Molecular and Cellular Endocrinology 528 (2021) 111243

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Inhibition of Aurora kinase A activity enhances the antitumor response of beta-catenin blockade in human adrenocortical cancer cells

Andrea Gutierrez Maria [a,b,*], Kleiton Silva Borges [b,1], R.C.P. Lira [b,2], Carolina Hassib Thomé [c], Annabel Berthon [a], Ludivine Drougat [a], Katja Kiseljak-Vassiliades [d,e], Margaret E. Wierman [d,e], Fabio R. Faucz [a], Vitor Marcel Faça [c], Luiz Gonzaga Tone [b], Constantine A. Stratakis [a,f]

[a] Section on Endocrinology & Genetics (SEGEN), Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD, 20892, USA
[b] Department of Pediatrics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, 14049-900, Brazil
[c] Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, 14049-900, Brazil
[d] Division of Endocrinology, Metabolism and Diabetes, University of Colorado School of Medicine, Aurora, CO, 80045, USA
[e] Research Service Veterans Affairs Medical Center, Denver, CO, 80045, USA
[f] Pediatric Endocrinology Inter-institute Training Program, Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD20892, USA

ARTICLE INFO

Keywords:
Adrenocortical cancer
Aurora kinases
Beta-catenin

ABSTRACT

Adrenocortical cancer (ACC) is a rare and aggressive type of endocrine tumor with high risk of recurrence and metastasis. The overall survival of patients diagnosed with ACC is low and treatment for metastatic stages remain limited to mitotane, which has low efficiency in advanced stages of the disease and is associated with high toxicity. Therefore, identification of new biological targets to improve ACC treatment is crucial. Blockade of the Wnt/beta-catenin pathway decreased adrenal steroidogenesis and increased apoptosis of NCI–H295 human ACC cells, *in vitro* and in a xenograft mouse model. Aurora kinases play important roles in cell division during the G1-M phase and their aberrant expression is correlated with a poor prognosis in different types of tumors. Hence, we hypothesized that inhibition of aurora kinases activity combined with the beta-catenin pathway blockade would improve the impairment of ACC cell growth *in vitro*. We studied the combinatorial effects of AMG 900, an aurora kinase inhibitor and PNU-74654, a beta-catenin pathway blocker, on proliferation, survival and tumor progression in multiple ACC cell lines: NCI–H295, CU-ACC1 and CU-ACC2. Exposure of ACC cells to the combination of AMG 900 with PNU-74654 decreased cell proliferation and viability compared to either treatment alone. In addition, AMG 900 inhibited cell invasion and clonogenesis compared to PNU-74654, and the combination showed no greater effects. In contrast, PNU-74654 was more effective in decreasing cortisol secretion. These data suggest that inhibition of aurora kinases activity combined with blockade of the beta-catenin pathway may provide a combinatorial approach for targeting ACC tumors.

1. Introduction

Adrenocortical cancer (ACC) is a rare and aggressive type of endocrine tumor derived from the adrenal cortex affecting 0.5 to 2 people per million per year worldwide. The prognosis of the disease is generally poor, with a survival rate of approximately 35% in 5 years after diagnosis (Mohan et al., 2018). The survival rates decrease to only to 13-6% in stage IV tumors (Paragliola et al., 2018; Mohan et al., 2018). Mitotane is currently the only therapy for advanced ACC approved by the U.S. Food and drug Administration and the European Medicines Agency. It has low therapeutic effectiveness in advanced stages of the disease and is associated with toxicity and side effects (Pittaway and Guasti, 2019). Despite the advances in treatment options for other cancers, the survival of patients with ACC has not improved over the past 40 years (Chandrasekar et al., 2019). Thus, identification of new biological targets for ACC treatment is crucial as the overall survival of patients diagnosed

* Corresponding author. 10 Center Drive, Building 10, NIH-Clinical Research Center, Room 1E-3216, Bethesda, MD, 20892, USA.
E-mail address: andreagutierrez.maria@nih.gov (A.G. Maria).
[1] Current address: Division of Endocrinology, Boston Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston, MA 02115, USA.
[2] Current address: Department of General Pathology, Federal University of Triângulo Mineiro, Uberaba, MG, 38025-180, Brazil.

https://doi.org/10.1016/j.mce.2021.111243
Received 18 June 2020; Received in revised form 6 March 2021; Accepted 8 March 2021
Available online 11 March 2021
0303-7207/Published by Elsevier B.V.

with ACC is still very low.

