Currently used and investigational drugs for Cushing’s disease

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ABSTRACT

Introduction: Cushing’s disease (CD) is caused by a corticotroph adenoma of the pituitary gland that secretes excess adrenocorticotropic hormone (ACTH) causing increased morbidity and mortality. Surgery is the treatment of choice, but is not always successful. Alternatives include radiotherapy, adenectomy, and pharmaceutical therapy. The latter is increasingly gaining momentum due to the recent development of compounds that reduce hypercortisolaemia or its symptoms, acting through different mechanisms.

Areas covered: In this article, the authors provide a complete overview of the treatment options for Cushing’s disease, including adrenal-directed, tumor-targeted, and peripheral therapies that are currently used or in development, and discuss their potential advantages and limitations.

Expert opinion: Considering the lack of long-term remission in up to half of the patients after surgery, and the delayed response to radiotherapy along with potential side effects, there is a strong need for an effective pharmaceutical treatment. Pasireotide, mifepristone, ketoconazole and metyrapone have been approved by regulatory authorities but their use remains limited due to considerable costs and side effects. Research in this field has focused recently on the improvement of pre-existing drugs and the development of safe new ones. However, few approaches aim at targeting the source of the disease, the ACTH-secreting adenoma.

1. Introduction

Cushing’s disease (CD) results from an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma and represents the most frequent cause of Cushing’s syndrome (CS), occurring in 60–70% of the diagnosed patients [1]. It is characterized by an excess of ACTH secretion and the consequent increase of cortisol production by the adrenal glands. This leads to several morbidities including central obesity, hypertension, hyperglycemia, osteoporosis, skin and muscle atrophy, and depression [2,3]. Transsphenoidal surgery is generally the initial treatment of choice, but remission rates in patients with microadenomas are in the range 65–90%, whereas in the case of macroadenomas they are lower than 65% [2]. Moreover, the risk of recurrence persists for at least 10 years after surgery [4,5]. When surgery is not effective, there are further possibilities for treatment that include repeat surgery, radiotherapy, pharmaceutical therapy, and adrenalectomy. However, repeat surgery is associated with a higher risk of hypopituitarism [6]. Radiotherapy, on the other hand, requires months or years to be effective, and its use has become rarer in recent years due to the increasing number of possibilities for medical treatment [3,7].

The majority of drugs available are steroidogenesis inhibitors that reduce cortisol levels in the adrenal glands. Among these, ketoconazole has been approved in Europe for the treatment of endogenous CS. However, lowering ACTH secretion by the corticotroph adenoma remains the main therapeutic goal for new drugs. Recently, the somatostatin analog pasireotide has received regulatory approval for cases in which surgery has failed or has not been considered as an option. Other therapeutic concepts include peripheral mechanisms such as the blockade of the glucocorticoid receptor (GR) with mifepristone, which received regulatory approval for hyperglycemia in patients with CS.

Adrenalectomy, on the other hand, even though it leads to definitive disease cure in the majority of CD patients, produces permanent adrenal insufficiency that requires hormone replacement and may be associated to development of Nelson’s syndrome, an aggressive corticotroph tumor that results from the complete lack of adrenal to pituitary feedback [3,8].

This paper provides a wide overview about the recent advances in the medical therapy. We will discuss the mechanism of action (Table 1), safety, and clinical efficacy of current possibilities for treatment (Table 2) with an outlook to the novel drugs developed and their targets.

2. Medical treatment of CD

2.1. Adrenal directed therapy

Adrenal directed therapies consist of drugs that act as steroidogenesis inhibitors in order to control cortisol excess (Table 1). Adrenal medical treatment can be used before and after surgery or radiotherapy, as a bridging therapy until the definitive control of hypercortisolism is reached. One
Cushing’s disease increases morbidity and mortality in patients if not treated. Surgery to remove corticotroph adenomas is the initial treatment of choice; when it is not effective further options are repeated surgery, radiotherapy, adrenalectomy, and/or medical treatment. Most of the investigational drugs act at adrenal level, inhibiting cortisol synthesis. Despite the reduction of cortisol, the accumulation of its precursors can also result in severe side effects. Moreover, treatment resistance often leads to increasing the drug dose. Pasireotide is the only approved drug that targets the ACTH-secreting pituitary adenoma, indicating that new compounds acting directly at the cause of the disease need to be developed. Results obtained with HSP90 inhibitors and retinoic acid agonists confirm the importance of the search for novel molecular pathways involved in CD.

### Article highlights
- Cushing’s disease increases morbidity and mortality in patients if not treated.
- Surgery to remove corticotroph adenomas is the initial treatment of choice; when it is not effective further options are repeated surgery, radiotherapy, adrenalectomy, and/or medical treatment.
- Most of the investigational drugs act at adrenal level, inhibiting cortisol synthesis. Despite the reduction of cortisol, the accumulation of its precursors can also result in severe side effects. Moreover, treatment resistance often leads to increasing the drug dose.
- Pasireotide is the only approved drug that targets the ACTH-secreting pituitary adenoma, indicating that new compounds acting directly at the cause of the disease need to be developed.

This box summarizes key points contained in the article.

The disadvantage of the use of these agents is the need to increase their doses due to an ‘escape phenomenon’ in order to maintain normal cortisol levels [3,6]. However, there is ongoing research to improve the efficacy of existing drugs. An alternative approach to normalize cortisol levels is inhibiting the steroidogenesis by blocking the response to ACTH at the adrenal glands through the blockage of the melanocortin-2 receptor (MC2R) [34].

#### 2.1.1. Cortisol synthesis inhibitors
Ketoconazole is an imidazole derivative that has been used for many years as an antifungal agent. It has shown inhibitory effect on 17 alpha-hydroxylase (CYP17A1) and 11 beta-hydroxylase (CYP11B) [36,37]. It may have direct effects on corticotroph tumors, as shown in rat anterior pituitary cells, in the mouse corticotroph tumor cell line AtT-20 and in human corticotroph cell cultures [32,36]. In a dose range between 200 and 1200 mg/day, ketoconazole can decrease cortisol production in CS patients with various etiologies [18]. It has been recently reported that ketoconazole induces biochemical remission in 50% of patients with CD [37]. However, potential serious side effects have to be considered. Hepatotoxicity has been described in patients treated with ketoconazole, therefore, it should be used under close liver enzyme monitoring [2]. An impairment of intestinal absorption is reported with the concomitant administration of proton-pump inhibitors due to the fact that the drug requires gastric acidity for its absorption [38,39]. Moreover, reduction of testosterone synthesis may lead to hypogonadism and gynecomastia, so the initial use of ketoconazole is not advised in men [2,36]. Since November 2014, ketoconazole has been approved by the European Medicines Agency (EMA) for the treatment of endogenous CS.

