Management of adrenocortical carcinoma: a consensus statement of the Italian Society of Endocrinology (SIE)

Medical Management of Adrenocortical Carcinoma: a Consensus Statement of the Italian Society of Endocrinology (SIE)

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Introduction

The rarity (0.5–2 cases per million per year) and aggressiveness of adrenocortical carcinoma (ACC) have limited our knowledge of the biological processes underlying its development and the design of specific and effective therapies. ACC is often associated to poor prognosis, with a mean 5-year survival rate between 16 and 47 %, dramatically dropping to 5–10 % in metastatic disease. Prognosis mainly depends on tumor stage and feasibility of radical surgery. At present, complete surgical removal of the tumor, possibly associated to adjuvant mitotane therapy, represents the best treatment option for ACC. Treatment of advanced ACC remains disappointing for limited efficacy and significant toxicity [1–3]. Management of ACC is challenging for both physicians and their patients and represents one of the most demanding tasks for clinical endocrinologists. However, endocrinologists should be involved in the management of patients with ACC because the disease and its treatment cause a profound derangement of the endocrine system that is harmful for patients and has a negative impact on prognosis. Moreover, endocrinologists play a unique role in the diagnostic process that is too often delayed because patients are not referred to physicians with specific expertise. Thus, presenting symptoms, that are often hormone-related, remain overlooked. On the basis of these considerations, the Italian Society of Endocrinology (SIE) appointed a panel of Italian experts in the field of adrenal diseases with the task to write a Position Statement whose intent was to review and synthesize currently available evidence regarding ACC and provide practical recommendations for the medical management of this devastating endocrine cancer. The authors have adopted in this Consensus Statement the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system [3]. This represents a practical and rigorous system, encouraged by Endocrine Society's Clinical Practice Guidelines program, which used two grades of recommendations (strong or weak) and four types of quality of the evidence (high, moderate, low, or very low). According to GRADE system, we used, along text, "recommend" for strong recommendations, and "suggest" for weak recommendations. In the Summary at the end of each section, we use the numbering 1 and 2 to express the recommendation degrees and the appropriate symbols for the level of quality of evidence according to GRADE system [3].

Hormonal Assessment

the signs and symptoms of pheochromocytoma, hyperaldosteronism, hyperandrogenism, and hypercortisolism on the basis of the patient's history and physical examination [1]. About 60 % of patients with ACC will show signs and symptoms of adrenal steroid excess, mainly hypercortisolism and/ or hyperandrogenism (in women hirsutism and oligomenorrhea), while symptoms of estrogen hypersecretion (gynaecomastia and testicular atrophy) are present in 5–10 % of male patients and are pathognomonic for ACC [2]. In some patients, severe hypertension and profound hypokalemia are the presenting symptoms, heralding aldosterone excess. However, a profound hypokalaemia may be also due to severe hypercortisolism since the highly increased cortisol levels activate mineralocorticoid receptors by overtaking the inactivating capacity of corticosteroid 11β-hydroxysteroid dehydrogenase isoenzyme 2 [1, 2]. A detailed endocrine assessment is recommended preoperatively since it may consent: (1) to establish the adrenocortical origin of the tumor excluding other differential diagnoses (i.e., lymphoma, sarcoma), and to suspect the malignant potential of the adrenal mass (i.e., estradiol excess in males, high concentration of dehydroepiandrosterone sulfate—DHEAS- or steroid precursors); (2) to evaluate the risk of a life-threatening post-operative adrenal insufficiency in patients with cortisol-secreting adrenal tumors; (3) to have a tumor marker for the post-operative follow-up that may help predicting persistence or recurrence of disease [2, 4]. In patients without overt steroid overproduction, an ACC may still secrete excessive amounts of adrenal steroid precursors due to decreased expression of several steroidogenic enzymes [5]. Increased secretion of urinary metabolites of several steroids, and precursors of androgens, glucocorticoids or mineralocorticoids can be detected even in the absence of a clinically or biochemically overt steroid excess by the use of sensitive methods such as gas chromatography/mass spectrometry. When applying this methodology, more than 95 % of all patients with ACC are found to secrete autonomously steroids or steroid precursors [6]. Therefore, we recommend a detailed hormonal assessment be done even in asymptomatic patients, to assess the presence of steroid excess. Overt hypercortisolism should be suspected in presence of easy bruising and/or facial plethora and/or proximal myopathy or muscle weakness and/or purple striae [7]. The 1 mg overnight dexamethasone suppression test (1-mg DST) has the highest sensitivity (95 % with a threshold of 1.8 μg/ dl), while measurement of 24-h urinary-free cortisol (UFC) excretion is less sensitive for the detection of mild hypercortisolism [8]. However, several conditions may lead to false positive and, less frequently, false-negative results. A review of the screening tests in overt hypercortisolism is beyond the scope of this Position Statement, and the reader is referred to the Endocrine Society Clinical Practice Guidelines [7]. The presence of altered biochemical parameters of cortisol secretion in the absence of the above-mentioned signs and symptoms of hypercortisolism is generally defined as "subclinical hypercortisolism". Due to the

lack of a specific clinical picture and the variability of cortisol secretion, the diagnosis of subclinical hypercortisolism is often difficult and the best strategy is currently debated [9–11]. However, we suggest that cortisol levels lower than 1.8 μg/dl following the 1-mg DST can reliably exclude subclinical hypercortisolism, whereas post-DST cortisol levels above 5 μg/ dl ascertain the condition [12], respectively. Intermediate cortisol values after 1-mg DST may require additional investigations (i.e., assessment of ACTH and UFC levels), particularly in presence of clinical conditions potentially associated with cortisol excess (i.e., osteoporosis, arterial hypertension, diabetes). We suggest that the presence of at least two out of the following alterations: cortisol after 1-mg DST above 3 μg/dl, ACTH below 10 pg/mL and increased UFC levels may suggest a subtle cortisol excess [13]. The presence of at least two out of the following alterations: cortisol after 1-mg DST above 3 μg/dl, ACTH below 10 pg/mL and increased UFC levels may suggest a subtle cortisol excess [13]. Diagnosing an asymptomatic cortisol excess may be useful to avoid post-operative adrenal insufficiency after adrenalectomy [14]. Aldosterone-producing ACC is rare and is generally associated with hypertension and severe hypokalemia [15]. In patients with a strong suspicion of malignant adrenal lesion, the diagnostic workup for hyperaldosteronism could be to avoid since it can lead to a delay of surgery [16]. The diagnostic approach of primary hyperaldosteronism is beyond the scope of this Position Statement, and the reader is referred to the Endocrine Society Guidelines [17]. Hypersecretion of sexual steroids is frequently observed in ACC patients. Estrogens hypersecretion, though rare, should be ascertained in males (particularly when presenting gynecomastia) and post-menopausal females. In many patients, the 17-OH progesterone levels are frequently increased, as well as androstenedione and, more often, DHEAS, which leads to increased plasma testosterone in females [18]. However, the predictive value of increased DHEAS levels to predict malignancy is rather low [19]. Fractionated metanephrines, along with steroid hormones assay, should be preliminarily performed in all patients affected by adrenal masses [20]. A comprehensive discussion of the best diagnostic approaches in patients with adrenal masses and suspected pheochromocytoma is beyond the scope of this Position Statement and the reader is referred to the Endocrine Society Guidelines [20].

