# New insights and future perspectives in the therapeutic strategy of adrenocortical carcinoma (Review)

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**Abstract.** Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with an incidence ranging from 0.7 to 2.0 cases/million people per year. Hypercortisolism represents the most common clinical presentation in many patients although, less frequently, some ACC secreting androgens and estrogens are even more pathognomonic compared to cortisol secretion. Currently, radical surgery, when feasible, is still the only curative therapy. Mitotane, an adrenolytic drug, is used in the adjuvant setting and in combination with chemotherapy drugs in metastatic disease. The use of radiotherapy remains controversial, being indicated only in selected cases. New targeted therapies, such as insulin growth factor-1 (IGF-1), mammalian-target of rapamycin (m-TOR), vascular endothelial growth factor (VEGF) inhibitors and others, have recently been investigated with disappointing clinical results. The partial effectiveness of current treatments mandates the need for new therapeutic strategies against this tumor.

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## 1. Introduction

ACC is a very rare tumor accounting for 0.7-2.0 cases/million people per year with an increased incidence in the first and fourth-fifth decades of life. By gender, females are the most affected (55-60%) (1). ACC is burdened by a poor prognosis with a mean 5-year survival rate between 16 and 47%, falling to 5-10% in the advanced stages (2). The cornerstones in the pathogenesis of ACC are considered to be the genetic alterations of the IGF-2, p53 and β-catenin molecular pathways (2,3). Additionally, other genes, such as ZNFR3, identified by a genome-wide study, appear potentially involved in the tumorigenesis of ACC (4). Comparative genomic hybridization (CGH) demonstrated several complex mutations in ACC with chromosomal gains at 4q, 4p16, 5p15, 5q12-13, 9q34, 12q13, 12q24, 19p and losses at 1p, 2q, 11q, 17p, 22p and 22q. Genes within these regions that are potentially involved in neoplastic transformation include fibroblast growth factor 4 (*FGF4*), cyclin-dependent kinase 4 (*CDK4*), and cyclin E1 (*CCNE1*) (1). A recent epigenetic study performed on 51 ACCs identified a promoter hypermethylation of the *H19, GOS2, PLAGL1* and *NDRG2* genes (1). However, it has been recently observed that the dysregulation of some miRNAs, such as the upregulation of miR-483 and the downregulation of miR-195 and miR-335, could play a substantial role in the ACC tumorigenesis (5).

When ACC manifests as a condition of steroid hormone excess, the clinical picture is dominated mainly by hypercortisolism and/or hyperandrogenism, whereas symptoms of estrogen hypersecretion such as gynaecomastia and testicular atrophy are pathognomonic in male patients (6). DHEA-S represents a possible hormonal marker of ACC, conversely a decreased serum DHEA-S concentration likely indicates an adrenal adenoma (7). Mineralocorticoid excess is a rare event, that occurs with severe hypertension and hypokalemia. Notably however, an excess of glucocorticoids could produce a similar effect (6). Although few ACC appear non-secreting, they may produce an excessive amount of adrenal precur-

Table I. Staging system for ACC proposed by the European Network for the Study of Adrenal Tumors (ENSAT).

| Stage |
| --- |
| 1. T1, N0, M0 |
| 2. T2, N0, M0 |
| 3. T1-T2, N1, M0; T3-T4, N0-N1, M0 |
| 4. any T, any N, M1 |

Classification criteria of the tumor stage according to the TNM system: Stage 1: T1, tumor ≤5 cm; N0, no positive lymph nodes; M0, no distant metastases. Stage 2: T2, tumor >5 cm; N0, no positive lymph nodes; M0, no distant metastases. Stage 3: T1, tumor ≤5 cm - T2, tumor >5 cm; N1, positive lymph node(s); M0, no distant metastases; T3, tumor infiltration into surrounding tissue - T4, tumor invasion into adjacent organs or venous tumor thrombus in vena cava or renal vein. N0, no positive lymph nodes - N1, positive lymph node(s). M0, no distant metastases. Stage 4: T1, tumor ≤5 cm - T2, tumor >5 cm; T3, tumor infiltration into surrounding tissue - T4, tumor invasion into adjacent organs or venous tumor thrombus in vena cava or renal vein; N0, no positive lymph nodes - N1, positive lymph node(s). M1, presence of distant metastasis.