From the racemic mixture of ketoconazole enantiomers, the purified 2S, 4R molecule, named levoketoconazole (COR-003), has superior effects on the integral enzymes in the cortisol biosynthesis pathway, possibly leading to higher efficacy. In vitro and in vivo studies showed that levoketoconazole might not be as damaging to the liver as racemic ketoconazole. It does not induce hypokalemia, Nelson’s syndrome, peripheral edema, hypertension, or endometrial hyperplasia but may cause diarrhea, fatigue, dizziness, and abdominal pain. In phase I and II trials, the drug has been evaluated.

### Table 1. Sites and mechanisms of action of the drugs tested in Cushing’s disease (CD).

| Site of action                  | Drug                  | Mechanism of action                  | Development status |
|--------------------------------|-----------------------|--------------------------------------|--------------------|
| Adrenal-directed therapy       | Glucocorticoid type   |                                      | Clinical practice   |
| Steroidogenesis                | II                    |                                      |                    |
|                               | Trilostane            | Block to the MC2R activated by ACTH  | Preclinical         |
|                               | Levoketoconazole      |                                      |                    |
|                               | Metyrapone            |                                      |                    |
|                               | Aminoglutetimide      |                                      |                    |
|                               | Triostane             |                                      |                    |
|                               | Etomidate             |                                      |                    |
|                               | Mitotane              |                                      |                    |
|                               | LCI699                |                                      |                    |
|                               | ATR-101               |                                      |                    |
|                               | GPS1573               |                                      |                    |
|                               | GPS1574               |                                      |                    |
| Cushing adenoma-directed       | Somatostatin receptor | Stimulate SSTRs, with more affinity  | Clinical practice   |
| therapy                        | Pasireotide           | for SSTRs (pasireotide) and 2, 4, 5  | Phase I             |
|                               |                      | (DG-3173)                            |                    |
| Dopamine receptor             | Cabergoline           | Stimulates D2 receptor               | Off-label           |
| Somatostatin and dopamine     | BIM23A760             | Stimulates SSTRs and D2 receptor     | Stopped             |
| receptor                       |                      |                                      |                    |
| PPAR-γ agonists               | Rosiglitazone         | Inhibits the mRNA expression of POMC | Stopped             |
| Retinoic acid receptor        | All-trans RA 9-cis RA| Inhibit cell proliferation and secretion | Phase II            |
|                               | 13-cis RA             |                                      |                    |
|                               | Gefitinib             |                                      |                    |
| EGFR                           | R-roscovitine         | Regulates POMC expression             | Preclinical          |
| Cycline-dependent kinases     |                      | Effects on cell cycle, and inhibits ACTH secretion by targeting | Phase II             |
|                               |                      | cyclin E2F1 pathway                  |                    |
| Peripheral therapy            | HSPP90C-terminal      | Restores glucocorticoid sensitivity  | Preclinical          |
|                               | domain                |                                      | Clinical practice    |
|                               | Glucocorticoid type   |                                      |                    |
|                               | II receptor           |                                      |                    |
|                               | Silibinin             |                                      |                    |
|                               | Mifepristone          |                                      |                    |
|                               |                      | Antagonist of GR                     |                    |

CYP17: cytochrome P45017A1 (17 alpha-hydroxylase); CYP11β: cytochrome P45011β (11 beta-hydroxylase); CYP11A1: cytochrome P45011A1 (side-chain cleavage of cortisol biosynthesis); CYP3B2: cytochrome P4503B2 (3-beta hydroxysteroid dehydrogenase/5A4 isomerase); ACAT 1: acetyl-CoA acetyltransferase 1; MC2 R: melanocortin-2 receptor; ACTH: adrenocorticotropic hormone; SSTR: somatostatin receptor; RA: retinoic acid; EGFR: epidermal growth factor receptor; POMC: proopiomelanocortin; HSP: heat shock protein; GR: glucocorticoid receptor.
Table 2. Overview on use, dose range, beneficial, and adverse effects of drugs currently used, tested, or under evaluation in clinical trials for CD.

| Developmental status | Drug & Molecular Targets | Potential clinical positioning | Dose range tested | Beneficial effects reported | Most common side effects reported | References |
|----------------------|-------------------------|--------------------------------|------------------|-----------------------------|----------------------------------|------------|
| Phase II             | Retinoic acid receptor agonists | Persistent or recurrent hypercortisolism after trans-sphenoidal surgery | 10–80 mg/day | Decrease of UFC | Severe adverse effects (potential teratogenicity, mucocutaneous toxicity, etc.) after long exposure to the efficacious concentrations | [9,10] |
| Phase III            | Levoketoconazole | Pre-post surgery, if surgery is contraindicated, waiting for the effect of radiotherapy | 150–600 mg/day | Higher effect than ketoconazole in decreasing enzymes in cortisol biosynthesis pathway | Diarrhea, dizziness, abdominal pain | [11–13] |
|                      | LCI699 | Pre-post surgery | 4–100 mg/day | Decrease of UFC | Gastrointestinal disturbances, headaches | [14–17] |
|                      | Ketoconazole | Pre-post surgery | 200–1200 mg/day | Decrease of cortisol production | Hepatotoxicity, may lead to hypogonadism/gynecomastia (not advised in men) | [18] |
| Clinical practice    | Mifepristone | For non-responders to multimodal therapies | 300–1200 mg/day (for CS) | Decrease in body weight, waist circumference, and body fat, increased insulin sensitivity | Nausea, fatigue, headache, decreased blood potassium, arthralgia, vomiting, endometrial thickening, peripheral edema | [19] |
|                      | Pasireotide | After surgery or in patients not eligible for surgery | 300–1200 µg/day | Decrease of UFC | Glucose-related adverse effects (not advised in patients with diabetes mellitus, impaired glucose tolerance) | [20–23] |
| Off-label            | Cabergoline | Mainly effective in patients with D₂ expression | 0.5–7 mg/week | Suppression of ACTH levels | Generally well tolerated | [3,24] |
|                      | Etomidate | Pre-post surgery, for patients not able to take oral medication | 0.2–0.6 mg/kg/h | Adrenal suppression after 30 min single dose application | Adrenal suppression, decrease in blood pressure, anaphylactic reactions, nausea, vomiting | [25–30] |
|                      | Metyrapone | Before surgery | 500–6000 mg/day | Decrease of cortisol levels | Adrenal insufficiency, increased androgen levels (not recommended in women, increased deoxycortisone levels) | [31] |
|                      | Mitotane | Pre-post surgery, need frequent monitoring | Up to 6 mg/day | Decrease of cortisol production | Gastrointestinal, neurological, and hepatic disturbances | [14,32,33] |
administered orally at a daily dose ranging from 150 to 600 mg. Levoketoconazole received an orphan drug designation by EMA in July 2012 and US Food and Drug Administration (FDA) in May 2012 for CS. In August 2014, a pivotal, open label, single-arm, international, phase III trial was started [11–13]. The primary endpoint is a comparison of urinary-free cortisol (UFC) after six months of treatment, relative to baseline levels. Secondary endpoints include body weight, cholesterol, blood pressure, and blood glucose levels. Levoketoconazole could be used to treat endogenous CS of any cause in adult patients who are unwilling to have surgery, prior to surgery, or in whom surgery has failed, or while waiting for the effects of radiotherapy to occur [13].

