

Treatment of Cushing's disease: a mechanistic update

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Abstract

Cushing's disease (CD) is characterized by an ACTH-producing anterior corticotrope pituitary adenoma. If hypothalamus–pituitary–adrenal (HPA) axis physiology is disrupted, ACTH secretion increases, which in turn stimulates adrenocortical steroidogenesis and cortisol production. Medical treatment plays an important role for patients with persistent disease after surgery, for those in whom surgery is not feasible, or while awaiting effects of radiation. Multiple drugs, with different mechanisms of action and variable efficacy and tolerability for controlling the deleterious effects of chronic glucocorticoid excess, are available. The molecular basis and clinical data for centrally acting drugs, adrenal steroidogenesis inhibitors, and glucocorticoid receptor antagonists are reviewed, as are potential novel molecules and future possible targets for CD treatment. Although progress has been made in the understanding of specific corticotrope adenoma receptor physiology and recent clinical studies have detected improved effects with a combined medical therapy approach, there is a clear need for a more efficacious and better-tolerated medical therapy for patients with CD. A better understanding of the molecular mechanisms in CD and of HPA axis physiology should advance the development of new drugs in the future.

Key Words

- ▶ cortisol
- ▶ Cushing's disease
- ▶ ACTH
- ▶ pasireotide
- ▶ mifepristone
- ▶ ketoconazole
- ▶ LCI699
- ▶ cabergoline

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Introduction

Cushing's disease (CD) is caused by an adrenocorticotropin (ACTH)-secreting pituitary tumor that stimulates cortisol production by the adrenal glands. Morbidity and mortality are significantly increased if hypercortisolemia is left untreated. Transsphenoidal surgery, performed by an experienced neurosurgeon, is currently considered the first-line treatment. Medical treatment is commonly used to control the deleterious effects of persistent chronic glucocorticoid excess and 24-h urinary free cortisol (UFC) normalization still represents the gold standard to evaluate the efficacy of most medical treatments.

Glucocorticoid exerts effects through the glucocorticoid receptor (GR) and as the GR is expressed in almost

every human tissue, conditions of glucocorticoid excess, such as CD, result in deleterious effects on cell metabolism. Pharmacological agents can be classified as adrenal steroidogenesis blockers, centrally acting drugs, and GR antagonists. Recent research has evaluated chimeric compounds that work synergistically through membrane interaction or dimerization of both somatostatin receptors (SSTRs) and dopamine D2 receptors in corticotrope cells. Although no available medical agents surpass the efficacy of surgical therapy, new treatments acting directly at the pituitary adenoma have been approved for use and highlight the importance of targeting the pituitary corticotrope. A review of the

mechanisms of current and future medical treatment modalities for CD is provided.

Overview of CD therapy

Cushing's syndrome (CS) is characterized by chronic overproduction of cortisol resulting in significant morbidity and, when left untreated, increased mortality (Newell-Price *et al.* 2006, Dekkers *et al.* 2007). CS is classified as ACTH-dependent and -independent. The most common etiology (70–80%) of CS is CD, caused by an ACTH-secreting pituitary adenoma or, more rarely, by ectopic ACTH or corticotropin-releasing hormone (CRH) production that may result in corticotrope hyperplasia (Newell-Price *et al.* 2006, Biller *et al.* 2008). Chronic cortisol excess leads to a typical clinical phenotype (Table 1). Although epidemiological data on CD are limited, population-based studies indicate an incidence of 1.2–2.4 per million (Arnardottir & Sigurjonsdottir 2011, Bolland *et al.* 2011) with a prevalence of approximately 39 per million population (Feelders *et al.* 2012). Compared with the general population or patients with other pituitary adenomas, patients with hypercortisolism have a four times higher

mortality risk if untreated and cardiovascular disease remains the leading cause of death (Newell-Price *et al.* 2006, Dekkers *et al.* 2007, Feelders & Hofland 2013).

Transsphenoidal surgery is the first line therapy achieving 65–90% disease remission for microadenomas (tumors <1 cm; Biller *et al.* 2008, Tritos *et al.* 2011, Fleseriu 2012), and lower remission rates (<65%) for macroadenomas (tumors >1 cm) (Aghi 2008). The risk of CD recurrence can reach 25%, 3 years after surgery (Patil *et al.* 2008). Second and third line therapies such as a second pituitary surgery (Patil *et al.* 2008, Fleseriu 2012), pituitary irradiation (conventional and/or stereotactic) (Estrada *et al.* 1997, Tritos *et al.* 2011) and bilateral adrenalectomy (Young & Thompson 2005, Assie *et al.* 2007, Chow *et al.* 2008, Ritzel *et al.* 2013) have been utilized with variable results and specific complications.

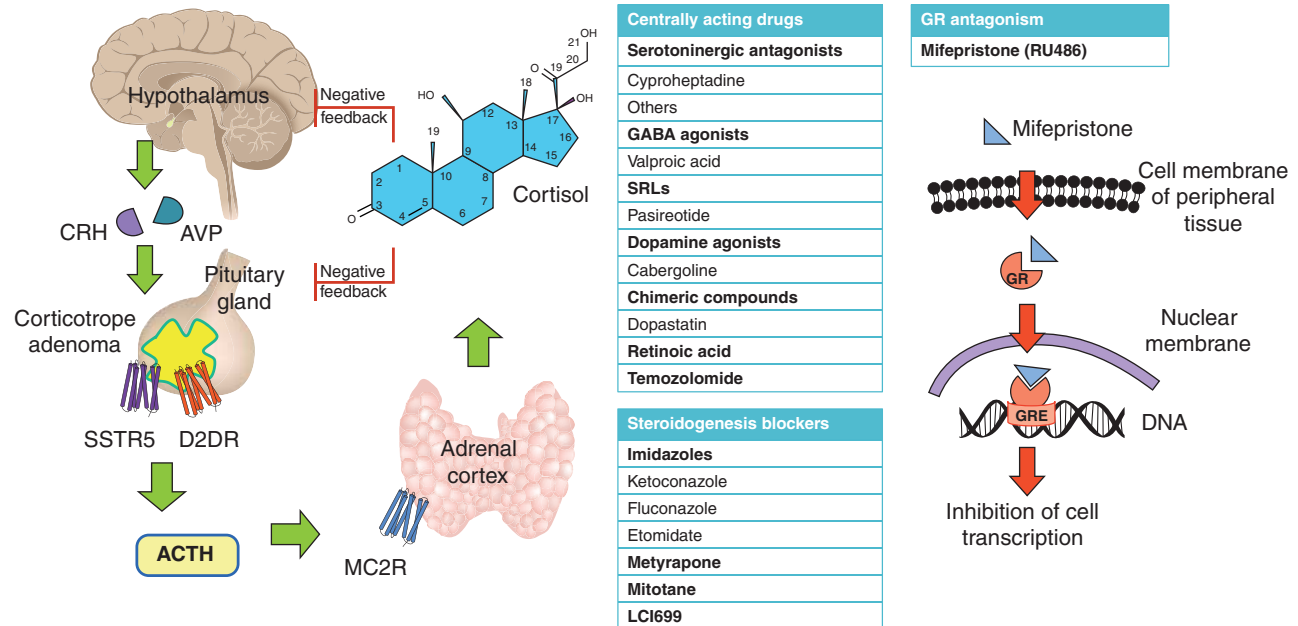
Targets for medical treatment of CD

The hypothalamus–pituitary–adrenal (HPA) axis is organized into three regions: the hypothalamus, pituitary gland, and adrenal glands (Fig. 1). CD is caused by a pituitary tumor; therefore, medical therapy should ideally

Table 1 Clinical features and associated morbidity observed in Cushing's syndrome

Clinical features	Frequency (%)
Increased weight (centripetal obesity, supraclavicular region, and upper back)	80–95
Skin changes (round face, facial plethora, and skin atrophy)	80–90
Decreased libido	25–90
Menstrual irregularity	75–80
Muscle proximal weakness	60–80
Hirsutism	70–75
Violaceous striae	55–65
Easy bruising	45–65
Associated morbidity	
Obesity	40–95
Hypertension	60–80
Glucose intolerance or diabetes mellitus	50–80
Osteoporosis	40–75
Psychiatric symptoms	50–70
Dyslipidemia	40–70
Increased infections and decreased wound healing	15–30
Renal calculi	15–20
Venous thromboembolism	10–20
Avascular necrosis in femoral head	5–10
Specific for Cushing's disease	
Headaches	0–37
Visual problems (bitemporal hemianopsia)	0–33
Other anterior pituitary hormone deficiencies	0–25
Alterations with severe hypercortisolism	
Weight reduction (with ectopic ACTH secretion by malignancy)	10–50
Hypoalbuminemia	15–35
Skin hyperpigmentation	10–15
Hypokalemia and metabolic alkalosis	4–10

Boscaro *et al.* (2001), Newell-Price *et al.* (2006), Biller *et al.* (2008), Bertagna (2006), and Greenman (2010).

