Activity and safety of temozolomide in advanced adrenocortical carcinoma patients

Deborah Cosentini^{1,*}, Giuseppe Badalamenti^{2,*}, Salvatore Grisanti¹, Vittoria Basile³, Ida Rapa⁴, Sara Cerri¹, Andrea Spallanzani⁵, Paola Perotti³, Emanuela Musso², Marta Laganà¹, Vittorio D Ferrari¹, Gabriele Luppi⁵, Alberto Dalla Volta¹, Lorena Incorvaia², Sandra Sigala⁶, Antonio Russo², Marco Volante⁴, Massimo Terzolo³ and Alfredo Berruti¹

¹Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, ASST Spedali Civili, Brescia, Italy, ²Section of Medical Oncology, Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy, ³Internal Medicine, Department of Clinical and Biological Sciences, San Luigi Hospital, University of Turin, Orbassano, Turin, Italy, ⁴Department of Oncology, San Luigi Hospital, University of Turin, Orbassano, Turin, Italy, ⁵Department of Medical Oncology, AOU di Modena, Modena, Italy, and ⁶Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

*D Cosentini and G Badalamenti contributed equally to this work

Correspondence
should be addressed
to A Berruti
Email
alfredo.berruti@gmail.com

Abstract

Objective: Temozolomide has shown a significant anti-proliferative activity on adrenocortical cancer (ACC) cells *in vitro*.

Design: On the basis of these results the drug was prescribed as second/third line in advanced metastatic ACC patients in four referral centers in Italy.

Methods: We retrospectively collected anagraphic, clinical and pathological data of patients with advanced ACC with disease progression to standard chemotherapy plus mitotane who were treated with temozolomide at the dose of 200 mg/m²/die given for 5 consecutive days every 28 days. The primary endpoint was the disease control rate, defined as objective response or disease stabilization after 3 months. Secondary endpoints were overall survival (OS), progression-free survival (PFS) and drug safety.

Results: Twenty-eight patients have been included in the study. Ten patients (35.8%, 95% CI: 17.8–53.8) obtained a disease control from temozolomide treatment. In particular, 1 patient had a complete response, 5 patients a partial response and 4 patients stable disease. Median PFS was 3.5 months and median OS was 7.2 months. Disease response was more frequently observed in patients with methylation of O6-methylguanine-DNA methyltransferase (MGMT) gene. Temozolomide therapy was well tolerated and most toxicities were limited to grade G1–2 according to WHO criteria.

Conclusion: Temozolomide was found active in the management of advanced ACC patients. The disease control rate obtained, however, was short-lived and the prognosis of treated patients was poor.

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Introduction

Adrenocortical carcinoma (ACC) is a rare and aggressive tumor with an incidence of 0.7–2 new cases per million populations per year (1). Surgery is the mainstay of therapy but a significant proportion of patients have non-resectable tumors at diagnosis; moreover, disease relapse occurs within 2 years in the majority of patients

who undergo radical resection (1). This is the reason why adjuvant mitotane therapy is recommended by recent guidelines (2, 3), despite the low evidence of efficacy (1, 2, 4, 5, 6).

The standard systemic treatment for advanced/metastatic ACC patients, not eligible to surgery, is

mitotane. This drug is administered either alone (1), or in combination with etoposide, doxorubicin and cisplatin (EDP-M regimen) (7). The efficacy of the EDP-M regimen, however, is limited as shown by the results of a randomized clinical trial reporting a disease response in about 25% of treated patients with a median survival of 14 months (8). No effective therapies are available for patients with disease progression after EDP-M (9). The combination of gemcitabine and capecitabine, which is recommended by currently available guidelines (2, 3), is poorly efficacious (10, 11). Other treatment strategies, including modern molecular target therapies and immunotherapy (12, 13, 14), failed to demonstrate significant activity. New therapeutic options are therefore needed.

Temozolomide is an alkylating drug initially used in the treatment of brain tumors (15). The drug has been demonstrated to be efficacious also in the management of neuroendocrine tumors (16) and malignant pheochromocytoma/paraganglioma (17). Creemers and colleagues recently published a preclinical study exploring the activity of temozolomide in ACC cells *in vitro*. Their data showed that the drug has cytotoxic and cytostatic effects through a strong inhibition of cell growth, apoptosis and cell cycle arrest (18). Moreover, a weak association was found between the *in vitro* cytotoxicity of temozolomide and the epigenetic silencing of the MGMT (O6-methylguanine-DNA methyltransferase) DNA-repair (MGMT) gene, which is associated with temozolomide efficacy in patients with glioblastoma (19, 20, 21) and advanced neuroendocrine tumors (16).