Summary

1. MostACCs secrete glucocorticoids, mineralocorticoids and sexual steroids in variable combination even in the absence of a suggestive phenotype.
2. A preoperative endocrine assessment allows to: i) establish the adrenocortical origin of the tumor and suspect its malignant potential, ii) evaluate the risk of peri-operative complications and post-operative adrenal insufficiency in patients with glucocorticoid excess; iii) have a marker of the persistence or recurrence of the disease during follow-up.
3. The following minimal endocrine work-up should be undertaken in patients bearing an adrenal mass:

- glucocorticoid excess (all patients):serum cortisol after 1-mgDST;
- mineralcorticoid excess (hypertensive and/or hypokalemic patients): potassium, aldosterone to renin ratio
- steroid precursor excess (all patients): DHEAS, 17OH-progesterone,
-androgen excess (symptomatic women): androstenedione, testosterone,
-estrogen excess (symptomatic men or postmenopausal women):17β-estradiol
-catecholamine excess (all patients except those with small, hypodense tumours): fractionated metanephrines in urine or free metanephrines in plasma.

Radiological Assessment

Imaging has a prominent role not only in diagnosis of ACC but also in staging, assessment of involvement of surrounding organs and vessels to evaluate the feasibility of radical surgery and during follow-up to monitor response to treatment.

Although the radiological characterization of adrenal masses and the differentiation between benign and malignant lesions has been the focus of a wealth of specialized literature over the years, many studies have suffered from important limitations. It is worth mentioning the retrospective nature of almost all studies, the use of different radiological equipments and scanning techniques as to timing, contrast volume, washout thresholds, the lack of a pathological reference standard, the limited number of ACC in the different series, the generic distinction between adenomas and non-adenomas without further differentiation among different tumor types. Moreover, criteria of benignity have been based mainly on densitometric data observed in small adrenal lesions (adenomas) without including larger benign lesions.

Unenhanced CT

On cross-sectional imaging, morphological features arising the suspicion of ACC in an adrenal lesion include tumor heterogeneity (due to necrosis or hemorrhage), lobulated shape, irregular margins, calcifications and a large size. None of these features can be considered a definitive sign of malignancy. Calcifications, present in about 30 % of ACC [21, 22], are rarely seen also in adenomas and are present in 10 % of pheochromocytomas [23] and either benign or

malignant adrenal lesions show heterogeneous density, particularly after intravenous contrast medium. Even the presence of well-defined margins does not indicate necessarily benignity [22]. Besides these features of the primary lesion, presence of venous thrombus and lymphadenopathy is clearly suggestive of malignancy; evidence of metastatic disease is a definitive proof of malignancy. Although tumor diameter is correlated with the risk of malignancy, it cannot be considered per se a useful discriminating criterium, due to the wide overlap in size among ACC, adenomas, metastases and pheochromocytomas [23–25]. Each of the size thresholds so far proposed as predictor for malignancy has shown inadequate diagnostic accuracy. The measurement of the attenuation of adrenal lesions on unenhanced CT has a relevant role in distinguishing between benign and malignant masses. The diagnostic value of CT densitometry rests on the concept of a lipidsensitive imaging procedure showing an inverse linear correlation between fat concentration and attenuation on unenhanced CT. Up to 70 % of adenomas are lipid-rich while malignant lesions are lipid-poor; thus, the higher the lipid content the lower the unenhanced CT density. A value of 10 HU has become the most widely used for the diagnosis of lipid-rich adenomas [26, 27]. Cumulative data for the identification of adrenal adenomas obtained subsequently indicate a sensitivity of 96–100 % and a specificity of 50–100 % in differentiating benign to malignant masses (mainly metastases) [12]. A lesion with an attenuation value >10 HU on unenhanced CT is categorized as indeterminate and worth of additional studies. In clinical practice, we recommend to perform a preliminary unenhanced CT attenuation measurement and subsequently a study after intravenous contrast scan following current technical recommendations for an optimal CT study of the adrenal glands [28]. Limitations to the diagnostic role of unenhanced attenuation value arise from the intrinsic characteristics of some pheochromocytomas that may exhibit values in the range of adenomas; moreover, about 30 % of adrenal adenomas are lipid-poor tumors that may show attenuation values >10 HU. In addition, differences in single-detector and multi-detector CT helical scanners can generate slightly different attenuation values that could produce different categorization [29]. A recent analysis of the German ACC registry on the largest number of adrenocortical cancers ever evaluated in any adrenal imaging report suggests that 13 HU is the most sensitive threshold to distinguish adenoma from carcinoma; although HU values below 21 most probably indicate a benign lesion, adrenal masses with HU values between 13 and 40 should be considered indeterminate requiring further evaluation [30].

Enhanced CT

On unenhanced CT, most lipid-poor lesions remain indeter- minate needing additional studies to be characterized. Over the last few years, many studies showed that the CT den- sitometry method could be used on delayed (10–30 min) images since adenomas, irrespective of fat content, show a rapid loss of contrast medium (contrast washout) at variance with malignant lesions showing a slower washout due to leaky capillaries [28, 31–34]. The washout can be expressed as absolute (the pre-contrast density is known) or relative (only a portal venous phase baseline is available). It has been reported that the absolute washout, including the consideration pre-contrast attenuation, allows better discrimination [28]. We recommend to perform an enhanced CT in lipid-poor lesions particularly when these show an increase of size. Adenomas are typically associated with a >60 % absolute washout (sensitivity 86–100 % and specific- ity 83–92 %) and >40 % relative washout (sensitivity of 82–97 %, specificity of 92–100 %) at 15 min after contrast administration. Although a high accuracy was reported also for a 10-min post-contrast delayed protocol [28, 32], a recent re-analysis in a large cohort of patients showed lower sensitivity of the 10-min protocol compared to pre- vious studies [35]. A limitation of these studies comparing washout in adenomas versus non-adenomas is the very lim- ited number of ACC, if any, in the different series. The two studies specifically evaluating the washout characteristics of ACC, which were similar to adrenal metastases, included only 7 and 11 patients, respectively [34].

A recent study by Zhang [22] including 42 pathologically ascertained ACC shows that the mean absolute and relative washout values do not significantly vary from values of other non-adenomas. Moreover, either absolute or relative washout values were in the range of adenomas in some cases.

ACC can extend into the adjacent vessels such as the renal veins and the inferior vena cava. This event is more common for right-sided ACC. Tumor invasion appears as a tumor thrombus, generally well encapsulated that can extend into the right atrium [36]. Involvement of inferior vena cava is not so rare being reported in about 15 % of patients with ACC [22, 36]. Although there are no specific comparison studies in ACC, MRI is generally preferred to CT for vascular assessment because of better resolution of soft tissues. Enhanced CT is extremely useful to assess organ invasion and metastatic spread. Metastases from ACC generally involve lungs, liver, regional and para-aortic lymph nodes and bones. Enhanced CT is the technique of choice for staging and assessment of response to treatment.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is overall as accurate as CT in the differentiation between benign and malignant adrenal masses. MRI relies on chemical shift imaging, a lipid-sensitive method that exploits the difference of resonant frequencies of water and fat protons so that they cancel each other out during out-of-phase sequences. The loss of signal intensity on out-of-phase images in relation to a refer- ence organ (generally spleen to avoid the confounding effect of liver steatosis) accurately demonstrates the presence of intra-cytoplasmatic fat, a phenomenon typical of adenomas due to their abundant lipid content. The normal adrenal is of low to intermediate signal on T1- and T2-weighted images. Adrenal adenomas are seen as homogeneous lesions, occa- sionally showing small areas of altered signal intensity due

