Clinical Advances in the Pharmacotherapy of Congenital Adrenal Hyperplasia

Alessandro Prete, Richard J. Auchus, Richard J Ross

1 Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK. 2 Department of Endocrinology, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK. 3 Departments of Pharmacology and Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, MI, USA. 4 Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK.

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Corresponding author and reprint request: Richard J. Ross, MD., FRCP, University of Sheffield, Sheffield, UK. Tel 0044 (0)114 2712386, Fax: 0044 (0)114 2712475, email: r.j.ross@sheffield.ac.uk

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Abstract

Background: Patients with 21-hydroxylase deficiency congenital adrenal hyperplasia (21OHD-CAH) have poor health outcomes with increased mortality, short stature, impaired fertility, and increased cardiovascular risk factors such as obesity. To address this there are therapies in development that target the clinical goal of treatment, which is to control excess androgens with an adrenal replacement dose of glucocorticoid.

Methods: Narrative review of publications on recent clinical developments in the pharmacotherapy of congenital adrenal hyperplasia.

Summary: Therapies in clinical development target different levels of the hypothalamo-pituitary-adrenal axis. Two Corticotrophin Releasing Factor type 1 (CRF₁) receptor antagonists, Crinecerfont and Tildacerfont, have been trialled in poorly controlled 21OHD-CAH patients, and both reduced ACTH and androgen biomarkers whilst patients were on stable glucocorticoid replacement. Improvements in glucocorticoid replacement include replacing the circadian rhythm of cortisol that has been trialled with continuous subcutaneous infusion of hydrocortisone and Chronocort, a delayed-release hydrocortisone formulation. Chronocort optimally controlled 21OHD-CAH in 80% of patients on an adrenal replacement dose of hydrocortisone which was associated with patient reported benefit including restoration of menses and pregnancies. Adrenal-targeted therapies include the steroidogenesis blocking drug abiraterone acetate, which reduced adrenal androgen biomarkers in poorly controlled patients.

Conclusions: CRF₁ receptor antagonists hold promise to avoid excess glucocorticoid replacement in those not controlled on standard or circadian glucocorticoid replacement such as Chronocort. Gene and cell therapies, are the only therapeutic approaches that could potentially correct both cortisol deficiency and androgen excess.
Background

The discovery of cortisone – and its use as replacement therapy for adrenal insufficiency – was life-saving for patients born with classic congenital adrenal hyperplasia (CAH)(1). Since the 1960s, however, there has been little innovation in the pharmacological treatment of patients with classic CAH, due to 21-hydroxylase deficiency (21OHD-CAH), and in the last decade, cohort studies have reported poor health outcomes in adults with 21OHD-CAH (2-5). These observational studies have stimulated the development of new pharmacotherapies to reduce the morbidity and mortality of patients with 21OHD-CAH. This review concentrates on the therapies that are now in clinical development.

21OHD-CAH, is a genetic disorder of steroidogenesis affecting ~1:15,000 live births (5). Lack of 21-hydroxylase causes cortisol deficiency and a counter-regulatory increase in pituitary adrenocorticotropic hormone (ACTH) secretion, which drives overproduction of adrenal androgens, and adrenal hyperplasia (Figure 1). Patients with 21OHD-CAH have two major problems: adrenal insufficiency and androgen excess. Adrenal insufficiency causes life threatening adrenal crises (5-7), while androgen excess causes atypical genitalia in 46,XX neonates, promotes abnormal growth, culminates in short adult stature, sometimes triggers precocious puberty, and in adulthood, virilization of women and infertility in both sexes (8). Treatment aims are to replace cortisol and, where required, aldosterone. Supra-physiologic doses of glucocorticoid are typically needed to lower ACTH and adrenal androgens, which chronically exposes patients to excess glucocorticoid treatment over time. Management of 21OHD-CAH involves balancing glucocorticoid doses to avoid glucocorticoid deficiency, risking adrenal crisis, and iatrogenic glucocorticoid excess, which predisposes to short stature, obesity, hypertension, osteoporosis and an adverse metabolic profile (Figure 2) (4-7,9,10).

Patients with 21OHD-CAH have increased mortality up to 5 times that of the healthy population (11,12), with adrenal crisis the leading cause of death (12). Patients with 21OHD-CAH have increased cardiovascular disease risk factors and metabolic morbidity as shown in the National Institute of Health (NIH) natural history study published in 2021 (13). This is the largest longitudinal study in 21OHD-CAH with patients followed from childhood with median follow up 18.6 years; doses of hydrocortisone at the beginning and end of the study were respectively 17.7 and 17.4 mg/m²/day, and exceeded those recommended for adrenal replacement of 8-14 mg/m²/day (14,15); adrenal androgens were only considered controlled...
at 28% of visits; 93% of patients were hypertensive at one or more visits; obesity was prevalent and increasing from childhood with 56% children 49% adults obese; and insulin resistance was observed in 94.7% patients at one or more visits. Adults with 21OHD-CAH are approximately 10 cm shorter than the population mean and have a higher prevalence of anxiety, depression, alcohol misuse, personality disorders, with suicides among male patients and adjustment disorders among female patients (5). Fertility is reduced in female and male adults with 21OHD-CAH. In women, androgen hypersecretion results in anovulation, and acyclic progesterone hypersecretion acts like a progestin-only contraceptive.

