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Efficacy of oral metformin in a patient with metastatic adrenocortical carcinoma: Examination of mechanisms and therapeutic implications

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Abstract

Although rare, adrenocortical carcinoma is among the most common tumors found in children with Li-Fraumeni syndrome and Li-Fraumeni-like syndrome, associated with germ-line mutations in the TP53 gene. In southern Brazil, one form of Li-Fraumeni syndrome, associated with childhood adrenocortical carcinoma, is caused by a mutation in the R337H TP53 tetramerisation domain and is attributed to a familial founder effect. Adrenocortical carcinoma is considered an aggressive neoplasm, usually of poor prognosis and is generally unresponsive to systemic chemotherapy. Optimal treatment regimens remain to be established. We report the case of a young woman with metastatic adrenocortical carcinoma, who achieved stable disease with mitotane, cisplatin, doxorubicin, and etoposide as first-line therapy, but then had an objective response to oral metformin that lasted 9 months. The presence of the R337H TP53 mutation suggests a mechanism for the observed response to metformin.

Keywords

Adrenocortical carcinoma, metformin, Li-Fraumeni syndrome

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Introduction

Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with an estimated annual prevalence of 0.5–2 cases per million population.[1] Most cases present as either locally advanced or metastatic disease.[1] Despite aggressive multimodality therapy, the prognosis of metastatic disease remains poor. The incidence of ACC is typically bimodal with most cases presenting in children <5 years of age or in adults in their fourth and fifth decades of life, affecting women slightly more often than men, with a ratio of 1.2–1.5:1.[2,3] Most ACC occurs as sporadic tumors, but a small percentage is associated with the rare hereditary Li-Fraumeni syndrome (LFS).[4] Recognizing a 10-fold higher incidence of ACC in southern Brazil, Ribeiro and co-investigators identified a unique germ-line mutation in TP53 that encodes for an arginine to histidine amino acid substitution at position 337.[5] Unlike the more common TP53 mutations that cluster in the DNA binding and transactivational domains, the R337H mutation occurs in the tetramerisation domain

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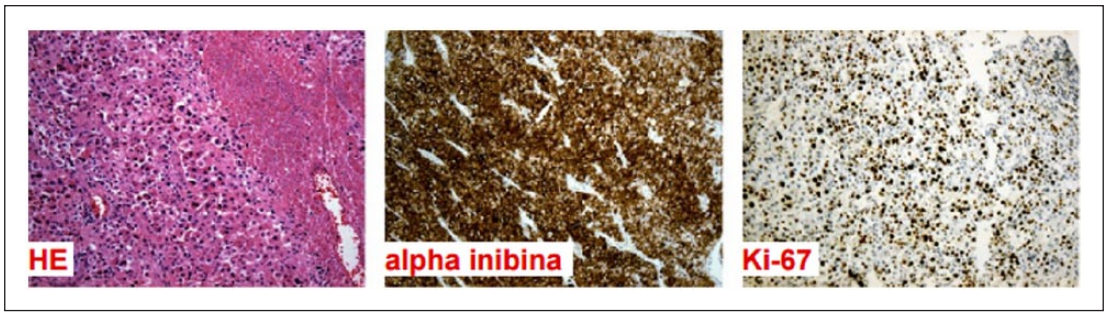

Figure 1. (a) Histopathology showing ACC with solid and diffuse architecture pattern, high nuclear grade, and extensive necrosis. (b and c) Immunohistochemistry showing positivity for alpha-inhibin and Ki-67 of 40%.

resulting in a conditional mutant protein whose function is pH dependent.[6,7]

The pathogenesis of ACC is not completely understood. Similar to benign adrenal tumors, ACC can be either hormonally active (functional) or hormonally inactive (nonfunctional) with approximately 70% of ACCs hormonally active, most often presenting with Cushing's syndrome.[8,9] When hormonally inactive, ACC commonly presents with abdominal pain.[10] Complete surgical resection, when feasible, is the optimal therapeutic strategy for improving survival and offers the only possibility for cure.[10] Despite treatment, patients with stage-IV ACC have a disease-specific 5-year survival of only 13%.[11] Stage-IV ACC is defined as metastatic disease as per the European Network for the Study of Adrenal Tumors (ENSAT) classification.[11] The limited efficacy of standard-of-care therapy has led to the search for new treatment options. As metformin has become the focus of a growing number of oncology trials, this class of drugs offered a novel approach for the management of a patient with advanced ACC.