to hemorrhage or cystic changes. On T1-weighted imag-ing, ACC is isointense or slightly hypointense to the liver but can appear heterogeneous both on T1- and T2-weighted imaging due to hemorrhage or necrosis, respectively. Con versely lipid-poor lesions, as metastases, pheochromocytomas or ACC, do not show any change in signal intensity on out-of-phase images. Sensitivity of MRI with chemical shift imaging in differentiating adenomas from non-adenomas is 84–100 % and specificity 92–100 %, reportedly similar to unenhanced CT [33].
It has been reported that CT with absolute and relative washout has higher sensitivity and specificity for lipid-poor adenoma than chemical shift MR, although the difference between the two imaging modalities is not significant [37].
Foci of mature fat can be found also in ACC determining irregular areas of loss of signal on chemical shift imaging [38, 39]. This finding, seen also in lipid-poor adenomas is different from the uniform loss of signal typical of lipid- rich adenomas.
Newer MRI techniques, such as three-dimensional technique for OP and IP 3-T MR imaging and MR adrenal spectroscopy, may reportedly offer further help to characterize the indeterminate adrenal masses. The 3D technique for OP and IP 3-T imaging in a series of 26 adrenal adenomas (9 lipid-poor) and 9 non-adenomas (4 ACC) showed better sensitivity and specificity than the 2D technique [40]. Preliminary data on MR adrenal spectroscopy show simi- larly promising result in the differentiation among adenomas, pheochromocytomas, ACC and metastases [41].

PET- PET/CT

Fluorine 18 fluorodeoxyglucose (18F-FDG) positron emis- sion tomography (PET) or PET/CT is nowadays widely employed in oncology for diagnosing and staging malignancy in different types of tumors. The procedure is based on the increased uptake of radiolabeled glucose by a tumor with respect to normal tissue. There is some debate on the analysis of FDG uptake whether quantitative assessment, using standardized uptake values (SUV) with respect to liver uptake, or qualitative assessment by visual inspection has greater accuracy [42]. However, an adrenal to liver max SUV ratio comprised between 1.45 and 1.8 has been identified to separate between benign and malignant adrenal tumor [42].
In the past decade, many studies have focused on the role of 18F-FDG PET in the characterization of adrenal masses. The combined use of PET/CT offers the advantage of combining functional information and anatomical defini- tion and allows incorporation into the analysis of CT densitometry. PET/CT performs better than PET alone and the combination of PET criteria with attenuation characteristics improves accuracy.
Previous studies, performed on patients with known primary cancer, showed an 18F-FDG PET or PET/CT sensitivity of 74–100 % and a specificity of 66–100 % [43]. Other studies including patients without a history of cancer reported also high sensitivity (89–100 %) and specificity (70–88 %) for detection of malignancy [44].
Available studies consistently show a high negative predictive value for 18F-FDG PET or PET/CT, meaning that it is accurate in excluding malignancy in tumors with indeterminate features on CT and might help avoiding unnec- essary surgery, in absence of other indications. However, the diagnostic role of 18F-FDG PET or PET/CT in adrenal lesions that remain uncategorized after conventional imaging has not been thoroughly evaluated.
Unfortunately, false negatives have been described due to small size of lesion (<1 cm), low FDG avidity of cer- tain cancer types (such as renal cancer or neuroendocrine tumors), or necrosis within the tumor. Apart from pheochro- mocytomas, some apparently benign adrenal adenomas may uptake FDG for unknown reasons (false positives) [42].
In patients with ACC, we suggest to use 18F-FDG PET in
staging disease and to evaluate patients for local recurrence and distant metastases. It is worth mentioning that small lung metastases have been missed by this test and that in a comparative study by Leboulleux et al. PET/CT was com- plementary to total-body CT in detection of metastatic sites of disease [43]. It is debated whether the intensity of FDG uptake correlates with survival. False-positive FDG uptake by the residual normal adrenal following extirpation of the contralateral adrenal has been transiently induced by treat- ment with mitotane [45].
11C-metomidate, a marker of 11 beta-hydroxylase, has
been recently introduced as a novel PET tracer for the identification of tumors of adrenocortical origin. It is taken up by ACC and adenomas and differentiates adrenocortical lesions from pheochromocytomas and metastases with a sensitivity and specificity of 89 and 96 % [46]. Necrosis and chemotherapy can lower the sensitivity of the test in ACC. However, it cannot distinguish between ACC and adenoma.
[123I]IMTO for single photon emission computed tomography (SPECT) and planar scintigraphy has proved a valu- able alternative to PET imaging with a sensitivity of 89 % and a specificity of 85 % for differentiating adrenocortical tumors from lesions of non-adrenocortical origin [47].
A recent report in a larger series of patients with adrenocortical cancer has shown that only a subset of ACCs is clearly positive by SPECT/CT iodometomidate imaging [48].

Fine Needle Aspiration Biopsy (FNAB)

In general, the role of percutaneous adrenal biopsy is lim- ited mainly in patients with known extra-adrenal malig- nancy where the identification of adrenal metastasis might change management. Studies reported a sensitivity of 81–96 % and specificity of 99–100 % to identify malignant masses. Inconclusive biopsies were reported in 6–50 % of samples. The rate of adverse events ranges from 2.8 to 14 % and the risk of morbidity/mortality is not negligi- ble [12].

FNAB should not have a role in the diagnostic approach to ACC due to the reportedly low accuracy in the differentiation between benign and malignant primary adrenal tumors and risk of inducing violation of the tumor capsule and needle track seeding of tumor cells [2]. We recommend against the use of FNAB in ACC. It is impor- tant to remember that any adrenal mass suspected to be an ACC should be operated on. FNAB has 95 % sensitivity in diagnosing an adrenal metastasis from extra-adrenal cancer in oncologic patients and only 44 % sensitivity in diagnos- ing an ACC [49]. Therefore, FNAB maintains an important role in the definitive diagnosis of adrenal metastases from an extra-adrenal tumor when the diagnosis may change the therapeutic plan.
Before FNAB is performed, the presence of a pheochro- mocytoma must be excluded by biochemical tests.

Summary

1. Unenhanced CT is the primary imaging test for dif- ferentiating benign from malignant adrenal lesions 1 ⊕⊕.
2. Enhanced CT may be useful to characterize masses of indeterminate dignity after unenhanced CT, but an adrenal-specific protocol with delayed washout assessment should be used 1 ⊕⊕.
3. FDG-PET or PET/CT is most useful to characterize indeterminate adrenal lesions or lesions suspected of malignancy after radiologic workup 2 ⊕⊕.
4. FNAB has no role in the differentiation of ACC from benign adenoma, but may be used only in selected patients with adrenal lesions suspected of being metas- tases of extra-adrenal cancer (after biochemical exclusion of pheochromocytoma) 1 ⊕⊕.
5. Total-body enhanced CT (with usual washout protocol) is mandatory for staging and assessment of response to treatment 1 ⊕⊕⊕.

Pathology diagnosis and Prognosis

Pathology diagnosis
Due to their broad histomorphological heterogeneity, accurate typing of adrenal tumors often poses a major diagnos- tic problem, and conventional histology frequently offers no conclusive diagnosis of the origin of an individual neoplasm.

SF-1
Already in 1995, Sasano et al. [50] suggested that steroido- genic factor-1 (SF-1) is a marker to differentiate between tumors of adrenocortical and non-adrenocortical origin. SF-1 is a transcription factor involved in the development of steroidogenic tissues and in the regulation of steroid bio- synthesis [51]. Recent studies have demonstrated that SF-1 is a highly valuable immunohistochemical marker to deter- mine the adrenocortical origin of an adrenal mass, with higher sensitivity and specificity than other immunohisto- logical markers [51–53].
We recommend immunohistochemical analysis of SF1 to identify the adrenocortical origin of a tumor, in particular in the case of differential diagnosis with metastatic lesion.

