table 2). During the extension phase of this study, investigators were able to relax the stringency of biomarker suppression, and average daily doses of 20 mg/day maintained excellent A4 control with modest elevations of 17OHP (38).

Alkindi Sprinkles (Hydrocortisone Granules)

In infancy and early childhood, smaller doses and incremental adjustments are required to reach a dose that is enough to prevent increased androgen production and exposure but not excessive to avoid causing hypercortisolism. This balance is very hard to achieve in practice, because the smallest US Food and Drug Administration (FDA)-approved commercial hydrocortisone formulation available in the United States had been, until 2020, a 5-mg tablet with a single score. This lack of an appropriate pediatric formulation led to various manipulations of the scored 5-mg hydrocortisone tablet to deliver prescribed doses under 2.5 mg.

Faltering growth, recurrent hypoglycemia (50) and iatrogenic Cushing syndrome (51, 52) have been described with hydrocortisone tablet manipulations such as splitting the 5 mg tablets to produce doses less than 2.5 mg (50), dispersing in water (51), and compounding small doses in capsules (52). Madathilethu et al reported that splitting 10-mg hydrocortisone tablets into halves and quarters produced unacceptable dose variations (53). Dispersing tablets in water to create a suspension and then drawing the prescribed dose over the course the day was the second most popular method to obtain prescribed doses of <2.5 mg according to the Pediatric Endocrine Society survey (54). As hydrocortisone is hydrophobic, sparingly soluble in water, and thus not equally distributed in the liquid, this approach can result in significant variability in dosing (55).

In 2020 the FDA approved Alkindi Sprinkles®, the first pediatric hydrocortisone formulation accommodating small incremental doses (<2.5 mg) since hydrocortisone cypionate suspension was taken off the market in 2001 (56) (Fig. 2). Alkindi Sprinkles are hydrocortisone granules with essentially identical PK profile with hydrocortisone tablets (57) and are well tolerated in neonates, infants, and children under 6 years of age with adrenal insufficiency (58). Granules are contained within transparent capsules and are available in dosages of 0.5, 1, 2, and 5 mg (57).The maximum diameter of the granules is 0.8 mm and has an inert cellulose capsule core, which is sprayed with hydrocortisone and is sealed with taste-masking excipients to reduce bitterness. To administer Alkindi, parents squeeze the bottom of the capsule and twist off the top of the capsule so that the granules can be sprinkled directly into the mouth of the child or onto soft food (59). Immediately following administration, the child should drink a liquid to ensure that all granules are swallowed.

A 2-year prospective study of 17 children with CAH and 1 with hypopituitarism aged from birth to 6 years recorded no adrenal crises during this period and normal growth profiles. In addition, the patients' doses of hydrocortisone decreased at the end of the study to the lower end of the recommended treatment range for CAH (39) (Table 2). Although Alkindi is the only FDA-approved way to administer doses less than 2.5 mg, most likely because of its higher cost, Alkindi's use has not been universally accepted by insurance companies, with prescriptions often requiring appeals.

Hydrocortisone Suspensions

Currently there are no commercially available hydrocortisone liquid formulations for the treatment of infants and young children with CAH that allow for the smaller doses (less than 2.5 mg) and incremental adjustments as small as 0.1 mg needed to control excess androgen production and

Table 2. Completed and ongoing trials of agents under development for 21OHD indication