sors (8). The diagnostic employement of chromatography/ mass spectrometry methods revealed that >95% of ACC patients are able to autonomously secrete steroids or steroid precursors (9). Imaging plays a key role in the diagnosis of primary ACC, in the involvement of surrounding tissues and in its spread to distance sites. Either computerized tomography (CT) or magnetic resonance imaging (MRI) exploiting particular features such as Hounsfield unit (HU) values or chemical shift imaging, respectively allow adequate diagnostic accuracy to be achieve (10,11) as suggested by a recent analysis of the German ACC registry showing that the value of 13 HU may be considered as the threshold for discriminating benign from malignant adrenal masses (12). More recently, the fluorine 18 fluorodeoxyglucose (^{18}F-FDG) positron emission tomography (PET) or PET/CT was introduced as a diagnostic tool for ACC (13). ^{11}C-metomidate, due to its particular ability to bind 11 β-hydroxylase, has been proposed for the identification of tumors of adrenocortical origin (14). The introduction of [^{123}I]IMTO for single photon emission computed tomography (SPECT) and planar scintigraphy has provided a diagnostic alternative to PET for the discrimination of adrenal masses from non-adrenal tissues (15).

The first official TNM classification for ACC was established only in 2004 by the International Union Against Cancer (UICC) and the World Health Organization (WHO) (16). It was based on the criteria described by MacFarlane (17) and later modified by Sullivan *et al* (18). A significant improvement in the prognostic assessment was due to the adoption of the ENSAT (European Network for the Study of Adrenal Tumors) ACC staging system, which proposes a careful prognostic differentiation among the stages (19) (Table I). The use of this system in recent years has greatly improved the diagnostic accuracy and the prediction of survival for stage compared to the criteria previously adopted (20).

## 2. Treatment options

ACC is a neoplastic disease with a poor prognosis. Current studies in this field have indicated the need for a multidisciplinary approach in the management of this tumor (21,22). Surgery remains the most effective treatment choice for the primary tumor or in for the removal of isolated metastases (1,23). The experience that at least one-third of patients show loco-regional recurrence or distant metastases even after a radical surgical excision introduced the concept of adjuvant therapy in these patients (24). Despite an extensive surgical resection, the survival rate of these patients is estimated as ~50% after 5 years (25). Although these data support the need for an adjuvant cancer therapy, the therapeutic options in ACC currently remain under debate. At present, mitotane represents the only drug approved in Europe and in the United States for ACC treatment; however opinions regarding its use in adjuvant settings are still highly discordant (26). Currently, chemotherapy is reserved for those cases of advanced disease with evidence of distant metastases unresponsive to mitotane treatment. Many efforts are directed to the development of targeted therapy in ACC. Several strategies have been developed *in vitro* and some clinical trials have been conducted with small molecules, such as inhibitors of tyrosine kinase receptors or serine/threonine kinase receptors and monoclonal antibodies.

## 3. Surgery

Surgery is the only truly effective therapy in the treatment of ACC. A complete surgical resection (R0) is the treatment of choice, avoiding tumor spread that is considered an adverse prognostic factor. The achievement of R0 resection status often requires a radical surgery with a wide dissection of the neighboring organs. It represents a predictor of long-term survival (27). The choice of an open approach vs laparoscopic approach is debated. Open adrenalectomy is classically the more secure treatment recommended in patients with localized (stage I-II) and locally advanced (stage III) ACC. Comparative data concerning the two surgical techniques are lacking and originate from retrospective data that involved selection bias (28,29). It is likely that laparoscopic surgery might be reserved only for selected cases with masses of small size. However, these statements must be confirmed by prospective trials. Regardless of the surgical option chosen, the surgical team must have proven experience in the oncologic ACC surgery.

Although lymphadenectomy has never been considered as a standard procedure in the adrenalectomy, recent studies show that lymph nodes dissection is significantly associated with a reduction of the relapse rate in patients with localized disease (30,31). However, confirmatory data are needed in order to standardize the surgical procedure.