Oligomenorrhoea and/or amenorrhea are reported in 45% of women with 21OHD-CAH compared to 13.6% in the healthy population (2,16), and women with 21OHD-CAH report only 0.25 live births per woman compared with 1.8 in the UK population; however, fecundity is normal in those wishing to get pregnant and receiving proper treatment (17). In men, ACTH excess drives the development of testicular adrenal rest tissue, which over time results in fibrosis and azoospermia, and adrenal-derived androgen hypersecretion suppresses the pituitary gonadal axis. In the UK cohort study, 37% of males had sought fertility support, of which only 67% of these were successful (2), and a separate study of 50 male clinic attendees with 21OHD-CAH reported severe oligospermia in 48% (18). In both women and men with 21OHD-CAH, fertility can be improved with supraphysiological doses of glucocorticoid, but this approach has the downside of all the complications from excess glucocorticoid exposure.

The majority of the poor health outcomes in patients with 21OHD-CAH result from the inability to precisely titrate currently available glucocorticoid preparations to both adequately replace the deficiency and sufficiently attenuate the adrenal-derived androgen excess. Undertreatment causes an increased risk of adrenal crisis and poor androgen control, and overtreatment causes iatrogenic Cushing’s syndrome. To understand the pathology of 21OHD-CAH and the impact of new pharmacotherapies on disease biomarkers, we need to understand how adrenal androgens are generated in patients with 21OHD-CAH (figure 1). In normal physiology, there are essentially three pathways to adrenal androgen production: the classic via dehydroepiandrosterone (DHEA), the 11-oxygenated via androstenedione (A4), and the alternative dihydrotestosterone (DHT) pathway via 17-hydroxyprogesterone (17OHP) that is usually only active in the foetus (19). In patients with 21OHD-CAH these pathways are deranged; the classic pathway via DHEA is downregulated and DHEA levels are normal or low, although the mechanism for this is not understood; With the exaggerated ACTH drive, 17OHP levels are elevated, and 17OHP is a key intermediate driving the three
androgen pathways to testosterone and 11-oxygenated androgens via A4 and by activating the normally quiescent alternative DHT pathway. 17OHP is therefore a key biomarker for assessing disease control in 21OHD-CAH. 17OHP levels in healthy individuals have a minimal circadian rhythm, and although there is some variation by gender and during the menstrual cycle, the Upper Limit of Normal (ULN) for 17OHP levels is ~12 nmol/L (400 ng/dl) in most quoted references ranges. In 21OHD-CAH, 17OHP levels can be very high in the early morning, often >50 nmol/L (1650 ng/dl), and it is recognised that to control 17OHP in to the reference range on standard glucocorticoid therapy risks over treatment (20). For this reason, endocrinologists have adopted a target optimal range for 17OHP < 3x ULN ~ 36 nmol/L (1200 ng/dl); patients having a morning 17OHP < 36 nmol/L (1200 ng/dl) are considered to have good disease control (2,3). This criterion is an important concept when we come to assess new therapies.

To address the poor health outcomes and unmet need for better treatment of 21OHD-CAH, researchers have targeted different levels of the hypothalamic-pituitary-adrenal axis from suppressing ACTH release from the pituitary to gene therapy at the adrenal (Figure 3). In this review, we report on the current status of the evolving pharmacotherapies for 21OHD-CAH that are being trialled in the clinic (Table 1).

**Corticotrophin releasing factor (CRF) receptor antagonists**

Elevated ACTH is the primary driver for adrenal androgen production in 21OHD-CAH, and therefore suppressing ACTH release is a rational approach to therapy in 21OHD-CAH. The primary regulator of ACTH synthesis and release is Corticotrophin Releasing Factor (CRF), released from the hypothalamus into the hypophyseal portal system, acting directly on specific receptors on pituitary corticotropes. Two different types of CRF receptors exist: the CRF type 1 receptor (CRF1), abundant in the pituitary, and the CRF type 2 receptor (CRF2), predominantly found in peripheral tissues (21). Small molecule CRF1 receptor antagonists have been synthesized and tested in patients with 21OHD-CAH (22), and two orally active, selective, non-steroidal CRF1 receptor antagonists are in development as therapies for 21OHD-CAH: Crinecerfont (Neurocrine Biosciences, Inc, USA) and Tildacerfont (Spruce Biosciences, USA).