**Figure 1**

The hypothalamus–pituitary–adrenal (HPA) axis and targets of drugs used for treating Cushing's disease. Under physiological conditions, cortisol synthesis and production are tightly regulated by the HPA axis. Adrenocorticotropin (ACTH)-producing cells in the anterior pituitary respond to hypothalamic corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP). After binding to melanocortin type 2 receptor (MC2R), ACTH induces the steroidogenic enzymes to increase the biosynthesis of cortisol and will decrease ACTH and CRH secretion. Pituitary corticotrope ACTH-secreting adenomas, however, function autonomously

and overstimulate cortisol production at the adrenal cortex. The pharmacotherapies for ACTH-secreting pituitary corticotrope adenomas are categorized by the site of action into three groups: i) centrally acting agents or neuromodulators, which inhibit ACTH release from pituitary adenomas, ii) adrenal steroidogenic inhibitors, which block one or several steps in cortisol biosynthesis and iii) the glucocorticoid receptor-blocking agent mifepristone. Green arrows indicate activation and red arrows/lines indicate inhibition.

target the corticotrope cell adenoma. However, as glucocorticoids represent the end hormone of the HPA axis and hypercortisolism induces comorbidities, steroidogenic inhibition was the first therapy used. Indications for medical therapy in patients with CD are summarized in Table 2.

Adrenal steroidogenesis blockers

Adrenal cortical atrophy was first documented in dogs treated with the insecticide dichlorodiphenyldichloroethane (DDD; Nelson & Woodard 1949). This observation led to the development of the use of *o,p'*-DDD or mitotane, initially for adrenal cancer and CS (Cueto & Brown 1958, Southren *et al.* 1961). Subsequently, amphenone B (Hertz *et al.* 1956, Thorn *et al.* 1956), aminoglutethimide (Camacho *et al.* 1967), metyrapone (Gower 1974), trilostane (Potts *et al.* 1978), and ketoconazole (Pont *et al.* 1982) were identified as steroidogenic inhibitors (Fig. 2 and Table 3).

Ketoconazole

Introduced as an antifungal agent, ketoconazole exerts endocrine side effects indicating its possible therapeutic efficacy in lowering cholesterol levels. Indeed, the imidazole derivative ketoconazole was noted to cause gynecomastia associated with lower plasma testosterone and cortisol values (Table 3; Pont *et al.* 1982). Ketoconazole was first used in the treatment of a patient with a cortisol-producing adrenal adenoma in 1983 (Engelhardt *et al.* 1983), and has been used off-label since then. Ketoconazole inhibits the side-chain cleavage complex (P450_{sc}, CYP11A1, or 20,22 desmolase), 11 β -hydroxylase, and 17 α -hydroxylase (Table 4) (Feldman 1986, Loli *et al.* 1986). As a result of effective inhibition of cholesterol side-chain cleavage and 17-hydroxylase/17,20 lyase activity, ketoconazole may reduce androgen synthesis; therefore, effects on hirsutism are favorable (Fig. 2). There are inhibitory effects on several cytochrome P450 enzymes, mainly CYP3A4, CYP2C9, and CYP1A2 (Feldman 1986, Felders *et al.* 2010a, Fleseriu & Petersenn 2012),

Table 2 Use of medical therapy in Cushing's disease – indications and needs

Before or after pituitary surgery
Preparation for surgery
Patients with contraindications for surgery
High operation risk
Patients unwilling to undergo surgery
After unsuccessful surgery
Amelioration or control of the metabolic effects of hypercortisolemia
Potentially life-threatening complications
Patients awaiting effects of pituitary radiation
Whenever a definitive treatment is delayed
Primary medical therapy
Low probability of surgical cure
Unfavorable localization
Invisible adenomas
Adenoma without optic chiasm compression

Fleseriu *et al.* (2007), Biller *et al.* (2008), Castinetti *et al.* (2008), Godbout *et al.* (2010), Fleseriu (2012), and Feelders & Hofland (2013).

but minimal effect on aromatase enzyme (Miller & Crapo 1993).

The first clinical reports on patients with CD described normalization of urinary or plasma cortisol values with ketoconazole doses of 600–1200 mg/day (Loli *et al.* 1986, Tabarin *et al.* 1991, Miller & Crapo 1993). Ketoconazole treatment is usually started at 200 mg twice a day, and biochemical effect is achieved at 600–1200 mg/day (Table 3; Nieman 2002, Fleseriu *et al.* 2012). It has been suggested that ketoconazole may also have inhibitory effects on ACTH secretion by corticotrope tumor cells as ACTH shows no significant increase despite confirmed reduction in cortisol levels (Loose *et al.* 1983, Loli *et al.* 1986, Jimenez-Rema *et al.* 1989, Tabarin *et al.* 1991). Escape from pharmacological control has been reported (Sonino *et al.* 1991). Administered as a monotherapy, ketoconazole decreases cortisol levels in 30–80% of patients (Sonino *et al.* 1991, Sonino & Boscaro 1999, Castinetti *et al.* 2008). Results from a recent large multi-center retrospective study ($n=200$) revealed normalization of UFC levels measured at the last follow-up in 49% of patients (Castinetti *et al.* 2014). Ketoconazole also blocks the GR at high concentrations in cultured hepatoma cells (Loose *et al.* 1983). Reportedly, hepatotoxicity (Sonino 1987, Sugar *et al.* 1987, Tabarin *et al.* 1991) was mild in the study and resolved after drug withdrawal (Castinetti *et al.* 2014). Ketoconazole has also been used in older patients (> 75 years of age) with good tolerance and disease control (Berwaerts *et al.* 1998).

The use of ketoconazole has been questioned after warnings from the European Medicine Agencies and the

US Food and Drug Administration (FDA) of potential hepatotoxicity (Castinetti *et al.* 2014). Long-term safety remains to be prospectively studied. In contrast to mitotane (which causes hypercholesterolemia), ketoconazole interferes with the conversion of lanosterol to cholesterol, leading to low cholesterol concentrations. Ketoconazole absorption requires an acidic environment, precluding the use of proton pump inhibitors or H₂ receptor blockers. Currently, drug availability is limited in many countries.

Fluconazole

Fluconazole appears to have similar effects to ketoconazole; however, data are limited. Fluconazole inhibits 11 β -hydroxylase and 17-hydroxylase activities (Fig. 2) and blocks cortisol production in primary cultures of human adrenocortical cells (van der Pas *et al.* 2012). Fluconazole has been shown to decrease 11-deoxycortisol production in H295R cells and reduce cortisol secretion in HAC15 cells and primary cultures (van der Pas *et al.* 2012). In cultures of normal adrenals, fluconazole suppressed corticosterone, 17-hydroxypregnenolone, and androstenedione levels, whereas concentrations of progesterone, deoxycorticosterone, and 11-deoxycortisol were increased (Fig. 2). Fluconazole slightly increased StAR protein mRNA expression (van der Pas *et al.* 2012). Results from clinical studies indicated that fluconazole at a dose of 100 mg twice a day successfully controlled UFC levels in two patients (Riedl *et al.* 2006). Together, the results of these *in vitro* and *in vivo* studies indicate that fluconazole can be used to control cortisol hypersecretion in patients with CD (Table 3).

Neither ketoconazole nor fluconazole were shown to affect the mRNA levels of steroidogenic enzymes or cell number; therefore, a single dose is unlikely to have a long-term effect (van der Pas *et al.* 2012).

Metyrapone

Metyrapone (SU-4885; Metopirone) inhibits 11 β -hydroxylase (Fig. 2) (Liddle *et al.* 1959) and 17 α -, 18-, and 19-hydroxylase (Table 4) (Gower 1974). Cortisol levels decrease after 2 h of the first dose (Verhelst *et al.* 1991). Doses range from 500 mg to 4.5 g/day, or 6 g/day in divided doses (usually starting with 250 mg four times daily, Table 3; Nieman 2002, Fleseriu 2012). Notably, and similar to ketoconazole, rapid up-titration of metyrapone is essential to achieve a full effect (Kamenicky *et al.* 2011, Castinetti *et al.* 2014). The strong cortisol-lowering effect of metyrapone can be accompanied by the loss of negative