The therapeutic option of removing metastases is founded on the observation that their excision is associated with long-term survival (32,33) and the consideration that many ACC are metastatic at the onset (34). Encouraging results from several retrospective studies show that the metastasectomy correlates with an improvement of progression-free and overall survival (35,36). Finally, although the objective of debulking surgery is to reduce either the compressive effect exerted by a large

Table II. Regimen protocols in the FIRM-ACT study.

| Berruti et al protocol (EDP/ M) (53) | Every 28 days: | | |
|---|---|---|---|
| | Day 1 | 40 mg/m$^2$ | Doxorubicin |
| | Day 2 | 100 mg/m$^2$ | Etoposide |
| | Days 3 and 4 | 100 mg/m$^2$ | Etoposide + 40 mg/m$^2$ cisplatin |
| | Daily | | Mitotane with a blood level between 14-20 mg/l |
| Khan et al protocol (Sz/ M) (54) | Every 21 days: | | |
| | Days 1-5 | 1 g | Streptozotocin |
| | Subsequently | 2 g | Streptozotocin |
| | Daily | | Mitotane with a blood level between 14-20 mg/l |

size mass, on surrounding organs or the hormonal excess secreted by the tumor, lacking in this surgical approach is an oncological rationale (23).

## 4. Mitotane

Currently, mitotane represents the only therapeutic option approved by the US Food and Drug Administration and European Medicine Executive Agency for the treatment of ACC (37). Mitotane is a derivative of the insecticide dichloro-diphenyldichloroethane (DDT) with adrenolytic and cytotoxic activity toward the fasciculata and reticularis adrenal areas. It inhibits steroidogenic pathways acting mainly at the level of the cholesterol side chain cleavage enzymes CYP11A1 and CYP11B1 (38,39). Mitotane metabolites (o',p-DDA and o',p-DDE, respectively) are the products of a hydroxylation that occurs in the liver and of which o',p-DDA represents the active form (40). It has indeed been shown, that o',p-DDA measurements reflect the mitotane response in treated patients (41). Drug administration is oral, with the aim of reaching the therapeutic target between 14 and 20 mg/dl (41,42) above which side effects involving the gastrointestinal tract and central nervous system frequently manifest (43). A recent study showed that blood mitotane concentrations ≥14 mg/l were associated with a prolonged recurrence-free survival (RFS) in patients following macroscopically radical surgery (44). Furthermore, the measurement of plasma mitotane levels in the management of patients with ACC is mandatory. Different treatment strategies have been proposed to achieve the therapeutic dose even if the high-dose regimen appears to be the most effective for reaching the target concentration more rapidly (43,45). Mitotane has been shown to be an inducer of hepatic cytochrome CYP3A4 and thus able to interfere with the metabolism of other drugs including chemotherapeutic agents (46). This drug property complicates the management of other codition-related treatments, such as antihypertensives, statins, antibiotics and others, that are also being used in ACC patients (47). Furthermore, future protocols involving mitotane and antineoplastic drugs in combination should consider this particular drug feature.

Several studies regarding mitotane use in the clinical setting of ACC have reported conflicting results. However, the retrospective nature of these studies exposes them to numerous biases, mainly related to the different concentrations used and the lack of mitotane plasma level measurements. In fact, the response rate, as evaluated in series in which mitotane treatment was given without considering plasma concentration and those with patients in whose the drug concentration had been assessed, ranged from 25 to 55% respectively (40,41,48,49). An extensive retrospective case-control study performed on two independent cohorts showed significantly improved RFS compared to untreated control patients (p<0.0001). Overall survival (OS) was 110 months in the mitotane group vs 52 and 67 months in the control group (p=0.01) (50).

These data have allowed the introduction of the concept of using mitotane in the adjuvant therapy of patients affected by ACC. Currently, an international, multicentric, prospective, randomized trial, called ADIUVO (http://www.adiuvo-trial. org/), designed to evaluate the effectiveness of mitotane in adjuvant therapy is ongoing.

The recent ESMO guidelines recommend adjuvant mitotane treatment in stage III patients with potential residual disease (R1 or Rx resection status) and Ki-67 >10%. For patients in stages I or II, R0 resection and Ki-67 <10%, adjuvant mitotane therapy is not considered mandatory. Mitotane should be administered progressively to reach the dose of 6 g/day over 4-6 days, adjusting the dosage according to the patient's tolerance and the plasma drug level (51).