Advances such as whole genome and exome sequencing to identify genetic drivers of ACC have greatly contributed to understanding tumor-specific outcomes and prognosis. Several transcriptome studies identified alterations in genes related to Wnt pathway, cell cycle regulation, chromatin remodeling and chromosome maintenance in ACC, with *ZNRF3*, a negative feedback regulator of Wnt/beta-catenin pathway, as the most frequently altered gene (Pereira et al., 2018, Zheng et al., 2016 and Assié et al., 2014). Beta-catenin mutations have been found in approximately 30% of ACCs (Salomon et al., 2015). *In vitro* studies showed that the blockade of Wnt/beta-catenin signaling in NCI–H295 cells increased apoptosis and decreased adrenal steroidogenesis (Gaujoux et al., 2013; Leal et al., 2015; Doghman et al., 2008). In mouse models, activation of the Wnt/beta-catenin pathway in the adrenal cortex alone cause hyperplasia (Berthon et al., 2010; Pignatti et al., 2020) and, when associated with loss of p53, leads to development of malignant adrenocortical tumors (Borges et al., 2020).

Genes involved in DNA damage and overexpression of kinases such as aurora kinases and cyclin-dependent kinase 1 (CDK1), among others, have also been identified as altered in ACC (Subramanian and Cohen, 2019). Aurora kinases are known to play an important role in cell division during G2-M phase. They are divided in three classes, aurora kinase A, B and C contributing differently to the progress of M phase despite their structural similarity (Carmena and Earnshaw, 2003; Glover, 2005, Willems et al., 2018). Aberrant expression of aurora kinases is correlated with poor prognosis in different types of tumors such as adrenocortical, breast, colorectal and prostate (Borges et al., 2013; Willems et al., 2018; Bertolin and Tramier, 2020). Alisertib, a selective aurora kinase A inhibitor showed promising efficacy in Phase I/II/III clinical trials; however, serious side effects were observed (Tayyar et al., 2017). NCI–H295 ACC cells treated with AMG 900, a pan-aurora kinase inhibitor, had increased apoptosis and chemosensitivity to anticancer drugs as mitotane, doxorubicin and etoposide (Borges et al., 2017). Supporting a tumorigenic role for this kinase, as a dysregulated target in ACC, is overexpression of aurora kinases A and B that are associated with poor prognosis in both pediatric and adult ACC (Borges et al., 2013; de Reyniès et al., 2009).

Considering that beta-catenin (*CTNNB1*) mutations are one of the common drivers in ACCs (Salomon et al., 2015) and aurora kinases play an important role in tumor progression (Damodaran et al., 2017), we sought to investigate whether inhibiting both pathways would be additive or syngergistc to impair ACC cell growth *in vitro*. Since aurora kinase inhibitors are being studied in clinical trials of multiple tumor types (Malumbres and Pérez de Castro, 2014), and there is no effective treatment for advanced ACCs, this study may potentially contribute to the identification of new combination therapeutic approachess for ACC patients.

2. Methods

2.1. Cell culture

NCI–H295 cells were grown in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12, A4192002, Gibco), supplemented with 2% fetal bovine serum (100–106, Gemini Bio Products), 1% antibiotic (Penicillin-Streptomycin – 15 140–148, Gibco) and 5 μg/ml insulin, 5 μg/ml transferrin and 5 ng/ml sodium selenite (1% ITS – 41400045, Thermo Fisher, Waltham, MA, USA). CU-ACC1 and CU-ACC2 cell lines were grown as previously described (Kiseljak-Vassiliades et al., 2018). Cells were incubated in a humidified atmosphere at 37 °C with 5% CO_2.