The development of Crinecerfont was based on a phase Ib study of NBI-77860, another CRF1 receptor antagonist, which afforded dose-dependent reductions of ACTH and 17OHP
in a single-dose study of 300 and 600 mg at bedtime (22). The phase 2 open-label, multiple-
dose, dose-finding study evaluated four Crinecerfont oral dosing regimens administered for
14 consecutive days: 50 mg at bedtime (n=8); 100 mg at bedtime (n=7); 100 mg once-daily
with an evening meal (n=8); and 100 mg twice-daily with meals (n=8) (23). The median daily
baseline glucocorticoid dose was ~25mg hydrocortisone dose equivalents, and patients were
generally poorly controlled with the median baseline morning 17OHP ranging from 5,000-
12,800 ng/dl (152-389 nmol/L) across the groups. Crinecerfont treatment delayed and
attenuated the morning ACTH rise and produced meaningful reductions in ACTH and 17-
OHP levels by 54% to 75% at all doses studied, but the median 17OHP remained above
2,000 ng/dl (60 nmol/L) in all groups (Figure 4A). There was a dose-related decrease in A4
levels, ranging from 21% to 64%, and in 3 of the 4 cohorts, the A4 median fell in the normal
range. The reduced adrenal androgen production induced by Crinecerfont was also
documented by a fall of testosterone levels in women and of the A4/testosterone ratio in men
ranging from 32% to 74% and from 33% to 59%, respectively. Treatment with Crinecerfont
was well tolerated with a favourable safety profile with no related severe adverse events
reported. Two ongoing phase 3 trials are assessing the long-term efficacy and safety of
Crinecerfont in both adult and paediatric patients with 21OHD-CAH (NCT04490915 and
NCT04806451).

Two phase 2 studies have assessed the efficacy and safety of Tildacerfont. Adults with
21OHD-CAH receiving stable glucocorticoid replacement and 17OHP at baseline ≥800 ng/dl
(24 nmol/L) were treated with oral Tildacerfont 200-1000 mg once daily (n=10) or 100-200
mg twice daily (n=9) for two weeks (study 1) and 400 mg once daily (n=11) for 12 weeks
(study 2) (24). In study 1, participants with poor disease control at baseline (defined as A4
>2x ULN) had reductions in ACTH (-59.4% to -28.4%), 17-OHP (-38.3% to 0.3%), and A4
(-24.2% to -18.1%), with no dose-response relationship. In study 2, patients with poor disease
control at baseline achieved ~80% maximum mean reductions in ACTH, 17OHP, and A4
(Figure 4B). ACTH normalized in 60% of participants and A4 in 40%. In both studies,
participants with good disease control at baseline (A4 ≤2x ULN) showed only minor changes
in ACTH, 17OHP, and A4 levels. Volume reduction of testicular adrenal rest tissue was
observed in two out of three male participants who had post-treatment testicular ultrasounds.
Adverse events were generally mild, and one participant discontinued Tildacerfont due to
grade 1 pruritus with grade 1 elevations in ALT/AST (<3x ULN) with no change to direct
bilirubin. Of note, a CYP3A4-mediated interaction between Tildacerfont and dexamethasone
was identified, resulting in approximately a 2-fold increased dexamethasone exposure. This finding is relevant as dexamethasone is commonly used in patients with 21OHD-CAH, and a switch to a different glucocorticoid may be required before starting Tildacotfont treatment. Currently, two phase 2b studies (NCT04457336, NCT04544410) are testing whether treatment with Tildacotfont can achieve long-lasting clinical benefit and allow reduction of the glucocorticoid dose while controlling relevant disease biomarkers.

In summary, the available evidence on CRF1 receptor antagonists shows that they can attenuate the exaggerated morning rise in ACTH in poorly controlled patients with 21OHD-CAH. The reduction of morning ACTH is associated with a fall in the key biomarkers of disease control, 17OHP and A4. Future studies of CRF1 receptor antagonists are now being planned to examine their clinical benefit in patients with 21OHD-CAH.

Optimised hydrocortisone dosing in children

The commonest cause of adrenal insufficiency (AI) in young children is congenital adrenal hyperplasia (CAH) (25). The recommended therapy in childhood is hydrocortisone given 3-4 times daily (26). Until 2018, the lowest available licenced preparations of hydrocortisone were 10 mg tablets in Europe and 5 mg tablets in the United States (US). As scored tablets are licensed to be divided into halves the lowest possible available dose was 5 mg (Europe) and 2.5 mg (US) respectively. However, these doses are not appropriate to treat neonates, infants and young children with adrenal insufficiency who require a daily dose of 10-15 mg/m² with single doses as low as 0.5 mg (27). Crushed hydrocortisone tablets suspended in water are often used in some countries (26), though accurate dosing is not possible as hydrocortisone does not dissolve well in water and may adhere to plastic material when applied with syringes (28,29). Another common practice in pharmacies is to compound hydrocortisone often mixed with sucrose to overcome the inherent bitterness of hydrocortisone. However, a German study demonstrated that up to 25% of compounded batches do not fulfil the acceptance criteria of the European Pharmacopeia in uniformity of net mass or drug content or are labelled inaccurately (30). In Europe, UK and US, Alkindi® hydrocortisone granules (development name Infacort, Diurnal Europe B.V., The Netherlands) have now become licensed for children with AI from birth to 18 years of age, and are available in low doses of 0.5, 1, 2 and 5 mg. They were developed to address the age group-specific needs of neonates, infants and young children (31,32). As part of the development
programme a single dose clinical trial was undertaken in neonates, infants and children under 6 years with AI, the majority of whom had CAH (33). The children were then invited to participate in a prospective follow-up study of continued treatment with hydrocortisone granules (34). Seventeen children with CAH aged from birth to 6 years, had their hydrocortisone medication changed from pharmacy compounded capsules to hydrocortisone granules. Patients were followed prospectively for 2 years. Median daily hydrocortisone dose varied with age groups and declined from entry to end of study: 9.9-12.0 to 8.6-10.2 mg/m²/d, respectively. There were no trends for accelerated or reduced growth. No adrenal crises were observed despite 193 treatment-emergent adverse events, which were mainly common childhood illnesses. This first prospective study of glucocorticoid treatment in children with AI and CAH demonstrated that accurate dosing and monitoring from birth results in hydrocortisone doses at the lower end of the recommended dose range, normal growth, without occurrence of adrenal crises. Hydrocortisone tablets at 1 mg, 5 mg and 10 mg are available in the Netherlands (Acecort®, hydrocortisone tablets, ACE Pharmaceuticals B.V.). The Netherlands public assessment report states that Acecort is indicated for the treatment of AI in patients in which other hydrocortisone containing products (prolonged release) cannot be used and/or in case of excessive physical and/or mental stress (adrenal crisis). There are no published studies of Acecort in children with CAH.