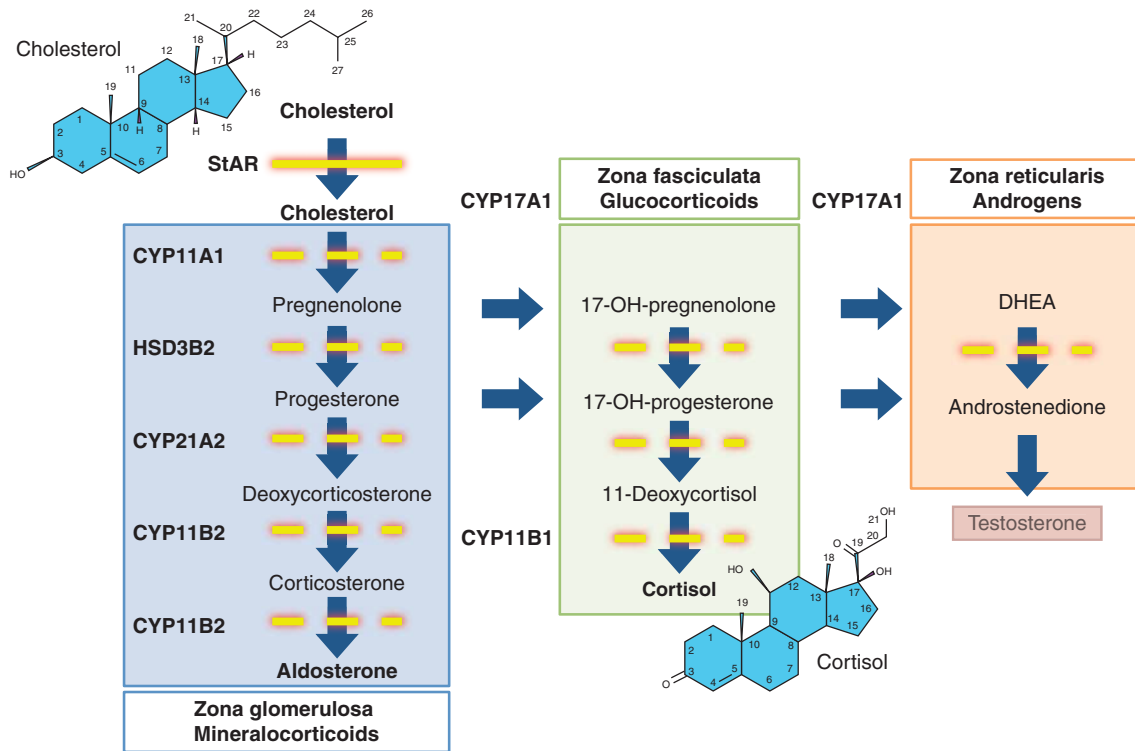


Figure 2

Adrenocortical steroidogenic pathways. A simplified diagram of adrenal steroidogenesis is depicted. Cushing's disease is almost always caused by a pituitary corticotrope adenoma that oversecretes corticotropin (ACTH). ACTH stimulates the adrenal gland to start steroid synthesis. After activation of MC2R by ACTH, the StAR protein is phosphorylated. Then, StAR facilitates cholesterol transport across the mitochondrial inner membrane. Cholesterol is the common precursor of the steroid molecules and, after several enzymatic reactions, is ultimately converted into

biologically active aldosterone, cortisol, or androstenedione that is further processed to testosterone in testicles. The zona glomerulosa, fasciculata, and reticularis are the three adrenal cortex histological zones, which synthesize steroid hormones with mineralocorticoid, glucocorticoid, or androgenic properties respectively. Enzyme nomenclature is given in detail in Table 1. CYP11A1, CYP11B1, and CYP11B2 are located in the mitochondria, and the remaining enzymes are located in the smooth endoplasmic reticulum.

feedback and ACTH inhibition (Fig. 1). Increments in ACTH secretion may override steroidogenic blockade that may occur during the first month following initiation of treatment. Nevertheless, prolonged metyrapone activity in patients with CD has been shown, despite a rise in plasma ACTH levels (Verhelst *et al.* 1991). ACTH also overstimulates production of adrenal androgen and mineralocorticoid precursors (e.g. desoxycorticosterone). Moreover, aldosterone biosynthesis is more severely affected than that of cortisol (Gower 1974). Therefore, several side effects related to metyrapone treatment limit its clinical use in patients with CD (Table 3). However, combination therapy has been proposed to improve tolerance and increase efficacy (Table 5; Verhelst *et al.* 1991, Kamenicky *et al.* 2011). Despite safety concerns about the use of this drug in pregnant women, metyrapone has been used sporadically during pregnancy (Lindsay *et al.* 2005, Karaca *et al.* 2010). Additional long-term studies with metyrapone

in patients with CD are warranted. Currently, metyrapone has limited availability in most countries.

Mitotane

1-(*o*-Chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD) (Lysodren; mitotane), an insecticide analog of dichlorodiphenyltrichloroethane has been extensively used to treat all forms of hypercortisolism (Boscaro *et al.* 2001). Mitotane's mechanism of action was initially described in animal studies as lipid accumulation and atrophy of fasciculate and reticularis regions of the adrenal cortex. Effects on the zona glomerulosa were only observed after prolonged therapy (Cueto & Brown 1958). Within 12 h of treatment in dogs, electron microscopy revealed rupture of mitochondrial cristae, followed by mitochondrial swelling, lysis, and cell death (Miller & Crapo 1993). Conversion of cholesterol to pregnenolone was markedly impaired indicating that

Table 3 Doses and relevant side effects of drugs used in the medical treatment for Cushing's disease

Steroidogenesis inhibitors		
Ketoconazole	400–1200 mg/day	Hepatitis, cholestasis, gynecomastia, gastrointestinal upset, edema, skin rash. Hypogonadism in men. Fluconazole may be better tolerated
Fluconazole	200 mg/day	
Metyrapone	0.5–4.5 g/day	Acne, hirsutism, lethargy, dizziness, ataxia, nausea, hypertensive crisis, hypokalemia, edema, and adrenal insufficiency
Mitotane	2–5 g/day	Gastrointestinal distress, impaired concentration and dizziness, gynecomastia, hepatitis, cholestasis, hyperlipidemia, prolongation of bleeding time, and adrenal insufficiency. Effect antagonized by spironolactone
Etomidate	Bolus 0.03 mg/kg i.v.; followed by 0.1–0.3 mg/kg per h	Nephrotoxicity, sedation, pain at the infusion site, anaphylactic reactions, coughing, hiccups, nausea, vomiting, and myoclonus
LCI699	4–100 mg/day (investigational drug)	Fatigue, nausea, headache, and hypokalemia
Centrally acting drugs		
Cabergoline	0.5–7 mg/week	Nasal congestion, nausea, postural hypotension, and headache. Gradual increase minimizes side effects
Bromocriptine	1–30 mg/day	
Pasireotide	750–2400 µg/day	
Retinoic acid	10–80 mg/day (research drug)	Gastrointestinal-related conditions, hyperglycemia, cholelithiasis, nausea, abdominal pain, fatigue, headache, hypotension, and adrenal insufficiency
Glucocorticoid receptor antagonist		
Mifepristone	300–1200 mg/day	Xerophthalmia and arthralgias
		Hypokalemia, worsening of hypertension, adrenal insufficiency, endometrial hyperplasia, fetal loss, gastrointestinal complaints, fatigue, nausea, headache, arthralgia, vomiting, and edema. Contraindicated in women planning pregnancy

Jeffcoate *et al.* (1977), Robinson *et al.* (1987), Verhelst *et al.* (1991), Castinetti *et al.* (2009), Fleseriu *et al.* (2012), Heyn *et al.* (2012), Nieman (2002), and Feelders & Hofland (2013).

side-chain cleavage of cholesterol was the major enzymatic step affected inhibiting the cytochrome P450 enzymes CYP11A1 (mitochondrial side-chain cleavage enzyme), 11 β -hydroxylation (CYP11B1), and 18-hydroxylation (CYP11B2), and non-P450 enzymes (3 β -hydroxysteroid dehydrogenase) (Fig. 2; Miller & Crapo 1993). Mitotane also stimulates CYP3A4 expression, reducing cortisol bioavailability (Kroiss *et al.* 2011). Therefore, mitotane has three effects: i) adrenocortolytic, ii) modification of steroid metabolism, and iii) direct inhibition of steroid biosynthesis. Although used to treat adrenocortical carcinoma, mitotane has proven effective in patients with CD (Bledsoe *et al.* 1964, Baudry *et al.* 2012). Mitotane displays a relatively slow onset of action compared with other steroidogenesis inhibitors and saturation can be expected 2–3 months after initiation of therapy (Luton *et al.* 1979, Fleseriu & Petersenn 2012). Overall, 80% of patients achieve normalization of urinary markers; however, 60% relapsed after therapy withdrawal indicating a low level of adrenolytic action (Luton *et al.* 1979, Schteingart *et al.* 1980). Sustained remission after mitotane discontinuation has been reported in 30% of patients measured at a mean follow-up of 37 months (Miller & Crapo 1993). Doses are approximately 4 g/day, with a gradual

increment from 0.5 to 1 g/day (Table 3; Luton *et al.* 1979). Results from a recent retrospective study revealed remission in 48 (72%) out of 67 patients treated for long-term CD after a median of 6.7 (5.2–8.2) months (Baudry *et al.* 2012) at lower doses compared with adrenal cancer.