Weiss score
The Weiss score is the cornerstone of pathological diag- nosis. Weiss score includes nine criteria of proliferation, nuclear abnormality and tumor extension [54] (Table 1). A Weiss score of 0–2 defines benign adrenal tumors, while tumors with a Weiss score ≥3 are considered malignant. Tumors with a Weiss score of 2 or 3 may eventually dis- play an undetermined behavior. A correct assessment of this morphological score is strictly dependent on individual expertise and an easier standardization is urgently needed. Tissier et al. recently validated a virtual approach for the diagnosis of ACC comparing Weiss score using microscopic and virtual (virtual slides on the computer) methods with an increase of intraobserver reproducibility. Also, 7 of 9 items (except venous invasion and sinusoidal invasion) of Weiss score were improved at the virtual reading [55]. Moreover, compared to the benign forms, ACC often show particular intratumor heterogeneity that needs a minute sampling because of the presence of areas with different phenotypes [2]. Therefore, a second evaluation by an expert pathologist is mandatory in patients operated in the community.
We recommend histological evaluation with the Weiss score to differentiate benign from malignant adrenal corti- cal lesions.

Prognosis
ACC shows a heterogeneous behavior as to clinical pres- entation and disease course. If ACC may be considered the prototype of aggressive endocrine tumors, in several cases tumor progression may be slower. However, an accurate prognostication remains an unmet need and this constitutes a barrier to the implementation of an effective, personalized treatment.

Pathologic prognostic parameters

Among pathologic parameters, the disease stage at initial diagnosis and a margin-free resection have been consid- ered the most important and validated prognostic factors [56–59] because it still represents the only curative treat- ment for ACC [60, 61]. Stage at diagnosis is a key prognos- tic factor also because feasibility of radical surgery depends heavily on disease extent.

Staging

Until 2004, no official TNM classification was available for ACC and different staging systems were used [56–58, 62, 63]. It was only in 2004 that the International Union Against Cancer (UICC) and the World Health Organization (WHO) published the first staging classification based on TNM criteria for ACC [64]. This classification was based largely on an earlier classification system proposed by Macfarlane [56] and later modified by Sullivan et al. [58]. Although largely used, this staging classification showed a limited prognostic value [65].

An important step toward a better prognostic assessment has been the development of an improved staging classi- fication, based on the analysis of disease-specific survival curves for each stage in larger series within the European Network for the Study of Adrenal Tumors (ENSAT) [65]. We recommend use of the ENSAT ACC staging system.

The ENSAT staging system (Table 2) allows obtaining a more precise prognostic differentiation among stages. In this system, tumor infiltration in surrounding tissues, tumor thrombus in caval or renal vein, and positive lymph nodes define stage III, whereas the presence of distant metastasis is the only criterion for stage IV [65]. In particular, a 5-year stage-dependent survival of 81, 61, 50, and 13 %, respec- tively, from stage 1 to stage 4 has been demonstrated [65].

During the last years, it was confirmed that the ENSAT staging system has improved prognostic accuracy for dis- ease-specific survival in ACC compared to precedent clas- sifications [59, 66].

We recommend use of the ENSAT staging system for prognostication.

Resection status

An incomplete resection results in a far worsen survival within the same stage [65] and this emphasizes the role of radical surgery as the treatment of choice for ACC [56–59]. Resection status is indeed a well-established prognostic factor, being Rx (unknown), R1 (microscopically positive margins) and R2 (macroscopically positive margins) associated with progressively reduced recurrence-free survival (RFS) irrespectively of other risk factors [59, 67].

We recommend that an experienced surgeon does sur- gical extirpation of ACC to have more chances to obtain complete resection and consequently better prognosis.

Proliferation index

The Weiss score may have also a prognostic stratification power. A Weiss score higher than 6 was significantly asso- ciated with shortened RFS and overall survival (OS) [68] confirming earlier demonstration that the total Weiss score has prognostic value [55, 69]. However, other studies did not find that Weiss score as a whole was a useful predictor for tumor recurrence after resection of the primary tumor [70–72]. There is insufficient evidence to recommend use of the Weiss score for prognostication.

One component of the Weiss score, mitotic activity, has been found to be the most significant determinant of survival. High mitotic activity was found to be a predictor of poor outcome either in localized or metastatic ACC [55, 73, 74]. A high mitotic rate [>5 per 50 high-power fields (HPF) and a lesion diameter ≥12 cm were associated with reduced RFS in patients with complete surgical removal of the tumor. Also, the presence of tumor necrosis and atypical mitotic figures have been associated with poor prognosis and advanced disease stage [71, 75–77], although they were less powerful predictors. In patients with stage IV ACC, multivariate analysis identified high mitotic index (>20 per 50 HPF) and the number of organs involved as the major factors influencing prognosis [76]. A recent pathological study showed that it was possible to stratify prognosis on the basis of stage and mitotic index of the primary tumor. Stage III/IV and mitotic index >9 per 50 HPF qualified the worst prognosis group [71]. These findings support the concept that a grading system based on mitotic count might help in the prognostic stratification of patients [78].

We recommend to consider a high mitotic index as a negative prognostic factor in patients with ACC.

Ki-67 is assessed by immunohistochemistry with the monoclonal antibody MIB1 and represents a validated index of cell proliferation being expressed in all stages of the cell cycle and absent in G0 phase. High expression of Ki-67 was found to be a predictor of reduced RFS regardless of Weiss score [68]. Conversely, a correlation between Ki-67 expression with reduced OS was less consistently found [68, 75]. In a multicentric study supported by ENSAT, a Ki-67 value at 10 % was found to separate patients as to risk of recurrence (low vs. high), with a hazard ratio of recurrence of 1.042 per each % increase in Ki-67. In a multivariate analysis, Ki-67 was the single best prognostic factor for RFS [79]. More recently, in a large cohort of patients with localized ACC identified from the German ACC registry, Ki-67 provided the single best prognostic value for RFS and OS, compared to other prognostic factors. In multivariable analysis including age, tumor stage, adjuvant mitotane treatment, and all standard histological parameters, the Ki67 index retained its outstanding prognostic power, with Ki-67 <10 % defining grade 1 tumors, Ki-67 10–19 % defining grade 2, and Ki-67 ≥20 % defining grade 3 tumors [80].We recommend the use of Ki-67 to identify patients at higher

risk of relapse (Table 3). However, assessment of Ki-67 is poorly standardized and shows a great inter-observer variability; thus, a consensus on technical aspects is urgently needed to allow a widespread application of this marker for diagnosis and prognostication.

Since the Weiss score is difficult to apply, subjective and time consuming, despite several attempts of revi- sion and implementation [75, 78, 81], Duregon et al. have introduced a new method, the "reticulin algorithm". This method identifies malignancy through detection of an altered reticulin framework evaluated using a specific stain- ing associated with 1 out of 3 parameters among necrosis, high mitotic rate and vascular invasion [53]. This method shows a better accuracy and higher reproducibility than the classic Weiss score among different pathologists [53] and may also represent a valid tool for identification of specific ACC variants, such as pediatric, oncocytic, myxoid, and sarcomatoid tumors. Further studies are needed to confirm its value for the pathological diagnosis.