Metyrapone is an inhibitor of CYP11β, used for the diagnosis of CS and for the management of hypercortisolism. Despite its widespread use since its discovery in 1958, there are scarce data regarding its actual efficacy, and the last studies have been published over 25 years ago, except for one recently published reporting a multicenter retrospective study in the United Kingdom [40]. It has been usually considered effective alone or in combination with other treatments, especially combined with ketoconazole for the rapid control of hypercortisolism. Doses range between 500 and 6000 mg/day. The most common side effects are adrenal insufficiency, acne, and hirsutism due to increase of androgen levels, hypokalemia, edema, and hypertension as a result of increased deoxycorticosterone levels [31]. Its use is not initially recommended in women for the increased androgen levels, and short term treatments are more advisable to reduce their effects. Metyrapone has been approved in 15 European countries through a mutual recognition procedure, which ended in April 2014, and was followed by national procedures for the granting of national approvals, with indications for the treatment of CS [3].

Aminoglutethimide inhibits the side-chain cleavage of cortisol biosynthesis (CYP11A1). It is more effective in combination with other drugs, such as metyrapone [6]. It has been used in adults and children in doses of 500–2000 mg/day. Generally, cortisol levels decrease gradually but some patients need glucocorticoid replacement. However, this effect is reversed when the treatment is interrupted [40]. Major side effects, including somnolence, dizziness, skin rash, and hypothyroidism, were observed in 5% of patients [1,41]. The drug is no longer available since 2007, when the manufacturer stopped producing it [32].

Trilostane is an androstane–carbonitrile derivate. It is a competitive inhibitor of 3-β hydroxysteroid dehydrogenase/Δ5–Δ4 isomerase (CYP3B2), the enzyme that blocks the early stage of steroidogenesis [25,42]. Trilostane has been found to be clearly effective in dogs, irrespective of whether CS derives from a pituitary or adrenal tumor, and is currently used in animals. In humans, it is not potent enough to block efficiently the biosynthesis of cortisol in patients with hypercortisolism. Therefore, remission rates were variable in the few studies performed [25,26]. Daily doses of trilostane range from 120 to 1440 mg. Side effects include asthenia, abdominal discomfort, nausea, vomiting, gastritis, diarrhea, paraesthesias, and an increased production of saliva. Escape phenomena due to increased ACTH-release may occur [25,27]. Trilostane is no longer used in the treatment of CD because of its limited efficacy in comparison to other steroidogenesis inhibitors [3].

Etomidate has been used originally as hypnotic, anesthetic, and occasionally for treatment of CD and CS based on its properties as a steroidogenesis inhibitor, blocking the 11-β–hydroxylaylation (CYP11β) of deoxycortisol to produce cortisol. It is mostly used in continuous infusion with non-hypnotic doses ranging between 0.2 and 0.6 mg/kg/h [25]. The most critical effect of etomidate is adrenal suppression, occurring approximately 30 min after application of a single dose and may last for approximately 24 h [28]. Side effects include pain at the injection site, anaphylactic reactions, minimal change in heart rate, reduction in mean arterial pressure, mild decrease of systolic blood pressure and cardiac index, myoclonus, nausea, and vomiting. Etomidate is the only drug available for CS and CD for parenteral administration in patients who are not able to take oral medication, but careful monitoring is needed to avoid excessive sedation [28–30].

Mitotane is used to treat advanced adrenal cortical carcinoma. Chemically, it is an ortho-derivative of the well-known insecticide dichlorodiphenyltrichloroethane (DDT). Apart from the suppression of cell growth, mitotane inhibits CYP11A1 and CYP11B, resulting in decreased cortisol production [33]. Mitotane, in a dose up to 6 g/day, can be effective in the treatment of CD; however, it has a slow onset of action and serum levels of mitotane should be monitored to achieve therapeutic concentrations and to prevent toxicity. Mitotane has serious gastrointestinal, neurological, and hepatic side effects. In addition, mitotane can induce adrenal insufficiency, hypercholesterolemia, and alterations in hormone-binding globulins [14,32,33].

LCI699 is a promising investigational drug for its effect on urinary cortisol levels. It potently inhibits CYP11B1 and CYP11B2 [14,15]. In a recent study, patients with moderate-to-severe CD (UFC levels >1.5 upper limit of normal) were treated with LCI699 for 10 weeks. The drug was initially administered at a dose of 4 mg/day, and then increased every 2 weeks to 10, 20, 40, and 100 mg/day until the normalization of UFC. After that, the dose was maintained till the end of the treatment. All of the 12 patients enrolled showed reduction in UFC levels or showed at least a 50% decrease from their baseline levels [15]. A phase III study with LCI699 in CD patients started in July 2014. The drug produced side effects such as gastrointestinal disturbances followed by headaches. At present, the recruitment of patients is still ongoing for a phase III, randomized, double blind, multicenter trial to test safety and efficacy of the drug in CD [16,17].