Case report

A 26-year-old previously healthy female sought medical attention in January 2015 with a 2-month history of weight gain, facial acne, and hirsutism. She was seen by an endocrinologist who obtained a 24-h urinary cortisol at 483 mcg/mL (normal range: 10–100 mcg/mL). Computed tomography (CT) scan of the thorax and abdomen revealed multiple lobulated pulmonary nodules of soft tissue density measuring between 5 and 15 mm as well as a solid heterogeneous mass in the left adrenal gland measuring 14 × 8 × 7 cm, with dystrophic calcifications, contrast enhancement, central area of necrosis, and extension into the left adrenal vein. The lesion was in contact with the superior kidney pole, displacing it inferiorly, but without signs of invasion. There were three nodular foci in the liver compatible with hemangiomas. Magnetic resonance imaging (MRI) of the abdomen confirmed a solid lobulated mass in the left adrenal, measuring 14 × 9 × 7 cm, with high signal on T2 sequences and heterogeneous contrast enhancement. There was invasion into the left adrenal vein with associated thrombosis. On 28 January 2015, left adrenalectomy with en bloc radical nephrectomy and adrenal vein tumor thrombus excision was performed. Postoperative recovery was uneventful. Histopathologic exam confirmed a 13 × 10 × 5 cm ACC with solid and diffuse architecture pattern, high nuclear grade, mitotic rate of more than 15 per 10 high power fields with atypical mitosis, extensive necrosis, clear cell component of 25%, the presence of capsular invasion, and neoplastic invasion into the left adrenal vein. Immunohistochemistry showed positivity for alpha-inhibin and synaptophysin, as well as a Ki-67 of 40% (Figure 1). The patient was first seen at our Oncology Center in February 2015. Due to the high mitotic count and Ki-67, she was started on mitotane combined with chemotherapy (doxorubicin, cisplatin, and etoposide). After three cycles, a positron emission tomography-CT (PET-CT) scan demonstrated stable disease. However, due to the side effects (especially nausea, vomiting, malaise, and alopecia), the patient decided to stop chemotherapy.

To explore additional treatment options, the patient travelled to California and underwent a surgical biopsy of a peripheral lung nodule that provided viable tissue for the ex vivo analysis of programmed cell death (EVA/PCD). This phenotypic platform provides drug response profiles that have been shown to correlate significantly with response, time to progression, and survival.[12] The platform has been the subject of prior review with methods described in previous publications.[13–16] In summary, sterile surgical specimens are submitted from the operating room in modified RPMI 1640 media. Following mechanical and enzymatic disaggregation, micro-spheroids of desired size (50–70 cells) are isolated by Ficoll-Hypaque density gradient. Cell suspensions are washed twice and adjusted to desired cell density with viability determined by initial Trypan blue. A volume of 90 µL of cell suspensions are then distributed into 96-well plates with cytotoxic agents distributed as 10 µL aliquots with continuous drug exposures for 72 h. Cell death events are examined using various endpoints including adenosine triphosphate (ATP) content by luciferase, mitochondrial metabolism by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), caspase