Given these results and the limited therapeutic strategies available in the management of advanced ACC, four Italian reference centers for this rare disease used temozolomide in ACC patients with disease progression to standard therapies. In this study, we did a retrospective assessment of temozolomide activity and toxicity in this peculiar clinical setting.

Patients and methods

Study design and patients' characteristics

This is a multicentric, retrospective study. Twenty-eight consecutive ACC patients treated with temozolomide from January 2016 to January 2018 at four Italian Institutions were included. Inclusion and exclusion criteria were considered also according to the summary of product characteristics of temozolomide. All patients met the following eligibility criteria: age >18 years; Eastern

Cooperative Oncology Group (ECOG) performance status 0–2; life expectancy of at least 3 months; pathological diagnosis of ACC; locally advanced or metastatic disease not suitable for surgery; at least one unidimensional (RECIST criteria) measurable lesion; adequate bone marrow reserve (neutrophils $\geq 1500/\text{mm}^3$ and platelets $\geq 100\,000/\text{mm}^3$, hemoglobin ≥ 9.0 g/dL); total bilirubin ≤ 1.5 times the upper limit of normal; serum creatinine ≤ 1.5 the upper limit of normal; effective contraception in premenopausal female and male patients. Exclusion criteria were history of prior malignancy, except for cured non-melanoma skin cancer, cured *in situ* cervical carcinoma, or other treated malignancies with no evidence of disease for at least 3 years; active clinically serious infections (greater than grade 2, Common Toxicity Criteria CTC V5.0); symptomatic metastatic brain or meningeal tumors; seizure disorder requiring medication (i.e. steroids or antiepileptics); decompensated heart failure (ejection fraction $\leq 45\%$); myocardial infarction or revascularization procedure during the last 6 months; unstable angina pectoris; uncontrolled cardiac arrhythmia; hypertension not controlled by medications; pregnant or breast-feeding patients; prior treatment with temozolomide; other anticancer chemotherapy or immunotherapy during the study or within 4 weeks of study entry; radiotherapy during study or within 3 weeks of study start (palliative radiotherapy was allowed); major surgery within 4 weeks of study start; concomitant treatment with another investigational drug. Before starting therapy all patients were examined at the Medical Oncology of ASST-Spedali Civili in Brescia. After assessing eligibility for temozolomide therapy, the drug was prescribed and a written informed consent for off-label temozolomide therapy was signed by each patient. Patients resident in other cities were referred to four referral oncology centers whose hospitals approved the off-label use of the drug. Temozolomide was distributed to the patients free of charge by the hospital pharmacy of all involved Institutions. The retrospective study was approved by the Ethical Review Board of ASST-Spedali Civili in Brescia (Protocol No. 3132).

Treatment consisted in temozolomide at the planned dose of $200\text{ mg}/\text{m}^2/\text{die}$ given for 5 consecutive days every 28 days. Maintenance of previous mitotane treatment was allowed but not mandatory and blood drug levels were monitored. Disease re-staging by CT scan and/or MRI was performed every three cycles. The following demographic, clinical and pathological data were collected: sex, age, medical history, physical examination, performance status, routine laboratory tests, endocrine work-up, chest

and abdominal CT scan, other imaging data (i.e. brain CT, MRI, bone scan) performed at baseline and during temozolomide treatment.

The study primary endpoint was the disease control rate, defined as the proportion of patients obtaining objective tumor response or stabilization at CT scan after 3 months of temozolomide therapy, using RECIST criteria, version 1.1. Secondary endpoints were the evaluation of progression-free survival (PFS), overall survival (OS) and treatment toxicity. PFS was defined as the time relapsing from the beginning of the treatment until disease progression or death whatever event occurred first. Non-progressing patients still alive were censored at the last follow-up examination. Overall survival was defined as the time interval between the date of treatment start and the date of death from any cause or the last known alive date. The mENSAT classification and GRAS parameters (22) were used to assess prognosis. In particular, GRAS parameters were considered favorable if grading defined by Ki67 was <20%, primary R0 resection status attained, age <50 years and absence of symptoms at diagnosis; unfavorable in case of age >50 years, or presence of symptoms at diagnosis; pejorative in case of grading as defined by Ki67 >20% and/or primary R1-2 resection status. The Common Toxicity Criteria CTC V5.0 were used to assess toxicity.