Circadian glucocorticoid therapies

ACTH rises over night from around 02:00-04:00h to provide the circadian rhythm of cortisol that peaks shortly after waking and falls to low levels in the evening (35). This rise in ACTH is exaggerated in 21OHD-CAH because of the failure in cortisol negative feedback as seen in children taking hydrocortisone (36). The issue being that hydrocortisone has a short plasma half-life, such that even when a dose is taken in the evening cortisol levels are low before the morning rise in ACTH (37). To address this consideration, clinicians have used a number of approaches including reverse circadian therapy, with a dose of longer acting synthetic glucocorticoids such as prednisolone taken at bedtime, intravenous and subcutaneous infusions of hydrocortisone to mimic the cortisol rhythm, and more recently the development of modified-release formulations of hydrocortisone.

Reverse circadian dosing is commonly used in adults with 21OHD-CAH. In two large cohort studies, >50% of patients were taking a glucocorticoid dose late at night (2,3). Despite the
different treatment regimens, optimal biochemical control, defined as 17OHP below three
times the upper limit of normal (<36 nmol/L, <1,200 ng/dl,) was only achieved in <55% of
patients. Reverse circadian dosing may improve control in patients, but it is evident from a
pharmacokinetic study in patients with 210HD-CAH that, despite taking prednisone and
dexamethasone late at night, ACTH and 17OHP still show an exaggerated rise in the morning
(38). There is also evidence that taking glucocorticoids later in the evening, when cortisol
levels are normally low, may result in metabolically adverse consequences (39). Thus,
reverse circadian dosing may improve control of androgens but has the downside of long-
term excess exposure to glucocorticoids. Dexamethasone in particular has been associated
with low bone mineral density and increased BMI (39) and prednisolone with increased
mortality in replacement of patients with primary adrenal insufficiency compared to
replacement with hydrocortisone (40).

The intravenous infusion of hydrocortisone via a pump can replicate the overnight rise in
cortisol, and in two poorly controlled patients with 210HD-CAH prevented the exaggerated
rise in 17OHP within the first 24 hours of pump therapy (41). Continuous subcutaneous
hydrocortisone infusion by pump was trialled in a 6 month phase 2 study in 8 poorly
controlled patients with 210HD-CAH (42). At study entry, all had elevated adrenal
androgens and one or more comorbidities. The subcutaneous infusion approximated
physiologic cortisol secretion and improved the control of 17OHP and A4 throughout the 24
hours. This treatment was associated with clinical benefits including improved quality of life,
restoration of menses in one of three amenorrhoic women, and reduction of testicular
adrenal rest tissue in one man. Five of the 8 patients; however, had skin infections at the site
of the infusion. Continuous subcutaneous infusion of hydrocortisone demonstrates that
replicating the cortisol circadian rhythm of cortisol can improve the biochemical control of
210HD-CAH and is associated with clinical benefit, but it is not a practical therapy for most
patients.

Plenadren® (Shire Services BVBA, Belgium) is a modified-release formulation of
hydrocortisone licensed in Europe for the treatment of adrenal insufficiency. It is a dual-
release tablet with an outer immediate-release hydrocortisone coating and an inner sustained-
release hydrocortisone core. Taken once daily, it provides daytime replacement of
hydrocortisone, but there is no overnight replacement of cortisol. Plenadren® has been used in
patients with 210HD-CAH (43), but reported morning levels of 17OHP have been very
elevated in most patients when taking Plenadren®, indicative of poor biochemical control
A clinical trial assessing its potential use in patients with 21OHD-CAH is ongoing (NCT03760835).

Efmody® (development name Chronocort, Diurnal Europe B.V., The Netherlands) is a modified-release formulation of hydrocortisone licensed in Europe and Great Britain for the treatment of 21OHD-CAH in patients 12 years old and above. Chronocort is a multi-particulate formulation of hydrocortisone with a delayed-release coating that allows for delayed and sustained absorption. When taken at bedtime and on arising, Chronocort replicates the overnight diurnal rise in cortisol (45). In a phase 2, switch study, in 16 adult patients with 21OHD-CAH, Chronocort, at a lower dose than standard treatment, improved control of 21OHD-CAH (46) and 94% of patients maintained good control (morning 17OHP <36 nmol/L, 1,200 ng/dl) during Chronocort therapy.