## 5. Chemotherapy

Among the different available treatment protocols for advanced ACC, chemotherapy is offered in combination with mitotane. The rationale of this combination is related to the ability of mitotane to overcome the drug-resistance induced by P-glycoprotein which is widely expressed in ACC (52). Several chemotherapeutic agents, such as adriamycin, cisplatin, doxorubicin, and others have been used alone or in combination with mitotane in the treatment of advanced ACC (53-56). Although variable percentages have been reported, the results from these studies demonstrate that cisplatin alone or in combination with etoposide have a higher effectiveness in advanced ACC (55,57-59). The First International Randomized Trial in Locally Advanced and Metastatic Adrenocortical Carcinoma Treatment (FIRM-ACT) clearly confirmed the advantage of the regimen of etoposide-doxorubicin-cisplatin (EDP) in combination with mitotane (Berruti et al protocol) (53) compared to streptozotocin plus mitotane (Khan et al protocol) (54) (Table II). In Berruti et al study, an overall response rate of 48.6% was achieved (53), whereas in the Khan et al regimen,

the response rate was 36.4% (54). According to these results, the International Consensus Conference on Adrenal Cancer of Ann Arbor recommended the use of these protocols as first-line regimens against metastatic ACC in 2003 (37). Despite the expectations, no significant differences were found in OS (median 14.8 vs 12 months, respectively). Similarly, quality of life and adverse events were comparable in patients receiving the two treatments, thus confirming the poor prognosis of patients with advanced ACC (60). Gemcitabine alone or in combination with mitotane demonstrated a good efficacy *in vitro* and its effectiveness was dependent on the sensitivity of the ACC cells to mitotane (61).

## 6. Radiotherapy

The effectiveness of radiotherapy in ACC has been extensively debated. A retrospective analysis from the German ACC Registry, demonstrated that adjuvant radiotherapy resulted in a significantly better 5-year RFS, but did not affect OS and disease-free survival (DFS) (62). In a recent retrospective study from the United States, radiotherapy was reported to decrease the risk of local failure 4.7 times compared with surgery alone (63). In contrast another retrospective study did not find a difference between adjuvant radiotherapy and surgery alone (64). Some *in vitro* studies support the potential combination of mitotane and radiotherapy. In fact, these studies reported an inhibitory effect of mitotane in association with ionizing radiations on ACC cell lines (65,66). Considering the current data, radiotherapy is intended for patients with R1 or Rx resection status with a high risk for local recurrence (67). However, the potential use of this method alone or in combination with other therapy should be investigated in future prospective clinical trials.

## 7. Targeted therapy

The failure of conventional therapies in advanced ACC and recent knowledge regarding the molecular pathways, involving oncosuppressor genes, such as *TP53, CDKN1C, CDKN2A* and *MEN1*, and oncogenes such as *IGF2, CTNNB1* and *RAS* involved in this malignancy have encouraged many efforts in developing new strategies against ACC (2).

*Insulin-like growth factor-2 mTOR pathway.* Overexpression of insulin-like growth factor-2 (IGF-2) is the most important molecular event occurring in >90% of ACCs (3). Its hypersecretion induces an uncontrolled activation of the PI3K/Akt/mTOR pathway by IGF-1R (68). Preclinical *in vitro* and *in vivo* studies on xenograft models showed that NVP-AEW541, a small molecule inhibitor, and IMC-A12, a human monoclonal antibody, were able to reduce cell proliferation, inhibiting the IGF-2 downstream pathway. The association of both molecules with mitotane sinergistically inhibited tumor growth (69,70). Two phase I studies have demonstrated the effectiveness of figitumumab, a monoclonal anti-IGF-1R antibody and linsitinib (OSI-906), a tyrosine kinase inhibitor binding IGF-1R inducing a clinical response in 57 and 33% of patients, respectively (71,72). Recently, the results of a phase III study to evaluate the therapeutic potential of OSI-906 were published, which were disappointing (73). An association was found

between *IGF2* overexpression, mTOR hyper-activation and reduced expression of miR-99a and miR-100 (74). A role of mTOR in normal and adrenal tumors has been demonstrated by several studies (74,75) and its inhibition by everolimus (RAD-001) leads to cell growth reduction both *in vitro* and *in vivo*, confirming the importance of microRNA regulation of the IGF-2/mTOR signalling cascade (74). Based on these data, a phase I trial tested the effects of temsirolimus (CCI-779), another inhibitor of mTOR in combination with cixutumumab, an anti-IGF-R1 monoclonal antibody, demonstrating a positive effect on tumor growth in 4 of 10 patients treated (76). A recent trial from the United States investigating the combination of cixutumumab and mitotane as first line treatment in patients with metastatic ACC reported effectiveness in 8/20 patients enrolled (77).