Assessment of MGMT promoter methylation in tumor samples

As ancillary study, we evaluated the predictive role of MGMT (O⁶-methylguanine–DNA methyltransferase) promoter methylation in a patient subset included in this study for which tumor samples were available. MGMT promoter methylation status was performed by means of pyrosequencing technique. Ten methylated CpG sites were analyzed, located in the promoter region (NG_052673.1-chr10:131 265 507-131 265 556) of MGMT gene exon 1 and involved in the regulation of gene expression. Genomic DNA (gDNA) was extracted using FFPE tissues after manual microdissection, for neoplastic cell enrichment (at least 50% of tumor cells), using the Maxwell® RSC instrument and tissue DNA Kit (Promega s.r.l.). A total of 500 ng of gDNA was modified by bisulfite conversion using a commercial available and certified CE-IVD kit (MGMT plus, Diatech Pharmacogenetics, Ancona, Italy) following manufacturer's instructions. Sequencing analysis was performed on PyroMark Q96MA apparatus (Biotage, Uppsala, Sweden) with PCR

and sequencing primers supplied in the MGMT plus kit according to the manufacturer's instructions.

Data analysis was performed using the PyroMarkCpG software (Biotage), obtaining a mean percentage of the ten CpG methylated islands for each case. A cut-off of 5% (mean of the CpG islands) of methylation was used to define 'methylated' (>5%) and 'unmethylated' (≤5%) samples. Methylated and unmethylated controls were properly used to take control of all workflow.

Statistical analysis

Descriptive statistics were used to analyze the patient clinical characteristics. Differences between categorical variables were assessed by a chi-square or the Fisher test when indicated. The PFS and OS curves were calculated with the Kaplan–Meier method and compared with the log-rank test. The primary end point of the study was to estimate the activity of the therapy in terms of proportion of patients attaining a clinical benefit. With 28 patients recruited, this study has a potency of 80% to refuse a clinical benefit rate of 15% (p0) and to assess the activity of the therapy as a disease control rate of 40%; given an alpha error of 0.05. Statistical significance was set at $P < 0.05$. SPSS v17.0 software was used for the statistical analyses (SPSS Inc.).

Results

Patients' characteristics

The characteristics of the 28 enrolled patients are summarized in Table 1. Median age at baseline was 54 years (range 31–72). Thirteen patients (46.4%) had a hormone-secreting tumor at diagnosis and ten of them (35.7%) had Cushing syndrome. Twenty-six patients (92.8%) underwent primary surgery as the first treatment and 20 of them (71.4%) obtained a complete resection (R0). Median disease-free survival of surgical treated patients was 19.9 months (range 5–49). Twelve patients (42.9%) received postoperative adjuvant mitotane.

Cisplatin alone or the EDP combination regimen, both administered in association with mitotane, were the first-line cytotoxic therapies adopted after the diagnosis of metastatic disease. At baseline conditions, before starting temozolomide, the majority of patients (71.4%) had a performance status ≤1. According to mENSAT classification, 16 patients (57.1%) had a stage IV-A disease, 9 (32.2%) stage IV-B, 3 (10.7%) stage IV-C. GRAS

Table 1 Patients characteristics.

