MANAGEMENT OF ENDOCRINE DISEASE

Adrenocortical carcinoma: differentiating the good from the poor prognosis tumors

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Abstract

Adrenocortical carcinoma (ACC) is a rare malignancy with a poor prognosis, the five-years overall survival being below 40%. However, there is great variability of outcomes and we have now a better view of the heterogeneity of tumor aggressiveness. The extent of the disease at the time of diagnosis, best assayed by the European Network for the Study of Adrenal Tumors (ENSAT) Staging Score, is a major determinant of survival. The tumor grade, including the mitotic count and the Ki67 proliferation index, also appears as a strong prognostic factor. The assessment of tumor grade, even by expert pathologists, still suffers from inter-observer reproducibility. The emergence of genomics in the last decade has revolutionized the knowledge of molecular biology and genetics of cancers. In ACC, genomic approaches – including pan-genomic studies of gene expression (transcriptome), recurrent mutations (exome or whole-genome sequencing), chromosome alterations, DNA methylation (methylome), miRNA expression (miRnome) – converged in a new classification of ACC, characterized by distinct molecular profiles and very different outcomes. Targeted measurements of a few discriminant molecular alterations have been developed in the perspective of clinical routine, and thus, may help defining therapeutic strategy. By individualizing patients’ prognosis and tumor biology, these recent progresses appear as an important step forward towards precision medicine.

Invited Author’s profile

Jérôme Bertherat, MD-PhD, is professor of Endocrinology at Paris Descartes University, Chief of the Endocrinology Department of Cochin Hospital in Paris, head of the National Center for Rare Adrenal Diseases, one of the Health Care Provider of the European Reference Network for Rare Endocrine Conditions. His research team ‘Genomics and Signaling of Endocrine Tumors’ in the Cochin Institute (INSERM U1016 & CNRS UMR8104), works on the pathophysiology of Cushing’s syndrome, the molecular genetics of adrenocortical tumors and the genetics of familial adrenal tumors and Carney complex. His research on adrenal tumors is developed in the background of national (COMETE) and international (European Network for the Study of Adrenal Tumors: ENSAT) networks.
Introduction

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy, developed from the adrenal cortex, with estimated incidence of one per million each year (1). The prognosis is generally poor with five-year overall survival (OS) below 40% in most series (1, 2, 3, 4, 5, 6, 7, 8, 9). However, the prognosis of ACC is also very heterogeneous and difficult to predict in clinical practice. Great variability in clinical presentations is observed, ranging from tumors with an indolent clinical course to aggressive tumors with fatal outcome.

Complete surgical resection represents the only curative treatment for ACC, which can be achieved only in tumors with no extensive locoregional or distant tumor dissemination. For patients with localized tumors, up to 70% will develop recurrence in the 3 years following radical surgery and the vast majority of patients with tumor recurrence will die from tumor progression (5, 6, 9). However, more recent series suggest that patients with a localized tumor radically resected in expert centers might recur in no more than 30% (10).

In patients with metastatic disease, medical therapies, such as mitotane or platinum-based chemotherapy regimens, offer limited efficacy (11, 12). Local treatment of metastases by surgical or non-surgical approaches – such as trans-arterial chemoembolization, radiofrequency ablation or radiotherapy – are offered in selected cases, but their benefit in terms on OS has been poorly evaluated (13, 14). Median OS of metastatic ACC patients varies between 10 and 20 months (2, 3, 5), with 5-year survival around 10% (3, 6, 8). However, some long-term survivors are described, including a few cases with synchronous metastases at the time of diagnosis (15, 16, 17, 18, 19, 20). These observations suggest that some ACC have a slow natural history, in contrast with the usual poor prognosis of this malignancy. Such different clinical presentations could be explained by distinct biology. In fact, genomic studies have demonstrated the existence of subgroups of ACC with heterogeneous molecular background, independent of clinical and pathological features (21, 22).

Prognostic stratification is important for clinical management of ACC patients (23). In patients with localized disease that underwent radical surgery, the estimation of the recurrence risk determines the prescription of adjuvant therapies and individualizes patient follow-up. In patients with non-removable tumor, prognosis assessment is also a major determinant of the therapeutic strategy. Mitotane and local treatments on metastases can be proposed to patients with indolent tumors, whereas those presenting aggressive disease are better candidates for mitotane and chemotherapy combination.

This review describes and discusses the main clinical and pathological established prognostic factors in ACC, as well as the new molecular markers derived from genomics that provide new perspective to understand ACC heterogeneity and to develop new ACC prognostication strategies.