Agent	Phase/NCT (Ref)	Primary endpoint(s)	Key results
Modified-release HC: Plenadren®	Phase 4/ NCT03760835	• CFB to months 6, 12, and 24 in total cholesterol and LDL	• Results not yet available
Modified-release HC hard capsules: Efmody®	Phase 3/ NCT02716818 (38)	• CFB to week 24 in 24-hour 17OHP SDS	• No difference between Chronocort and conventional GCs in 24-hour 17OHP SDS • Superior reduction of morning 17OHP and A4 with Chronocort vs conventional GCs
	Phase 3 extension/ NCT03062280 (38)[a]	• Efficacy, safety, and tolerability	• Median HCe dose decreased to 20 mg/d at month 18 • 80% of participants achieved morning 17OHP <1200 ng/dL at month 18 • 96% of participants achieved morning A4 ≤ULN at month 18 • Incidence of adrenal crisis similar to population estimates
HC granules: Alkindi Sprinkles®	Phase 3 extension/ NCT02733367 (39)	• Safety	• Most common AEs: pyrexia (56%), gastroenteritis (50%), and viral upper respiratory tract infection (44%) • 78% (14/18) of participants reported a total of 193 AEs including 9 SAEs • No adrenal crises, severe TEAEs, TEAEs leading to discontinuation, or deaths • Median daily HC dose decreased from 9.9-12.0 mg/m^2/day at BL to 8.6-10.2 mg/m^2/day at end of study
Flutamide + letrozole	Phase 4/ NCT00001521	• Adult height at age 13 years (girls) or 14 years (boys)	• Results not yet available
Tildacerfont no longer under development	Phase 2/ NCT03257462 and NCT03687242 (40)[b]	• CFB in morning ACTH, 17OHP, A4 • Safety	• Mean reductions from BL to week 2 in ACTH (28-59%), 17OHP (0-38%), and A4 (18-24%) with tildacerfont 200-1000 mg QD in participants with BL A4 > 2× ULN; no clear dose-response relationship • Maximum mean reductions from BL to week 12 of ~80% in ACTH, 17OHP, and A4 with tildacerfont 400 mg QD • Most common AEs: headache (7.1%), upper respiratory tract infection (7.1%), contusion (5.4%), diarrhea (5.4%), pruritus (5.4%)
	Phase 2b/ NCT04457336 (41)[a]	• CFB to week 12 in morning A4	• No significant placebo-adjusted CFB in A4 • Approximately one-half of participants reported <80% drug compliance
	Phase 2b/ NCT04544410	• Reduction in daily GC dose at 24 weeks	• Primary endpoint of glucocorticoid reduction not met
	Phase 2/ NCT05128942 (41)[a]	• Safety	• No SAEs or AEs leading to study withdrawal reported • 73% (22/30) achieved A4 or GC reduction at week 12 • No clear dose response observed
Crinecerfont: Crenessity™	Phase 2/ NCT03525886 (42)	• CFB to day 14 in morning 17OHP	• Median reductions of >50% in morning ACTH (54-66%), 17OHP (53-64%), and A4 (21-64%) at doses from 50 mg QHS to 100 mg BID at day 14 • Substantial reductions in elevated T (females) and A4-to-T ratios (males)
	Phase 2/ NCT04045145 (43)	• CFB to day 14 in morning 17OHP	• Median reductions of >50% in morning ACTH (57%), 17OHP (60%), and A4 (58%) with 50 mg BID at day 14 • Substantial reductions in elevated T (females) and A4-to-T ratios (males)
	Phase 3/ NCT04806451 (44)	• CFB to week 4 in morning A4	• Substantial decreases with crinecerfont but not placebo at week 4 for A4 (LSM CFB: -197 vs. +71 ng/dL; LSMD: -268 ng/dL; p=0.0002) and 17OHP (LSM: CFB: -5865 vs. +556 ng/dL; LSMD: -6421 ng/dL; p<0.0001) • GC dose reduction with crinecerfont but not placebo at week 28 (LSM %CFB: -18% vs. +6%; LSMD: -24%; p<0.0001), while A4 was maintained or improved relative to baseline • Increased percentage of participants achieving GC dose ≤11 mg/m^2/day HCe with crinecerfont vs placebo (30% vs 0%) at week 28, while a4 was maintained or improved relative to baseline
	Phase 3/ NCT04490915 (45)	• Percent CFB to week 24 in GC dose while maintaining A4 control	• Greater GC dose reduction with crinecerfont compared to placebo at week 24 (LSM %CFB: -27% vs. -10%; LSMD: -17%, p<0.0001), while A4 was maintained or improved relative to baseline

(*continued*)

Table 2. Continued

Agent	Phase/NCT (Ref)	Primary endpoint(s)	Key results
			• Increased percentage of participants achieving GC dose ≤11 mg/m^2/day HCe with crinecerfont vs placebo (63% vs 18%) at week 24, while A4 was maintained or improved relative to baseline • Substantial decrease in A4 with crinecerfont but not placebo at week 4 (LSM CFB: -299 vs. +46 ng/dL; LSMD: -345 ng/dL; p<0.0001) • Substantially greater decrease in 17OHP with crinecerfont compared to placebo at week 4 (LSM CFB: -5994 vs. -156 ng/dL; LSMD: -5838 ng/dL; nominal p<0.0001 [secondary endpoint])
Anastrozole	Observational (46)	• Change in bone age	• Average bone age SDS decreased from 4.3 at BL to 1.9 at end of study • No difference in BMD between groups
Atumelnant	Phase 1/ NCT06048887 (47)	• Safety	• Most common AEs: GC deficiency (17.5%), headache (9.5%), and contact dermatitis (7.9%) • No SAEs or AEs leading to study discontinuation • Dose-dependent suppression of ACTH-stimulated serum cortisol and A4
	Phase 2/ NCT05907291 (48)[a]	• Safety • CFB to week 12 in morning A4	• Most common AEs: fatigue (30%), headache (20%), and upper respiratory tract infection (20%) • 70% of participants reporting any AE • Reductions in morning A4 (96%) and morning 17OHP (94%) at week 12
Lu AG13909	Phase 1/ NCT05669950 (49)	• Safety and pharmacokinetics	• Results not yet available