Despite its effectiveness, mitotane therapy is complicated by several side effects (Table 3). Owing to an accelerated metabolism of exogenous steroids, especially hydrocortisone, replacement doses must be increased to avoid adrenal crises (Robinson *et al.* 1987). Mitotane may lead to restoration of gonadal function and fertility; therefore, effective contraception is advisable for female patients (Miller & Crapo 1993). Mitotane is stored in adipose tissue for 2 years after administration ends and should not be used in women considering pregnancy within 5 years of discontinuation (Leiba *et al.* 1989). Owing to the stimulatory effect of mitotane on cortisol-binding globulin levels (Nader *et al.* 2006), serum cortisol measurements are not useful and monitoring of urinary or serum free cortisol is the best index of response (Alexandraki *et al.* 2010). Replacement therapy requirements are higher than normal, due to interference of hormone-binding proteins as well. Mitotane combined with pituitary irradiation was

Table 4 Enzymes that catalyze the initial reaction in the pathways of steroid hormone biosynthesis

Localization and common name	Nomenclature ^a		Inhibitors	References
	Previous	Current		
Mitochondrial StAR protein	StAR	StAR	Imidazoles ^b Imidazoles Etomidate Mitotane Aminoglutethimide	Walsh <i>et al.</i> (2000) Camacho <i>et al.</i> (1967), Schulte <i>et al.</i> (1990), and Miller & Crapo (1993)
Side-chain cleavage (sc) enzyme or 20,22 desmolase	P450scc	CYP11A1		
11 β -hydroxylase	P450 C11	CYP11B1		
Aldosterone synthase, 18-hydroxylase	P450 C11AS	CYP11B2	Mitotane Aminoglutethimide Metyrapone	Camacho <i>et al.</i> (1967), Gower (1974), Feldman (1986), Loli <i>et al.</i> (1986), and Miller & Crapo (1993) Camacho <i>et al.</i> (1967), Gower (1974), Miller & Crapo (1993), and Calhoun <i>et al.</i> (2011)
Smooth endoplasmic reticulum 17 β -hydroxysteroid dehydrogenase, 3 β -hydroxysteroid dehydrogenase	3 β -HSD	3 β -HSD	Trilostane	Potts <i>et al.</i> (1978), Dewis <i>et al.</i> (1983), and Engelhardt & Weber (1994)
17 α -hydroxylase/17,20 lyase	P450 C17	CYP17	Imidazoles Aminoglutethimide Metyrapone	Camacho <i>et al.</i> (1967), Gower (1974), Feldman (1986), Loli <i>et al.</i> (1986), and Miller & Crapo (1993)
21-Hydroxylase	P450 C21	CYP21A2	Aminoglutethimide	Camacho <i>et al.</i> (1967)
Aromatase	P450 aro	CYP19	Aminoglutethimide	Camacho <i>et al.</i> (1967)

^aP450scc or CYP11A1, i.e. gene family 11, subfamily A, polypeptide 1.

^bImidazoles = ketoconazole, fluconazole, econazole, and/or miconazole.

shown to be efficacious in non-controlled clinical trials (Luton *et al.* 1979, Schteingart *et al.* 1980).

Etomidate

The imidazole anesthetic agent etomidate (Amidate or Hypnomidate) was observed to decrease postoperative cortisol values in patients during anesthesia (Feldman 1986). Subsequently, it was discovered that etomidate inhibits the 11 β -hydroxylase enzyme (CYP11B1), 17 α -hydroxylase (CYP17A1), and the cholesterol side-chain cleavage complex (P450scc, or CYP11A1, or 20,22 desmolase), thus blocking multiple steps of steroidogenesis (Fig. 2). It is the only parenteral steroidogenesis inhibitor available and provides rapid hypercortisolemia control (Allolio *et al.* 1988, Schulte *et al.* 1990). An i.v. bolus injection of etomidate at a low non-hypnotic dose (0.03 mg/kg) followed by constant infusion of 0.3 mg/kg per h for 24 h (Table 3) decreases serum cortisol in a dose-dependent manner with significant suppression after the first 5 h with a maximum effect after 11 h (Allolio *et al.* 1988, Schulte *et al.* 1990). Glucocorticoid replacement to prevent adrenal insufficiency is warranted after 24 h of etomidate infusion. Clinical use of etomidate in CS has been limited by sedative side effects, but could be safe and effective in significant biochemical

disturbance, sepsis, severe psychosis, and in preoperative instability (Heyn *et al.* 2012, Preda *et al.* 2012).

LCI699

First characterized as an aldosterone biosynthesis inhibitor for primary aldosteronism and essential hypertension, LCI699 is an 18-hydroxylase (aldosterone synthase) inhibitor blocking the conversion of hydroxycorticosterone to aldosterone. It also inhibits 11 β -hydroxylase (CYP11B1) in a similar manner to the R-enantiomer of fadrozole (FAD286) that blocks the hydroxylation of deoxycortisol to cortisol as well as CYP11B2 blocking the conversion of deoxycorticosterone to corticosterone (Fig. 2) (Calhoun *et al.* 2011). LCI699 is currently under investigation as a treatment for CD (Tritos *et al.* 2011, Feelders & Hofland 2013). In a phase II proof of concept study of LCI699, rapid UFC normalization in 11 out of 12 patients with CD, all achieving >50% reduction in baseline UFC, was observed. Doses ranged from 4 to 100 mg/day for 10 weeks (Bertagna *et al.* 2014). As expected, ACTH increased; 45% of cases had ACTH more than twice that of baseline. Most adverse events were mild or moderate (Table 3). Based on these promising results, a larger phase III trial is awaited.

Table 5 Overview of agents that have been tested as combination drug therapy for Cushing's disease. Doses: cabergoline from 0.5 to 3 mg/week, ketoconazole 200 to 1200 mg/day, pasireotide 300 to 750 µg/day, mitotane 3 to 5 g/day, metyrapone 3 to 4.5 g/day, and octreotide 300 to 1500 µg/day. The studies are described in the text

References	Compounds	Patients (n)	UFC normalization (%)	
			Monotherapy	Combination
Barbot <i>et al.</i> (2014)	Ketoconazole + cabergoline	14	20–30	80
Feelders <i>et al.</i> (2010b,c)	Pasireotide + cabergoline + ketoconazole	17	30	50 P+C 90 P+C+K
Vilar <i>et al.</i> (2010)	Cabergoline + ketoconazole	12	25	70
Total/average	–	43	27	73
Severe hypercortisolism/aggressive tumor				
Vignati & Loli (1996)	Ketoconazole + octreotide	4	0	0
Bode <i>et al.</i> (2010)	Temozolomide + pasireotide	1	0	100
Kamenicky <i>et al.</i> (2011)	Mitotane + metyrapone + ketoconazole	11 CS (4 CD)	0	100
Total/average	–	16	0	66

UFC, urinary free cortisol.

Trilostane (Vetoryl), an inhibitor of 3β-hydroxysteroid, was withdrawn from human use in the USA in 1994 (Potts *et al.* 1978, Dewis *et al.* 1983, Engelhardt & Weber 1994). Aminoglutethimide (Cytadren; Camacho *et al.* 1967, Misbin *et al.* 1976) is rarely used in the treatment of patients with CD. The mechanisms of action, common doses, and main adverse effects of aminoglutethimide are listed in Tables 2 and 3.

Centrally acting drug treatments

Human corticotrope adenomas display responsiveness to neurohumoral influences such as dexamethasone suppression, lysine vasopressin, thyrotropin-releasing hormone, and metyrapone administration. Dexamethasone is a synthetic glucocorticoid extensively used to demonstrate the sensitivity of HPA axis negative feedback to glucocorticoids for differential diagnosis of CS. If the HPA axis is normal, any supraphysiological dose of dexamethasone is sufficient to suppress pituitary ACTH secretion. ACTH secretion can also be suppressed in most patients with CD as pituitary corticotrope adenomas are only relatively resistant to inhibition of glucocorticoid negative feedback. Hypophyseal ACTH secretion can be influenced by serotonin antagonists, dopamine or gamma-amino butyric acid (GABA) agonists, somatostatin receptor ligands (SRLs), retinoic acid, and temozolomide (Fig. 1). In addition to biochemical effects, CD has been associated with periodic hormonogenesis (cyclical CD) or spontaneous remission, indicating that some cases of CD may be due to altered neuroregulatory influences (Van Cauter & Refetoff 1985, Dickstein *et al.* 1991, Colao *et al.* 1997, Nieman 2002).

Somatostatin receptor ligands

Somatostatin Somatostatin (SST) is a cyclic peptide with a circulating half-life of <3 min (Brazeau *et al.* 1973). The name SST originates from a supposed 'specific' function as an inhibitor of somatotropin (growth hormone (GH)) release, thus somatotropin release-inhibiting factor. In the CNS, SST has a key inhibitory action in the secretion of GH (Brazeau *et al.* 1973), prolactin (PRL; Vale *et al.* 1974), thyrotropin (Siler *et al.* 1974), and ACTH (Richardson & Schonbrunn 1981, Lamberts *et al.* 1989a,b) from the anterior pituitary gland. At the peripheral nervous system level, SST plays a regulatory role in the gastrointestinal tract inhibiting flow from the gallbladder, bowel motility and gastric emptying, smooth muscle contraction, and nutrient absorption from the intestine as well as in the exocrine pancreas. SST also inhibits the release of glucagon (Boden *et al.* 1986), insulin (Alberti *et al.* 1973), and pancreatic polypeptide (Koerker *et al.* 1974).

SST exerts action through five G protein-coupled receptor subtypes: SSTR1–SSTR5 (Batista *et al.* 2006, Ben-Shlomo *et al.* 2010, Cuevas-Ramos & Fleseriu 2014). The majority of corticotrope adenomas (>85%) express *SSTR2* and *SSTR5* mRNA, and to a lesser extent *SSTR1* (63%) (Miller *et al.* 1995). The membrane density of SSTR subtypes, particularly *SSTR2*, is affected by hypercortisolism (Schonbrunn 1982, Stalla *et al.* 1994, de Bruin *et al.* 2009). In contrast, *SSTR5* expression appears to be relatively unaffected by high cortisol levels. Therefore, in patients with active CD, *SSTR5* is predominantly expressed compared with *SSTR2* (de Bruin *et al.* 2009).