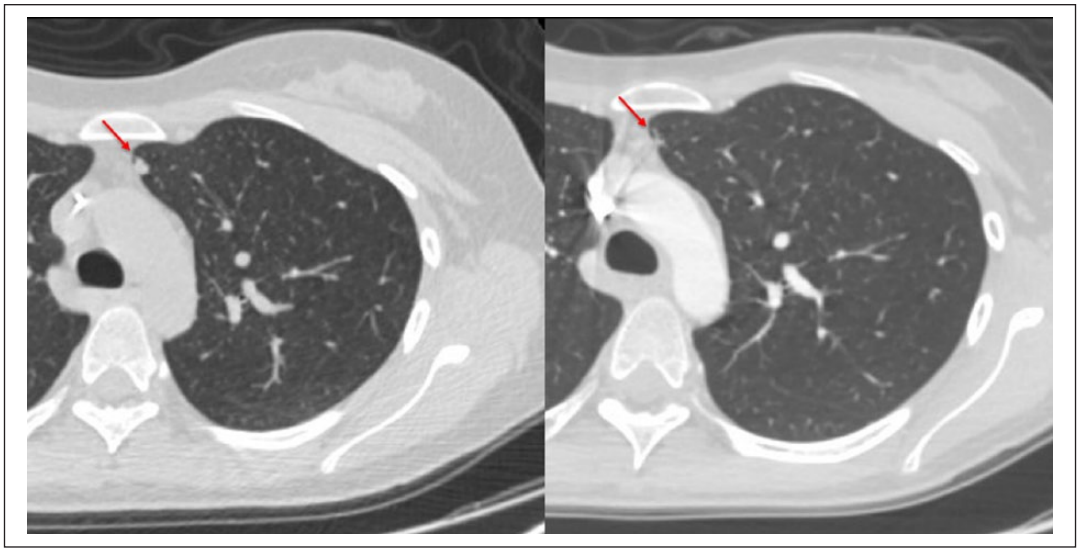

Figure 2. CT scan of the lungs showing reduction in the size of a left lung nodule.

activation, and delayed loss of membrane integrity measured by staining and morphology. As metformin actions are metabolic in nature, we utilize the membrane integrity endpoint to measure its activity to prevent possible artifacts being introduced by non-lethal perturbations in mitochondrial signal.

Phenformin had previously been selected in our platform as the index biguanide. Active concentration ranges (500–31 μM) have been determined in training sets and calibrated to provide clinically relevant signals in over 350 prior phenformin human tumor analyses. Five-point dose–response curves, in duplicate, were averaged and interpolated to provide the patient LC50 value of 39.7 μM. This fell in the most sensitive range for phenformin as determined by modified *Z*-score. The results also identified activity for the cyclin-dependent kinase 4/6 inhibitor, palbociclib, and the phosphoinositol α-specific kinase inhibitor BYL-719 with moderate activity for everolimus and sunitinib. Phenformin was included in this patient's tumor as a comparator molecule in ongoing research being conducted at the time on a structurally unrelated inhibitor of mitochondrial complex I (unreported observation). Based upon this favorable finding with phenformin, the structurally similar and clinically available biguanide, metformin, was recommended.

In June 2015, the patient was started on oral metformin at 500 mg twice daily and continued on mitotane. After 3 months, a repeat CT scan of the thorax showed marked reduction in the size of the dominant nodules, with complete disappearance of several smaller nodules (Figures 2 and 3). The measurable response continued for approximately 9 months.

With her family history positive for a retroperitoneal sarcoma (mother) and a lung cancer (grandmother), the patient was referred for genetic counseling and tested positive for LFS as a pathogenic mutation was found in TP53: c.1010G>A (p.Arg337His) (het). Exon codons and the exon–intron boundaries of the CHEK2 (22q12.1) and TP53 (17p13.1) genes were amplified and processed for next-generation sequencing (NGS). The sequencing results were analyzed in the Variant Caller software (Ion Torrent; Life Technologies) and compared with the version of the GRCh37/HG19 genome. The nomenclature of the detected mutations was made following the recommendations of HUman Genome Organization (HUGO) and Human Genome Variation Society (HGVS). The reference sequences of the detected genes were NM_000546.5 (TP53) and NM_007194.3 (CHEK2). In this analysis, a pathogenic mutation was detected according to the information deposited in the consulted databases (ClinVar, dbSNP, and/or IARC LOVD).