Clinical prognostic parameters

Among the clinical parameters, age and functioning activity of the tumor have been more consistently associated with poorer prognosis, although data are not completely uniform [70, 82–88]. Conversely, gender has not been associated with survival [70, 83, 85, 87]. The correlation between older age and adverse prognosis was found in some studies dealing with early or all stage ACC patients [83, 85, 87] but not all [63, 70, 75]. Earlier studies did not find evidence that the functional status of the tumor may influence outcome [83, 84]; however, in different series [87, 89], patients with cortisol-secreting ACC had a worse outcome. In a very recent multinational study of patients with completely resected ACC, the presence of clinical or biochemical signs of cortisol excess was a negative prognostic factor either for RFS and OS, after adjustment for other recognized prognostic factors [88]. The mechanism underlying the negative effect of cortisol excess on prognosis remains unclear. Although in patients with metastatic ACC, cortisol excess may lead to increased mortality due to derangement of metabolic profile, immunosuppression, catabolism, and infection [87, 89], this is not the case for patients who underwent complete resection and attained a complete remission of the endocrine syndrome [88]. Therefore, it may be argued that cortisol excess is associated with a more aggressive tumor behavior, although no correlation was found between cortisol excess and mitotic index in that study [88]. Other mechanisms in addition to tumor proliferative activity may be operative. In this line, it was recently suggested that the expression of the gene for serum glucocorticoid kinase 1 (SGK1), a kinase involved in multiple cellular functions, is inversely associated with cortisol hypersecretion and that low SGK1 represents a negative prognostic factor in ACC [90].

We recommend considering hypercortisolism due to ACC with as a negative prognostic factor.

Genetic and molecular prognostic markers

Molecular profiling of ACC using expression arrays per- formed on large cohorts of clinical–pathological-annotated biopsies has recently moved from diagnostic to prognostic purposes [91–96]. Independent transcriptional profiling enabled to classify ACC in two groups at different prognosis, according to a differential expression of a specific pattern of genes reflecting tumor proliferation [91, 94–96]. The majority of the up-regulated genes in the bad progno- sis groups belongs to transcription factors, genes involved in proliferation and cell cycling. In particular, inactivating mutations in the anti-oncogene TP53 or activating mutations in beta catenin gene (CTNNB1) were found in the poor-outcome group only [94]. Combined expression of BUB1B and PINK1 genes was found to be one of the best predictors of overall survival [93]. Interestingly, cluster- ing based on gene expression profiles provides prognostic information independently of tumor mitotic rate and stage, thus representing an additional potential prognostic marker. Some immunohistochemical markers were shown to be independent predictors of disease-specific survival. In particular, patients with a strong glucose transporter 1 (GLUT1) staining showed a considerably higher over- all mortality (HR 6.34) compared with patients with no GLUT1 staining, without any difference between early or advanced stage [97]. Moreover, nuclear overexpression of steroidogenic factor 1 (SF-1) was found to correlate with a poor prognosis. In the original observation by Sbiera et al., expression of SF-1 was not correlated to sex, age, tumor stage, and hormone secretion [51]. In the study by Duregon et al., high SF-1 expression was positively correlated with advanced ENSAT stage, high proliferation and mitotic

index, and high Weiss score [98].

MicroRNAs, small noncoding RNAs regulating gene expression at post-transcriptional level in a sequence- specific manner, are emerging as potential diagnostic and prognostic markers in different tumors. The combination of high levels of miR-483-5p and low levels of miR-195 detected in ACC tissues [99, 100] and in bloodstream of ACC patients [100] were associated with shorter RFS and OS. These findings hold promise that miRNAs may represent a promising tool for enhanced prognostic stratification of ACC patients. Another possible future tool could be the evaluation of

circulating tumor cells (CTC). Preliminary results suggest a possible prognostic role of this technique[101]. The recent advent of high-throughput genome analysis techniques applied to large cohorts of ACC samples obtained by collaborative international research consortia made it possible the discovery of new driver genes and more efficacious molecular classification of ACC. Indeed, a recent ENSAT study identified new driver genes, such as ZNRF3, DAXX, TERT and MED12 by integrated exome sequencing and SNP array applied to a discovery cohort of 45 ACCs integrated by a 77 ACC validation cohort of samples. Moreover, by integration of wide genome analy- sis, DNA methylation analysis, miRNA expression array and miRNA sequencing, these authors further identified prognostic ACC profiles which may be useful for a better patient management [102].
At present, no data about the superiority of molecular markers in a large consecutive cohort of patients have been published, therefore reducing their additional value in prognostic prediction.

Summary

1. SF-1 should be incorporated in the diagnostic process 1 ⊕⊕.

2. Pathological diagnosis should be done with the Weiss score 1 ⊕⊕⊕.

3. Staging should be done with the ENSAT system 1 ⊕⊕⊕.

4. Ki67 represents the best single prognostic histopatho- logic parameter for RFS and OS 1 ⊕⊕⊕.

Treatment Concept

Prognosis of ACC patients appears to be rather disap- pointing. It is now well established that treatment of ACC requires a multidisciplinary management. Surgery is the key therapeutic option in ACC, and is the only one to offer possibility of cure. Adjuvant therapy concepts in ACC derived from the observation that at least one-third of patients show loco-regional recurrence or distant metasta- ses after an apparent complete surgical excision. However, recurrence was reported to be much higher in some series with long-term follow-up [103–105]. Despite en bloc, com plete resection of tumor in patients without evidence of metastatic disease, the 5-year survival rate is only approximately 50 % [84, 106–108]. Although these findings make a strong case in favor of the use of adjuvant therapy in ACC patients, this therapeutic option for patients with stage I– III ACC following radical surgery remains debated. Mitotane represents the only drug approved by international pharmaceutical agencies for ACC treatment. Although the effectiveness of this drug is quite recognized [67], its use in adjuvant setting remains somewhat debated [109, 110].

Surgery

Surgery is the first therapeutic option in the ACC, and is the only one with potential for cure.

Open approach versus laparoscopic approach

Laparotomic approach is recommended in patients with localized (stage I-II) and locally advanced (stage III) ACC. A disease-free resection margin (R0) is essential in predicting long-term survival [85]. In previous series, the percentage of relapse was probably overestimated (85 %) [85, 107, 111–113]; however, also recent studies show that this event is not infrequent (50 %) [114]. The achievement of R0 resection is a key objective for the surgeon. It must be achieved by avoiding tumor spread in the abdominal cavity since this eventuality is considered an unfavorable prognostic factor. Thus, the need to per- form radical surgery often requires the resection of adjacent organs such as ipsilateral kidney, spleen, and partial pancreatectomy for left adrenal cancer and partial hepatectomy for right adrenal cancer [113]. It is mandatory that surgery must only be performed by a highly skilled surgical team.
The role of the laparoscopic approach is still matter of debate. The relative effectiveness compared to the “open” approach is unknown and there are currently no prospective trials that may justify its use [113]. Literature data are rather conflicting even if the most recent studies failed to show any difference in the oncologic outcome between laparoscopic and laparotomic approaches in patients with ACC [105, 115, 116]. However, these are retrospective studies that may suffer from selection bias.

Role of lymphadenectomy

Lymphadenectomy has never been considered a standard of care in the ACC. Retrospective data from the National Cancer Data Base (NCBD) and the German ACC Registry report a lymph node positivity rate of 26 % and show that lymphadenectomy is significantly associated with reduction in tumor recurrence and death in patients with local- ized ACC [117]. Considering that the most frequent lymph nodes involved in ACC are para-aortic/paracaval and hilar/ peri-renal, Gaujoux and Brennan recommend to perform regional lymphadenectomy including peri-renal, celiac and aortocaval nodes [118]. Even though the reported data, the observation that the positivity of lymph nodes is not always associated with a poor outcome. From the NCBD, a relative survival of 42 % in patients with negative and 14 % with metastatic lymph nodes has been reported [119]. Prospective trials are mandatory to establish the therapeutic significance of this surgical procedure.