ATR-101 is a novel molecule in development for the treatment of adrenocortical carcinoma. It is an inhibitor of acetyl-CoA acetyltransferase 1 (ACAT 1). Studies in dogs with spontaneous CD have proved that ATR-101 leads to a rapid and dose-dependent decrease in ACTH-stimulated cortisol levels in 90% of the subjects and was well tolerated [43]. A phase I study is currently ongoing in patients with advanced adrenocortical carcinoma whose tumors have progressed on standard therapy [43,44].
2.1.2. Melanocortin-2 receptor antagonists

The blockade of MC2R activation by ACTH is expected to decrease cortisol levels and adrenocortical androgen secretion, while preserving mineralocorticoid hormone secretion [45]. Selective antagonists of the MC2R could therefore be a new treatment for CD. One of the first agonists described is ACTH₁₁⁻₂₄, which was shown to inhibit corticosterone release by rat primary adrenal cells but results were contrasting about the effect on ACTH-stimulated cAMP production [46–48]. Moreover, a peptide with substitution of tryptophan by N-methyltryptophan at the ninth position in ACTH₁₁⁻₂₄ had antagonist activity [49]. The analogs GPS1573 and GPS1574 were found to antagonize MC2R in vitro at lower concentrations than the previously reported MC2R antagonists, but the effect was not exclusively specific for MCR2 [50]. Despite the initial promising results the activity of these two compounds in vivo, in response to ACTH administration, did not match their in vitro potency [50].

2.2. Cushing adenoma-directed therapy

The concept of targeting directly the Cushing’s adenoma corrects the pathogenic source of the disease. Due to the fact that little is known about the molecular alterations that lead to the development of corticotroph tumors, there are currently few drugs based on direct mechanisms (Table 1). In this category of drugs, only the somatostatin analog pasireotide has been approved for the treatment of CD, whereas other drugs have been tested without demonstrating acceptable efficacy or safety in a large population of patients.

2.2.1. Somatostatin analogs

Pasireotide is a somatostatin analog with affinity for all the somatostatin receptors (SSTR), especially for SSTR5, which is commonly the most abundant SSTR in human corticotroph adenomas [51]. Its functional activity is much higher than octreotide for all of the SSTRs, except for SSTR2 [20,52]. It triggers antiserotonin and antiproliferative signaling cascades, inhibiting tumor growth and reducing tumor size in vitro and vivo [21]. In vitro studies showed that pasireotide (10 nmol/l) significantly inhibited ACTH secretion by 30–40% in 60% of human primary cultures from corticotroph adenomas [51]. Pasireotide was capable of controlling CD in dogs. Corticotroph adenomas in dogs predominantly express SSTR2 in contrast to humans; thus, the SSTR2 receptor subtype could also mediate the drug’s inhibitory action on ACTH [21]. The low expression of SSTR2 in human corticotroph adenomas is considered a result of the high circulating levels of cortisol, which explains the poor effectiveness in CD of octreotide and lanreotide, two drugs that act on SSTR2 [22,23].

Pasireotide has been administered to patients at doses ranging from 300 to 1200 µg/day. During phase II and phase III studies, 76% and 50% of patients showed decreased UFC levels after the treatment period [20,53]. Important to note is that moderate-to-severe drug-related adverse effects have been reported in these studies. Among these, the most serious ones were glucose-related in 36% and 73% of patients in the respective studies. In some cases, this fact led to the discontinuation of the treatment [20,53]. The preexistence of diabetes mellitus or impaired glucose tolerance increased the risk for manifestation of hyperglycemia-related effects. Therefore, there is a strong need of a frequent monitoring of blood glucose levels in pasireotide-treated patients [20,23]. The FDA approved pasireotide for treatment of CD in 2012, considering, however, surgery as the first line therapy and assuming that the drug would be useful in patients not eligible for surgery or with recurrence. In parallel, new formulations of pasireotide are under investigation. A phase III, double-blind, randomized, multicenter study has started to determine the efficacy and safety of two doses of a long-acting release version of pasireotide in CD patients administered once every 28 days. Completion of the trial is expected in February 2017 [54].

DG-3173 is another somatostatin analog that selectively binds SSTR subtypes 2, 4, and 5 with affinity similar to pasireotide [16,55]. DG3173 has completed phase I trials in CD demonstrating fewer metabolic and gastro-intestinal side effects than pasireotide [55].

2.2.2. Dopamine agonists

Cabergoline is a dopamine agonist with higher affinity for the dopamine D2 receptor and fewer side effects than bromocriptine (Br). It has been tested in patients with CD based on the expression of D2 receptors in some corticotroph tumors. The D2 receptor was found to be expressed and functional in 60% of the corticotroph tumors in vitro. One study with cabergoline showed effectiveness in 40% of the cases, and therefore the use of dopaminergic drugs was proposed for the treatment of CD [56]. This was also demonstrated on dogs, with an initial favorable response in 60% of the cases, being ultimately useful in 42.5% of treated dogs [57]. The effectiveness of cabergoline could as well be related with corticotroph adenomas with origin in pars intermedia or distalis as a result of negative regulation by dopamine [57]. In human patients, it has been administered orally at doses ranging from 0.5 to 7 mg/week [3]. Some cases were reported in which suppression of ACTH levels and amelioration of disease symptoms were observed in absence of important adverse effects. However, cabergoline is effective in only a small subset of CD patients, and this may possibly be explained in connection with the tumor size, the origin of the corticotroph adenoma or the molecular characteristics of the D2 receptor [24,56].

2.2.3. Somatostatin–dopamine chimeric compounds and combined therapies

Chimeric molecules may represent a promising category of compounds for CD management. Somatostatin and dopamine receptors are expressed in corticotroph tumors, and the somatostatin–dopamine chimeric compound BLM23A760 has been designed to act on both targets. Functional heterodimerization of somatostatin and dopamine receptor subtypes might occur in vivo, as has already been shown to occur in vitro [58]. Through this potential mechanism, this type of treatment could result in greatly enhanced efficacy of these compounds in controlling hormone secretion and tumor growth in corticotroph pituitary tumors [59]. With chronic administration,
however, BIM-23A760 was found to produce a metabolite with dopaminergic activity that gradually accumulated and interfered with the activity of the parent compound. Consequently, efforts are currently under way to produce a second-genera-
tive chimeric compound for solving this issue [60].