*Angiogenesis.* Most solid tumors display marked angiogenesis and substantial data highlight the vascular endothelial growth factor (VEGF) overexpression in ACC (70). Despite expectations for the inhibitors of this pathway, the results of clinical trials have been quite disappointing. A monoclonal VEGF antibody in combination with capecitabine administered in a series of 10 patients affected by advanced ACC did not show any positive results (78). A partial response with capecitabine at a dose of 200 mg/die has been described only in one case, a 40-year-old patient with chemoresistant ACC (79). Both sorafenib and sunitinib, tyrosine kinase inhibitors able to target VEGF, produced poor results also (70). A phase II trial consisting of the administration of sunitinib in 38 patients with unresponsive ACC recorded a progression-free survival ranging from 5.6 to 12.2 months (80). A phase I trial described a positive response in two patients affected by advanced ACC who received sorafenib in combination with tipifarnib, a farnesyltransferase inhibitor (81). Moreover, only in a single case report, a regression of metastatic ACC with sorafenib administration has been observed (82). Recently, a phase II study investigating the combined effect of sorafenib with metronomic paclitaxel did not show any clinical improvement, contradicting the obtained *in vitro* results (83). A partial response to sunitinib in a patients with metastatic ACC, after chemotherapy treatment, has been described (84). Moreover, Gangadhar *et al* reported a partial response, in a patient with advanced ACC, who received combination treatment with sirolimus, an mTOR inhibitor, and sunitinib (85). Finally, a role for heparanase-1 in ACC angiogenesis has been hypothesized thus representing a new selective therapeutic target in ACC (86).

*Tyrosine kinase inhibitors.* Microarray transcriptome analyses have provided new knowledge regarding the hyperactivated molecular pathways involved in ACC, thereby suggesting new potential target molecules for treatment strategies to address (3,87). Frequently, these targets are represented by growth factors and therefore the therapeutic concept is based on the inhibition of protein kinases involved in signal transduction, often tyrosine kinase receptors (70). A clinical study performed on 10 patients with advanced disease treated with erlotinib, an EGFR inhibitor, in combination with gemcitabine demonstrated very limited effectiveness (88). Similarly, in another study, that investigated treatment with gefitinib as a

second-line monotherapy in a series of 19 patients with unresectable ACC, no response was obtained (89). In a phase II study, treatment with imatinib mesilate, a PDGFR inhibitor, was associated with disease progression in 75% of patients with severe side effects (90). It is likely that the failure of these therapies is related to the low presence of these receptors in ACC. Interestingly, no mutation of the EGFR gene has been detected (91).

*MDR/P-glycoprotein*. The chemoresistant properties of ACC have been classically related to the overexpression of the multidrug resistance protein MDR-1 (P-glycoprotein, Pgp), a debated drug efflux pump (52). Even with results from an *in vitro* study suggesting that mitotane enhances doxorubicin activity by interfering with Pgp (92), the exact role of MDR-1 protein in ACC needs to be elucidated. Furthermore, a clinical trial using doxorubicin, vincristine, and etoposide in combination with mitotane failed to demonstrated therapeutic effectiveness (93).

*PPAR-γ antagonists*. Thiazolidinediones effects are somewhat different in the oncologic field (94). The PPAR-γ receptor is widely expressed in normal and in tumor adrenal tissue. It is involved in the IGF-2/IGF-1R signaling pathway, by inhibiting Akt activation (95,96). It has been shown that these compounds are able to inhibit cell proliferation in ACC cell models and in xenograft models (95-98). In particular, it has been shown that rosiglitazone, a thiazolidinediones class member, activating both PPAR-γ-dependent and -independent pathways, led to a growth arrest, cell death and reduction of *VEGF* expression involved in adrenocortical angiogenesis (95,98).