Written informed consent for patient information and images was obtained from the patient before publication. Hospital Alemão Oswaldo Cruz does not require ethical approval for reporting individual cases or case series.

Discussion

Surgery is the only curative treatment for ACC. Despite aggressive treatment, 70%–85% of the patients who undergo adrenalectomy develop local recurrence or distant metastases resulting in a 5-year overall survival of 16%–35% for those with complete resection which falls to less than 1 year for patients with incomplete resection.[17] The most common sites of distant metastases are lungs and liver.[17]

Mitotane is currently a cornerstone in the management of metastatic ACC. Most experts recommend its use either as monotherapy or combined with cytotoxic chemotherapy.[18,19] Although the optimal therapeutic strategy is not yet well

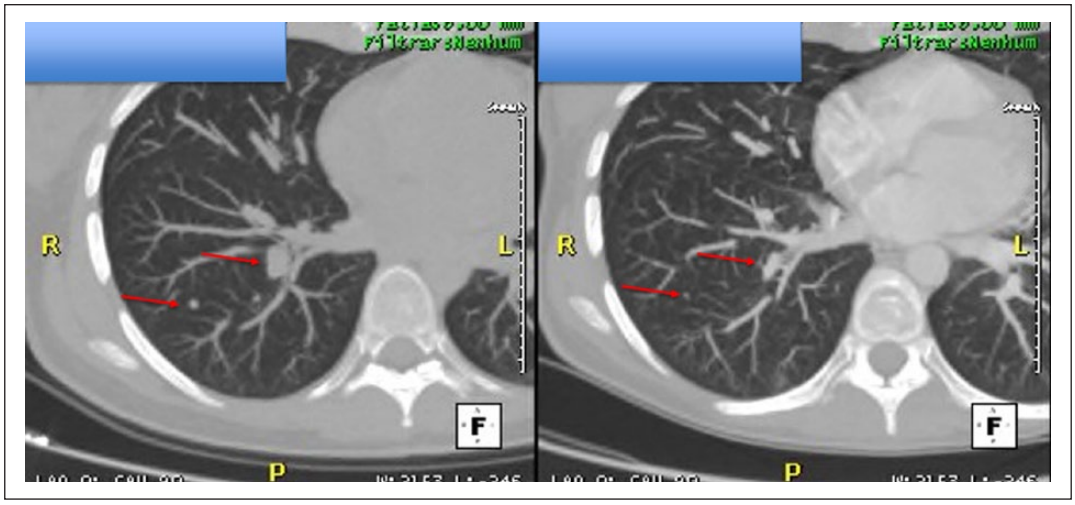

Figure 3. CT scan of the lungs showing reduction in the size of two right lung nodules.

established due to the rarity of this disease, the combination of etoposide, doxorubicin, and cisplatin (EDP) with or without mitotane is among the most widely used regimen. In a phase-II trial of 72 ACC patients,[20] this combination provided a response rate of 48%, while another small study reported response in 15 of 28 (53%) ACC patients.[21] In a subsequent phase-III trial (FIRM-ACT), 304 patients were randomized to receive mitotane either in combination with EDP or with streptozocin. Patients in the EDP–mitotane group achieved a significantly higher response rate than those in the streptozocin–mitotane group (23.2% vs 9.2%, $p<0.001$) and longer median progression-free survival (5.0 months vs 2.1 months, $p<0.001$), although there was no significant difference in overall survival between the groups (14.8 months vs 12.0 months, $p=0.07$).[22]

The limited efficacy of available systemic chemotherapy has led to a search for new treatment options based on the underlying molecular mechanisms involved in ACC. Several targets and agents have been explored.[23] Unfortunately, the epithelial growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib did not show efficacy as a single agent in ACC.[24] Similarly, sunitinib exhibited only modest activity as a single agent in mitotane-exposed ACC patients.[25] Other pathways such as those involving fibroblast growth factor receptor (FGFR) and Wnt-β-catenin signaling cascades and loss of p53 function have been implicated in ACC tumorigenesis.[26] The development of strategies targeting these pathways should be exploited in the future.