An interesting combination therapy has been reported in the literature. In 2010, a prospective, open-label, multicenter trial used a stepwise approach for the medical treatment of CD, with pasireotide as the initial form of treatment and the sequential addition of cabergoline and low-dose ketoconazole [61]. This treatment was based on the use of three drugs that differentially target SSRT5 and D2 in the corticotroph adenoma and steriodogenic enzymes in the adrenal. The treat-
ment started with pasireotide as monotherapy in a dosage of 100 mg s.c. thrice daily. After 10 days, this dosage was increased to 250 mg s.c. thrice daily in case UFC excretion remained elevated. UFC excretion was measured again at day 28; in case of persistent hypercortisolism, cabergoline (1.5 mg every other day) was added to pasireotide. If UFC remained elevated at day 56, ketoconazole (600 mg daily) was added to pasireotide and cabergoline. Using this treatment regimen, 88% biochemical remission was achieved after 80 days [61,62].

2.2.4. PPAR-γ agonists

Activation of the transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ) results in the pituitary in apop-
totic and anti-secretory effects [33]. Among the PPAR-γ glita-
zone agonists, the more effective one was rosiglitazone, which has been proved to be efficacious in an animal model of CD [63]. It decreased tumor growth, increased apoptosis, and reduced ACTH secretion by inhibiting mRNA expression of proopiomelanocortin (POMC) [64,65]. Subsequently, the effect-
iveness of rosiglitazone was investigated in clinical studies that failed to prove a consistent reduction UFC, plasma ACTH, or serum cortisol levels, possibly due to the low proliferative-
potential of human ACTH-secreting pituitary tumors [66]. The drug had been withdrawn from the medical market because of the discovery of hepatotoxicity and cardiotoxicity in the countries in which it has initially been approved [67].

2.2.5. Retinoic acid receptor agonists

There are several recent reports in the literature about retinoic acid for the therapy of CD. Treatment of corticotroph tumors in vitro and in vivo leads to the inhibition of cell proliferation and hormone secretion and also reduced tumor growth [68]. Adenoma shrinkage and amelioration of clinical signs were also found after treatment of dogs with spontaneous CD with-
out adverse events or signs of hepatotoxicity [69]. All-trans-
retinoic acid (ATRA) and 9-cis retinoic acid (9-cis-RA) are the most biologically active retinoic acid isomers, whereas 13-cis-retinoic acid (13-cis-RA) is considered less active and may require isomerization to ATRA in order to exert biologic func-
tion [70,71]. ATRA has a plasma half-life shorter than 1 h and induces its own degradation, while 13-cis-RA is cleared from the plasma in more than 13 h without inducing its metabo-
ism. A key limitation of retinoid therapy is that the concentra-
tions required for being effective might cause teratogenicity, mucocutaneous toxicity, defects in liver function, conjunctivi-
tis, mucositis, and severe photosensitivity. However, reducing

the exposure of the treated patients to light might reduce these side effects. In a prospective proof-of-concept study performed in seven patients with CD, the efficacy and safety of escalating dosages of retinoic acid (10-80 mg/day orally) was evaluated over a period of 6-12 months. Fifty percent of patients had a decrease or normalization of UFC levels after 6 months; 42.9% of patients had full disease control, whereas the 28.6% had partial disease control. The treatment was generally well tolerated, except for mild and mostly transitory mouth and conjunctival dryness, arthralgia, diarrhea and abdominal discomfort, headache, and worsening of leukocy-
tosis [9]. It was recently reported that a combination of 9-cis RA and Br might be of advantage. 9-cis RA is a natural ligand that binds with high affinity to the retinoid X receptor and also to the family of retinoic acid receptors. In nearly 45% of corticotroph adenoma-derived primary cultures, the combined administration of 9-cis RA and Br lowered the steady-state level of the ACTH precursor POMC more efficiently than either drug alone. As demonstrated in the AtT-20 cell line, 9-cis RA induced a significant increase in the efficacy of Br by upregu-
lation of the D2 receptor expression [72]. A recent prospective study evaluated the efficacy and safety of 13-cis RA in CD patients with persistent or recurrent hypercortisolism after transsphenoidal surgery. Normalization of UFC and midnight salivary cortisol occurred in 37.5% of patients, and UFC reduc-
tions were also found in nonresponsive subjects. Minimal side effects (conjunctival irritation, cheilitis, nausea, headache, and arthralgias) were reported by ~44% if patients but they were mild and often transient [10]. In addition, after promising results obtained in phase II and III studies in different types of diseases such as cutaneous T-cell lymphoma, bexarotene, a synthetic retinoid analog with affinity for the retinoic acid receptor X, is currently being clinically tested in CD [73,74].

2.2.6. EGFR blockers

The epidermal growth factor receptor (EGFR) has been shown to be involved in the secretion of POMC and its regulation by ubiquitin specific peptidase 8 (USP8) is part of a pathogenic mechanism in Cushing adenomas [75]. POMC expression and ACTH secretion are enhanced when EGFR is activated. A laboratory study demonstrated that inhibition of the EGFR with gefitinib, an inhibitor of EGFR tyrosine kinase activity, caused a decrease in POMC expression and ACTH secretion. Moreover, the compound was able to reduce tumor growth and size by preventing the proliferation of tumor cells and prompting apoptosis in corticotroph tumor cells [76,77].

2.2.7. CDK inhibitors

Preclinical studies showed that the cyclin-dependent kinase (CDK 2/cyclin E) inhibitor R-roscovitine is able to reverse corti-
tocortroph tumor growth in zebrafish xenograft embryos and to suppress ACTH and corticosterone in a mouse model of ACTH-secreting pituitary tumor, suggesting that regulatory mechan-
isms in addition to the effects on cell cycle are also affected [78]. This compound also inhibits human pituitary corticotroph tumor ACTH secretion by targeting the cyclin E2F1 pathway [79]. It has been tested in phase I and II clinical trials for non-
small cell lung cancer and its safety profile raises some con-
cern [80,81]. However, there is currently a phase II study going
on for CD patients for which recruitment of patients is still active [82].