Abbreviations: 17OHP, 17-hydroxyprogesterone; A4, androstenedione; ACTH, adrenocorticotropic hormone; AE, adverse event; BID, twice daily; BL, baseline; BMD, bone mineral density; CFB, change from baseline; GC, glucocorticoid; HC, hydrocortisone; HCe, hydrocortisone equivalents; LDL, low-density lipoprotein; LSM, least-squares mean; LSMD, least-squares mean difference; QD, once daily; QHS, once daily at bedtime; SAE, serious adverse event; SDS, standard deviation score; T, testosterone; TEAE, treatment-emergent adverse event.
[a]Based on available preliminary or interim data.
[b]Results from the 2 phase 2 studies were presented together in a single publication.

avoid the negative effects of overtreatment. A new drug application for ET-400, a hydrocortisone oral solution developed by Eton Pharmaceuticals, Inc., is under review by the FDA. The last commercially available liquid formulation that allowed for smaller doses, hydrocortisone cypionate suspension, was removed from the market in 2001 following a study that showed that patients on the cypionate suspension required higher total daily (mg/m^2/day) hydrocortisone, had higher A4 levels, and had increased weight gain and hypertension compared to those taking the hydrocortisone tablets (56). Though the authors indicated their study results were specific only to hydrocortisone cypionate, this led to the recommendation that any type of liquid suspension formulation should be avoided (60), even noncypionate formulations. In a report based on a survey administered by the Pediatric Endocrine Society Drug and Therapeutic Committee to 187 pediatric endocrine providers in the United States, nearly all used 5 mg hydrocortisone tablets and recommended splitting the tablet multiple times or creating a suspension by crushing the tablet in water to achieve small doses ranging from 0.5 to 4.5 mg (54), all of which can lead to inconsistent dosing (50, 51, 53, 55) in the treatment of children with CAH <10 years of age.

In the absence of commercially available liquid formulations, pharmacy-compounded liquid formulations of hydrocortisone have been used to treat young children with CAH. Alcohol-free hydrocortisone liquid formulations of either 2 mg/mL (61) or 1 mg/mL (62), which are compounded in a suspension vehicle, have been shown to be stable when stored in amber plastic bottles at 4 °C or 25 °C for 90 days with excellent dose repeatability. A study in children with CAH that compared the bioavailability of extemporaneously compounded alcohol-free hydrocortisone suspension to hydrocortisone tablets found that the absorption and PK parameters were similar (63). In addition, adrenal steroid concentrations, weight gain, and growth were comparable between children on tablets and suspension. In another study, Al-Rayess et al reported that children with CAH treated with an alcohol-free hydrocortisone compounded suspension had decreased androgen exposure, as shown by lower bone age Z-scores, and lower average and cumulative daily hydrocortisone dose compared to hydrocortisone tablets (64).

While compounding is an important part of the healthcare system, compounded drugs are less regulated than licensed medications by the FDA and are exempt from good manufacturing practice regulations, which can be concerning for inconsistent quality among different compounding pharmacies (65).

Nonglucocorticoid Treatment Options

Mitotane and Nevanimibe

Mitotane is primarily used in the treatment of adrenocortical cancer with variable success. Mitotane has some effect to inhibit steroidogenesis but is also adrenolytic, which is the primary treatment goal in adrenal cancers. While mitotane has not been studied as primary therapy in 21OHD, the drug has been successfully employed to regress TART and to improve sperm production for cryopreservation in men with 21OHD (32) (Table 1, Fig. 2). Although the related compound dichlorodiphenyltrichloroethane is a known teratogen,

pregnancy outcomes with sperm obtained from mitotane-treated patients have not been reported.

Nevanimibe, which is structurally unrelated to mitotane, causes adrenocortical destruction in dogs after a week of therapy. Both drugs inhibit sterol-O-acyltransferase 1, which esterifies intracellular cholesterol for storage as cholesteryl esters in adrenal cells (66, 67). A common pathogenic mechanism has been suggested, in which the inhibition of sterol-O-acyltransferase 1 raises free cholesterol and leads to cellular toxicity. A trial of nevanimibe in 21OHD found some reduction in biomarkers but no dose-response up to 1000 mg twice daily (33), and development of the agent was terminated (Table 1, Fig. 2).