Methods

A systematic search on ACC prognosis was performed using the following search terms on PubMed until December 2017: ‘adrenocortical carcinoma' (Mesh) and ‘prognosis' (Mesh). A total of 380 records were screened. Original articles and reviews were considered, and their reference lists were screened for additional relevant publications.

Clinical prognostic factors

Tumor stage

Tumor stage represents the main prognostic factor of ACC. Different staging classifications have been proposed through years and tumor stage has been recognized as a consistent and independent predictor of survival in ACC (3, 4, 5, 9, 11, 24, 25, 26).

As for other cancer types, ACC staging is based on the tumor, node and metastasis (TNM) classification, which assesses the local extension of the primary tumor, the lymph node involvement and the presence of distant metastasis. The aim of this classification is to provide a robust prognostic tool for both disease-free (DFS) and disease-specific survival (DSS) in cancer patients. The first TNM classification in ACC was proposed in 2004 by the International Union Against Cancer (UICC) and the American Joint Committee on Cancer. This scheme was largely based on previous classification system proposed by Macfarlane (27) and modified by Sullivan (28). Tumors limited to the adrenal gland were classified as stage I (T1N0M0) if <5 centimeters (cm) or stage II (T2N0M0) if >5 cm. Advanced tumors were categorized as stage III in case of tumor infiltration into surrounding tissue (T3N0M0) or lymph node invasion (T1–2N1M0) and as stage IV in case of tumor infiltration into surrounding tissue and lymph node invasion (T3N1M0), tumor invasion into adjacent organs (T4N0–1M0) or metastatic disease (T1–4N0–1M1).
The European Network for the Study of Adrenal Tumors (ENSAT) evaluated the prognostic value of 2004 UICC classification, and showed that distant metastases were associated with decreased survival, compared to other advanced stages without metastases (2) (DSS hazard ratio, HR = 2.93). A modification of the 2004 UICC-staging system was proposed in consequence (Fig. 1): the 2008 ENSAT staging system reclassified stage III as all locally advanced tumors (T3–4N0M0 or T1–4N1M0). Conversely, the ENSAT stage IV included only tumors with distant metastases (T1–4N0–1M1) (2). The prognostic value of ENSAT-staging classification was validated by another team in North America population (29), which confirmed the better survival stratification of the ENSAT classification compared to the 2004 UICC classification. ENSAT-staging system is now considered as a standard prognostic factor for ACC management (23). A modified ENSAT (mENSAT) classification was then proposed in order to precise the prognosis of advanced ACC, with new definitions of stage III (invasion of surrounding tissues/organs or the vena renalis/cava) and stage IVa, IVb, IVc (2, 3 or >3 metastatic organs, including lymph node invasion, respectively) (30).

One limit of the current staging system is the distinction between stage I and stage II. The cutoff of 5 cm in tumor size is rather arbitrary, and no significant difference in DSS was shown between stage I and stage II (2, 29). Of note, the number of patients with stage I tumors is small – only 5% of all ACC (2) – which limits substantially the survival analysis. However, tumor size alone is not consistently found as a prognostic factor in ACC (5, 26, 31) and the use of different cutoffs in tumor size does not improve prognosis stratification of patients with stage II compared with those with stage I disease (29).

**Cortisol secretion**

ACC cause steroid hormones excess in 50–75% of the cases (6, 26, 32), including androgens, estrogens, mineralocorticoids and corticosteroids. Cortisol-secreting tumors are the most common secreting tumors. However, the impact of cortisol secretion on survival in ACC remains uncertain. In patients that underwent surgery, cortisol secretion appeared as an adverse prognostic factor of recurrence and death in ACC (OS HR = 1.5) (6, 26, 32, 33), although this effect was not always significant after adjustment on patient and disease characteristics (33). In addition, cortisol secretion failed to demonstrate prognostic value in other series involving localized (34) or metastatic ACC patients (3).

Although patients with non-functional tumors might be often diagnosed latter than those with steroid excess, several hypotheses can be raised about the poor prognostic value of cortisol-secreting tumors. First, patients with cortisol-secreting tumors commonly present with severe Cushing’s syndrome. Those patients are more likely to experience a postoperative complication compared to patients with non-functional tumors (33). Moreover, cortisol secretion may favor escape from immune surveillance by blunting the cellular immune response (35), which could result in tumor growth and recurrence. Independently of the hormonal consequences of cortisol excess, it is also possible that the biology of cortisol-secreting tumor is associated with specific growth characteristics.

**Age**

ACC can occur at any age of life. In some series, childhood ACC displays a better prognosis than adult’s ACC (36, 37). Younger patients presented lower tumor stages, whereas adults were more likely to show aggressive tumors with shorter prognosis. After complete tumor resection, 5-year DFS was above 80% for children under 3 years but only 40% for children over 13 years, similar to that of adults (37). However, pathology and molecular biology suggest that pediatric ACC are different tumors than adults ACC (38).