Somatostatin receptor ligands The potent antisecretory properties of SST have made it an important

pharmacological target for hormonal hypersecretion treatment. However, its short half-life, the multiple and simultaneous actions in different organs, and the need for parenteral administration hampered its clinical use. SRLs have originated after manipulation of SST key structural characteristics, which allowed several reductions of the above disadvantages. Recently, an update on SRLs and SRL's resistance has been published (Cuevas-Ramos & Fleseriu 2014).

Octreotide and lanreotide are SSTR2 SRLs with low activity in suppressing ACTH in patients with CD (Lamberts *et al.* 1989a,b, Biller *et al.* 2008). After eucortisolemia restoration, SSTR2 expression recovers, becoming similarly abundant to SSTR5, thus improving treatment responsiveness (Feelders *et al.* 2010a, van der Pas *et al.* 2013). The SSTR2 mRNA expression, however, did not correlate with protein levels. The discrepancy between mRNA and protein levels may be caused by persistent cortisol-induced disturbed translation of SSTR2 mRNA and GR on the adenoma cells still exposed to sufficiently high levels of circulating cortisol to suppress the transcription/translation of the SSTR2 gene (van der Pas *et al.* 2013). The recovery of SSTR2 expression may have therapeutic implications, as it may allow sequential treatment with SSTR2 ligands after induction of partial or complete remission with other drugs. Nevertheless, the predominant expression of SSTR5 mRNA in cultured human corticotrope adenoma cells prompted an alternative approach using a SSTR5 ligand (Batista *et al.* 2006).

Pasireotide

Pasireotide (SOM230; Signifor) is an SRL that principally binds to SSTR5, in addition to SSTR1, SSTR2, and SSTR3 (Ben-Shlomo *et al.* 2010). Pasireotide has a 40-fold higher affinity for SSTR5 than octreotide (Murray *et al.* 2004). After pasireotide treatment, in primary cultures of human corticotrope tumors, cell proliferation and ACTH secretion were suppressed (Batista *et al.* 2006). Notably, dexamethasone pre-treatment did not reduce cell sensitivity to pasireotide, indicating that SSTR5 is resistant to negative control by glucocorticoids (Hofland *et al.* 2005). Moreover, SSTR2 mRNA expression was reduced after dexamethasone treatment, whereas SSTR5 mRNA was not significantly affected (van der Hoek *et al.* 2005). As pituitary corticotrope ACTH-secreting adenomas predominantly express SSTR5, results obtained using primary cell cultures (Hofland *et al.* 2005, Batista *et al.* 2006), animal models (Silva *et al.* 2005, Ben-Shlomo *et al.* 2010), and clinical studies have confirmed ACTH suppression after pasireotide treatment.

Overexpression of SSTR2 or SSTR5 in mouse AtT20 corticotrope adenoma cells resulted in pasireotide suppressive effects through SSTR5 but not SSTR2 (Ben-Shlomo *et al.* 2009). In a phase II study, proof-of-concept, open-label, single-arm, and multicenter study, using s.c. pasireotide at 600 µg twice a day for 15 days, patients who displayed normalized UFC levels with pasireotide treatment showed a 1.8-fold higher plasma concentration and 1.3-fold higher plasma exposure in comparison with non-responders, indicating that the clinical response required a plasma pasireotide level above a certain threshold for an optimal response (Boscaro *et al.* 2009). A subsequent double-blinded, randomized, multicenter phase III trial with pasireotide 600–900 µg twice a day revealed UFC reduction in most patients (Colao *et al.* 2012) and normalization in approximately one-quarter of patients. The rate of UFC normalization was higher (~50%) in patients with mild CD. Responders can be identified within approximately 2 months of treatment (Colao *et al.* 2012). Serum and salivary cortisol and plasma ACTH levels were also reduced with notable clinical improvement including blood pressure, lipids, and quality of life (Colao *et al.* 2012). An interesting observation that warrants further investigation is the reduction in tumor volume after 12 months of high-dose pasireotide (900 µg twice daily) treatment in patients with CD (Colao *et al.* 2012).

Except for a higher degree and frequency of hyperglycemia, adverse effects are similar to those of other SRLs (Table 3). Hyperglycemia-related adverse events occurred in 73% of patients and 6% of patients discontinued the study treatment because of such events. Glucose and HbA1c levels increased soon after the initiation of treatment with pasireotide, necessitating the administration of medications to manage these complications. Of the patients who did not have diabetes at baseline 48% had a HbA1c level of 6.5% or more at the end of the study.

The mechanism of pasireotide-induced hyperglycemia in healthy volunteers is mediated by incretin hormone, glucagon-like peptide 1 (GLP1) and gastric inhibitory polypeptide, reduction and therefore insulin secretion decline. Glucagon secretion, however, seems to be mildly inhibited (Henry *et al.* 2011). A dipeptidyl peptidase 4 inhibitor or a GLP1 agonist was shown to be particularly effective in countering increased glucose levels in healthy volunteers; however, further studies in CD are required (Henry *et al.* 2011, Colao *et al.* 2014).

The absence of expression of SSTR5 mRNA in 40% of ACTH-secreting pituitary adenomas (de Bruin *et al.* 2009) might explain the different responses to pasireotide. It remains to be determined whether any correlation exists

between the degree of SSTR2/SSTR5 receptor expression and the *in vivo* response to pasireotide in patients with CD.

Pasireotide was approved (in the USA and in Europe) for the treatment of patients with CD for whom surgery has failed or is not possible (Fleseriu & Petersenn 2012, Feelders & Hofland 2013, Colao *et al.* 2014).

Additionally, somatropin (DG3173) is a novel SRL that selectively binds SSTR2, SSTR4, and SSTR5. It has a well-demonstrated effect in GH suppression (Plöckinger *et al.* 2012, Fleseriu & Petersenn 2013). Further research is necessary to evaluate the effects on ACTH.

Dopamine agonists

Dopamine is the main catecholamine neurotransmitter in the human brain involved in the regulation of locomotor activity, food intake, and endocrine function (Missale *et al.* 1998). The dopamine receptor (DR) family consists of five receptor subtypes, two with preferentially stimulatory effects (D1 and D4) and three with inhibitory properties (D2, D3, and D5) (Missale *et al.* 1998). D2 (DRD2) and, to a lesser extent, D4 (DRD4) are the DR subtypes that are expressed in the anterior pituitary gland. The D2 subtype has a long (D2long) and a short (D2short) isoforms and both variants are expressed in lactotrope and melanotrope cells (Pivonello *et al.* 2004). Inhibition of PRL secretion is the most well-known action of dopamine in the anterior pituitary. However, results of recent studies have indicated that the D2 receptor is expressed in 80% of corticotrope adenomas (Lamberts *et al.* 1980, Pivonello *et al.* 2004, Gatto & Hofland 2011). The D2 receptor is not negatively regulated by cortisol (de Bruin *et al.* 2009). Consistently, dopamine agonists, bromocriptine, and cabergoline have shown inhibitory effects *in vitro* on ACTH secretion in corticotrope tumor cells (Pivonello *et al.* 2004) and apoptosis in an ACTH-secreting mouse cell line; therefore, *in vivo* effectiveness can also be suspected (Lamberts *et al.* 1980, Yin *et al.* 1994). In the pituitary gland, pro-opiomelanocortin (POMC) is cleaved by prohormone convertases (PCs) 1 and 2 (Bertagna 1994). PC1 (PCSK1) is expressed in both the anterior and intermediate pituitary lobes, whereas PC2 (PCSK2) expression is restricted to the intermediate lobe, which is believed to regress soon after birth and, therefore, is absent in adults (Bertagna 1994, Iino *et al.* 2010). Lamberts and colleagues showed that D2 receptor agonist-responsive tumors located in the region consisted of basophilic adenomatous tissue or multiple microadenomas accompanied by hyperplastic cell nests (Lamberts *et al.* 1980, 1982). PC2 expression at the intermediate lobe, and the phosphorylation of its cell-signaling molecule Akt, have been identified as clinical

markers of an increased rate of tumor growth in CD (Iino *et al.* 2010). Compared with corticotrope pituitary tumors that were histologically classified as pure adenomas, tumors identified as adenomatous hyperplasia or hyperplasia of ACTH-producing cells have higher PC2 expression and, although not statistically significant, higher neurofilament (NF) protein expression. Together, these results led to the hypothesis that CD may originate not only from corticotrope adenomas of the anterior lobe, but also from an adenoma or adenomatous hyperplasia of ACTH-producing cells of the intermediate zone because the PC2 enzyme is predominantly expressed in this region of the pituitary gland (Iino *et al.* 2010). In addition, increases in the levels of alpha-melanocyte-stimulating hormone, which is a PC2 processing product, in the inferior petrosal sinus were higher in these patients. Finally, increased NF expression indicates close proximity of these lesions to the posterior pituitary lobe. PAX7, a protein restricted to the melanotrope lineage of the intermediate pituitary lobe that is not expressed in the anterior corticotrope cells, was identified as a key regulator that drives POMC-positive pituitary cells toward melanotrope differentiation (Budry *et al.* 2012). In the intermediate lobe, cells of rat pituitary glands lacking PAX7 showed downregulation of melanotrope-specific genes (e.g. *POMC*, *PC2*, and *D2R (DRD2)*) and these cells switched towards the corticotrope lineage. Similar observations were made using AtT20 mice corticotrope adenoma cells (Budry *et al.* 2012). The hypothesis that adenomatous hyperplasia or corticotrope hyperplasia is particularly sensitive to dopamine D2 receptor agonists (Lamberts *et al.* 1982), and SSTR5 expression appears to be lower than that in the pure adenomas awaits further confirmation (Budry *et al.* 2012).