Androgen Receptor Antagonists

The first trial of nonglucocorticoid therapies in 21OHD was started at the National Institutes of Health in the 1980s. In a 6-month cross-over pilot study in children with 21OHD, treatment with an androgen receptor antagonist (flutamide) and a first-generation aromatase inhibitor (testolactone) along with reduced hydrocortisone dose resulted in greater reductions in linear growth rate velocity and bone maturation compared to treatment with conventional dosing of hydrocortisone alone (Table 1, Fig. 2) (34). In a subsequent 2-year randomized study in children with 21OHD, better control of growth velocity and bone maturation was observed with low-dose hydrocortisone combined with flutamide and testolactone, despite increases in 17OHP, A4, and T during the experimental treatment (35). These findings illustrate that extra-adrenal metabolism of precursor steroids to active androgens and estrogens drives skeletal maturation in children with 21OHD. These studies were the first demonstrations that clinical parameters of disease control could be achieved despite glucocorticoid dose reduction toward a physiologic regimen when combined with drugs that target the pathophysiology of 21OHD. A similar treatment combination of reduced hydrocortisone with flutamide and letrozole, a potent nonsteroidal aromatase inhibitor, has been investigated in a long-term study that followed children until adult height (NCT00001521), but final results have not yet been published (Table 2, Fig. 2).

Abiraterone Acetate

All pathways to androgens require the activities of cytochrome P450 17A1 (CYP17A1; steroid 17-hydroxylase/17,20-lyase). Therefore, inhibition of CYP17A1 is a potential strategy to reduce excess adrenal androgens. Abiraterone acetate (AA), a prodrug of abiraterone, is a very potent (~1 nmol/L affinity) CYP17A1 inhibitor, which is approved in many countries to treat prostate cancer. When combined with medical castration and prednisone, AA lowers A4 and T to undetectable values in most patients with prostate cancer (68), and 11oAs decline in parallel (7). Based on impressive androgen reduction in prostate cancer patients, AA has been investigated as a treatment for 21OHD (Fig. 2). In a phase 1 dose-escalation study in women with 21OHD and elevated serum A4 [>1.5 × upper limit of normal (ULN)], who were receiving 20 mg/day of hydrocortisone therapy, serum A4 and T were normalized in 3 or 5 of 6 women after 6 days of AA treatment at doses of 100 or 250 mg/day, respectively (36); serum levels of T and 11oAs, as well as urinary androgen metabolites, also declined sharply from baseline (Table 1) (7, 36). Two phase 1 studies of AA in children with 21OHD were initiated. The studies have been either suspended (NCT02574910) or withdrawn (NCT03548246), however, for logistical reasons rather than safety or efficacy concerns and are unlikely to be completed.

Tildacerfont and Crinecerfont

The HPA axis drives steroidogenesis in 21OHD through hypothalamic corticotropin-releasing factor (CRF) binding to its type 1 receptor (CRF_1) on corticotropes in the anterior pituitary, which stimulates ACTH release. Consequently, interruption of the HPA axis through antagonism of CRF_1 to reduce ACTH secretion and to decrease downstream androgen production might offer a new approach for treating 21OHD (Fig. 2). Similar to studies with AA or with flutamide and testolactone, addition of a CRF_1 antagonist to attenuate adrenal androgen production might be achieved while also reducing glucocorticoid dosing to a physiologic range, thereby potentially mitigating the negative consequences of long-term supraphysiologic glucocorticoid treatment. After the cloning of genes encoding the CRF receptors (69, 70), several CRF_1 antagonists were developed and trialed for mood disorders, yet none were advanced into clinical practice (71). To test the efficacy of repurposing these agents for 21OHD, an exploratory study in 8 female patients with 21OHD found that a single dose of the CRF_1 antagonist NBI-77860 at bedtime (300 or 600 mg) delayed and decreased the overnight rise in ACTH and 17OHP, while the first-morning dose of glucocorticoid was withheld until 1400 (72). This proof-of-concept data then led to phase 2 trials of other CRF_1 antagonists in 21OHD using a similar study design.