In adults, several studies have shown that older age is associated with decreased OS (5, 6, 8, 9, 26, 30, 32, 39). Asare et al. have even proposed a novel staging system, where stage I and stage II ACC were differentiated by age (≤55 years for stage I vs >55 years for stage II) and not by size, like it is done in the ENSAT-staging system (40). This new classification based on age showed significant
differences in OS between stage I and stage II (40). However, the majority of studies including multivariate models did not found age as a predictor of recurrence (6, 32, 34) or cancer-specific mortality (29). These data suggest that older age could be associated with an increase in all-cause mortality due to an increase in treatment-related mortality or in non-specific mortality. Thus, in adult’s ACC, older age appears more as a confounding factor of frailty and comorbidities, than as an independent pejorative prognostic factor.

**Pathological prognostic factors**

**Tumor grade: from mitoses to Ki67**

Besides tumor stage, tumor grade represents another major prognostic factor for ACC management (23).

Mitotic count is a fundamental criterion in the diagnosis of ACC as part of the Weiss score, which gathers 9 histologic criteria associated with malignancy in adrenal tumors (41). These parameters include high nuclear grade, mitotic count >5 per 50 high-power fields (HPF), atypical mitoses, cytoplasm ≤25% clear cells, diffuse architecture, necrosis, venous invasion, invasion of sinusoidal invasion and capsular invasion. Adrenal tumors with a Weiss score >3 are considered malignant (ACC) and those with a score ≤2 as benign (adenomas). Moreover, Weiss score was developed by comparing metastatic and non-metastatic adrenal tumors and was also associated with prognostic value (41, 42). Among all features of the Weiss score, mitotic count was the only criterion consistently associated with patient prognosis (3, 42, 43, 44, 45, 46). A cut-off of 20 mitotic figures per 50 HPF was proposed to separate low from high-grade ACC (42, 44). Some limitations exist for using mitotic count as a prognostic factor in clinical routine: it is notably time consuming and subject to inter-observer variability, although inter-observer variability may decrease with trained pathologists (47).

The Ki67 immunohistochemistry represents an alternative technique for assessing proliferation in ACC. Several studies have reported the prognostic value of Ki67 index in ACC (30, 34, 48, 49, 50, 51, 52). A large study in localized ACC identified Ki67 as the most powerful prognostic factor of DFS after complete surgery. This study suggested a tumor grading based on three levels of Ki67 index: grade 1 tumors with Ki67 <10%, grade 2 with Ki67 10–19% (DFS HR=1.94) and grade 3 tumors with Ki67 ≥20% (DFR HR=2.58). Likewise, the Ki67 index was also an independent prognostic factor of OS (34). In recent series, evaluating both mitotic count and Ki67, the Ki67 proliferation index proved to be the most powerful tool to predict patient’s survival (34, 51) (Fig. 2). However, Ki67 index was not consistently associated with OS in stages III and IV ACC (30).

Compared to mitotic count, Ki67 proliferation index is less time consuming but also confronted to limited reproducibility. First, preanalytical protocol – including time to fixation, type of fixative, time in fixative and method for long-term storage – is not standardized and might affect Ki67 measurement (53). Ki67 results also depend on the assay that was used for immunostaining. Mouse anti-human Ki67 monoclonal antibody, MIB1 clone, is the most commonly used assay and is now considered as a gold standard for Ki67 immunostaining. However, even with the same preanalytical protocol and the same antibody, interpretation of Ki67 staining and scoring may be subjected to controversies: how many cells or high-power fields have to be included for evaluation? Do we need to score Ki67 into hot spot areas, or average

![Figure 2](https://viafreeaccess.com/)

**Figure 2**

Recurrence-free (A) and overall (B) survival stratified on the Ki67 index in stages I–III ACC after R0 surgery. From Beuschlein et al. (34).
score across the section, including or excluding hot spot area? A recent study has evaluated the inter-observer variability of Ki67 scoring assessment in ACC among 14 trained endocrine pathologists. Low levels of inter-observer and intra-observer concordance were observed (54), especially for intermediate Ki67 indexes (10–30%). This may be a concern for clinical practice since the clinically relevant cutoff values are in this range. However, the development of digital microscopy-enabled methods could increase reproducibility and improve reliability for clinical setting (54).

In 2014, Pennanen and coworkers have introduced a novel prognostic scoring system – the Helsinki score – that uses 2 Weiss criteria – mitotic index (3 points if >5/50 HPF) and necrosis (5 points) – plus Ki67 index in the most proliferative area of the tumor measured by computer-assisted image analysis. This score was developed by combining the most discriminative parameters for metastatic disease in a large cohort of adrenocortical tumors. For malignant tumors, a score >17 was associated with poor OS (45). The prognostic value of Helsinki score for ACC was validated in an independent cohort (46).