In the phase 3 Chronocort study (47), 122 adult patients with 21OHD-CAH were randomised to continue either standard therapy or switch to Chronocort with a dose taken at bedtime and on arising. Standard therapy consisted of a variety of treatment regimens including hydrocortisone, prednisolone, and dexamethasone (both singly and in combination), and 84% of patients were taking standard glucocorticoids after 18:00h in a reverse circadian fashion. Dose titrations were made for both treatment groups, using identical rules, by 2 independent physicians blinded to all data except the 24-hour 17OHP and A4 profiles and an investigator-completed checklist screening for clinical signs and symptoms of possible glucocorticoid over- or under-treatment. Patients who enrolled in the phase 3, and the previous phase 2 studies were invited to enrol in an open-label extension study of Chronocort treatment (47). In the extension study, dose titration was performed by the local investigators as in a “real world” experience. At the end of the phase 3 trial, patients who received Chronocort had superior hormonal control during the morning and early afternoon compared to those receiving standard therapy, and this advantage was sustained during 18 months follow up (Figure 4). The trial failed to meet its primary endpoint, because the difference between the two groups in the morning did not translate into a difference over 24 hours at 6 months. The prespecified methods for data analysis obscured the impact of Chronocort in the morning and early afternoon (Figure 5A). The raw data showed significant improvement of the clinically relevant endpoint of morning biochemical control, with reduced AUC and 17OHP amplitude in patients receiving Chronocort (Figure 5A,B,C). At baseline in the phase 3 study, patients were taking a median hydrocortisone dose equivalent of 25 mg, and after 6 months, both groups had been titrated up to ~30 mg. In the extension study, where clinicians titrated the
Chronocort dose, the median daily dose fell from 30 mg to 20 mg over time (Figure 5D). The improvement in biochemical control compared to standard treatment was maintained at 18 months, with 80% displaying good control (morning 17OHP < 36 nmol/L, <1200 ng/dl) for 17OHP and 96% for A4 (in the reference range) versus 52 and 45% at baseline, respectively. The improved disease control was observed despite a reduction in hydrocortisone dose by 33%, to doses typically used for adrenal replacement therapy (15-25 mg/day). Chronocort therapy was associated with patient-reported clinical benefit including menses restoration in 8 patients (1 on standard therapy), and 3 patient and 4 partner pregnancies (none on standard therapy); one of these male patients had a history of testicular adrenal rest tissue with documented sperm count improvement (<0.1 million/mL prior to Chronocort and 10.3 million/mL during Chronocort treatment). During the extension study, quality of life, which was good at baseline, was maintained, and no weight gain was observed. In the phase 3 study, no patients experienced adrenal crises in the Chronocort group compared with 3 in the standard group, and in the extension study, there were 4 patients with an adrenal crisis over 18 months. In the phase 3 study, no severe adverse event was considered related to the study intervention, and in the extension study, severe adverse events were reported for 14 participants one of which, hypokalaemia, was considered related to Chronocort.

The Chronocort development programme included the first randomized study of glucocorticoid therapy and afforded some key learning points. At entry into the phase 3 study, patients were relatively well controlled receiving standard treatment, but with intense monitoring, using 24-hour hormone profiles and blinded titrators, control was improved with standard treatment that included long-acting glucocorticoids, with the majority of patients taking a bedtime dose of glucocorticoid. Nevertheless, the standard glucocorticoid treatment could not prevent the fluctuations and excessive rise of adrenal androgens seen in the morning, and the monitoring regime used in the study is not practical for routine clinical care. By delivering the pre-awakening rise in cortisol, Chronocort controlled the 17OHP into the optimal and, frequently, the reference range throughout the 24 hours abolishing the fluctuations in 17OHP in most patients. At the end of the phase 3 study, the median daily dose of hydrocortisone equivalents was ~30 mg (15.8 and 17.0 mg/m²/day for Chronocort and standard group, respectively), similar doses to those reported in cohort studies (15-18 mg/m²/day) where biochemical control was worse than that in the Chronocort trial (2,3,48,49). In the extension study, with local clinicians performing dose titrations, biochemical control was maintained at a reduced daily Chronocort dose of 20 mg consistent
with that recommended for replacement in adrenal insufficiency; 15-25 mg daily (14). The
maintenance of control despite dose reduction suggest that lower cortisol exposure is
necessary when circadian replacement regimens are used chronically, perhaps related to
reduction in size of the hyperplastic adrenals.