A phase 2, open-label trial of the CRF_1 antagonist tildacerfont (Spruce Biosciences) at 200 to 1000 mg once daily or 100 to 200 mg twice daily in adults with 21OHD and A4 > 2.5 × ULN showed reductions in ACTH, 17OHP, and A4 after 2 weeks of treatment, with no clear dose-response relationship (40) (Table 2). In a second phase 2 trial of tildacerfont 400 mg/day for 10 to 12 weeks, maximum mean reductions of 84% (ACTH), 82% (17OHP), and 79% (A4) were achieved (40). Based on qualitative differences in responses to tildacerfont from patients with poor or good disease control, 2 phase 2b trials were initiated, primarily to test androgen reduction in the poor control group or glucocorticoid dose reduction in the good control group. In the randomized double-blind placebo-controlled trial for the poor control group, the placebo-adjusted reduction from baseline in A4 did not reach statistical significance after 12 weeks of treatment with tildacerfont 200 mg once daily (41), and the trial was terminated. This result might have been due to low treatment adherence, as only 50% of participants reported drug compliance of ≥80%, as might be anticipated in this population. The other randomized double-blind placebo-controlled phase 2b trial of tildacerfont in adults with A4 control on a stable glucocorticoid regimen (NCT04544410) also failed to meet its primary endpoint of glucocorticoid dose reduction. Consequently, development of tildacerfont for 21OHD has been terminated, and the open-label dose-finding phase 2 trial in children with 21OHD has also been stopped (NCT05128942).

Promising results were also reported in 2 phase 2 trials of the CRF_1 antagonist crinecerfont (Neurocrine Biosciences) (Table 2). In the open-label study in adults with 21OHD (CAHlibrate) (42), median reductions of >50% in ACTH and 17OHP were observed after 2 weeks of treatment with 4 dosing groups, ranging from 50 mg at bedtime to 100 mg with the morning and evening meals. In parallel, dose-dependent reductions in A4 from 20% to over 60% were

also observed, particularly in the early morning hours while the first-morning dose of glucocorticoid was intentionally delayed. In a similar trial for adolescents with 21OHD (CAHlibrate Pediatric), > 50% reductions in ACTH, 17OHP, and A4 were also demonstrated after 2 weeks of crinecerfont treatment at 50 mg with the morning and evening meals (43). Moreover, after 2 weeks of crinecerfont treatment, substantial reductions in elevated T levels in female participants and in the A4-to-T ratios in male participants were observed in both studies (42, 43). These data informed phase 3 trial designs of crinecerfont for children and adults with 21OHD.

The 2 phase 3 multinational, randomized crinecerfont studies in pediatric (CAHtalyst Pediatric) (44) and adult (CAHtalyst Adult) (45) participants represent the largest interventional studies conducted to date in 21OHD. In the pediatric study, children (2-17 years of age) with 21OHD who were taking stable glucocorticoid regimens [>12 mg/m^2/day hydrocortisone equivalent (HCe)] and had both A4 greater than the midpoint of the reference range and 17OHP >2× ULN were randomized (2:1) to either crinecerfont (25, 50, or 100 mg twice a day based on weight) or placebo for 28 weeks. Glucocorticoid dosing was maintained stable for 4 weeks, then adjusted from 4 to 28 weeks based on A4 levels to a minimum dose of 8 to 10 mg/m^2/day HCe, provided that A4 level was controlled (protocol-specified target of ≤120% of baseline or ≤ ULN). At baseline, the mean glucocorticoid dose was 16.4 mg/m^2/day HCe, with elevated mean A4 (431 ng/dL, 15.0 nmol/L) and 17OHP (8682 ng/dL, 263 nmol/L). At week 4, A4 was substantially reduced in the crinecerfont group (−197 ng/dL, −6.9 nmol/L) but increased in the placebo group (+71 ng/dL, +2.5 nmol/L) (Table 2). Similarly, 17OHP decreased substantially from baseline to week 4 with crinecerfont (−5865 ng/dL, −178 nmol/L) and increased with placebo (+556 ng/dL, +16.9 nmol/L). These decreases in biomarkers allowed for substantial and clinically meaningful reductions in glucocorticoid doses while maintaining A4 control after 28 weeks in the crinecerfont group (-18.0%), compared to an increase in the placebo group (+5.6%). Although not a prespecified endpoint, A4 decreased from baseline at Week 28 in the crinecerfont group (mean −94 ng/dL [−3.3 nmol/L]), despite the reduction in glucocorticoid dose; in contrast, A4 increased at Week 28 in the placebo group (+147 ng/dL [+5.1 nmol/L]), despite the increase in glucocorticoid dose. Moreover, 30% of participants randomized to crinecerfont achieved a protocol-defined physiologic glucocorticoid dose of ≤11 mg/m^2/d HCe (95th percentile of normal cortisol production rate) (73, 74) vs 0% randomized to placebo, with changes in glucocorticoid dose set to zero if protocol-defined A4 control was not maintained. Changes in body mass index standard deviation scores and in homeostatic model of insulin resistance scores also improved in the crinecerfont group relative to the placebo group at week 28. The open-label phase of CAHtalyst Pediatric is completed, and the extension phase is ongoing.