Cabergoline

Cabergoline (Caberlin, Dostinex, or Cabaser) is a D2 receptor agonist, currently approved in the USA to treat hyperprolactinemia. It has a long half-life and a very high affinity and specificity for D2 receptors. Cabergoline has been evaluated as a potential therapy in patients with CD who have failed surgery (de Bruin *et al.* 2009). Initial response is up to 75% (Pivonello *et al.* 2009); however, 30–40% of these patients will have a sustained response over a 2-year period (Pivonello *et al.* 2009, Lila *et al.* 2010). A retrospective study reported complete response to cabergoline therapy in 37% of cases using an initial dose of 0.5–1 mg/week with a further increment up to 6 mg/week. At a mean dose of 2.1 mg/week, 30% (nine out of 30 cases) persisted with sustained control after 3 years (Godbout *et al.* 2010). Acute cabergoline response does not predict long-term disease control.

Approximately 25% of patients present with cabergoline escape phenomenon at 2–5 years. Doses range from 0.5 to 7.0 mg/week (median 3.5 mg) raising potential concerns about long-term safety (Table 3). By activating valvular fibroblasts through serotonin receptor 2B, valve calcification was observed in patients with prolactinomas treated with cabergoline. However, this finding was not associated with cardiac valve dysfunction after 2 years of follow-up (Delgado *et al.* 2012). Long-term studies, however, are required to elucidate potential cardiac involvement at long-term high doses.

Bromocriptine

The effectiveness of bromocriptine (Parlodel, Cycloset, or Brotin) was initially reported in small studies of patients with CD (Lamberts & Birkehaager 1976, Miller & Crapo 1993). Out of 23 patients with CD treated with 1.25–30 mg/day for 3–180 weeks, approximately 40% achieved urinary or plasma glucocorticoid levels normalization, and ACTH levels decreased by more than 50% in 20% of patients (Table 3; Miller & Crapo 1993). Subsequent consecutive studies on the effects of bromocriptine in patients with CD have not confirmed relevant efficacy (Biller *et al.* 2010).

Chimeric compounds

Corticotrope cells coexpress both SSTRs and D2 receptors, which work synergistically through membrane interaction or dimerization (Fig. 3; Rocheville *et al.* 2000, Ren *et al.* 2003, de Bruin *et al.* 2009). Studies on different tumor models, including pituitary adenomas, revealed a higher potency of BIM-23A779, BIM-23A760, and BIM-23A781 (chimeric molecules containing both SST and dopamine structural elements) in controlling tumor cell growth (Ferone *et al.* 2013). Further studies of patients with CD are required.

Retinoic acid

Retinoic acid type II nuclear receptors are important drug targets for cancer therapy. In animal studies and *in vitro* experiments, an effective inhibitory effect has been shown on corticotrope tumor growth, *POMC* transcription, plasma ACTH, and corticosterone secretion from human ACTH-secreting pituitary tumors, but not in normal cells (Fig. 1). Retinoic acid also increased caspase-3-activity-induced cell death (Labeur *et al.* 2009). Retinoic acid exhibits peripheral activity at the level of the adrenal and induced bone morphogenetic protein 4 (BMP4), a member of the

transforming growth factor beta superfamily that plays a central role during pituitary organogenesis and transcription (Labeur *et al.* 2009). The antiproliferative action of retinoic acid is considered to be mediated by BMP4. Such preliminary results promoted a randomized treatment with retinoic acid vs ketoconazole in dogs with CD (Labeur *et al.* 2009). Recently, seven patients with CD were treated with 10–80 mg of retinoic acid in a longitudinal, non-controlled, non-randomized, multicenter study (Pecori Giraldi *et al.* 2012). After 6–12 months, three patients displayed normalized UFC with mild adverse effects (Table 3). These results need further confirmation in larger studies.

Temozolomide

Temozolomide (Temodar, Temodal, or Temcad) is an orally administered second-generation alkylating chemotherapeutic agent. It is a methyl-triazenoimidazole-carboxamide derivative that methylates DNA at the O6 position of guanine causing a mismatch with thymine during the next DNA replication cycle, leading to cell apoptosis (Fig. 1). Drug efficacy relies on O6-methylguanine-DNA methyltransferase (MGMT) enzyme activity. MGMT reverses alkylation, and it has been initially suggested that low MGMT expression could predict a better response to temozolomide. After treatment with temozolomide, tumors become soft and friable, and display better differentiation, less mitoses, and a lower Ki-67 index, indicative of surgical benefits as well as a reduction in cell proliferation (Syro *et al.* 2011). Temozolomide could be an efficacious drug in cases of aggressive CD, but the predictive role of MGMT expression remains to be determined (Syro *et al.* 2011, Dillard *et al.* 2011).

Cyproheptadine

Cyproheptadine (Periactin or Peritol) has anti-serotonergic, anti-histaminic, and anti-cholinergic properties, and it has been studied for treatment of CD and Nelson's syndrome since 1975 (Krieger *et al.* 1975, Nieman 2002, Fleseriu 2012). In normal subjects, cyproheptadine may alter the feedback regulation of ACTH to different stimuli such as metyrapone and insulin-induced hypoglycemia (Cavagnini *et al.* 1975). These responses are independent of the effects on basal ACTH levels, and patients treated with cyproheptadine therapy have documented normalization of low-dose dexamethasone suppression, metyrapone response, insulin tolerance test, CRH response, and diurnal cortisol patterns (Ferrari *et al.* 1977). It has been postulated that ACTH secretion was

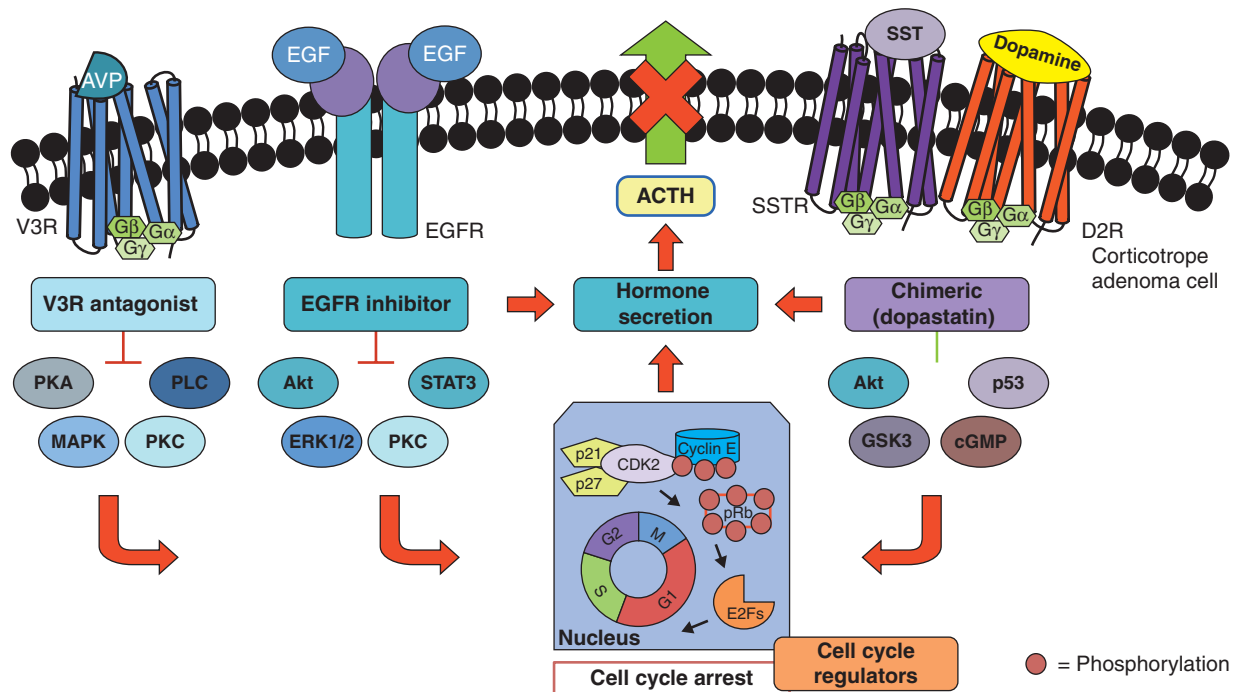


Figure 3

Potential novel therapeutic targets for CD. From left to right; the effect of V3 receptor-specific antagonists that could lead to a new class of agents that can suppress ACTH secretion in corticotrope adenomas (Ferone *et al.* 2013). After blocking of EGFR, a tyrosine kinase receptor, POMC expression is attenuated, and inhibition of corticotrope cell proliferation and apoptosis can be induced (Fukuoka *et al.* 2011). Through membrane interaction or dimerization (Rocheville *et al.* 2000), the G-protein-coupled somatostatin receptor (SSTR) and dopamine D2 receptor (D2R) have a synergistic effect on controlling tumor cell growth and ACTH secretion (de Bruin *et al.* 2009, Ferone *et al.* 2013). The main proteins of each receptor

signaling pathway are depicted. The leading effect of such pathways is cell cycle arrest with a decrease in tumor growth. Interestingly, using a cyclin-dependent kinase 2 (CDK2)/cyclin E inhibitor in animal models, the ACTH and corticosterone levels were suppressed, and xenografted pituitary tumor growth was restrained (Liu *et al.* 2011b). Red lines/arrows indicate inhibition, whereas green lines/arrows indicate induction. PKA, protein kinase A; PLC, phospholipase C; PKC, protein kinase C; Akt, protein kinase B; ERK, extracellular signal-regulated kinases; GSK3, synthase kinase 3 beta; Rb, retinoblastoma protein; E2F, E2 transcription factors.

also under serotonergic control and a direct inhibitory effect on CRH and arginine vasopressin (AVP) secretion from the hypothalamus has been confirmed (Suda *et al.* 1983). However, clinical studies showed disappointing results (Nieman 2002, Biller *et al.* 2008). Control of CD activity with cyproheptadine has been described in case reports (Tanakol *et al.* 1996) at doses varying from 12 to 24 mg/day. Main side effects are somnolence, hyperphagia, and weight gain. Other serotonin antagonists, metergoline, ketanserin, and ritanserin, have been evaluated in very few patients with limited results (Cavagnini *et al.* 1975, Sonino *et al.* 1992, Miller & Crapo 1993).