Characteristics	Values
Total number of patients	28
Age, years	54 (31–72)
Sex, <i>n</i> (%)	
Males	17 (60.7)
Females	11 (39.3)
Secreting at diagnosis, <i>n</i> (%)	13 (46.4)
Cortisol excess	10 (35.7)
Androgen excess	3 (10.7)
Non secreting tumors	15 (53.6)
Histological characteristics at diagnosis	
Ki67, %	20 (2–50)
Ki67 <20%	15 (53.6)
Ki67 ≥20%	13 (46.4)
Mitotic Rate (n/50HPF)	10 (2–66)
Weiss Score	6.5 (3–9)
GRAS*, <i>n</i> (%)	
Favorable	6 (21.4)
Unfavorable	9 (32.2)
Pejorative	13 (46.4)
Primary surgery, <i>n</i> (%)	26 (92.8)
R0	20 (71.4)
Adjuvant mitotane, <i>n</i> (%)	12 (42.9)
Disease free survival, months	19.9 (5–49)
First-line metastatic treatment, <i>n</i> (%)	
EDP-M	21 (75)
Cisplatin only	7 (25)
Second-line metastatic treatment, <i>n</i> (%)	
Cisplatin only	2 (7.1)
Gemcitabine plus capecitabine	15 (53.6)
Mitotane in advanced setting, <i>n</i> (%)	28 (100)
ECOG PS**, <i>n</i> (%)	
0–1	20 (71.4)
>1	8 (28.6)
mENSAT***, <i>n</i> (%)	
Stage IV-A	16 (57.1)
Stage IV-B	9 (32.2)
Stage IV-C	3 (10.7)

Data are expressed as median and range if not otherwise specified.

*GRAS parameters are considered favorable if grading defined by Ki67 is <20%, primary R0 resection status performed, age <50 years and absence of symptoms at diagnosis. GRAS parameters are classified unfavorable in case of age >50 years or presence of symptoms at diagnosis. GRAS parameters are classified as pejorative in case of grading as defined by Ki67 >20% and/or primary R1-2 resection status. **ECOG PS Performance Status sec ECOG assessed at the beginning of temozolomide therapy.

***mENSAT stage assessed at the beginning of temozolomide was defined as IV-A when tumor involves two organs, IV-B in case of three tumor organs, IV-C in case of >3 tumor organs.

parameters were favorable in 6 (21.4%), unfavorable in 9 (32.2%) and pejorative in 13 (46.4%) patients.

Treatment administered and activity

Temozolomide was administered as second-line therapy in 11 patients (39.3%), third-line therapy in 15 patients (53.6%) and fourth-line approach in 2 (7.1%). All patients

continued mitotane therapy during temozolomide administration and the drug levels were within the therapeutic range in 10 (35.7%) of them.

Median dose of temozolomide was 250 mg/die given for 5 consecutive days and median number of temozolomide cycles was 4 (range 2–16). One patient (3.6%) obtained complete clinical response evaluated by RECIST 1.1 criteria, while partial response was observed in 5 patients (17.9%), and 4 patients (14.3%) obtained disease stabilization. However, 18 patients (64.2%) had disease progression (Fig. 1). The overall response rate was 21.5% (95% CI: 6.5–27.5) and a disease control was obtained in 10 patients (35.8%) (95% CI: 17.8–53.8) (Table 2).

At disease progression, further chemotherapy was administered in six patients; in particular, four patients received gemcitabine and capecitabine, whereas two patients received cisplatin as single agent.

Patients' outcome

Median PFS was 3.5 months (range: 1.2–24.2) (Fig. 2A) and OS was 7.5 months (range 2–38.8) (Fig. 2B). No significant difference in PFS was seen stratifying patients on the basis of whether they received temozolomide as second or further line of treatment (median PFS 3.9 months, range 1.4–24.2 vs 3.5 months, range 1.2–15.8, respectively,

Figure 1

Waterfall plot of temozolomide responses. PD, progressive disease, defined by RECIST criteria as at least a 20% increase in the sum of the longest diameter (LD) of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. SD, stable disease, defined by RECIST criteria as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started. PR, partial response, defined by RECIST criteria as at least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD.

Table 2 Treatment response.

Complete response	1 (3.6%)
Partial response	5 (17.9%)
Stable disease	4 (14.3%)
Progressive disease	18 (64.2%)
Overall response rate	6 (21.5%, 95% CI: 6.5–27.5)
Clinical benefit	10 (35.8%, 95% CI: 17.8–53.8)

$P=0.62$). In addition, the attainment of a clinical benefit did not have any positive impact on OS: median 8.1 (range 3.6–38.8) in patients attaining a clinical benefit vs 7.1 months (range 2–33.3) in those who did not ($P=0.78$).