In the adult study (45), participants ≥18 years of age with 21OHD who were receiving stable glucocorticoid regimens (>13 mg/m^2/day HCe) and had normal or elevated A4 were randomized (2:1) to crinecerfont (100 mg twice a day) or placebo for 24 weeks. Glucocorticoid dosing was maintained stable for 4 weeks, followed by a scheduled glucocorticoid down-titration over 8 weeks—independent of A4 control—to a targeted dose of 8 to 10 mg/m^2/day. Glucocorticoid doses were then increased as needed during the last 12 weeks to maintain A4 at the protocol-specified target of ≤120% of baseline or ≤ ULN. At baseline, the mean glucocorticoid dose was 17.6 mg/m^2/day HCe, with elevated mean A4 (620 ng/dL, 21.6 nmol/L) and 17OHP (9467 ng/dL, 287 nmol/L). At week 4, A4 was substantially reduced in the crinecerfont group (−299 ng/dL, −10.4 nmol/L) but increased in the placebo group (+45.5 ng/dL, + 1.6 nmol/L) (Table 2). Similarly, 17OHP decreased substantially from baseline to week 4 with crinecerfont (−5994 ng/dL, −182 nmol/L) but changed minimally with placebo (−156 ng/dL, −4.7 nmol/L). These decreases in androgens allowed for substantial and clinically meaningful reductions in glucocorticoid doses—with changes in glucocorticoid doses set to zero if protocol-defined A4 control was not maintained—after 24 weeks in the crinecerfont group (−27.3%) or −9.1 mg/day HCe, compared to the placebo group (−10.3%). At week 24, mean A4 in the crinecerfont group was maintained below baseline (−17 ng/dL [−0.6 nmol/L]). By comparison, mean A4 rose in the placebo group at Week 24 (+190 ng/dL [+6.6 nmol/L]). The inability of the investigators to prevent a rise in A4 after glucocorticoid reduction in the placebo group further magnifies the effects of crinecerfont in this trial. Moreover, 63% of participants randomized to crinecerfont achieved the protocol-defined physiologic glucocorticoid dose of ≤11 mg/m^2/day HCe vs 18% on placebo while maintaining the protocol-specified A4 control. Body weight declined more in the crinecerfont group than the placebo group at week 24, although the difference did not reach statistical significance. The open-label phase of CAHtalyst Adult is completed, and the extension phase is ongoing.

Based on the successful phase 2 and phase 3 clinical trials, crinecerfont was approved in December 2024 as an adjunctive treatment to glucocorticoid replacement to control androgens in pediatric and adult patients (4 years of age and older) with classic CAH.

Third-generation Aromatase Inhibitors: Anastrozole and Letrozole

Children with CAH due to 21OHD are exposed to chronically elevated adrenal androgen, which through aromatization to estrogen leads to estrogen-mediated accelerated bone maturation and early growth plate fusion contributing to short adult stature, below their target height (75-77). As epiphyseal fusion in both females and males is estrogen driven, aromatase inhibitors (AIs) have been suggested to delay growth plate fusion and prolong duration of growth by inhibiting aromatization of androgen to estrogen. The aromatase (P450 19A1, estrogen synthase) enzyme is encoded by the *CYP19A1* gene and catalyzes the conversion of T and A4 (C_{19} steroids) to estradiol and estrone, respectively. Aromatase is expressed in several tissues including ovary, adipose tissue, liver, muscle, bone, brain, and adrenals.

Potent, oral third-generation AIs are FDA approved for postmenopausal women with metastatic breast cancer (78). These AIs have been effectively used off-label either alone or in combination with GH and/or GnRH analogues in treating children with short stature due to idiopathic short stature (79), constitutional delay of puberty (80, 81), disorders of puberty and advanced bone age (82, 83), and males with GH deficiency (84). Anastrozole (1 mg) and letrozole (2.5 mg), the commonly used third-generation AIs, both achieve potent tissue aromatase blockade, 96.7% and >99.1%, respectively (85), and are mostly metabolized by the liver. Since letrozole can cause a ~25% greater increase in T compared to anastrozole, and both produce the desired deceleration in bone age maturation, anastrozole may be a better choice in children with 21OHD (85).