Insulin growth factor 2 (IGF-2) is known to be upregulated in ACC. IGF-2 signaling is mediated through its interaction with the IGF-1 receptor (IGF-1R), which leads to downstream activation of mechanistic target of rapamycin (mTOR).[26] Cixutumumab is a new, fully human IgG1 monoclonal antibody directed against the IGF-1R. An ongoing phase-I, dose-expansion study with 26 patients with heavily pretreated metastatic ACC is evaluating the combination of temsirolimus and cixutumumab.[27] Preliminary results indicate that 11 patients (42%) had durable (>6 months) stable disease. Although there were no partial or complete responses, this trial suggests that there might be a role for targeting the IGF-2 pathway in ACC.[27] However, an initial single-arm phase study for safety evaluation with cixutumumab and mitotane which should be followed by a randomized phase was terminated before randomization due to slow accrual and limited efficacy. Although therapeutic effects were observed in 8 of 20 patients, including one partial response and seven stable diseases, the relatively low response rate casts doubt on the efficacy of this regimen.[28]

The biguanides (metformin, phenformin, and buformin) are organic compounds, originally extracted from the French lilac (*Galega officinalis*), that were introduced into the treatment of diabetes mellitus in the 1950s. Metformin, the only commercially available biguanide, is a well-established and effective agent for the management of type 2 diabetes mellitus. Its hypoglycemic and insulin-lowering properties may play a role in its anticancer activity since insulin has mitogenic and pro-survival effects. In addition, tumor cells often express high levels of the insulin receptor, indicating a potential sensitivity to the growth-promoting effects of the hormone.[29,30] Although the mechanisms of action of metformin are not fully understood, it has been suggested that inhibition of mitochondrial complex I may downregulate mTOR via the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK).[31] The observed tumor regression in this case may also reflect inhibition of the IGF-2 pathway, as this is commonly upregulated in ACC.

Metformin has been shown to induce significant growth inhibitory effects in several cancer cell and mouse tumor models.[32] In cell culture, metformin inhibits the proliferation of a range of cancer cells including breast, endometrial, ovarian, prostate, colon, pancreatic, gastric, and glioma.[32] The effects of metformin on cancer cell proliferation are

associated with AMPK activation, reduced mammalian target of rapamycin (mTOR) signaling, and protein synthesis, as well as a variety of other responses including decreased EGFR, Src, and mitogen-activated protein kinase (MAPK) activation, decreased expression of cyclins, and increased expression of p27.[32]

P53-null (p53(−/−)) colon cancer cells are presumed to be susceptible to metformin-induced apoptosis owing to their inability to undergo the metabolic alterations imposed by metformin in the absence of p53 which is a crucial controller of several aspects of metabolism.[33] In a study with HCT116 $p53^{+/+}$ and $p53^{-/-}$ cell lines, metformin-treated cells compensated for the suppression of oxidative phosphorylation (OXPHOS) by increasing their rate of glycolysis in a p53-dependent manner. It suggests that metformin treatment forces a metabolic conversion that p53(−/−) cells are unable to execute, highlighting the therapeutic potential of metformin in the treatment of p53-deficient tumors.[33] In a mouse model of LFS that expresses mutant p53, metformin inhibited OXPHOS mitochondrial respiration, providing an intriguing evidence of how this drug may operate in inhibiting cancer progression in p53-inactivated tumors related to LFS.[34] Mechanistically, inhibition of mitochondrial function in this mouse model increased autophagy and decreased the aberrant proliferation signaling caused by mutant p53.[34]

Other investigators have reported that functional P53 is essential for the metformin to induce growth inhibition, senescence, and apoptosis in breast cancer cells.[35] They used nutlin-3a and CP/31398 to reactivate p53 and enhance the anti-tumor effect of metformin. Results in prostate cancer that combined metformin with 2-deoxyglucose also identified the p53 dependence of metformin-induced apoptosis.[36] This raises an alternative mechanism of action for the biguanides that may be operative in the patient reported.