Resection status

The effect of margin status on outcome has been well established for several cancers but only few retrospective studies have evaluated the effect of the margin status in ACC prognosis (5, 8, 55).

Among patients that underwent surgical resection, 20–30% presented positive margins – i.e. R1 (microscopically positive resection margins) or R2 (macroscopic residual disease) resection. R1 and R2 resection are associated with higher risk of recurrence (8) and death (5, 55) (adjusted OS HR=2). These results highlight the critical role of surgical expertise to achieve R0 resection and to improve outcome.

P53 and β-catenin (CTNNB1) immunohistochemistry

The tumor stage and the resection status have long been the only widely accepted prognostic factor of ACC. However, these features do not capture the wide heterogeneity in outcome for a given stage, leading to the development of several prognostic markers based on immunohistochemistry through years (56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70). Most of these markers are derived from small retrospective series, mostly without adjustment to other prognostic factors and without external validation. Among those, CTNNB1 and P53 staining retain attention as they may be used as surrogates for molecular alterations in the two major signaling pathways of ACC (60). Indeed, activation of the Wnt/CTNNB1 pathway is associated with CTNNB1 nuclear staining and correlates with high mitotic rate (58, 59) and poor survival (56, 57, 58, 59, 60). Somatic mutations or loss of heterozygosity of TPS3 result in aberrant P53 expression and are linked with aggressive phenotype (57, 61, 62, 71), with higher tumor stage and poorer DFS. However, these markers did not show independent prognostic value in multivariate analyses including tumor grade (59).

Genomic studies and molecular prognostic markers

The development of the genomics methods during the last decade has allowed to study gene expression, genetic and epigenetic alterations at the pan-genomic level in numerous cancer types. In ACC, genomic studies led to identifying subgroups of tumors with distinct biology and different outcome. These recent progresses also allow the development of prognostic molecular markers derived from genomics (Table 1).

Transcriptome and mRNA prognostic markers

Unsupervised cluster analyses of transcriptome data have identified two major subgroups of ACC based on gene expression profiles. Four studies reported a strong prognostic value of the transcriptome considering DFS (72, 73) and OS (73, 74, 75). Our team has named the ACC transcriptome clusters ‘C1A’ and ‘C1B’, the latter being associated with better outcome (73). Giordano et al. also observed that genes differentially expressed in the two clusters of ACC are mostly involved in cell-cycle progression and proliferation and that transcriptome signatures are in agreement with tumor grade (74). However, the prognostic value of the transcriptome-based classification remained significant independently of tumor stage and tumor grade (73, 74), suggesting that transcriptome did not only reflect tumor grade.