The literature on the use of AIs in children with CAH, including 11-hydroxylase deficiency, is sparse and are mainly case reports (86-89). The first formal trial was a 2-year study of 28 children with 21OHD using testolactone, a first-generation AI, administered 4 times a day in conjunction with flutamide and reduced daily hydrocortisone dose as discussed earlier (35) (Table 1). During the study, no growth acceleration or rapid bone age maturation was observed, although adrenal androgens were significantly elevated. A second study in 25 children with 21OHD reported that anastrozole significantly decreased bone age Z-scores from 4.3 to 1.9 after an average of 5.2 (±2.2) years of use (Table 2) (46). Children in this study were all prepubertal with an age range from 3.2 to 13.9 years at initiation of therapy, which underscores the impact of aromatization of adrenal androgens in advancing the bone age. Bone mineral density remained normal while on treatment with anastrozole (46). A retrospective, longitudinal review of growth and bone maturation outcomes in 60 children with classic CAH due to 21OHD spanning up to an 8-year period, including 2 years prior and 6 years on anastrozole, found that the overall mean predicted adult height Z-score increased from −2.1 at the start of therapy to 0.18 at 6 years of treatment, which resulted in a significant gain in growth potential, as demonstrated by an average of 13 cm increase in the predicted adult height in girls and 17 cm in boys over the 6 years of treatment (90). The mean bone age Z-score was 4.2 at the time of initiation of anastrozole therapy and decreased at each subsequent measured time point (1, 2, 4, and 6 years) to a 1.3 bone age Z-score at the end of 6 years. There was no significant difference in hydrocortisone dose from the beginning to the end of anastrozole treatment [12.1 (3.4) vs 12.8 (2.6), respectively]. A longitudinal randomized controlled clinical trial is still needed to assess long-term clinical outcomes.

There is also a report on the efficacy of anastrozole as a monotherapy in 3 females with nonclassic 21OHD who had bone age advancement, early pubarche, and growth acceleration but normal adrenal cortisol production (91). The average bone age Z-score at the initiation of therapy was +4.43 and decreased to +0.77 after stopping. All 3 achieved adult heights comparable to their genetic potential. No adverse outcomes including early precocious puberty, liver dysfunction, or abnormal bone mineral density were noted (46). Of note, a study using stable isotopes of leucine to examine the impact of full GnRH axis suppression vs estrogen suppression using anastrozole in healthy eugonadal young men showed a substantial decrease in rates of whole-body protein synthesis and increased adiposity with GnRH suppression, whereas no significant differences were detected in these metrics when only estrogen was suppressed with AI therapy (92).

Overall, third-generation AIs have been shown to slow bone age maturation and cause a net height gain in children with short stature (85). However, unlike CRF_1 antagonists that can be used as an adjunct therapy to control elevated androgen and prevent the onset of bone age advancement, the use of aromatase inhibitors is recommended when bone age Z-scores are more than 2 SDs advanced.

Atumelnant

Another potential strategy to reduce excess androgens is via antagonism of the melanocortin type 2 receptor (or ACTH receptor) (Fig. 2). Preclinical studies in rodents demonstrated dose-dependent acute suppression of plasma corticosterone and reversal of adrenal hypertrophy, which led to phase 1 dose-escalation studies of atumelnant (CRN04894, Crinetics Pharmaceuticals) in normal volunteers (NCT06048887). Atumelnant (10-80 mg once daily) was well tolerated and was associated with dose-dependent reduction of morning serum cortisol, A4, and aldosterone with blunting of the response to cosyntropin (47) (Table 2). Based on these data, atumelnant has been advanced to phase 2 trials for 21OHD and ACTH-dependent Cushing syndrome. Interim results from an ongoing phase 2 dose-finding study (TouCAHn study; NCT05907291) in adults with 21OHD and A4 > 1.5 × ULN showed reductions of 94% and 96% in A4 and 17OHP, respectively, after 12 weeks of treatment with atumelnant at the 80 mg/day dose (48).

Anti-ACTH Antibody Lu AG13909

Lu AG13909 (Lundbeck; previously ALD1613, Alder BioPharmaceuticals) is a specific, high-affinity, neutralizing monoclonal antibody to ACTH (Fig. 2). Preclinical studies in rodents and cynomolgus monkeys found dose-dependent reductions in plasma corticosterone or cortisol, respectively (93, 94). An open-label, phase 1, dose-finding study (NCT05669950) is currently underway in adults with 21OHD to evaluate safety, tolerability, PK properties, and PD effects (49) (Table 2).