Additional analyses were done to explore the potential prognostic significance of several patient and tumor characteristics. The patients with ECOG PS 0-1 had longer OS than those with PS 2 (median: 10.5 months, range: 2.6–38.8 vs 3.2 months, range: 2.0–7.5,

respectively, $P=0.001$) (Fig. 3A). Favorable GRAS score was associated with a longer OS (median: 12.2 months, range: 5.5–33.3) than unfavorable/pejorative GRAS (median: 6.9 months, range: 2.0–38.8), although the difference was not statistically significant ($P=0.17$) (Fig. 3B). OS curves were similar in patients with unfavorable and pejorative GRAS score (data not shown). However, mENSAT failed to be associated with OS (mENSAT A: 7.5 months, range: 2.0–38.8, vs mENSAT B-C: 6.9 months, range: 2.6–33.3, $P=0.82$); and the prognostic significance of mENSAT was not improved by the addition of GRAS (mENSAT A+GRAS A median OS: 12.2 months, range: 5.5–24.2, vs mENSAT+GRAS B-C: 7.5 months, range: 2.0–38.8,

Figure 2

(A) Progression-free survival in patients treated with temozolomide. (B) Overall survival in patients treated with temozolomide.

Figure 3

(A) Overall survival on the basis of ECOG performance status ($P 0.001$). (B) Overall survival on the basis of GRAS parameters: favorable vs unfavorable or pejorative ($P 0.17$).

$P=0.56$) (data not shown). Finally, stratifying patients according to mitotane plasma concentrations, PFS and OS were 4.4 months (range: 2.9–24.2) and 12.7 months (range: 4.4–33.3), respectively in patients in which blood mitotane was within the therapeutic range (14–20 $\mu\text{g/L}$), while they were 3.3 months (range: 2.4–15.8) and 6.9 months (range: 2.4–38.8) respectively in those in which the drug was below 14 $\mu\text{g/L}$ ($P=0.36$ for PFS, $P=0.37$ for OS) (Fig. 4).

Treatment toxicity

Patients were evaluated after each cycle with both clinical examination and blood chemistry (complete blood count, liver and renal function). The observed temozolomide toxicities are summarized in Table 3. As expected, nausea and vomiting were the most frequent side effects, occurring in 35.8 and 25% of patients, respectively. These symptoms were classified as grade 3 in 14.3% and in 10.7% of patients, respectively. Liver toxicity was observed in 25% of patients, being grade 3 in 7.2% of cases. Only 1 patient (3.6%) developed grade 3 neutropenia. The other observed toxicities were renal impairment and thrombocytopenia, involving 25% and 21.4% of patients, respectively. A 1-week delay in the start of a new cycle was needed in 12 patients due to toxicity, whereas no dose reduction was prescribed.

Figure 4

Overall survival on the basis of circulating mitotane levels ($P=0.37$).

MGMT status and relevant correlation with drug activity

MGMT status was evaluated in 15 patients, eight of them showing MGMT promoter methylation. In the methylated MGMT group, 4 patients out of 8 (50.0%) obtained a disease response, in particular, 1 had a complete response and 3 had a partial response. Conversely, in the non-methylated MGMT group, only 1 patient out of 7 (14.3%) had a partial response, whereas 1 patient (14.3%) had a stable disease and the others had progressive disease (Table 4). No differences in PFS or OS were observed comparing patients with (median PFS: 4.4 months, range: 2.1–24.2; median OS: 6.9 months, range: 3.1–12.7, respectively) or without (median PFS: 3.9 months, range: 2.1–15.8; median OS: not reached, range: 3.2–38.8) MGMT methylation ($P=0.55$, $P=0.08$ respectively) (data not shown).

Discussion

Alkylating agents have shown some activity in ACC. Our group observed a clinical benefit of the administration of oral cyclophosphamide on a metronomic schedule in two heavily pre-treated patients (23). Moreover, streptozotocyn (Sz) demonstrated a response rate of 35% in 40 advanced ACC assessed retrospectively in Sweden (24). On the basis of these results, Sz was considered as the best treatment to be tested against EDP in the FIRM-ACT trial (8). Since this combination appeared inferior to EDP, Sz is not generally used as first-line approach. However, it is still recommended as a possible second-line therapy by currently available guidelines (2, 3).