Another key learning point is that when 17OHP was well-controlled, A4 levels were low.
This finding can be explained because not only is 17OHP an inefficient precursor of A4, but
also the classic 17-hydroxypregnenolone-to-DHEA pathway is down-regulated in 21OHD-
CAH (38). This analysis is important because, if the clinicians had depended on A4 to titrate
patients, they would have reduced the dose of Chronocort below that required for adrenal
replacement therapy. In the extension study, clinicians were able to titrate dosing using once
or twice daily sampling during clinic times and independent of dosing, as 17OHP levels show
little fluctuation whilst patients take Chronocort. Controlling the overnight rise in 17OHP
with Chronocort also reduces the output of all adrenal androgen pathways including the 11-
oxgenated androgen pathway and the alternative DHT pathway (50). The importance of
controlling the fluctuations in 17OHP in the morning are shown by the patient-reported
clinical benefit from Chronocort, with restoration of menses and fertility in both men and
women. In the extension study, 4 patients had an adrenal crisis with a frequency of 6.2 crises
per 100 treatment years, similar to population estimates of 5 to 10 adrenal crises/100 patient-
years (51-53), and providing confidence that the safety profile of Chronocort does not differ
from that of immediate-release hydrocortisone.

Adrenal-Targeted Therapies

The defect in 21OHD-CAH is the deficiency of the adrenal enzyme, 21-hydroxylase, so it
makes sense to target therapy at the adrenal steroidogenic pathway. Two drugs, abiraterone
acetate (Janssen Research & Development, Raritan, NJ, USA) and Nevanimibe (Millendo,
USA), that block different levels of the adrenal steroidogenic pathway have been trialled in
patients with 21OHD-CAH.

Abiraterone acetate is a prodrug, which is metabolized to abiraterone, a potent active site-
directed inhibitor of CYP17A1 (17α-hydroxylase/17,20-lyase). Abiraterone acetate added to
medical castration suppresses circulating testosterone and improves survival in castration-
resistant prostate cancer (54). As androgen biosynthesis requires CYP17A1 activities, it is a
rational approach to use Abiraterone acetate to control the androgen excess in 21OHD-CAH.
and obviate the need for supraphysiological glucocorticoids. In a phase 1, dose-escalation study, 6 adult females with 21OHD-CAH were administered abiraterone acetate 100 or 250 mg every morning with 20 mg/day hydrocortisone for 6 days (55). With 100 mg/d abiraterone, mean pre-dose A4 fell from 764 to 254 ng/dL (26.7–8.9 nmol/L). At 250 mg/d, mean A4 normalized in five participants (83%) and decreased from 664 to 126 ng/dL (23.2–4.4 nmol/L). Mean A4 declined further during day 6 to 66 and 38 ng/dL (2.3 and 1.3 nmol/L) at 100 and 250 mg/day, respectively. Hypertension, hypokalaemia, and peripheral oedema, secondary to 11-deoxycorticosterone accumulation are regularly seen in patients with prostate cancer treated with abiraterone but were not observed in patients with 21OHD-CAH because of the defective conversion of progesterone to 11-deoxycorticosterone. In addition to the potential benefit of lower glucocorticoid requirements, the reduction of androgen and oestrogen biosynthesis secondary to abiraterone could be beneficial in prepubertal children with 21OHD-CAH to normalise linear growth, a hypothesis currently being tested in clinical trial NCT02574910. Because of the effect on gonadal steroidogenesis and potential adverse foetal outcomes seen in preclinical studies, abiraterone is not suitable for chronic therapy in adult males or women of childbearing age.

Nevanimibe potently inhibits acyl-coenzyme A:cholesterol O-acyltransferase 1 (ACAT1, or sterol O-acyltransferase 1 [SOAT1]), the principal enzyme that catalyses the esterification of free cholesterol to cholesteryl esters for storage in adrenal cortex cells. At lower concentrations, Nevanimibe reduces adrenal steroidogenesis across all 3 adrenocortical steroid pathways. In a phase 2 single-blind, dose-titration study, n=10 adults with 21OHD-CAH poor disease control received the lowest dose of Nevanimibe (125 mg twice daily) for 2 weeks followed by a single-blind 2-week placebo washout (56). Nevanimibe was gradually titrated up (up to 1,000 mg twice daily) if the primary outcome measure (17-OHP ≤2x ULN) was not met. Two subjects met the primary endpoint, and 5 others experienced 17-OHP decreases ranging from 27% to 72%. The most common side effects were gastrointestinal (30%), and one subject discontinued the study because of a severe adverse event (enteritis). Further development of Nevanimibe for 21OHD-CAH was halted after the interim review of a phase 2b study, which showed insufficient efficacy to continue the programme.

**Therapies Targeting Peripheral Androgen Action**
Suboptimal treatment outcomes due to hyperandrogenism or glucocorticoid excess can potentially be addressed by giving physiological glucocorticoid replacement doses with antiandrogens. This strategy was tested in a 2-year trial of 28 children with 21OHD-CAH randomized to standard hydrocortisone therapy or to a combination of reduced hydrocortisone dose + flutamide (androgen receptor antagonist) + testolactone (aromatase inhibitor to prevent androgen-to-oestrogen conversion) (57). The combination therapy normalised growth and bone maturation, and the results of a long-term study testing the efficacy and safety of this treatment strategy are awaited (NCT00001521).