Gene Therapy BBP-631

The potential of gene therapy to restore endogenous 21-hydroxylase activity was first explored 25 years ago. The injection of an adenovirus expressing the human *CYP21A2* mRNA directly into adrenal glands of *Cyp21*$^{-/-}$ mice induced 21-hydroxylase expression and activity, which lowered ACTH and adrenal-derived progesterone (95). In subsequent preclinical studies, intravenous delivery of an adeno-associated virus (AAV) vector to *Cyp21*$^{-/-}$ mice also induced CYP21A2 expression in the adrenal cortex and reduced progesterone levels for over 15 weeks (96), and intramuscular injection of an AAV-based *Cyp21a1* transgene achieved long-lasting extra-adrenal enzyme expression, with reduced progesterone levels that were sustained through 7 months (97). In contrast, AAV-mediated transgene expression in the adrenal cortex lasted only 8 weeks in another study (98), which was attributed to the centripetal renewal of the adrenal cortex from stem cells near the capsule migrating through to the medulla (99). Most recently, systemic AAV-mediated delivery of *CYP21A2* restored circulating aldosterone, reduced circulating progesterone and adrenal size, and increased but did not normalize circulating corticosterone in *Cyp21*$^{-/-}$ mice after 28 days (100). The authors attributed the changes in steroid patterns to the high and durable transgene expression in the liver, rather than the low and time-limited expression in the adrenal cortex, which was 300- to 800-fold lower than *Cyp21a1* expression in wild-type mice. The authors did not provide assessment of 11-deoxycorticosterone, which would be the initial product from hepatic 21-hydroxylation of adrenal-derived progesterone, nor did the study demonstrate durable corticosterone production after renewal of the adrenal cortex lacking the transgene.

A phase 1/2 clinical trial of gene therapy with escalating doses of BBP-631 (Adrenas Therapeutics), an AAV5 vector containing the human *CYP21A2* transgene, has been enrolling participants (NCT04783181) (Fig. 2). A preclinical study in cynomolgus monkeys found dose-dependent, human *CYP21A2* RNA expression through 12 weeks and expression of vector genome copies for up to 24 weeks after

intravenous delivery of BBP-631 (101). Preliminary results in 7 adults with 21OHD suggest that BBP-631 is well tolerated, with increases in 11-deoxycortisol and some cortisol production observed at the higher doses (37) (Table 2); however, the study has been closed to enrollment. Higher doses of this vector will not be tested, and further development of BBP-631 for 21OHD has been halted.

Cell Therapy

Another potential approach for restoring endogenous cortisol production for patients with 21OHD and other forms of cortisol deficiency is the transplantation of adrenal or adrenal-like cells with the capacity to produce aldosterone and cortisol in response to angiotensin II or ACTH, respectively (102) (Fig. 2). For xenotransplantation, encapsulation of the adrenal cells has been used to prevent T-cell mediated rejection yet preserve the permeability to nutrients, tropic hormones, and steroids. In a preclinical study, transplantation of bovine adrenocortical cells encapsulated in alginate into adrenalectomized rats resulted in ACTH-driven increases in circulating cortisol (103). In addition, investigators have generated adrenal-like cells appropriate for cell therapy applications from mouse and human induced pluripotent stem (iPS) cells. Human iPS cells derived from blood, urine, and skin have been successfully reprogrammed into cells, which recapitulate steroidogenesis in the fetal (104) or definitive adrenal cortex, the latter of which were viable in mice for up to 3 weeks following intra-adrenal or kidney capsule implantation (105). The adrenal-like cells generated from human iPS cells produced cortisol in response to ACTH stimulation and could be expanded to quantities large enough for use in cell-based therapy (106).

Conclusions

The management of patients with classic 21OHD remains a difficult balance between supraphysiologic glucocorticoid exposure vs suboptimal disease control with adrenal-derived androgen excess in children and adults. The poor PK and limited dose range of hydrocortisone tablets, coupled with limited monitoring options, conspire to create a high burden of disease and poor outcomes. Improved oral and parenteral hydrocortisone delivery methods and nonglucocorticoid cotherapies have been trialed, and many of these treatment options are in or nearing clinical use. Frequent testing strategies of adrenal biomarkers would provide information on the effect that changing the amount, time, and frequency of glucocorticoid dose will have on an individual's PD steroid response over a 24 hour period and advance our understanding of how to adjust glucocorticoid dosing to optimize chronic androgen and cortisol exposures. With improved monitoring and a wider range of adrenal biomarkers, endocrinologists might soon deliver superior care with less disease burden and improved outcomes tailored to each patient's needs.

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Data Availability

The data described in this manuscript is publicly available from the citations as indicated.

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