Treatment of metastatic disease

Many cases (30–40 %) of ACC are metastatic at the onset [75]. Unfortunately, also patients with an R0 resection show a recurrence (40–65 %) within two years [70, 119]. Surgical treatment of pulmonary or hepatic metastasis is associated with a long-term survival [120, 121]. Different retrospective studies reported a positive role of metastasectomy in patients affected by primary ACC with an overall survival reaching up to 41 % [122]. Erdogan et al. showed in a series of 154 patients, with local and metastatic dis- ease occurring after 12 months with a R0 resection, an improvement of progression-free and overall survival [123]. These studies suggest the use of metastasectomy to prolong patients' survival and attribute a prognostic signifi- cance at the time of first recurrence (6–12 months) and at R0 resection.

Debulking surgery

The criteria for debulking surgery are essentially due to the need to remove a large mass producing mechanical signs and reduce the hormonal excess produced by the tumor. However, the significance of this therapy on the median survival appears to be less than 12 months [81, 112].

Mitotane

Although randomized, controlled trials on the use of adju- vant mitotane in ACC patients following radical surgery are still unavailable, a large retrospective case–control study reported convincing data to support the use of mito- tane in an adjuvant setting [70]. The median RFS was sig- nificantly prolonged to 42 months in the adjuvant group as compared with 25 and 10 months in the control groups, respectively ($p < 0.0001$). OS was 110 months in the adju- vant group vs. 52 and 67 months in the control groups, respectively ($p = 0.01$). In multivariate analysis, mitotane treatment maintained a RFS and OS advantage after adjust- ing for other prognostic factors. Mitotane daily dose was 3 g as average and the median duration of treatment was 29 months. Adverse events were graded 1–2 in most of cases.

Since its publication, this study raised a strong debate. Many criticisms were moved to the study but at now this study remains the most important ever published on this topic and represents a reference point for decision mak- ing in ACC patients. Some authors suggest to use adjuvant mitotane therapy only in patients at high risk of recurrence [110, 124]. In particular, Wangberg et al. [110] suggested to combine an aggressive surgical approach with the use of adjuvant mitotane. The benefit of mitotane in terms of disease-specific survival was evident for patients with high-stage ACC and circulating mitotane levels higher than 14 mg/l. Grubbs et al. recently reported that surgery in skilled centers without adjuvant mitotane would be associated with similar RFS rates as those observed in the original study in the mitotane-treated group [125]. However, these findings are biased by mitotane treatment in some patients of the no-mitotane group. Moreover, patients treated with adjuvant mitotane had a better RFS although they were not treated by reference surgeons [125].

Finally, the 2012 ESMO guidelines recommend, on the basis of the conclusions of a panel of international experts [59], the adjuvant use of mitotane in high-risk surgically treated ACC patients, as defined by stage III, Ki-67 >10 %, R1 or Rx resection. For low-risk patients, who are char-
acterized by stage I or II, R0 resection and Ki-67 $\leq$10 %,
adjuvant mitotane therapy is not mandatory. An international, multicentric, prospective, randomized trial (ADI- UVO trial) is now available to enroll low-risk patients, to definitely establish the effectiveness of adjuvant mitotane in this subgroup of patients. A recent retrospective analysis
demonstrated that blood mitotane concentrations $\geq$14 mg/l
were associated with a prolonged RFS in patients treated adjuvantly with mitotane following macroscopically radical surgery [126]. Thus, maintenance of target mitotane concentration may represent a predictor of response to adju- vant treatment. A recent experimental study provided either in vivo or in vitro evidence that expression of ribonucleo- tide reductase large subunit 1 (RRM1) was linked to mitotane activity. RRM1 expression was able to predict sensi- tivity to

the cytotoxic effects of mitotane in ACC cell lines and RFS in patients treated with adjuvant mitotane [127]. These findings propose RRM1 as a candidate predictive marker that may merit further investigation in an adjuvant setting.
According to the ESMO guidelines [59], mitotane therapy should be administered following a high-dose regimen with the aim of reaching a daily dose of 6 g/daily rather soon and then adjust the dose according to tolerability and mitotane levels. Monitoring of blood mitotane concentrations plays a pivotal role and treatment should target levels of 14–20 mg/l. Duration of adjuvant mitotane therapy has not been established, but it is reasonable to continue ther- apy for at least 2 years if tolerated.
We recommend use of mitotane in an adjuvant setting and the monitoring of its concentration.

Radiotherapy and chemotherapy

In a retrospective analysis from the United States, adjuvant radiotherapy was reported to decrease 4.7 times the risk of local failure compared with surgery alone [128]. In a retrospective analysis from the German ACC Registry, radio- therapy in an adjuvant setting resulted in a significant better

5-year RFS, but did not affect OS and disease-free survival [129]. However, no difference between surgery plus radio- therapy and surgery alone was found in another retrospective study done in the United States [130]. A review of the literature concluded that adjuvant radiotherapy should be considered in patients with incomplete, or R1 resection, or Rx resection, who are at high risk for local recurrence [131]. A total dose of >40 Gy with single fractions of 1.8 to 2 Gy should be administered. However, prospective investigations are required and no definitive conclusions are avail- able at the moment.
Currently, we suggest that radiation therapy may be of some benefit in certain cases of ACC after surgery.
As far as chemotherapy is concerned, limited data are available. A paper published data on 3982 ACC patients from the National US Cancer Data Base (NCDB), revealing that adjuvant chemotherapy was performed in 10 % of cases. By comparing these subjects with those treated with surgery only, OS was not different, while no RFS analy- sis was reported [119]. Anecdotal cases reported a more favorable outcome after an adjuvant etoposide—cisplatin- based chemotherapy [132]. A phase II clinical trial reported that the combination of mitotane plus streptozotocin was effective in an adjuvant setting. However, the study design does not allow to discriminate the relative merits of the two drugs [133]. We recommend against the use of adjuvant chemotherapy.

Summary
1. Open surgery is recommended in patients with ACC stages I–III. 1 ⊕⊕⊕⊕.

2. An adjuvant use of mitotane is recommended in patients at high risk for recurrence. 1 ⊕⊕.

In low-risk patients, consider inclusion in the ADIUVO trial or individualize decision.

3. A monitored approach should be employed targeting drug concentrations of 14–20 mg/l. 1 ⊕⊕.

4. Adjuvant radiotherapy of the tumor bed is suggested in patients with incomplete surgical resection (R1/ Rx).2 ⊕⊕.

5. The use of chemotherapy, or any other systemic or loco-regional therapy, in an adjuvant setting is cur- rently not recommended. 2 ⊕.

Management of Advanced ACC

Metastatic or advanced ACC does not have an effective therapy. Unfortunately, advanced ACC is rapidly progres- sive with a limited prognosis [2]. However, a few patients may be responsive to treatment showing a prolonged sur- vival [134]. The difficulty in addressing management of advanced, metastatic disease stems from limited clinical series due to the rarity of ACC and its rapid unfavorable evolution.