*Wnt/β-catenin pathway*. The constitutive activation of this pathway is found in 85% of ACC (99). Moreover, nuclear localization of β-catenin represents a worse prognostic factor in ACC (99,100). Preclinical *in vitro* studies were performed with PKF115-584, a small molecule inhibitor of the T-cell factor (Tcf)/β-catenin complex, on β-catenin-dependent transcription and proliferation processes in H295R ACC cells, that harbored *CTNNB1* gene mutations. As a results, this treatment was able to inhibit cell growth and induce apoptosis in the H295R adrenocortical cell line but not in HeLa, a human epitheliod cervical carcinoma cell line, confirming that the Wnt/β-catenin pathway is an useful target in ACC (101). Another *in vitro* study demonstrated that β-catenin silencing inhibited cell proliferation and induced apoptosis in the H295R cell line (102). A phase I study with CWP232291, a compound recognized for its ability to promote β-catenin degradation with activity in several multiple myeloma cell lines, is currently ongoing in patients with relapsed or refractory acute myeloid leukemia (103). However, no clinical study is currently underway for ACC.

*Steroidogenic factor-1*. Steroidogenic factor-1 (SF-1) is a nuclear transcription factor involved in the steroidogenic tissue development (104). It is frequently overexpressed in pediatric ACCs, whereas in the adult population some abnormalities on chromosome 9 have been described. However, a higher nuclear SF-1 expression level has been associated with a worse prognosis in ACC (104) and is positively correlated with

advanced ENSAT stages, and a higher mitotic index and Weiss score (105). An increased SF-1 dosage was observed to stimulate proliferation, decrease apoptosis in adrenocortical cells, and induce tumorigenesis in transgenic mice (106). SF-1 silencing affected TGF-β and Wnt/β-catenin signaling, suggesting crosstalk between these pathways in a study performed on the H295R adrenocortical cell line. Moreover, SF-1 knockdown showed a significant reduction of cell proliferation be interference with S-phase of the cell cycle (106,107). Two members of the alkyloxyphenol class, AC-45594 and OOP, the synthetic SF-1 inverse agonists have been shown to inhibit proliferation in both H295R and SW13 adrenocortical cell lines through an SF-1 non-selective mechanism. In contrast, SID7969543 (IsoQ A) and the compounds numbered 31 and 32, members of the IsoQ class, induced a selective inhibition of cell proliferation when SF-1 was increased strongly suggesting that the IsoQ molecules targeted SF-1-related genes (108).

*Gene therapy and immunotherapy*. The rationale of gene therapy lies in correcting the gene regulation, reactivating oncosuppressor genes and/or inhibiting oncogenes during tumorigenesis. Systemic therapy with antisense oligonucleotides represents an innovative approach for ACC treatment. A construct, composed of the herpes simplex virus thymidine kinase (HSV-TK) gene driven by the CYP11B1 promoter with a P450scc enhancer element, increased the chemosensitivity in a Y1 mouse ACC cell line (109). Immunotherapy represents another therapeutic approach that relies on the stimulation of the immune system against specific target proteins of neoplastic cells. This approach, using dendritic cells, was effective in stimulating the immune response (110), inducing antigen-specific Th1 immunity in a study performed on two patients with advanced secreting ACC. However, no clinical benefit has been shown (111).

In the cytokine family, interferon-β (INF-β) showed an inhibitory effect *in vitro* on ACC cell lines and primary ACC human cell cultures. Furthermore, when co-administered INF-β, sensitized ACC cells to mitotane (112). Recently, a phase I clinical study was conducted with interleukin-13-*Pseudomonas* exotoxin in patients affected by advanced ACC, exploiting the rationale that the α2 receptor of interleukin 13 (IL13Rα2) is more highly expressed in ACC than in normal adrenal tissue. This study showed a low disease stability rate lasting a few months before the ACCs progressed (113).