Preclinical Therapy Development

Various other therapies are in preclinical development and include anti-ACTH monoclonal antibodies (58), ACTH receptor antagonists (59), gene-based therapy (60-63), and cell-based therapy approaches (64). Whilst most of these treatment strategies do not eliminate the need for glucocorticoid replacement, gene- and cell-based therapies have the anticipated hope that replacing the 21-hydroxylase enzyme itself will allow both replacement of cortisol and control of androgens (60). Adrenocortical-like, steroid-secreting cells have been generated from fibroblasts, blood-, and urine-derived cells through cellular reprogramming and were viable when transplanted into the mouse adrenal gland or kidney capsule (64). Also, the impaired steroidogenesis of reprogrammed cells derived from patients with 21OHD-CAH was reversed through lentiviral delivery of the wild-type 21-hydroxylase-encoding gene (64). Gene-based therapy has also been tested and has shown promising results in temporarily reverting the 21OHD-CAH-like phenotype of 21-hydroxylase-deficient mice (60-62). Currently, there is a trial proposed for gene therapy to provide functional copies of the 21-hydroxylase-encoding gene using an adeno-associated virus (NCT04783181).

Conclusions

The last ten years have seen great advances in our understanding of the pathophysiology of 21OHD-CAH and with it a recognition that our current management is suboptimal. This impetus has led to a variety of different pharmacological approaches to improve the control of 21OHD-CAH. CRF1 receptor antagonists and adrenal enzyme blockers have shown they can reduce androgen biomarkers in patients with poorly controlled 21OHD-CAH, and further
448 studies are planned to test whether they can improve health outcomes. Improved
449 glucocorticoid delivery to provide accurate dosing in children and better replicate the
450 circadian rhythm of cortisol either by infusion of hydrocortisone or treatment with
451 Chronocort, a delayed-release formulation of hydrocortisone, can control androgen
452 biomarkers using an adrenal-replacement glucocorticoid dose, and patients report restoration
453 of menses and pregnancies. The only treatments in development that have the potential to
454 cure 21OHD-CAH and both control adrenal androgens and replace glucocorticoid treatment
455 altogether are gene- and cell-based therapies.
Legends

**Figure 1: Pathophysiology of CAH due to 21-hydroxylase deficiency.** Simplified representation of the steroid hormone biosynthesis, with a focus on androgen generation. 21-hydroxylase deficiency causes defective secretion of aldosterone and cortisol. The latter leads to excessive ACTH secretion from the pituitary, which results in adrenal androgen excess. Androgens can be generated through three pathways: the classic androgen pathway through dehydroepiandrosterone (DHEA); the 11-oxygenated androgen pathway through androstenedione (A4); the alternative pathway to dihydrotestosterone (DHT) through androsterone. The Alternative DHT pathway is active in the testis during male development in the foetus but not active in childhood and adults. The accumulation of 17OHP-progesterone (17OHP) in CAH (circled in red) increases atypical conversion of 17OHP to A4 by CYP17A1 17,20-lyase activity, which physiologically has a much higher preference for the conversion of 17OHP-pregnenolone to DHEA. Accumulating 17OHP also drives increased androgen production by the alternative DHT pathway, and increased A4 feeds enhanced 11-oxygenated androgen pathway activity. The Classic pathway via DHEA is downregulated in CAH.

**Figure 2: Challenges of CAH treatment.** The current standard of care for patients with CAH is glucocorticoid therapy, which targets both the cortisol deficiency and the adrenal androgen excess.

**Figure 3: Pharmacotherapies for CAH target different levels of the HPA axis.** The hypothalamus controls ACTH release from the pituitary through CRF and ACTH in turn stimulates cortisol release from the adrenal that feeds back at the hypothalamus and pituitary in a classic endocrine feedback loop.

**Figure 4: Effect of treatment with CRF1 receptor antagonists on CAH biomarkers. (A)** Percentage fall in 17OHP and androstenedione (A4) after 14 days of Crinecerfont oral dosing: 50 mg at bedtime (n=8); 100 mg at bedtime (n=7); 100 mg once-daily with an evening meal (n=8); and 100 mg twice-daily with meals (n=8). **(B)** Percentage fall in 17OHP and A4 during Tildacerfont treatment in patients with poor control of CAH at baseline.

**Figure 5: Chronocort phase 3 and extension study. (A)** 24-hour profile of 17OHP levels at 24 weeks comparing titrated standard treatment and Chronocort showing Chronocort improves morning control of 17OHP. **(B & C)** 24-hour profiles of 17OHP and A4 at baseline.
and after 24 weeks showing that Chronocort normalises 17OHP levels and this is associated with low levels of A4. (D) 0900h 17OHP levels during Chronocort treatment from the phase 3 through the 18 month extension study compared to median hydrocortisone dose showing that during the extension study the hydrocortisone dose came down to a median dose of 20mg and control of 17OHP was maintained (note right y axis represents current hydrocortisone dose range used in patients with CAH). Hormone levels shown as geometric mean ± 95% confidence (Adapted from Merke et al., The Journal of Clinical Endocrinology & Metabolism, Volume 106, Issue 5, May 2021, Pages e2063–e2077, https://doi.org/10.1210/clinem/dgab051)