Consequently, molecular prognostic markers were set by translating global gene expression profiles to focus assessments for clinical practice. Our team has developed a simple prognostic marker based on the differential expression of two genes (BUB1B-PINK1) measured by quantitative reverse transcription PCR (RT-qPCR) on tumor RNA. These two gene predictors of survival have
### Table 1  Main studies on prognostic molecular markers derived from genomics in ACC.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Molecular marker</th>
<th>Patients for prognostic study, n</th>
<th>Targeted measurement</th>
<th>Prognostic value – univariate analysis</th>
<th>Prognostic value – multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA markers</td>
<td>Clusters based on expression of 14 genes including ISGF3G and IL2RG</td>
<td>13</td>
<td>None</td>
<td>Recurrence-free survival</td>
<td>NA</td>
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<tr>
<td>(72)</td>
<td>SNAI1</td>
<td>26</td>
<td>RT-qPCR IHC</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(65)</td>
<td>Clusters based on expression of 60 genes</td>
<td>11</td>
<td>None</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(73)</td>
<td>BUB1B-PINK1 differential expression</td>
<td>35</td>
<td>RT-qPCR</td>
<td>Disease-specific survival</td>
<td>NA</td>
</tr>
<tr>
<td>(63)</td>
<td>SF-1</td>
<td>33 (genomics)</td>
<td>IHC</td>
<td>Recurrence-free survival</td>
<td>Overall survival</td>
</tr>
<tr>
<td>(76)</td>
<td>BUB1B-PINK1 differential expression</td>
<td>14</td>
<td>RT-qPCR</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(77)</td>
<td>PTTG1</td>
<td>35</td>
<td>RT-qPCR</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(70)</td>
<td>TOP2A and EZH2</td>
<td>61</td>
<td>IHC</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(78)</td>
<td>EZH2</td>
<td>149</td>
<td>RT-qPCR</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(79)</td>
<td>VAV2</td>
<td>126 (genomics)</td>
<td>IHC</td>
<td>Recurrence-free survival</td>
<td>Overall survival</td>
</tr>
<tr>
<td>(80)</td>
<td>VAV2</td>
<td>171 (IHC)</td>
<td>IHC</td>
<td>Recurrence-free survival</td>
<td>Overall survival</td>
</tr>
<tr>
<td>Non-coding RNA markers</td>
<td>miR-195 and miR-483-5p</td>
<td>18</td>
<td>RT-qPCR</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(94)</td>
<td>miR-503, miR-1202 and miR-1275</td>
<td>25</td>
<td>RT-qPCR</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(97)</td>
<td>Serum miR-483-5p and miR-195</td>
<td>23</td>
<td>RT-qPCR</td>
<td>Recurrence-free survival</td>
<td>Overall survival</td>
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<tr>
<td>(99)</td>
<td>miR-9</td>
<td>28</td>
<td>RT-qPCR</td>
<td>Recurrence-free survival</td>
<td>Overall survival</td>
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<tr>
<td>(69)</td>
<td>miR-210</td>
<td>51</td>
<td>RT-qPCR</td>
<td>Overall survival</td>
<td>Not independent of mitotic count</td>
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<td>(100)</td>
<td>PRINS IncRNA</td>
<td>20</td>
<td>RT-qPCR</td>
<td>Recurrence-free survival</td>
<td>NA</td>
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<tr>
<td>(107)</td>
<td>Chromosome alterations markers</td>
<td>Clusters based on aberrations in 10 chromosomes</td>
<td>25</td>
<td>None</td>
<td>Overall survival</td>
</tr>
<tr>
<td>(83)</td>
<td>Clusters based on whole genome aberrations</td>
<td>46</td>
<td>None</td>
<td>Overall survival</td>
<td>Not independent of tumor stage</td>
</tr>
<tr>
<td>(84)</td>
<td>Clusters based on aberrations in 12 chromosomes</td>
<td>22</td>
<td>None</td>
<td>Overall survival</td>
<td>Not independent of tumor stage and Ki67</td>
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<tr>
<td>Methylation markers</td>
<td>Clusters based on CpG islands methylation</td>
<td>51</td>
<td>MS-MLPA</td>
<td>Overall survival</td>
<td>NA</td>
</tr>
<tr>
<td>(88)</td>
<td>PAX5, PAX6, PYCARD, GSTP1</td>
<td>203</td>
<td>MS-MLPA</td>
<td>Recurrence-free survival</td>
<td>Overall survival</td>
</tr>
<tr>
<td>(91)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Independent of tumor stage and Ki67</td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification; NA, not available; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.
been validated in an independent cohort in our institution (73) and by another team (76), and showed an excellent prognostic value – with 5 years OS <20% in the ‘poor prognosis’ subgroup vs >80% in the ‘good prognosis’ subgroup.

Other prognostic markers were proposed based on transcriptome studies (63, 65, 70, 77, 78, 79, 80). For instance, SF-1 expression – which is usually used as a diagnostic tool for the adrenocortical origin of a tumor – was investigated as a prognostic marker in ACC and demonstrated prognostic value on DFS and OS, independently of tumor stage, sex, age and hormone secretion (63). A recent analysis showed that VAV2 expression, which is a SF-1 dosage-dependent target, was also an independent prognostic factor in multivariate analysis including age, sex, tumor stage and Ki67 (79, 80). Finally, a recent meta-analysis of transcriptome data identified the histone methyltransferase EZH2 as overexpressed in ACC. High EZH2 expression was also associated with proliferation and poor prognosis (78). Of note, another team demonstrated that EZH2 staining also carries prognostic value in stages I–II ACC (70). The major limit of these studies is that EZH2 expression was not confronted to other prognostic factors of ACC, such as tumor stage.

Exome and genes with recurrent mutations

Before genomics studies, several publications focused on specific candidate genes in ACC, including TP53 and CTNNB1. Recently, three pangenomic studies have investigated instead exome sequencing and SNP arrays to identify somatic mutations, homozygous deletions and high-level amplifications in adult’s ACC (21, 22, 81). These studies converged on a short list of genes with recurrent alterations, occurring in about 50% of ACC. The most frequent alterations were CTNNB1 and TP53 mutations and ZNRF3 and CDKN2A homozygous deletions.