In line with our findings of successful treatment with metformin, Italian investigators have previously reported the activity for metformin in two ACC cell lines, H295R and SW13.[37] Using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, they identified an LC50 in the H295R cell line between 23.8 and 58.6 mM, similar to the LC50 identified in this patient's primary culture of 39.6 mM. In H295R, metformin was shown to induce inhibition of extracellular-signal-regulated kinase (ERK) ½ and mTOR, associated with the stimulation of AMPK that led to apoptosis as measured by Annexin V and caspase-3, a reduction in Ki-67 and growth inhibition in a xenograft mouse model. Of interest, the H295R cell line carries p53 deletions in codons 8 and 9 that determine a frame shift at codon 261 resulting in a stop codon at position 271.[38] In the H295R cell model, it appears that metformin exerts its actions independent of p53 function.

Recently, morphoproteomic analysis with the use of bright-field microscopy and immunohistochemistry directed against various protein analytes was performed for a case of a young woman with chemoresistant ACC who had metastatic disease to the liver (resected) and left lung.[39] Morphoproteomic profiling suggested a combination of metformin, melatonin, and a statin as maintenance therapy following resection, which she used for 2 years, when a new metastatic lesion was found in the right lung out and biopsied. By comparison with the pretreatment specimen from the liver, the metastatic tumor from the right upper lobe of the lung with the patient on maintenance therapy with metformin and melatonin showed molecular and morphometric evidence of growth inhibition. Whether this patient had LFS was not reported.[39]

Our case differs from the aforementioned report in which we have observed apoptotic morphology in our patient's ACC cells in primary culture following 72 h of exposure to phenformin, the closely related biguanide. It was on the basis of observed degree of apoptotic morphology in the patient's tumor micro-spheroids that metformin was recommended for therapy. While the exact mechanisms by which metformin exerted its anti-tumor effects in this patient remain unclear, the choice of metformin represents an interesting clinical application of a phenotypic platform for the process of drug selection. As this approach is mechanism agnostic, it has the capacity to discern clinically relevant drug activities regardless of their putative modes of action. In this instance, the end result, tumor cytolysis, provided the needed insight for treatment selection resulting in the effective application of a novel and well-tolerated therapy for a patient with a rare malignancy for which few good treatment options exist. To our knowledge, this is the first report of response to metformin in a patient with metastatic ACC and LFS.

In conclusion, the course of our patient was remarkable for the means by which metformin was identified and for the impressive and durable degree of cytoreduction achieved with this simple, non-toxic intervention. Our results support a role for metformin in the treatment of metastatic ACC, consistent with the studies reported by Poli et al.[37] Clinical trials applying metformin in patients with ACC will enable investigators to better interrogate mechanism of action and the p53 dependence of metformin activity in patient samples.

As ACC occurs at demonstrably higher incidence in the southern Brazilian provinces of Parana and Sao Paulo, metformin may offer a unique and well-tolerated intervention for patients who carry the TP53 R337H mutation, and further studies in ACC are warranted. Discussions are underway to investigate the clinical study of metformin in other patients in southern Brazil who are afflicted with this rare form of LFS-associated ACC.