Due to the demonstrated activity of alkylating agents in the management of ACC and the preclinical findings showing that temozolomide exerted a potent antitumor effect on ACC cells *in vitro* (18), four Italian reference centers obtained the authorization for the off-label administration of the drug at dose of 200 $\text{mg/m}^2/\text{die}$ in ACC patients with disease progression after first-line chemotherapy with cisplatin-containing regimens plus mitotane. In this retrospective evaluation of these patients, temozolomide was as a whole well tolerated and toxicities were manageable. In particular, toxicities were less frequent than previously seen in other tumors, except for thrombocytopenia which was found in a higher number of patients (21.4 vs 13.7%) (16, 25).

Tumor responses according to RECIST criteria were observed in 20% of patients, whereas about 35% had a

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Table 3 Temozolomide toxicity.

	Toxicity, n = 28 (100%)			
	All grades	G1	G2	G3
Nausea	10 (35.8%)	4 (14.3%)	2 (7.2%)	4 (14.3%)
Vomiting	7 (25%)	1 (3.6%)	3 (10.7%)	3 (10.7%)
Liver toxicity	7 (25%)	4 (14.3%)	1 (3.6%)	2 (7.2%)
Renal toxicity	7 (25%)	4 (14.3%)	3 (10.7%)	0 (0%)
Thrombocytopenia	6 (21.4%)	4 (14.3%)	2 (7.2%)	0 (0%)
Neutropenia	5 (17.9%)	2 (7.1%)	2 (7.2%)	1 (3.6%)

disease control. Although comparisons between different trials have to be considered with caution, these results seem to be better in terms of response rate than those obtained by the association gemcitabine plus capecitabine (about 4%) while similar data were seen in terms of disease control rate (10, 11). Unfortunately, disease response and stabilization to temozolomide were short-lived and did not influence patients survival, which was only of 7 months on average. This finding is in contrast to what it is commonly observed in chemotherapy trials, in which disease response usually discriminates two patient populations with different prognosis (26).

In these pre-treated ACC patients, poor performance status was the strongest negative prognostic factor, whereas mENSAT stage failed to correlate with PFS and OS. Favorable GRAS score was associated with better outcome in terms of PFS and OS without attaining the statistical significance than either unfavorable and pejorative scores. The low number of patients enrolled conferred to our series a low potency to test these prognostic factors.

It was found in other malignancies that the therapeutic benefit of temozolomide depends on its ability to alkylate/methylate DNA. This methylation damages the DNA, thus

triggering cell death by apoptosis. Primary resistance to temozolomide in glioblastoma and in neuroendocrine tumors is often directly related to high MGMT expression (16, 19). So MGMT inactivation by methylation is a marker of temozolomide tumor cell sensitivity. In our series, we were able to assess MGMT expression in 15 cases. Despite the low numbers, the objective response rate was of 50% in methylated ACC vs 14% in non-methylated ACC, and this finding is consistent with the potential role of MGMT inactivation in favoring temozolomide cytotoxicity in ACC patients.

This study provides for the first time data on activity of temozolomide in ACC patients, the retrospective design and the small number of patients included are the main limitations.

In conclusion, temozolomide, administered as second-line approach in ACC, induced a significant tumor shrinkage in about one out of five patients. Despite this non-negligible activity, the drug failed to demonstrate to be efficacious, since the responses observed were short-lived and did not influence patient survival. On the basis of these data, we believe that temozolomide should not be recommended as second-line therapy in unselected ACC

Table 4 MGMT methylation assessment.

n = 17	MGMT methylation (% sites of methylation)	MGMT methylation with kit-score	Objective response	OS	PFS
1	5	NO	SD	38.8	15.8
2	2	NO	PD	33.3	7.0
3	12	YES	PD	12.7	6.6
4	3	NO	PD	3.2	2.5
5	6	YES	PR	3.1	2.1
6	58	YES	PR	8.6	4.5
7	10	YES	PD	12.2	2.9
8	7	YES	PD	6.0	7.1
9	3	NO	SD	8.1	3.5
10	2	NO	PD	20.4	3.9
11	5	NO	PD	20.5	3.9
12	6	YES	PD	6.9	2.8
13	8	YES	PR	4.4	4.4
14	13	YES	CR	24.2	24.2
15	2	NO	PR	5.1	2.1

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; UND, undetermined, objective response.

patients. However, the drug could be a potential option in patients with good PS bearing MGMT methylated ACC, and this may represent a first step toward a personalized medicine approach in advanced ACC.

Declaration of interest

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

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