About 20% of ACC harbored gain-of-function mutations in CTNNB1 (21, 22, 81). Another 20% presented ZNRF3 deletions (21, 22) – a negative regulator of Wnt/CTNNB1 – which results in the activation of the Wnt/CTNNB1 pathway. Thus, CTNNB1 mutations correlated with nuclear CTNNB1 staining (57, 58, 60). Moreover, CTNNB1 mutations were associated with higher mitotic count (58), poor prognosis transcriptome cluster (21, 57) and poor survival (57, 58, 60). However, the prognostic value of CTNNB1 was not tested after adjustment on tumor grade. A trend toward OS was also noted for ZNFR3 deletions (81).

Loss-of-function mutations of TP53 occurred in about 20% of adult’s ACC (21, 22), often associated with loss of heterozygosity (LOH) of the 17p13 region, on which TP53 is located (61, 71). TP53 mutations were almost mutually exclusive from CTNNB1 mutations (21, 22, 81). TP53-mutated tumors were associated with aberrant P53 staining (57, 61, 62), advanced stage (61), poor prognosis transcriptome cluster (21, 57) and shorter DFS (61). No significant association with OS was reported (61, 62, 81). Germline TP53 mutations have also been linked with the development of ACC, as part of the Li-Fraumeni syndrome. In pediatric ACC, germline TP53 mutations were indeed very frequent, representing up to 70% of tumors and were also associated with advanced stage and shorter DFS (38).

Chromosomal alterations

Comparative genomic hybridization (CGH) and single-nucleotide polymorphism (SNP) arrays showed recurrent chromosomal alterations in ACC genome, including gains (chromosome 5, 7, 12, and 19) and losses (chromosome 1, 2, 13, 17, 22) (82, 83, 84, 85, 86). ‘Chromosomal’ profiles showed the highest frequency of whole chromosome arm gains and losses, with extended LOH. ‘Noisy’ profiles were characterized by high number of chromosomal breaks. ‘Quiet’ profiles displayed a very limited number of large alterations. In terms of prognosis, noisy tumors showed decreased DFS, compared with chromosomal and quiet tumors. Moreover, in the subgroup of chromosomal tumors, whole genome doubling event was associated with worse prognosis.

The prognostic value of clusters based on chromosomal alterations has been validated in independent cohorts (22, 84, 86). However, some limitations need to be taken in account before using chromosomal alterations as a prognostic marker in clinical practice. On the one hand, single alterations cannot account for significant prognostic information, making DNA chips necessary to obtain a large combination of alterations for ACC prognostication (84). Our team has recently developed a new process, using NGS to determine chromosomal alterations profiles by sequencing a limited number of SNP on selected chromosome arms (87). On the other hand, the prognostic value of chromosomal alterations needs to be evaluated after adjustment on other prognostic factors.
in ACC, including tumor stage and tumor grade, and requires further prospective validations in independent centers.

**Methylation and methylation prognostic marker**

Three studies have investigated genome-wide DNA methylation profiles in ACC and showed that a subset of ACC presents hypermethylation of CpG islands that are located in gene promoter regions (88, 89, 90). The CpG islands methylator phenotype (CIMP) resulted in transcriptional inactivation of tumor suppressor genes (88, 89, 90) and was associated with poor prognosis in ACC (88).

Our team has recently developed a targeted prognostic marker measuring methylation of 4 genes (PAX5, PAX6, PYCARD, GSTP1) (91). These genes were selected for their strong correlation to methylation profiles (CIMP or non-CIMP) and for association with survival. The methylation of selected genes was measured with a commercial kit by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), a technique based on methylation-sensitive restriction enzyme and PCR. This methylation marker demonstrated strong prognostic value on DFS (HR = 2.8) and OS (HR = 2.4), independently of ENSAT tumor stage and Ki67 proliferation index (91).

In the perspective of moving to clinical practice, targeted measurements by MS-MLPA represent a fast and cost-effective approach compared to methylation arrays. In contrast to the previous RNA marker derived from the transcriptome study, the methylation marker is based on DNA analysis, which is easier to obtain in routine practice. In the future, the prognostic value of methylation has also to be confronted with other molecular markers, including those derived from transcriptome, chromosomal alterations and miRNome studies.

**MiRNome and miRNA prognostic markers**

MicroRNA (miRNA) are small non-coding RNA (18–25 nt), regulating the gene expression at the post-transcriptional level by RNA-interference (92). Several hundreds of miRNA have been identified, and some of them may be good biomarkers for cancer diagnosis and prognosis (93). MiRnome studies identified several miRNA differentially expressed between ACC and normal adrenal or benign tumors: among the most consensual ones, miR-483-3p, miR-483-5p, miR-210 and miR-503 were upregulated, while miR-195 and miR-335 were downregulated in ACC (21, 94, 95, 96, 97, 98, 99, 100, 101). Unsupervised classifications of ACC based on miR expression showed 2 or 3 subgroups of ACC, which are associated with different outcomes (21, 94, 97). MiRNA clusters were correlated with transcriptome subgroups, with rather miRNA overexpression in the transcriptome ‘C1A’ cluster.