*miRNA*. Recent biological advances concerning microRNA dysregulation in all cancers including ACC highlights the hypothetical consideration of these small non-coding RNAs as potential target molecules for anti-cancer treatment (114). Because miRNAs may function as tumor suppressors, the assumption of replacement miRNA cancer therapy must not disregard the identification of an miRNA deficiency. In a previous analysis of miRNA expression in adrenocortical tumors, miR-7 was the most significantly under-expressed miRNA when compared to normal adrenal tissue (115). Glover *et al* provided the first demonstration of the effectiveness of the nanoparticle systemic delivery of miR-7 in the reduction of cell growth in both cell lines and in an ACC xenograft model, respectively. Furthermore, they demonstrated that miR-7 functions as a tumor suppressor in ACC leading to the
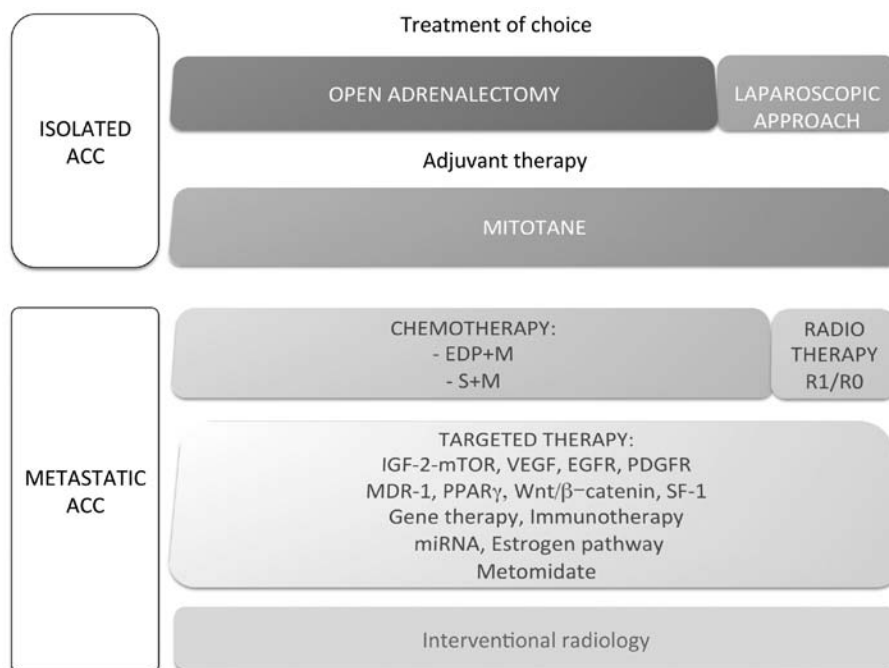
Figure 1 Treatment of choice in isolated and metastatic adrenocortical carcinoma (ACC).

repression of several genes involved in the pathogenesis of ACC, including *RAF-1, mTOR* and *CDK1* (116).

*Estrogen pathway*. Recent advance confirm an estrogenic pathway in normal adrenal tissue and in adrenal tumors. A differential expression of estrogen receptors (ER) α and β has been demonstrated in ACCs (117). Moreover, Barzon *et al* showed an increased aromatase activity in ACC, hypothesizing a paracrine estrogenic effect at the tumor level (118). An *in vitro* study demonstrated that hydroxytamoxifen, increasing the pro-apoptotic factor FasL expression, reduced H295R cell proliferation by ERα downregulation and ERβ upregulation, respectively (119). ERα activation may occur by an 17-β estradiol (E2)-dependent mechanism or alternatively by IGF-II/IGF1R in a ligand-independent manner, activating proliferative pathways *in vitro*, such as IGF1R/AKT signaling, in H295R cell lines. Furthermore, in the same study, hydroxytamoxifen, an active metabolite of the estrogen antagonist tamoxifen, reduced IGF1R protein levels and cell proliferation induced by E2 and IGF-II both *in vitro* and in an ACC xenograft model (120). These data indicate a crucial role of the estrogenic pathway in ACC and support the possibility of using antiestrogens in the treatment of ACC. A recent interesting study elucidated the ability of a non-steroidal G-protein-coupled estrogen receptor (GPER) agonist to exert a growth inhibitory effect, mediated by activation of the ERK1/2 pathway, both in the H295R cell line and in xenograft ACC (121). Finally, the compound XCT790, an inverse agonist of the transcription factor estrogen-related receptor α (ERRα) (122), an orphan member of the nuclear hormone receptor superfamily with a similar sequence to ERα involved in cellular metabolism and mitochondrial biogenesis (123), was able to reduce cell growth in both the H295R cell line and in an ACC xenograft model, with impaired mitochondrial functioning leading progressively to cell death (124).

*Metomidate*. [123I]IMTO single-photon emission CT imaging has been recently introduced as a novel tracer for the identification of adrenocortical tumors (15). Treatment options with [131I]IMTO depend on the [123I]IMTO uptake in the lesion potentially related to ACC (15). A recent experience in 11 patients with advanced ACC receiving ≤20GBq [131I]IMTO, showed a low progression-free survival in six responders (125).