Mitotane
Currently, mitotane represents the mainstay of therapy in advanced ACC. Mitotane is an isomer of the insecticide DDT with adrenolytic activity. Mitotane inhibits gene expression of a number of enzymes involved in the steroidogenetic

pathway in the mitochondria of steroidogenic cells decreasing both plasma and urine hormone levels [135–137]. Mitotane produces focal degeneration of the zona fasciculata and the zona reticularis, while the effects on the glomerular area are relatively scarce [138]. Mito- tane needs an enzymatic activation in the liver, by α- and β-hydroxylation, producing the metabolites o′,p-DDA and o′,p-DDE, respectively. The metabolite o′,p-DDA represents the active compound [139] and it has been shown that the measurement of o,p′-DDA may add to measurement of mitotane levels in predicting drug response in patients affected by advanced ACC [140]. Administration is oral aiming to target concentrations between 14 and 20 mg/L, which have been associated with anti-tumoral activity and represent the so-called "therapeutic window" [140, 141]. Different regimens have been proposed and recent data seem to favor a high-dose strategy that is able to get the target concentrations more rapidly [141, 142]. However, mitotane dose is fixed only for the starting phase of treatment and thereafter is guided by results of blood monitoring and tolerability. Mitotane may interfere with pharmacokinetics of concomitantly given drugs. Recent evidence demonstrated that mitotane induces enzymatic activity of CYP3A4, thus increasing the metabolism of several drugs, including steroids and chemotherapeutics [143]. This rep- resents a very important piece of evidence limiting the use of conventional therapeutics molecules such as antihypertensive drugs, antibiotics, statins [144]. The evidence con- cerning mitotane-due CYP3A4 induction leads to consider this aspect in the future development of oncological trial involving chemotherapy in combination with mitotane.

The widespread access to mitotane monitoring in Europe has facilitated the management of treatment. However, no data from prospective studies in patients with advanced disease are currently available. Although there are reports of patients with advanced disease (likely low-grade ACC) who attained a long-term control of the disease with mitotane [67], the benefits of a monotherapy with mitotane are usually scarce and patient survival is poor [76, 139, 140].

The response rate, in patients treated with mitotane at different concentration, is estimated on average at 25 %

in different studies coming mostly from old retrospective series without reliable response criteria [76, 139, 140]. On the contrary, this percentage reaches the average of 55 % in those studies in which therapeutic levels of the drug were observed. This suggests that the drug monitoring is essential for the maintenance of plasma levels including 14–20 mg/l [145, 146].

These data, although not conclusive, suggest to consider the use of adjuvant mitotane therapy in patients with residual disease (R1 or Rx resection) together with a Ki67 level greater than 10 %. On the contrary, the choice to undertake therapy with mitotane is not mandatory in patients with stage I or II disease with histological R0 resection and Ki67 less than 10 % in tumor cells [59, 147].

Therefore, mitotane monotherapy in advanced ACC may be recommended in those patients with metastatic involve ment of only a few organs, low-grade mitotic index, and relapse after years from surgery [76].

Chemotherapy

Chemotherapy in combination with mitotane was used fol- lowing the concept that mitotane inhibits the MDR-1/P-glycoprotein, a multidrug resistance protein functioning as a drug efflux pump, widely expressed in ACC [148]. Chem-otherapy drugs used alone or in combination in the treatment of advanced ACC patients include cisplatin, etopo- side, doxorubicin/adriamycin, vincristine, 5-fluorouracil, and streptozotocin [89, 133, 149, 150]. Although results are variable, there is some evidence that cisplatin-based reg mens exert a positive effect in advanced ACC. Bukowski et al. evaluated the effectiveness of cisplatin and mitotane in combination, achieving a positive response in 30 % of cases [149]. In another study, Bonacci et al., using a regi- men that included the combination of cisplatin, etoposide, and mitotane, obtained an overall response of 33 % [151] while Burgess et al., with a combination of cisplatin and etoposide without mitotane, reached a response rate of 46 % [152]. Williamson et al. administered the same drugs (cisplatin plus etoposide) without mitotane to patients with advanced or metastatic ACC and achieved a lower rate of response [153]. The most promising studies of association between chemotherapy and mitotane were those of Khan et al. and Berruti et al. that proposed the combination of mitotane with streptozotocin [133] or with etoposide, doxorubicin and cisplatin (EDP) [89], respectively. The results achieved by the Berruti's study, based on a large study in 72 patients affected by ACC not amenable to surgery, showed a complete response in 5 and a partial response in 30 patients, totaling an overall response rate of 48.6 % [89]. The Khan's study, achieved a complete response in 1 patient and partial response in 7 patients with an over- all response rate of 36.4 % [133]. Due 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 [113]. The First International Randomized Trial in Locally Advanced and Metastatic

Adrenocortical Carcinoma Treatment (FIRM-ACT) started in April 2004, with the aim to establish the gold standard in advanced ACC not amenable to radical surgical resection comparing the two most promising protocols (Table 4). The study randomized 304 patients with advanced ACC and showed the superiority of EDP- mitotane compared to streptozotocin-mitotane in terms of tumor response (23 vs. 9 %, $p < 0.001$) and median progression-free survival (5 vs. 2.1 months, hazard ratio, 0.55; 95 % confidence interval, 0.43–0.69, $p < 0.001$). Unfor- tunately, no significant difference was observed in overall survival (median 14.8 vs. 12 months) confirming the poor prognosis of patients affected by advanced ACC. No differences were found in the quality of life and adverse events recorded in patients receiving the two therapeutic regimens [154]. Regretfully, results of the two regimens in the FIRM-ACT study were much lower than in the original studies and this is likely due to the fact that not all patients enrolled in those studies had progressive tumors. However, the cross-over design of the FIRM-ACT study may have hampered demonstration of a survival advantage associated with EDP-mitotane that was more active than streptozo- tocin-mitotane even as second-line treatment after failure of streptozotocin-mitotane in first-line.

Targeted therapies

Recent advances in the understanding of genetic alterations involved in ACC onset and progression led to the identification of several potential therapeutic targets. Several genetic modifications involving oncosuppressor genes, such as TP53, CDKN1C, CDKN2A and MEN1, and oncogenes such as IGF2, CTNNB1 and RAS were considered in an anti-tumor strategy to contrast ACC. Currently, many other molecular pathways are under investigation to understand their usefulness for development of new therapeutic options [155]. The present section addresses only the therapeutic strategies that have been tested in clinical studies.

Overexpression of insulin-like growth factor-2 (IGF-2) represents the most important molecular event occurring in ACC [93]. IGF-2 binding the IGF1R activates the PI3K/ Akt/mTOR pathway [156]. Recently, two phase I studies have shown the effectiveness of figitumumab, a monoclonal anti-IGF-1R antibody and OSI-906, a small molecule tyrosine kinase inhibitor directed against IGF-1R, inducing a partial response in 57 and 33 % of patients, respectively [157, 158]. An international phase III study to evaluate the therapeutic perspectives of OSI-906 in patients affected by ACC has been recently completed even if the results were mainly negative [159]. Recent studies showed an associa- tion between IGF2 overexpression, m-TOR hyperactivation and reduced expression of miR-99a and miR-100 [160]. On these data, a phase I trial evaluated the effects of temsirolimus (CCI-779), an inhibitor of m-TOR in combination with cixutumumab, an anti-IGF-R1 recombinant monoclonal antibody, demonstrating a tumor growth inhibition in 4 out of 10 patients affected by advanced ACC [161].