2.2.8. HSP90 inhibitors

We have recently reported that ACTH secreting adenomas show a strong overexpression of heat shock protein 90 (HSP90) and heat shock factor 1. Inhibitors of HSP90 targeted at its N-terminal domain such as 17-AAG or targeted at its C-terminal domain such as novobiocin and silibinin strongly reduced AtT-20 cell proliferation and altered the distribution of these cells among the phases of the cell cycle in a dose-dependent manner. However, while N-terminal inhibitors induced the release of the GR in an immature state from the HSP90 complex, resulting in its degradation, the treatment with the C-terminal inhibitor silibinin increased GR activity due to the release of a higher number of mature GR from the chaperone complex. This mechanism restores glucocorticoid sensitivity in vitro and leads to the suppression of ACTH production. In an allograft model of CD, silibinin treatment reduced tumor growth and hormone levels and alleviated typical symptoms of the disease [83]. Taking into account that silibinin has a good safety profile in humans for liver disease and poisoning treatment [84], the results reported showed that these compounds might be effective in restoring glucocorticoid sensitivity in subjects with CD.

2.2.9. Alkylating chemotherapeutic agents

Temozolomide (TMZ) is an alkylating chemotherapeutic drug, which can be used to treat malignant brain tumors. TMZ promotes apoptosis of target cells and induces massive cell shrinkage and necrosis, depleting the DNA repair enzyme O6-methylguanine-DNA-methyl transferase (MGMT) in different cell types. Multiple studies reported that intra-tumor levels of MGMT might predict responsiveness to TMZ [85]. Several studies described a variable response to TMZ in CD at a dose range of 150–200 mg/m²/day, with relatively mild side effects (moderate nausea, vomiting, fatigue) [86–89]. Emerging evidences indicate that aggressive corticotroph tumors that exhibit or develop resistance or are refractory to conventional treatment, and typically have a poor prognosis, show either a partial or complete response to TMZ therapy [88–91]. Long-term follow-up studies are needed to assess the effectiveness and durability of treatment [87,88].

2.3. Peripheral therapy

The concept of treating CD with a peripheral targeted therapy is based on the idea that the symptoms might be reduced by blocking the activation of GR directly at the target tissues, preventing in this way all the effects of hypercortisolism (Table 1).

Mifepristone is a glucocorticoid type II receptor antagonist, approved by regulatory authorities after a 6 months open-label, multicenter clinical study evaluating its efficacy and safety [19]. Administered at a daily dose of 300–1200 mg/day, the drug significantly decreased body weight, waist circumference, and body fat, and increased insulin sensitivity. Clinically significant improvements were seen in 87% of patients, although ACTH and cortisol levels were increased. The most common side effects were nausea, fatigue, headache, decreased blood potassium, arthralgia, vomiting, endometrial thickening, and peripheral edema. Glucocorticoid receptor antagonism with mifepristone may offer a new approach to control the clinical manifestations of endogenous hypercortisolism in patients who have not responded to multimodal therapies. In order to further evaluate efficacy and safety after a long-term treatment, phase III clinical trials are still ongoing [38]. A further option in this category of drugs might be ISIS-gccrxx, a glucocorticoid receptor antagonist currently under development for moderate-to-severe type 2 diabetes, obesity, and/or elevated triglyceride and cholesterol levels [13].

3. Conclusion

CD is a complex disease that requires a rapid medical intervention, but currently surgery and approved therapeutic options are not efficacious in a considerable proportion of patients. Therefore, there is still a pressing need to develop new and better compounds for the pharmaceutical treatment of CD. Most of the efforts in research have focused on controlling the production of cortisol acting directly on the adrenal glands, without affecting directly the source of the disease, the pituitary ACTH-secreting adenoma. However, new molecular targets have been found and promising new compounds are in the development phase but the search for new ones remains still open.

4. Expert opinion

Almost a century after its first characterization, CD continues to be a big challenge for researchers and physicians. In contrast to more aggressive forms of cancer, Cushing adenomas are characterized by small tumors with low cell proliferation rate and excessive ACTH secretion that is partially resistant to feedback control. The high levels of ACTH are responsible for the elevated morbidity of this disease, while tumor growth is not a major concern in most cases. For these reasons, conventional cancer treatments are not effective.

The particular pathogenic mechanism of these tumors is still in the process of being unveiled at the biochemical and genetic level. Recent studies suggest protein homeostasis as the major biological process in the pathogenesis of these tumors. The aberrant hormone secretion in corticotroph adenomas is regulated by HSP90 and other chaperones that control protein folding in response to cellular stress on the one hand and USP8 triggering protein degradation by the proteasome on the other hand. However, more genetic and functional studies are needed to fully understand the mechanisms that control corticotroph tumors’ growth and hormone secretion. A specific challenge in the field of Cushing adenomas is their heterogeneity. This fact limits the possibility to extrapolate the results obtained with small series of tumor samples or patients to the whole
patient population. In addition, Cushing adenomas are relatively rare and they do not proliferate in vitro, which limits the nature and number of experiments that can be done with each tumor sample. Therefore, more and better cellular and animal models need to be developed to facilitate the development of pharmaceutical compounds.

The main objective for medical treatment is to reduce disease-associated risks caused by the metabolic and cardiovascular repercussions, which impact survival. Considering the different possible approaches to achieve this goal through reduction of ACTH secretion, cortisol synthesis, or blocking the actions of these hormones on their target organs, most experts in the field favor the direct targeting of the tumor. However, the absence of specific validated targets that control ACTH secretion in most of the patients makes this approach difficult at the moment.

Most of the compounds designed and tested so far act at the level of the adrenal glands to normalize cortisol levels. Some of these drugs have limited use because of serious side effects. The most frequently prescribed is ketoconazole, which despite side effects and warnings continues to help manage hypercortisolaemia. Newer drugs based on similar mechanisms seem safer and very promising, such as LCI699. At the level of the corticotroph adenoma itself, ongoing research is focusing on new options to reduce ACTH secretion in order to give physicians a novel tool to treat patients whose surgery might be contraindicated or not consented. Due to the recent availability of pasireotide for the treatment of CD, patients have come to expect a pharmaceutical treatment for their condition. Unfortunately, somatostatin receptors are expressed and play important roles in other organs, in particular the pancreas, which inherently produces unwanted side effects. This encourages researchers in this field to pursue the identification of new tumor targets expressed in corticotroph adenoma cells and better compounds to regulate them.