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Conflict of interest

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References

1. Kebebew E, Reiff E, Duh QY, et al. Extent of disease at presentation and outcome for adrenocortical carcinoma: have we made progress? *World J Surg* 2006; 30(5): 872–878.
2. Wajchenberg BL, Albergaria Pereira MA, Medonca BB, et al. Adrenocortical carcinoma: clinical and laboratory observations. *Cancer* 2000; 88(4): 711–736.
3. Dackiw AP, Lee JE, Gagel RF, et al. Adrenal cortical carcinoma. *World J Surg* 2001; 25(7): 914–926.
4. Loncar Z, Djukic V, Zivaljevic V, et al. Survival and prognostic factors for adrenocortical carcinoma: a single institution experience. *BMC Urol* 2015; 15: 43.
5. Ribeiro RC, Sandrini F, Figueiredo B, et al. An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *Proc Natl Acad Sci U S A* 2001; 98(16): 9330–9335.
6. DiGiammarino EL, Lee AS, Cadwell C, et al. A novel mechanism of tumorigenesis involving pH-dependent destabilization of a mutant p53 tetramer. *Nat Struct Biol* 2002; 9(1): 12–16.
7. Giacomazzi J, Selistre S, Duarte J, et al. TP53 p.R337H is a conditional cancer-predisposing mutation: further evidence from a homozygous patient. *BMC Cancer* 2013; 13: 187.
8. Icard P, Goudet P, Charpenay C, et al. Adrenocortical carcinomas: surgical trends and results of a 253-patient series from the French Association of Endocrine Surgeons study group. *World J Surg* 2001; 25(7): 891–897.
9. Tauchmanovà L, Colao A, Marzano LA, et al. Adrenocortical carcinomas: twelve-year prospective experience. *World J Surg* 2004; 28(9): 896–903.
10. Lee JE, Berger DH, el-Naggar AK, et al. Surgical management, DNA content, and patient survival in adrenal cortical carcinoma. *Surgery* 1995; 118(6): 1090–1098.
11. Fassnacht M, Johanssen S, Quinkler M, et al. Limited prognostic value of the 2004 International Union Against Cancer staging classification for adrenocortical carcinoma: proposal for a Revised TNM Classification. *Cancer* 2009; 115(2): 243–250.
12. Apfel C, Souza K, Hornuss C, et al. Accuracy and clinical utility of in vitro cytometric profiling to personalize chemotherapy: preliminary findings of a systematic review and meta-analysis. *J Clin Oncol* 2013; 31(Suppl. 15): e22188.
13. Nagourney RA. Ex vivo programmed cell death and prediction of response to chemotherapy. *Curr Treat Options Oncol* 2006; 7(2): 103–110.
14. Nagourney RA, Link JS, Blitzer JB, et al. Gemcitabine plus cisplatin repeating doublet therapy in previously treated, relapsed breast cancer patients. *J Clin Oncol* 2000; 18(11): 2245–2249.
15. Nagourney RA, Brewer CA, Radecki S, et al. Phase II trial of gemcitabine plus cisplatin repeating doublet therapy in previously treated, relapsed ovarian cancer patients. *Gynecol Oncol* 2003; 88(1): 35–39.
16. Nagourney RA, Blitzer JS, Shuman RL, et al. Functional profiling to select chemotherapy in untreated, advanced or metastatic non-small cell lung cancer. *Anticancer Res* 2012; 32(10): 4453–4460.
17. Veytsman I, Nieman L and Fojo T. Management of endocrine manifestations and the use of mitotane as a chemotherapeutic agent for adrenocortical carcinoma. *J Clin Oncol* 2009; 27(27): 4619–4629.
18. Terzolo M, Zaggia B, Allasino B, et al. Practical treatment using mitotane for adrenocortical carcinoma. *Curr Opin Endocrinol Diabetes Obes* 2014; 21(3): 159–165.
19. Fassnacht M, Kroiss M and Allolio B. Update in adrenocortical carcinoma. *J Clin Endocrinol Metab* 2013; 98(12): 4551–4564.
20. Berruti A, Terzolo M, Sperone P, et al. Etoposide, doxorubicin and cisplatin plus mitotane in the treatment of advanced adrenocortical carcinoma: a large prospective phase II trial. *Endocr Relat Cancer* 2005; 12(3): 657–666.
21. Berruti A, Terzolo M, Pia A, et al. Mitotane associated with etoposide, doxorubicin, and cisplatin in the treatment of advanced adrenocortical carcinoma. Italian Group for the Study of Adrenal Cancer. *Cancer* 1998; 83(10): 2194–2200.
22. Fassnacht M, Terzolo M, Allolio B, et al. Combination chemotherapy in advanced adrenocortical carcinoma. *N Engl J Med* 2012; 366: 2189–2197.
23. Almeida MQ, Fragoso MC, Lotfi CF, et al. Expression of insulin-like growth factor-II and its receptor in pediatric and adult adrenocortical tumors. *J Clin Endocrinol Metab* 2008; 93(9): 3524–3531.
24. Samnotra V, Vassilopoulou-Sellin R, Fojo AT, et al. A phase II trial of gefitinib monotherapy in patients with unresectable adrenocortical carcinoma (ACC). *J Clin Oncol* 2007; 25(Suppl. 18): 15527.
25. Kroiss M, Quinkler M, Johanssen S, et al. Sunitinib in refractory adrenocortical carcinoma: a phase II, single-arm, open-label trial. *J Clin Endocrinol Metab* 2012; 97(10): 3495–3503.
26. Demeure MJ, Bussey KJ and Kirschner LS. Targeted therapies for adrenocortical carcinoma: IGF and beyond. *Horm Cancer* 2011; 2(6): 385–392.
27. Naing A, LoRusso P, Fu S, et al. Insulin growth factor receptor (IGF-1R) antibody cixutumumab combined with the mTOR inhibitor temsirolimus in patients with metastatic adrenocortical carcinoma. *Br J Cancer* 2013; 108(4): 826–830.
28. Lerario AM, Worden FP, Ramm CA, et al. The combination of insulin-like growth factor receptor 1 (IGF1R) antibody cixutumumab and mitotane as a first-line therapy for patients with recurrent/metastatic adrenocortical carcinoma: a multi-institutional NCI-sponsored trial. *Horm Cancer* 2014; 5(4): 232–239.