The vascular endothelial growth factor (VEGF) is over- expressed in ACC. The demonstration that its expression level falls after tumor removal confirms the hypothesis that it may represent an effective therapeutic target in the ACC [162]. Despite the significance of this pathway, the results obtained from clinical trials in ACC are discouraging. Recently, a study performed on 10 patients with advanced ACC treated with bevacizumab, a monoclonal antibody directed against VEGF, in combination with capecitabine did not show any positive effect. On the contrary, this regimen resulted in two severe adverse events that required discontinuation of therapy [163]. A positive remark was observed only in a case report in which the administration of thalidomide at a dose of 200 mg/die induced a partial response in a chemo-resistant ACC [164]. Disappointing results have been achieved in clinical trials with tyrosine kinase molecule inhibitors targeting VEGFR, such as sorafenib and sunitinib [162]. Administration in a phase II study of sunitinib in 38 patients with unresponsive ACC managed to stabilize the disease only in 5 patients with a progression-free survival ranging from 5.6 to 12.2 months [165]. A phase I trial demonstrated the efficacy of sorafenib in combination with the farnesyltransferase inhibitor tipifarnib in two cases of advanced ACC [166]. Moreover, in a single case report, a regression of metastatic lesion in a stage IV ACC after sorafenib was observed [167]. How- ever, a recent phase II study investigating the effects of sorafenib in combination with metronomic paclitaxel failed to demonstrate any synergistic effect of the two drugs despite encouraging in vitro findings [168]. No response or disease stabilization was observed and the trial was stopped early before schedule after observing a seemingly fastened progression in some cases.

A partial response to sunitinib has been reported in a patient with metastatic ACC, after failure of chemotherapy which prompted initiation of a phase II study with sunitinib in monotherapy for refractory ACC [169]. The combination of sirolimus, an m-TOR inhibitor, and sunitinib, attained a partial response in a patient affected by advanced ACC [170]. Microarrays and transcriptome analysis allowed to identify several signaling pathways that are hyper-activated by overexpression of growth factors or increased activity of their receptors [96]. Novel therapeutic strategies have been addressed after identification of tyrosine kinase inhibitors involved in signal transduction. The use in 10 patients of

erlotinib, an EGFR inhibitor, associated with gemcitabine showed a very limited effectiveness [171]. Likewise, treatment with gefitinib as a second-line monotherapy showed a response rate of 0 % in a series of 19 patients with unresectable ACC [172]. Similarly, in a phase II study including 4 ACC patients, treatment with imatinib mesylate, a PDGFR inhibitor, resulted in disease progression in three cases and in severe side effects in the remainder [173]. A hypothesis to justify the failure of these therapies is a low expression of these receptors in ACC. Notably, mutations in the EGFR gene have not been identified [174].

ACC resistance to chemotherapy has been related to overexpression of the multidrug resistance protein MDR-1 (P-glycoprotein, Pgp), although there are not convincing evidence, which is an ATP-dependent drug efflux pump [148], The concept that some inhibitors of MDR-1 may allow chemotherapy drugs to remain longer within the cell and exert more prominent toxic effect is the basis of this therapeutic strategy. A clinical trial with doxorubicin, vincristine, and etoposide in combination with MTT failed to demonstrate the effectiveness of this strategy [175].

The rationale of immunotherapy is based on the stimulation of the immune system against antigens of the neoplastic cell. The attempt to "stimulate the immune system" with autologous dendritic cells of two patients with ACC metastatic secreting induced antigen-specific Th1 immunity did not produce any clinical benefit [176]. Probably, the main limitation in this type of approach consists in the identification of a specific tumor antigen.

Interventional radiology

In oncology, interest has been recently focused on mini- mally invasive procedures as an alternative to surgery. Radio frequency thermal ablation (RFA) is one of these proce- dures that may be part of the management of patients with advanced ACC in combination with systemic medical treat- ments. RFA represents a viable alternative to surgery to improve results of medical treatments, since an integrated use of multiple techniques may allow a better control of the disease in patients with advanced ACC. RFA has shown promising results for treatment of other cancers and has been proposed also for stage IV ACC patients. However, there is little evidence on the use of this technique [121, 177].

In a study by Wood and coworkers, RFA was safely performed without any side effect except one delayed abscess in 8 patients with 15 ACC recurrences or metast ses. In eight of 15 (53 %) lesions, a post-treatment loss of enhancement was observed and the lesions stopped growing on a follow-up CT scan after 6 months [177]. Despite the limited evidence, RFA can be used in the treatment of ACC lesions in the liver, kidney, retroperitoneum, lymph nodes and lung since it is a reliable and inexpensive technique implying minimal morbidity and a short recovery. Results in terms of local disease control may be comparable with those of surgery [177, 178].

Percutaneous laser ablation is another minimally invasive procedure that may be viewed as an alternative to sur- gery. Percutaneous laser ablation has been shown to pro- duce local tissue destruction in a rapid, predictable, and inexpensive manner with minimal morbidity and a short recovery time, although the effect on survival remains unclear [179]. The combination of embolization using various chemotherapeutic agents can possibly improve the effi- cacy of percutaneous ablation techniques. Chemoembolization has been used for liver metastasis of ACC [180]. A promising alternative may be the use of transcatheter arterial chemoembolization that, in the experience at the Gustave Roussy Institute, was associated with a median survival of 11 months in twenty-nine patients with progressive ACC and liver metastatization [180].

Summary

1. Monotherapy with mitotane is recommended in patients after incomplete surgical resection or in patients not fit for surgery or chemotherapy 1 ⊕⊕.

2. Monotherapy with mitotane may be recommended in advanced ACC with involvement of few organs and low-grade mitotic index, particularly when RFS after removal of the primary tumor has been longer than 12 months 1 ⊕.

3. The chemotherapeutic regimen EDP in combination with mitotane is recommended in most patients with advanced or metastatic ACC 1 ⊕⊕⊕⊕.

4. There are insufficient data to recommend a particular targeted therapy in patients with advanced ACC beyond ongoing clinical trials.

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Compliance with ethical standards

Conflict of interest The authors AS, IC, RG, AF, LC, SDC, PL, ML, FM declare no conflicts of interest. MT declares the following conflicts of interest: Advisory board, HRA and Atterocor, Speaker honoraria HRA and Corcept.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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Table 1 - Staging System for ACC proposed by the European Network for the Study of Adrenal Tumors (ENSAT) (Fassnacht et al., 2009).

Stage	
I	T1, N0, M0
II	T2, N0, M0
III	T1-T2, N1, M0; T3-T4, N0-N1, M0
IV	any T, any N, M1

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); M0, no distant metastases; M1, presence of distant metastasis.

Table 2 – The Weiss score (Weiss et al., 1989).

Nuclear atypia
Atypical mitoses,
Mitotic rate >5 in 50 HPF
Character of cytoplasma
Architecture of tumor cells
Necrosis
Invasion of venous structure
Invasion of sinusoidal structure
Invasion of the capsule of tumor

Table 3 – Factors influencing patient's outcome

Before surgery

- Patient age*
- Staging by ENSAT classification
- Tumor hormonal secretion

After surgery

- Re-staging by ENSAT classification
- Tumor margins

(R0=free, R1=microscopic involvement, R2=macroscopic involvement, RX= unknown)

- Ki-67 staining
- Mitotic index
- Weiss score*

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*Age and Weiss score are disputed prognostic factors

Table 4 – Treatment protocols in the FIRM-ACT study. Both

Berruti's protocol (EDP-M) given every 28 days:			
-	day 1	40 mg/m^2	Doxorubicin
-	day 2	100 mg/m^2	Etoposide
-	day 3, 4	100 mg/m^2	Etoposide + 40 mg/m^2 Cisplatin
-	daily		Mitotane targeting blood levels of 14 – 20 mg/L
Khan's protocol (SZ-M) given very 21 days:			
-	day 1-5	1 g	Streptozotocin
-	subsequently	2 g	Streptozotocin
-	daily		Mitotane targeting blood levels of 14 – 20 mg/L

EDP-M includes etoposide, doxorubicin, cisplatin and mitotane

SZ-M includes streptozotocin and mitotane