Valproic acid (valproate)

The neurotransmitter GABA has an inhibitory effect on ACTH release (Maraka & Stark 1974). GABA inhibits the functioning of the HPA axis *in vivo*, reducing CRH levels in hypophyseal portal blood after i.c.v. administration

(Fig. 1; Plotsky *et al.* 1987). Valproic acid (sodium valproate or valproate) is an antiepileptic agent that inhibits GABA aminotransferase. Patients with seizures treated with valproate showed suppression of ACTH. Valproate blocks GABA reuptake enhancing GABA inhibition of hypothalamic CRH release. Clinical reports of valproate-responsive patients with CD have emerged (Cavagnini *et al.* 1984, Koppeschaar *et al.* 1986, Colao *et al.* 1997). However, chronic administration and placebo-controlled studies failed to confirm the ACTH-lowering effects of valproate (Reincke *et al.* 1988, Colao *et al.* 1997).

Peroxisome proliferator-activated receptor gamma ligands

Peroxisome proliferator-activated receptor gamma (PPAR γ) is a nuclear receptor that functions as a transcription factor. It is highly expressed in

ACTH-secreting adenomas (Heaney 2004, Mannelli *et al.* 2010). PPAR γ ligands, thiazolidinediones, have been shown to increase insulin sensitivity and also inhibit the growth of many tumors, including breast, colon, and prostate cancer cells (Mannelli *et al.* 2010). In addition, antiproliferation with G0/G1 cell cycle arrest, and apoptotic effects in murine cell model of corticotrope adenoma cells and inhibition of *POMC* transcription were demonstrated using PPAR γ ligands (Heaney *et al.* 2002). However, the antiproliferative effects were observed at very high doses. The PPAR γ agonist rosiglitazone was evaluated as a possible therapy for patients with CD. Despite initial results indicating that rosiglitazone could be an effective treatment for patients with CD (Ambrosi *et al.* 1990), further reports confirmed that PPAR γ ligands have no efficacy (Kreutzer *et al.* 2009), a sustained ACTH decrease is rare, and ACTH levels may rebound (Suri & Weiss 2005, Morcos *et al.* 2007). Current information is insufficient to support the routine clinical use of rosiglitazone or pioglitazone in patients with CD (Biller *et al.* 2008, Mannelli *et al.* 2010). Moreover, side effects such as edema, hypertension, weight gain, somnolence, hirsutism, and bruisability are significant, and there have also been concerns related to possible increased cardiovascular disease risks (Nissen & Wolski 2010).

Alpha 1 adrenergic receptor antagonists

Alpha 1 adrenergic receptor antagonists have been suggested as a novel therapy for pituitary adenomas (Fernando & Heaney 2005). In murine pituitary tumor cells, doxazosin (Cardura, Cardura XL) reduced phosphorylated retinoblastoma protein levels and induced G0–G1 cell cycle arrest with decreased tumor growth and reduced plasma ACTH levels (Fernando & Heaney 2005). Further research is required to support the observations.

GR antagonism

As almost every human cell expresses GRs, conditions with glucocorticoid excess, such as CD, result in the deleterious effects on cell metabolism. Therefore, blocking GRs seem to be a promising and attractive approach to treat CD. GRs are classically classified as type I with mineralocorticoid effects and type II with glucocorticoid effects. GR protein is encoded by the *NR3C1* gene, which is located on chromosome 5 (5q31). Two isoforms of the GR have been identified, GR α and GR β (Bamberger *et al.* 1996).

Mifepristone

Mifepristone is an 11 β -[*p*-(dimethylamino)phenyl]-17 β -hydroxy-17-(1-propynyl)estra-4,9-dien-3-one competitive antagonist of androgen, progestin, and GRs (Bertagna *et al.* 1984, Bertagna *et al.* 1986). Mifepristone is the first potent glucocorticoid antagonist available for clinical use, which has affinity for GRs and very little agonist effect (Fig. 1). In normal subjects, mifepristone inhibits dexamethasone suppression and raises endogenous cortisol and ACTH values (Bertagna *et al.* 1984). It has been suggested that mifepristone may upregulate SSTR2 on corticotrope adenomas, which may then be treated with a SSTR2 ligand (octreotide or lanreotide; de Bruin *et al.* 2012). Symptomatic improvement was reported at doses of 5–20 mg/kg per day (Nieman *et al.* 1985), including prompt reversal of neuropsychiatric symptoms (Van der Lely *et al.* 1991, Johanssen & Allolio 2007). Early in the 2000s, a few patients with CS had been treated with mifepristone and the first patient with CD was treated with mifepristone in 2001 (Chu *et al.* 2001). Results of further studies of treatment of additional CD cases with mifepristone confirmed clinical improvement (Castinetti *et al.* 2009). In a large prospective study (Fleseriu 2012), mifepristone showed clinical efficacy at doses of 300–1200 mg/day (mean 900 mg) for over 6–4 months (Table 3). Diabetes control improved significantly, with reduction in the mean HbA1c (7.4–6.3%) in addition to an overall decrease in antidiabetic medications (Fleseriu *et al.* 2012). Body weight and waist circumference also improved significantly after 24 weeks and mean percentage total body fat also declined. As a result of the mechanism of action, cortisol concentrations (up to sevenfold) and ACTH (up to twofold) tend to increase (Sonino & Boscaro 1999, Fleseriu *et al.* 2012). Side effects are described in Table 3 (Fleseriu 2012). The authors of the main study concluded that the drug had an acceptable benefit–risk profile (Fleseriu 2012, Fleseriu & Petersenn 2013). Nevertheless, some physicians consider mifepristone use only in specific situations such as in patients with severe hypercortisolism, when the chance of surgical cure is low, and in those waiting for the maximal efficacy of radiotherapy (Castinetti *et al.* 2009, Feelders & Hofland 2013). The development of a selective GR without antiprogesterin effects could also represent an important step in the long-term treatment of women with CD. Mifepristone should not be administered with drugs that are metabolized by CYP3A or CYP2C (e.g. simvastatin, cyclosporine, fentanyl, ciprofloxacin, non-steroidal anti-inflammatory drugs, and warfarin) because of an increased toxicity risk (Fleseriu & Petersenn 2013). In the USA, in 2012,

mifepristone (Korlym) was FDA approved for the treatment of glucose intolerance or diabetes in patients with CS.

Mechanistic approach for combination therapy

Combination medical therapy is an option that is gaining momentum and increased attention (Feelders *et al.* 2010*b,c*, Vilar *et al.* 2010, Kamenicky *et al.* 2011). It is possible that drug doses may be decreased with fewer adverse events and synergistic or additive effects on ACTH-secretion inhibition (Sonino & Boscaro 1999, Feelders & Hofland 2013) are possible; however, there is no treatment strategy valid for all cases (Table 5). Owing to coexpression of SSTR2 and D2 receptor that frequently occurs in corticotrope pituitary adenomas (Fig. 3), there is a rationale for treating patients with CD with a combination of drugs that target these receptors (Table 5). A prospective study is going on (Fleseriu *et al.* 2014).

Medical therapy should be selected according to drug properties and each patient's particular clinical situation (Table 2). A ketoconazole and octreotide effect was shown to be additive with improved clinical features and reduced cortisol production; however, a minimal proportion of cases exhibited normalized UFC (Vignati & Loli 1996). In two studies, after 3–6 months of combined therapy, a complete response was shown in 67% and 90% of patients with a stepwise combination of pasireotide and cabergoline or pasireotide, cabergoline, and ketoconazole respectively (Feelders *et al.* 2010*b,c*, Vilar *et al.* 2010). In contrast to previously reported cases during long-term cabergoline monotherapy, patients on combined pasireotide–cabergoline treatment did not exhibit escapism (Feelders & Hofland 2013). It would seem that UFC normalization in combination therapies remains at approximately 80% regardless of which drug is administered first: cabergoline with ketoconazole or, ketoconazole with cabergoline (Barbot *et al.* 2014).