A common caveat in all possibilities of available treatments is that none of them is effective and safe in the majority of cases. For instance, somatostatin and dopamine receptor agonists as well as retinoic acid seem to be effective in between 20% and 30% of the patients, reflecting the heterogeneity of the response to the treatment in these tumors. Focusing on targets that are more central to the physiological function of the corticotroph cell, such as HSP90 and USP8, might result in the development of drugs that are efficacious on a bigger proportion of patients.

As efforts continue to detect tumor-specific mutations, eventually, other targets will be identified that participate in the development of Cushing adenomas. Transgenic technology will then likely produce animal models that can recapitulate this disease and might be used to test systematically and in relatively large-scale different concepts for treatment. In the meantime, the pipeline is populated by a variety of promising compounds in development that are based on different therapeutic concepts.

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References
Papers of special note have been highlighted as either of interest (+) or of considerable interest (++) to readers.
1. Bertagna X, Guignat L, Groussin L, et al. Cushing’s disease. Best Pract Res Clin Endocrinol Metab. 2009;23(5):607–623.
2. Biller BMK, Grossman AB, Stewart PM, et al. Treatment of adrenocorticotropin-dependent Cushing’s syndrome: a consensus statement. J Clin Endocrinol Metab. 2008;93(7):2454–2462.
3. Pivonello R, De Leo M, Cozzolino A, et al. The treatment of Cushing’s disease. Endocr Rev. 2015;36(Aug):385–486.
4. Tritos NA, Biller BM, Swearingen B. Management of Cushing disease. Nat Rev Endocrinol. 2011;7:279–289.
5. Rutkowski MJ, Flanigan PM, Aghi MK. Update on the management of recurrent Cushing’s disease. Neurosurg Focus. 2015;38(Feb):E16–19.
6. Liu JK, Fleseriu M, Delashaw JB, et al. Treatment options for Cushing disease after unsuccessful transsphenoidal surgery. Neurosurg Focus. 2007;23(3):E8.
7. Hamrahian A, Yuen K, Hoffman A. AAACE/ACE disease state clinical review: medical management of Cushing disease. Endocr Pract. 2014;20(7):746–757.
8. Assié G, Bahurel H, Coste J, et al. Corticotroph tumor progression after adrenalectomy in Cushing’s disease: a reappraisal of Nelson’s syndrome. J Clin Endocrinol Metab. 2007;92(1):172–179.
9. Pecori Giraldi F, Ambrogio AG, Andrioli M, et al. Potential role for retinoic acid in patients with Cushing’s disease. J Clin Endocrinol Metab. 2012;97(10):3577–3583.
10. Vilar L, Albuquerque JL, Lyra R, et al. The role of isotretinoin therapy for Cushing’s disease: results of a prospective study. Int J Endocrinol. 2016;2016:8173182.
11. Cortendo’s Normocort: a Cushing’s syndrome newcomer enters phase III. [cited 2014 Aug 18]. Available from: https://healthcare.globaldata.com/resources/expert-insights/pharmaceuticals/cortendos-normocort-a-cushings-syndrome-new-comer-enters-phase-iii.html
12. Cortendo AB First patient enrolled into NormoCort phase 3 SONICS trial. Following a successful EU investigator meeting. [cited 2014 Aug 12]. Available from: http://otc.nmf.no/public/news/14650.pdf
13. MarketResearch.com [Internet]. Cushing’s Syndrome-opportunity analysis and forecast to 2018. GlobalData. [cited 2015 Dec 12]. Available from: http://www.marketresearch.com/product/sample-8941833.pdf
14. Morris D, Grossman A. The medical management of Cushing’s syndrome. Ann N Y Acad Sci. 2002;970(1):119–133.
15. Bertagna X, Pivonello R, Fleseriu M, et al. LCI699, a Potent 11β-hydroxylase Inhibitor, normalizes urinary cortisol in patients with Cushing’s disease: results from a multicenter, proof-of-concept study. J Clin Endocrinol Metab. 2014;99(4):1375–1383.
A complete report about the types of medical treatment available for Cushing’s disease.

Castillo V, Theodoropoulou M, Stalla G, et al. Effect of SOM230 (Pasireotide) on corticotrophic cells: action in dogs with Cushing’s disease. Neuroendocrinology. 2011;94(2):124–136.

Lamberts SW, Uitterlinden P, Klijn JM. The effect of the long-acting somatostatin analogue SMS 201-995 on ACTH secretion in Nelson syndrome and Cushing’s disease. Acta Endocrinol (Copenh). 1989;120(6):760–766.

Stalla GK, Brockmeier SJ, Renner U, et al. Octreotide exerts different effects in vivo and in vitro in Cushing’s disease. Eur J of Endocrinol. 1994;130(2):125–131.

Lamberts S, McLoad R. Regulation of prolactin secretion at the level of the lactotroph. Physiol Rev. 1990;70:279–318.

Igaz P, Tombol Z, Szabò PM, et al. Steroid biosynthesis inhibitors in the therapy of hypercortisolism: theory and practice. Curr Med Chem. 2008;15(26):2734–2747.

Komanicky P, Sprak R, Melby J, et al. Experience with Trilostane in Nelson syndrome as a remission-maintaining treatment. Orphanet J Rare Dis. 2015;10:1–9.

Fleseriu M, Petersenn S. Medical management of Cushing’s disease: a retrospective multicenter study in 195 patients. J Clin Endocrinol Metab. 2015;100(11):4146–4154.

Feelders RA, Hofland LJ. Medical treatment of Cushing’s disease. J Clin Endocrinol Metab. 2013;98(2):425–438.

Feelders RA, Hofland LJ, de Herder WW. Medical treatment of Cushing’s disease: a randomized, double-blind, multicenter study of pasireotide in Cushing’s disease. J Clin Endocrinol Metab. 2010;95(18):671–677.

Kapas S, Cammas FM, Hinson JP, et al. Agonist and receptor binding properties of adrenocorticotropic peptides using the cloned mouse adrenocorticotropic receptor expressed in a stably transfected HeLa cell line. Endocrinology. 1996;137(8):3291–3294.

Szalay KS, De Wied D, Stark E. Effects of ACTH(11–24) on the corticosteroid production of isolated adrenocortical cells. J Steroid Biochem. 1989;32(2):259–262.

Hofmann K, Montibeller JA, Finn FM. ACTH antagonists. Prog Natl Acad Sci USA. 1974;71(1):80–83.