*Interventional radiology*. Minimally invasive procedures such as radiofrequency thermal ablation (RFA), or transarterial chemoembolization (TACE) represent an alternative to surgery in advanced metastatic malignancies. The same approach was adopted also in the treatment of lesions in the liver, kidney, lymph nodes and lung for stage IV ACC patients (126). Wood *et al* observed that RFA induced a growth arrest in 8 of 15 lesions after 6 months of follow-up. The procedure was safe and was not associated with any particular side effects (126). TACE allows the selective infusion of high doses of cytotoxic drugs in the metastatic lesion reducing the systemic toxicity. In a French study, this technique was associated with a median survival of 11 months in 21 patients with liver metastatic disease (127). These procedures provide palliative benefits, are safe and inexpensive while implying minimal morbidity and a short recovery, however, none of these methods have been supported by a clinical trial.

**8. Conclusion**

ACC in the past was considered an orphan disease for which surgery represented the only feasible therapy. Over the years the focus on this aggressive endocrine malignancy has gradually grown, capturing the interest of many investigators. Despite the enormous progress achieved in the biological knowledge of this tumor, the ACC remains an oncological disease burdened by a high mortality. Surgery

is still the first therapeutic option and the only potentially curative treatment. Mitotane has represented the first drug in the treatment of ACC since 1959. Subsequently, knowledge regarding its mechanism of action has increased substantially while its clinical use has become much more controlled and appropriate. Currently, mitotane is the only drug approved by international pharmaceutical agencies for ACC treatment. Although mitotane treatment in the adjuvant setting is still debated, the recent ESMO guidelines recommend its use in adjuvant setting after surgery in patients with incomplete resection status. The optimal chemotherapy regimen is considered to be etoposide-doxorubicin-cisplatin (EDP) in combination with mitotane in patients with advanced metastatic disease, although this regimen is burdened by substantial side effects. The current -OMICs approach has permitted the discover of different molecules belonging to pathways potentially involved in the pathogenesis of the ACC. The therapeutic option described in isolated and meta-static ACC are summarized in Fig. 1. Future efforts should be made not only to explore new frontiers but also to investigate innovative therapies in the clinical field.

ACC urgently requires new therapeutic strategies. The clinical translation of new research products *in vitro* and in preclinical studies may help improve the standard of care in these patients. Achieving this objective in a rare disease such as ACC will require the carefully selection of the clinical series to be devoted to experimentation and an increasead collaborative network of research centers involved in the study of this malignancy.

*Future directions*. Recent contributions made by applying -OMICs to large-scale analyses such as the genomics, tran-scriptomics, proteomics and epigenetics of tumor samples had steadily increased the scientific knowledge concerning the heterogeneity of ACC. However, despite recent progress achieved in this field, the prognosis of this cancer remains poor. Beyond the surgery that is considered the standard of care, current therapeutic options include mitotane in adjuvant therapy and the use of different chemotherapeutic agents in combination, among which EDP plus mitotane is the prevailing combination, in the treatment of advanced ACC. In the coming years, the working agenda includes defining the current therapeutic protocols by prospective trials to: i) optimize the surgical techniques and procedural strategies; ii) validate mitotane use in the adjuvant setting by ADIUVO trial; and iii) evaluate post-surgery radiotherapy effectiveness. Additional efforts to manage and treat this aggressive tumor must be pursued by clinical, basic and genetic research studies. The main purpose of these studies should be aimed at testing new therapeutic targets. The final hope is to prompt multi-center clinical trials for the investigation of the most promising molecules to fight ACC.

## 9. Bullet points

• Open surgery represents the first therapeutic choice in ACC.
• Mitotane treatment is recommended in patients at high risk for recurrence, whereas low-risk patients can be recruited into the ADIUVO trial or followed according to an individual management plan.

• Monotherapy with mitotane is useful in patients after incom-plete surgical excision or in metastatic disease with limited metastatic spread.
• The 'therapeutic window' of 14-20 mg/l should be consid-ered in monitoring blood mitotane concentration.
• Post-surgery radiotherapy of the tumor bed is suggested in patients with R1/RX resection status.
• The combination chemotherapeutic treatment of etoposide, doxorubicin and cisplatin plus mitotane is currently consid-ered the most suitable regimen for ACC metastatic disease.
• Several target molecules have been identified; currently however, none have shown established effectiveness due to the lack of confirmatory clinical data.

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