29. Belfiore A and Frasca F. IGF and insulin receptor signaling in breast cancer. *J Mammary Gland Biol Neoplasia* 2008; 13(4): 381–406.
30. Mulligan AM, O'Malley FP, Ennis M, et al. Insulin receptor is an independent predictor of a favorable outcome in early stage breast cancer. *Breast Cancer Res Treat* 2007; 106(1): 39–47.
31. Pryor R and Cabreiro F. Repurposing metformin: an old drug with new tricks in its binding pockets. *Biochem J* 2015; 471(3): 307–322.
32. Dowling RJ, Goodwin PJ and Stambolic V. Understanding the benefit of metformin use in cancer treatment. *BMC Med* 2001; 9: 33.
33. Buzzai M, Jones RG, Amaravadi RK, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 2007; 67(14): 6745–6752.
34. Wang PY, Li J, Walcott FL, et al. Inhibiting mitochondrial respiration prevents cancer in a mouse model of Li-Fraumeni syndrome. *J Clin Invest* 2017; 127(1): 132–136.
35. Li P, Zhao M, Parris AB, et al. p53 is required for metformin-induced growth inhibition, senescence and apoptosis in breast cancer cells. *Biochem Biophys Res Commun* 2015; 464(4): 1267–1274.
36. Ben Sahra I, Laurent K, Giuliano S, et al. Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res* 2010; 70(6): 2465–2475.
37. Poli G, Cantini G, Armignacco R, et al. Metformin as a new anti-cancer drug in adrenocortical carcinoma. *Oncotarget* 2016; 7(31): 49636–49648.
38. Sampaoli C, Cerquetti L, El Gawhary R, et al. p53 stabilization induces cell growth inhibition and affects IGF2 pathway in response to radiotherapy in adrenocortical cancer cell. *PLoS ONE* 2012; 7: e45129.
39. Brown RE, Buryanek J and McGuire MF. Metformin and melatonin in adrenocortical carcinoma: morphoproteomics and biomedical analytics provide proof of concept in a case study. *Ann Clin Lab Sci* 2017; 47(4): 457–465.