Nensey NK, Bodager J, Gehrland AL, et al. Effect of novel melano-cortin type 2 receptor antagonists on the corticosterone response to ACTH in the neonatal rat adrenal gland in vivo and in vitro. Front Endocrinol (Lausanne). 2016;7:233.

Hofland Li, van der Hek J, Feelders R, et al. The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5. Eur J Endocrinol. 2005;152:645–654.

Schmid HA, Schoeffler P. Functional activity of the multiligand analog SOM230 at human recombinant somatostatin receptor subtypes supports its usefulness in neuroendocrine tumors. Neuroendocrinology. 2004;80(Suppl. 1):47–50.

Colao A, Petersenn S, Newell-Price J, et al. A 12-month phase 3 study of pasireotide in Cushing’s disease. N Engl J Med. 2012;366 (10):914–924.

Ligueros-Saylan M, Zhang Y, Newell-Price J, et al. Evaluation of the efficacy and safety of pasireotide LAR in patients with mild-to-moderate Cushing’s disease: a randomized, double-blind, multicenter, phase III study design [abstract]. Endocrine Abstrs. 2012;29(P1542:1).

Plößking U, Hoffmann U, Geese M, et al. DG3173 (somatoprim), a unique somatostatin receptor subtype 2-, 4- and 5-selective analogue, effectively reduces GH secretion in human GH-secreting pituitary adenomas even in Octreotide non-responsive tumors. Eur J Endocrinol. 2012;166:223–234.

Pivonello R, Ferone D, de Herder WW, et al. Dopamine receptor expression and function in corticotropic pituitary tumors. J Clin Endocrinol Metab. 2004;89(5):2452–2462.

Castillo V, Gomez N, Cabrera M, et al. Cushing’s disease in dogs: caborcine treatment. Res Vet Sci. 2008;85:26–34.

Rocheville M, Lange DC, Kumar U, et al. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science. 2000;288(5463):154–157.

De Bruin C, Feelders R, Lamberts SWJ, et al. Somatostatin and dopamine receptors as targets for medical treatment of Cushing’s Syndrome. Rev Endocr Metab Disord. 2009;10(2):91–102.

Culler MD. Somatostatin-dopamine chimeras: a novel approach to treatment of neuroendocrine tumors. Horm Metab Res. 2011;43 (12):854–857.
This study demonstrates the effectiveness of targeting EGFR in corticotrophs adenomas both in vitro and in vivo.

77. Lau D, Rutledge C, Aghi MK. Cushing's disease: current therapies and molecular insights guiding future therapies. Neurosurg Focus. 2015;38(2):E11.

78. Liu NA, Jiang H, Ben-Shlomo A, et al. Targeting zebrafish and murine pituitary corticotroph tumors with a cyclin-dependent kinase (CDK) inhibitor. Proc Natl Acad Sci USA. 2011;108(20):8414–8419.

79. Liu NA, Araki T, Cuevas-Ramos D, et al. Cyclin E-mediated human proopiomelanocortin regulation as a therapeutic target for Cushing disease. J Clin Endocrinol Metab. 2015;100(7):2557–2564.

80. Benson C, White J, De Boni J, et al. A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-Roscovitine), administered twice daily for 7 days every 21 days. Br J Cancer. 2007;96(1):29–37.

81. Le Tourneau C, Favier S, Laurent N, et al. Phase I evaluation of seliciclib (R-roscovitine), a novel oral cyclin-dependent kinase inhibitor, in patients with advanced malignancies. Eur J Cancer. 2010;46(18):3243–3250.

82. ClinicalTrials.gov [Internet]. Treatment of Cushing’s disease with R-roscovitine. ClinicalTrials.gov. [cited 2015 Feb 25]. Available from https://www.clinicaltrials.gov/ct2/show/NCT02160730.

83. Riebold M, Kozany C, Freiburger L, et al. A C-terminal HSP90 inhibitor restores glucocorticoid sensitivity and relieves a mouse allograft model of Cushing disease. Nat Med. 2015 Mar;21(3):276–280. doi:10.1038/nm.3776. Epub 2015 Feb 9. 

This study demonstrates the effectiveness of targeting HSP90 in corticotrophs adenomas both in vitro and in vivo.

84. Saller R, Meier R, Brigioni R. The use of silymarin in the treatment of liver diseases. Drugs. 2001;61(14):2035–2063.

85. Pozza C, Giazzadi C, Giannetta E, et al. Management strategies for aggressive Cushing’s syndrome: from macroadenomas to ectopics. J Oncol. 2012;2012:685213.

86. Mohammed S, Kovacs K, Mason W, et al. Use of temozolomide in aggressive pituitary tumors: case report. Neurosurgery. 2009;64(4):E773–E774; discussion E774.

87. Raverot G, Sturm N, de Fraincourt F, et al. Temozolomide treatment in aggressive pituitary tumors: case report. Endocrinology. 2012;150(6):863–875.

88. Levin AA. Receptors as tools for understanding the toxicity of retinoids. Toxicol Lett. 1995;82:83–91.

89. Kim YW, Sharma RP, Li JK. Characterization of heterologously expressed recombinant retinoic acid receptors with natural or synthetic retinoids. J Biochem Toxicol. 1994;9(5):225–234.

90. Occhi G, Regazzo D, Albiger NM, et al. Activation of the Dopamine Receptor Type 2 (DRD2) promoter by 9-cis retinoic acid in a cellular model of Cushing’s disease mediates the inhibition of cell corticotroph-to-melanotroph transdifferentiation. Endocrinology. 2014;155(9):3538–3549.

91. Ashok VS, Ogilvie GK, Anghel H, et al. Bexarotene be a candidate drug for the medical therapy of Cushing’s disease? Endocrine Abstracts. 2014;35:211.

92. Farol LT, Hynes KB. Bexarotene: a clinical review. Expert Rev Anticancer Ther. 2004;4(12):180–188.

93. Reincke M, Shibera S, Hayakawa A, et al. Mutations in the deubiquitination gene USP8 cause Cushing’s disease. Nat Genetics. 2015;47(1):31–38.

Dominant mutations in USP8 activate EGFR signaling, which is associated with Cushing’s adenomas.

94. Fukuo H, Cooper O, Ben-Shlomo A, et al. EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas. J Clin Invest. 2011;121(12):4712–4721.

95. This study demonstrates the effectiveness of targeting EGFR in corticotrophs adenomas both in vitro and in vivo.