MiRNome dysregulation in ACC has led to investigate the expression of the miRNA processing machinery, such as DICER1 enzyme and its cofactor TARBP2, as potential diagnostic and prognostic markers in adrenocortical tumors (102, 103). Thus, DICER1 immunochemistry was found as an independent predictor of DFS (HR = 2.6) but not of OS (103).

Chabre et al. also investigated the prognostic value of circulating miRNA (99). Low miR-195 and high miR-483-5p levels in the serum of ACC patients were associated with shorter DFS and OS. However, this result was obtained on 12 ACC patients and was not confirmed by another small series (104).

**Long non-coding RNA**

Long non-coding RNA (lncRNA) are RNA transcripts longer than 200 nt that may act in epigenetic silencing, transcriptional and splicing regulation (105). LncRNA dysregulation has been involved in the development of several neoplasms (106). SNP array analysis identified recurrent deletions in the locus of LINC00290, suggesting a role of lncRNA in ACC pathogenesis (21). One study focused on lncRNA expression profiling in ACC (107). LncRNA microarrays showed distinct patterns of expression between ACC and normal adrenal or benign tumors. Moreover, expression of the lncRNA PRINS was associated with DFS and its prognostic value was validated by RT-qPCR in an independent cohort of 20 ACC (107).
Molecular classification

Genomics studies showed major differences between aggressive and non-aggressive ACC. Further step was to generate global genomics classification by integration of transcriptome profiles with exome or whole-genome sequencing, SNP array analysis, methylome and miRnome. Integrated genomics has drawn a new classification of ACC, with distinct molecular subgroups that are associated with very different outcome. Omics-based classifications have been generated in two independent international cohorts: one from the ENSAT network (21) in Europe and one from The Cancer Genome Atlas (TCGA) (22) consortium in America, Europe and Australia. These two studies converged in a highly consensual global molecular classification.

![Figure 3](https://www.eje-online.org)

**Figure 3**
Integrated genomics of ACC. From Assié et al. (A and B) and Zheng et al. (C and D). (A) Integrated genomics of the ENSAT cohort. Tumors were divided into 2 groups from mRNA expression. DNA methylation, miRNA expression clusters, mutation rate and alterations in key genes and pathways converge with transcriptome clusters. (B) Overall survival stratified on the ENSAT genomic classification. (C) Integrated genomics of the TCGA cohort. Tumors were divided into 3 groups by clustering the clusters from mRNA expression (red), chromosome alterations profiles (black), DNA methylation profiles (blue); miRNA expression (purple). (D) Event-free survival stratified on the TCGA genomic classification.
strong agreement between the different omics allowed to define two main molecular subgroups, corresponding to ACC of ‘good’ and ‘bad’ prognosis.

Our team as part of the ENSAT network has proposed a classification based on the two transcriptome signatures ‘C1A’ and ‘C1B’ (Fig. 3A and B). The molecular group of ‘good’ prognosis ACC was characterized by ‘C1B’ cluster, low mutation rate, very rare mutations in ACC driver genes and no hypermethylation (non-CIMP). Conversely, the molecular group of aggressive ACC corresponded to ‘C1A’ transcriptome signature and harbored high mutation rate and frequent alterations on the recurrent ACC driver genes. A subset of aggressive tumors was also hypermethylated and associated with poorer outcome compared with non-hypermethylated tumors. Therefore, methylation pattern defined one subgroup of poorer prognosis (C1A and CIMP) and another subgroup of intermediate prognosis (C1A and non-CIMP) into this group of aggressive ACC (21).

In the TCGA study, the unsupervised clustering of omics clusters – including transcriptome, miRnome, methylome and chromosomal alterations profiles – established a molecular classification into three groups (Fig. 3C and D). The first group corresponded to ‘good’ prognosis ACC and was characterized by transcriptome ‘C1B’, ‘chromosomal’ SNP profile and no hypermethylation. The two other groups corresponded to aggressive ACC and showed a ‘C1A’ transcriptome cluster profile. The second group showed intermediate levels of hypermethylation (CIMP-intermediate), and ‘chromosomal’ SNP profile, and was associated with intermediate outcome, whereas the third group of tumors was characterized by high levels of hypermethylation (CIMP-high), ‘noisy’ SNP profile and poorer prognosis (22).