Table 1: Novel treatment strategies for congenital adrenal hyperplasia that are available for clinical use or currently undergoing clinical evaluation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Administration</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF1 receptor antagonists</td>
<td>Crinecerfont*</td>
<td>• Oral administration.</td>
<td>• Lack of long-term efficacy and safety data.</td>
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<tr>
<td></td>
<td>Oral, twice daily.</td>
<td>• Effectively reduced ACTH and adrenal androgen levels in a phase 2 trial</td>
<td>• Need for concomitant glucocorticoid replacement.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(unpublished results).</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Possibly leads to reduced glucocorticoid requirements.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Favourable safety profile (unpublished results).</td>
<td></td>
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<tr>
<td></td>
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<td>• Possibly leads to reduced glucocorticoid requirements.</td>
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<td></td>
<td></td>
<td>• Favourable safety profile.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>• Associated with testicular adrenal rest tissue reduction.</td>
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<tr>
<td></td>
<td>Tildacerfont*</td>
<td>• Oral administration.</td>
<td>• Lack of long-term efficacy and safety data.</td>
</tr>
<tr>
<td></td>
<td>Oral, once daily.</td>
<td>• Effectively reduces ACTH and adrenal androgen levels.</td>
<td>• Need for concomitant glucocorticoid replacement.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Possibly leads to reduced glucocorticoid requirements.</td>
<td>• Drug-drug interactions (including increased bioavailability of Dexamethasone).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Favourable safety profile.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Associated with testicular adrenal rest tissue reduction.</td>
<td></td>
</tr>
<tr>
<td>Glucocorticoid replacement</td>
<td>Modified-release hydrocortisone</td>
<td>• Oral administration.</td>
<td>• Reduced bioavailability</td>
</tr>
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<td></td>
<td>Plenadren®</td>
<td>• 5 and 20mg tablets.</td>
<td>• Does not replace overnight cortisol levels.</td>
</tr>
<tr>
<td></td>
<td>Oral, once daily.</td>
<td>• Provides once daily dosing.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Favourable safety profile.</td>
<td></td>
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<tr>
<td>Modified-release hydrocortisone</td>
<td>Efmodo®; development name</td>
<td>• Oral administration.</td>
<td>• Cannot be used for sick day dosing (patients should be provided with</td>
</tr>
<tr>
<td></td>
<td>Chronocort)</td>
<td>• Provides once daily dosing.</td>
<td>immediate-release glucocorticoid formulations, e.g. hydrocortisone or</td>
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<td></td>
<td></td>
<td>• Favourable safety profile.</td>
<td>cortisone acetate).</td>
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<td></td>
<td></td>
<td></td>
<td>• Not recommended in patients with increased gastrointestinal motility due</td>
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<td></td>
<td></td>
<td>to the risk of impaired cortisol absorption.</td>
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<td></td>
<td>• Needs to be taken on an empty stomach (at least 1 hour before and 2 hours</td>
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<td></td>
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<td>after any food).</td>
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<tr>
<td>Treatment</td>
<td>Administration</td>
<td>Key Points</td>
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<td>-----------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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</tbody>
</table>
| Hydrocortisone pump                           | Continuous subcutaneous infusion                | • Mimics the natural cortisol rhythm.  
• Effectively controls androgen excess.  
• Associated with clinical benefit (quality of life, restoration of menses, testicular adrenal rest tissue reduction)  
• Relies on technology that is already available (insulin pump).  
• Evidence derived from one small-scale study.  
• Requires continuous device wear and high engagement by the patient and health professionals.  
• Possibility of malfunction.  
• Available hydrocortisone formulations are not designed for subcutaneous use.  
• Possible injection site and systemic reactions. |
| Alkindi® hydrocortisone granules (development name Infacort, Diurnal Europe B.V., The Netherlands) | Oral, 2-4 times daily                            | • Oral administration.  
• Paediatric appropriate dosing available as 0.5, 1.0, 2.0, and 5mg.  
• Taste masking.  
• Favourable safety profile.  
• Does not replace overnight cortisol levels. |
| Accort® hydrocortisone tablets                | Oral, 2-4 times daily                            | • Oral administration.  
• Dosing available at 1.0, 5.0, and 10mg.  
• Colour coded  
• Does not replace overnight cortisol levels. |
| CYP17A1 inhibitor Abiraterone acetate*         | Oral, once daily                                | • Oral administration.  
• Effectively controls androgen excess.  
• Favourable safety profile.  
• Need for concomitant glucocorticoid replacement.  
• Inhibits gonadal sex steroid biosynthesis and could be used only in patients who do not desire fertility.  
• Potential adverse foetal outcomes.  
• Potential concern for expansion of testicular adrenal rest tissue in men.  
• Concerns around liver toxicity.  
• Needs to be taken on an empty stomach (at least 1 hour before and 2 hours after any food).  
• Drug-drug interactions. |
| Androgen receptor antagonist Flutamide*        | Oral, three times daily                          | • Oral administration.  
• Associated with normal linear growth and reduced glucocorticoid requirements in a small-scale study.  
• Need for concomitant glucocorticoid +/- aromatase inhibitor treatment.  
• Concerns around liver toxicity.  
• Contraception is recommended in women of reproductive age. |

* Currently used in patients with 21OHD-CAH only as part of clinical trials.
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225x153mm (150 x 150 DPI)
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225x256mm (150 x 150 DPI)
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