A triple-drug combination has been recently evaluated as an alternative to urgent adrenalectomy (Kamenicky *et al.* 2011). A group of 11 patients with severe CD were simultaneously treated with mitotane (3–5 g/day), metyrapone (3–4.5 g/day), and ketoconazole (400–1200 mg/day, Table 5). Within days, UFC decreased significantly and clinical improvement permitted pituitary surgery in five patients. Although side effects were present, they were tolerable (Kamenicky *et al.* 2011). Ketoconazole with metyrapone might cause mineralocorticoid hypertension. Blood pressure should be monitored if this therapeutic approach is applied.

Pasireotide with temozolomide administered over 12 months revealed sustained control of tumor growth and ACTH secretion in a pituitary carcinoma, indicating that such a combination may be a promising option for aggressive pituitary tumors (Bode *et al.* 2010).

The degree of hypercortisolism at baseline could predict the doses needed to control cortisol excess (Vilar *et al.* 2010, Feelders & Hofland 2013). Combination treatments that have been evaluated for CD treatment are summarized in Table 5.

Novel targets for treatment of CD

Epidermal growth factor receptor inhibitors

Epidermal growth factor receptor (EGFR) has been studied as a therapeutic target for ACTH-secreting pituitary adenomas (Fukuoka *et al.* 2011). Normal pituitary and corticotrope adenomas express EGFR, which controls POMC expression. After blocking of EGFR with gefitinib, a tyrosine kinase inhibitor, POMC expression was attenuated, with inhibition of corticotrope cell proliferation and induced apoptosis (Fig. 3). The results were confirmed using canine and human corticotrope tumors as well as an athymic nude mouse model, indicating that inhibiting EGFR signaling with gefitinib may be a novel strategy for treating CD (Fukuoka *et al.* 2011). However, confirmation of efficacy in patients with CD requires clinical trials.

Cell cycle regulators

Animal and cell models have provided insights into mechanisms underlying the pathogenesis of ACTH-secreting pituitary adenomas, mostly due to cell cycle disruption. A classic example indicating the association of cell cycle regulators and pituitary tumorigenesis is derived from the heterozygous Rb mouse (Melmed 2003). The Rb (*Rb1*) gene encodes a tumor suppressor that controls the G1/S cell cycle checkpoint. Rb phosphorylation by cyclin-dependent kinases (CDKs) releases the E2F transcription factor enabling S-phase progression (Fig. 3) (Liu *et al.* 2011*a,b*). Inhibitors of CDK4 (INK4) type (p16, p15, p18, and p19) and CIP/KIP type (p21, p27, and p57) suppress the action of CDK. Sequential activation and inactivation of protein kinase complexes regulate cell cycle progression (Melmed 2003). Heterozygous *Rb*^{+/-} mice developed intermediate lobe POMC cell tumors at 12 months with 100% penetrance. Deletion of *p27*^{Kip1} (*Cdkn1b*) or *p21* (*Cdkn1a*) enhances intermediate lobe tumorigenesis in *Rb*^{+/-} mice (Park *et al.* 1999). These results motivate the

pharmacological assessment of a CDK2/cyclin E inhibitor, R-roscovitine (seliciclib; CYC202) in corticotrope pituitary adenomas. In transgenic POMC-pituitary tumor-transforming gene (*PTTG* (*PTTG1*)) zebrafish embryos and in a mouse model of ACTH-secreting pituitary adenomas, R-roscovitine suppressed ACTH expression/production and inhibited tumor growth (Liu *et al.* 2011b). Molecular analyses *in vitro* and *in vivo* showed upregulation of p27, p21, and p57 (*CDKN1C*), and downregulation of cyclin E expression (Fig. 3). The results indicate that use of selective CDK inhibitors may effectively target corticotrope tumor growth and hormonal secretion (Liu *et al.* 2011b).

Therapeutic role of microRNA's

MicroRNAs (or miRNAs) are noncoding, single-stranded RNAs constituting a novel class of gene regulators. miRNAs control diverse biological processes including cell growth, differentiation, and apoptosis by post-transcriptional regulation of target gene expression (He & Hannon 2004). miRNA mutations or misexpression correlate with several human cancers indicating that miRNAs can function as tumor suppressors. Real-time PCR in corticotrope adenomas compared with normal pituitary tissue revealed downregulation of several miRNAs, including *miR-15a* (*MIR15A*), *miR-16*, and *Let-7a* among others (Amaral *et al.* 2009). Interestingly, *miR-15a* and *miR-16* genes are colocalized with the Rb tumor suppressor on chromosome 13q14, which is commonly deleted in corticotropinomas. *Let-7* miRNA negatively regulates high-mobility group A2 protein, which is highly expressed in pituitary adenomas, increasing aggressiveness of corticotrope adenomas and, therefore, contributing to pituitary tumorigenesis and progression (Qian *et al.* 2009). Genetic manipulation of miRNAs may induce tumor regression and decrease the activity of corticotrope adenomas.

Potential future therapeutic targets

Several transgenic mouse models have contributed important knowledge to understanding of human pituitary disease. Transgenic mice with metallothionein (mMT)-promoter-driven overexpression of CRH exhibited disruption of the HPA axis with elevated plasma ACTH and glucocorticoid levels, and development of CS (Stenzel-Poore *et al.* 1992).

Desmopressin (1-desamino-8-D-arginine vasopressin, DDAVP), a long-acting synthetic AVP analog, stimulates ACTH secretion from human pituitary corticotrope adenomas through V3 pituitary receptor stimulation.

The V3 pituitary receptor is located mainly in the anterior pituitary gland (Fig. 3) and has been previously classified as the V1b AVP receptor. Transgenic mice expressing the human AVP V3 receptor under the control of rat *Pomc* promoter sequences showed increased basal concentrations of corticosterone; however, no corticotrope tumors developed (Rene *et al.* 2002). AVP antagonists (vaptans) showed mainly antidiuretic effects, as they are selective V2 renal receptor antagonists. However, development of V3-receptor-specific antagonists could lead to a new class of agents that suppress ACTH secretion in corticotrope adenomas (Fig. 3; Ferone *et al.* 2013).

Manipulation of leukemia inhibitory factor (LIF) and the ATPase subunit of the chromatin remodeling Swi/Snf complex Brg1 may be good therapeutic targets for controlling hypercortisolism. LIF is a pleiotropic cytokine that enhances *POMC* transcription, ACTH secretion, and corticotrope cell proliferation, and is an essential protein for *POMC* repression (Auernhammer & Melmed 2000). Brg1 stabilizes the interaction between GR and Nur77 (NGFI-B), a nuclear orphan receptor that is also essential for *POMC* transrepression (Bilodeau *et al.* 2006).

Testicular orphan nuclear receptor 4 (TR4) has been recently identified as another potential target for CD treatment. TR4 (NR2C2) was overexpressed in human corticotrope tumors and in human and mouse corticotrope tumor cell lines (Du *et al.* 2013). Consistently, TR4 overexpression in human and murine tumor cells increased *POMC* transcription, ACTH secretion, cellular proliferation, and tumor invasiveness. TR4 activated *POMC* transcription through a MAPK-mediated pathway (Du *et al.* 2013).

Vascular endothelial growth factor receptor 2 (VEGFR2 (KDR)), *PTTG*, and fibroblast growth factor expressions are associated with extrasellar extension and greater pituitary adenoma proliferative potential. These data provide useful information for novel targeted treatments. For example, treatment of a corticotrope carcinoma with bevacizumab, a monoclonal antibody that blocks VEGFRs, stopped tumor progression and induced CD control (Ortiz *et al.* 2012).

The rapamycin (mTOR) pathway is upregulated in pituitary adenomas. Human ACTH-secreting pituitary adenomas, however, express mTOR and its active phosphorylated isoform at levels similar to those of normal pituitary tissue. In addition, AtT-20 mice corticotrope adenoma cells were shown to be resistant to rapamycin. Interestingly, such resistance can effectively be overcome using octreotide as a cotreatment. If CD is treated first with an SRL, and then with an mTOR inhibitor, control of

hypercortisolism may be more easily achieved (Ferone *et al.* 2013).

Summary

CD is a severe pituitary endocrine disorder with related long-term complications and increased mortality if not appropriately treated. Transsphenoidal surgery is the first-line treatment of choice; however, surgery does not result in a long-term cure for many patients. Recently, approved medical therapies target the corticotrope adenoma itself (i.e. pasireotide) or block cortisol effects in the periphery (i.e. mifepristone), thus providing new approaches and treatment options. The most commonly utilized clinical inhibitors of steroid biosynthesis are ketoconazole, metyrapone, and mitotane. Mitotane's mechanism of action seems to be multifactorial. Other steroidogenesis inhibitors interfere with cytochrome-P450-catalysed reactions. This mechanism of action has little selectivity and extra-adrenal adverse effects are likely. Daily doses should be titrated to maintain urinary and serum cortisol in the normal range, avoiding excessive suppression of adrenal function. However, glucocorticoid replacement is sometimes recommended with a 'block and replace' approach. A relatively new adrenal steroidogenesis inhibitor, LCI699, seems to be efficient and well tolerated; clinical trials are ongoing. Finally, in recent years, new molecular targets have been identified on corticotrope adenomas including EGFR, cell cycle regulators, and miRNAs.

Declaration of interest

D C-R declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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