A global molecular classification of ACC emerges from the studies described in this review (Fig. 4). As stated before for individual omics studies, the molecular classification could theoretically be summarized to a combination of targeted measurements for ACC prognostication in clinical setting. However, the combination of targeted molecular markers should both be as simple as possible for routine use and should

Figure 4
Global molecular classification of ACC. Gene expression, chromosome alterations and DNA methylation profiles divided ACC into 3 prognostic groups. These groups are also associated with some other molecular events – including miRNA expression and somatic mutations – and clinical characteristics. Gene expression profiling defines poor/intermediate prognostic ‘C1A’ and better prognostic ‘C1B’ clusters. The ‘C1A’ group of poor prognosis is also characterized by ‘noisy’ chromosome profile (numerous chromosome losses and gains) and hypermethylation in CpG islands (CIMP). The ‘C1A’ group of intermediate prognosis displays ‘noisy’ profile or ‘CIMP’ but not both. The ‘C1B’ group shows a small number of molecular events – the most common alteration being a downregulation of the DLK1-MEG3 miR cluster (14q32.2) – and a better prognosis.
demonstrate prognostic value independently of tumor stage and tumor grade.

**Summary and perspectives**

Due to rarity of the disease, prognostic factors in ACC are established on small series, mostly in retrospective design. The tumor stage still represents the main prognostic factor in ACC. Among all classifications that were proposed, the ENSAT-staging system provides the best survival discrimination. This classification highlights the poor outcome of metastatic disease (stage IV), that are now individualized from locally advanced tumors (stage III) (2).

Beyond tumor stage, the tumor grade also shows a strong prognostic value. The mitotic count tends to be replaced by the Ki67 proliferation index (34). Tumor grade assessment still suffers from inter-observer reproducibility, although new computer-assisted techniques seem promising (54).

Molecular classification provides new insights in ACC biology. Distinct molecular subgroups have emerged from genomics, associated with different outcomes (21, 22). This clearly explains why prognosis of this aggressive cancer might nevertheless vary among tumors. On the short term, this could help to develop new molecular prognostic markers for patient management. On the long term, this will inevitably stimulate the development of specific targeted therapies based on the tumor biology driving these molecular classes.

To develop molecular markers, gene and miRNA expression can be assayed by targeted measurements using RT-qPCR. Focal measurements of methylation can be done with qPCR based techniques – such as MS-MLPA – or with targeted NGS after bisulfite treatment (87). Alterations of driver genes and chromosomal profiles can both be determined by targeted NGS (87). However, additional studies are needed to precise the best set of molecular markers for prognosis and to confront their prognostic value to the already validated prognostic markers, especially ENSAT stage and Ki67 index. Moreover, the reproducibility of the discriminant molecular tools within a single tumor has also to be evaluated. Finally, the molecular markers will have to be simple and cost-effective for transfer to clinical routine.

Individualizing patients’ prognosis and tumor biology appears as a necessary step for personalized medicine. In addition to tumor stage and tumor grade, the genomic classification may precise the risk stratification and thus help defining therapeutic strategy.

In adjuvant setting, it would be of particular interest to determine the individual risk of recurrence. Patients with molecular ‘good’ prognosis ACC could probably be proposed a simple follow-up after radical ‘R0’ resection and benefit from loco-regional treatments in case of recurrence. By contrast, patients with molecular aggressive ACC could benefit from closer follow-up and adjuvant systemic therapies like mitotane or even platinum chemotherapy.

Liquid biopsies represent another perspective for patients’ follow-up. Circulating miRNA (99, 104, 108, 109) and circulating tumor DNA (110, 111) are measurable in patients’ blood. Larger validation cohorts are needed to precise their ability for early detection of recurrence.

For advanced disease, one future direction will be the pharmacogenomics of ACC. Although overall response rate remains below 25% (12), a few patients show deep and durable responses to mitotane or to platinum-based chemotherapy. Additional studies are needed to precise whether some molecular markers can predict response to treatment and identify this minority of responders.

Genomics may also help identifying new therapeutic targets. Wnt/β-catenin pathway inhibitors (112) and cycle cell inhibitors, such as CDK4/CDK6 or Wee1 inhibitors (113), are currently in development and could offer new therapeutic strategies for ACC with alterations in these pathways. Demethylating agents could be proposed for hypermethylated tumors. Indeed, Suh et al. showed that decitabine inhibits proliferation in ACC cells (NCI-H295R) (114). Demethylating agents have mostly shown efficacy in hematological diseases, whereas they induced at best a stable disease in patients treated for solid tumors (115). However, their efficacy in CIMP ACC has not been evaluated so far. RNA interference therapy represents another promising approach to target molecular alterations. A recent preclinical study reported the first hopeful results of miRNA replacement in ACC, showing that systemic miR-7 administration reduces ACC xenograft growth (116).

Such strategies based on the molecular classification would need prospective validation in clinical trials.

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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