2.2. Inhibitor compounds

AMG 900 (catalog number S2719), Alisertib (catalog number S1133) and PNU-74654 (catalog number S8429) were purchased from Selleckchem (Houston, TX). Dilutions were performed following the manufacturer's protocol.

2.3. Cell cycle syncronization

NCI–H295 cells were seeded at a 70% confluence and incubated for 48 h. Cell culture medium was replaced by medium containing 2 mM of thymidine blocker for 24 h. After incubation, medium was replaced by fresh medium for 3 h followed by another medium replacement with 10 μM of colchicine for 12 h. Medium was removed and cells were treated with DMSO or AMG 900 50 nM.

2.4. Gene expression analysis

NCI–H295 cells were seeded into 6-well plates at a density of 4×10^5 cells per well. After 48 h of incubation, cells underwent to cell cycle synchronization and AMG 900 treatment for 6 and 24 h as described above. Total RNA was isolated using Trizol reagent (Invitrogen, USA) and cDNA was generated from 1 μg of RNA using High Capacity Kit (Applied Biosystems, USA). The human genes *CTNNB1* (Hs00170025_m1) and *MYC* (Hs99999003_m1) were amplified by qRT-PCR using TaqMan gene assays and the Quant Studio 12 K flex system (Thermo, USA). All samples were analyzed in triplicate and normalized to the endogenous reference human gene *GUSB* (cat. nº 4326320E; Applied Biosystems, USA). Untreated cells (control) were used as reference samples and the relative expression was determined by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

2.5. Protein expression analysis

NCI–H295 cells were seeded into 6-well plates at a density of 4×10^5 cells per well. After 48 h of incubation, cells underwent to cell cycle synchronization and AMG 900 treatment for 24 h as described above. After DMSO or AMG 900 treatment, cells were washed with PBS and resuspended in 50 μl of ice-cold lysis buffer (Tris-HCl 10 mM, pH 7,5, NaCl 150 mM, EDTA 1 mM, EGTA 1 mM, SDS 0.1%, Nonidet P-40 1%) containing a cocktail of protease and phosphatase inhibitors (PPC1010, Sigma-Aldrich, USA). The collected cells were incubated for 30 min on ice and centrifuged for 15 min at 4 °C, 13 000 rpm. Total protein concentration of supernatant was determined by a PierceTM BCA Protein Assay (23 227, Thermo Scientific), following the manufacturer's protocol. After quantification of protein extracts, 50 μg of total protein were separated by electrophoresis in 10% polyacrylamide gel under denaturing conditions (SDS-PAGE). Proteins were transferred to a nitrocellulose membrane (1620115, BioRad, USA) and Western Blot was performed using antibodies against beta-catenin (8480, Cell Signaling, USA), c-Myc (5605, Cell Signaling, USA) diluted 1:1000, MPM-2 (14 581, Abcam, USA) diluted 1:1000, phospho-histone H3 (9701, Cell Signaling, USA) diluted 1:1000 and GAPDH (SC-32233, Santa Cruz Biotechnology, USA) diluted 1:2000. Fluorescent secondary antibodies (827–08364 IRDye 800CW Goat anti-Mouse and 926–68073 IRDye 680RD Donkey anti Rabbit, LiCor, USA) diluted 1:20 000 and Odyssey CLx Imaging System (Licor, USA) were used to acquire the signal of the bands. Densitometric quantification was performed using ImageJ software and a ratio between the intensity of beta-catenin or c-myc and GAPDH bands was calculated. Image of the blots were treated using Image Studio Lite software (Licor, USA) for gray scale color.

2.6. Cell viability assay

NCI–H295 and CU-ACC2 cells were seeded into 96-well plates at a density of 1×10^4 cells per well, CU-ACC1 cells were seeded at a density of $1{,}2 \times 10^4$ cells per well. Cells were treated with DMSO (control), AMG 900 50 nM, Alisertib 50 nM or PNU-74654 50 μM or the combination of AMG 900 and PNU-74654 or Alisertib and PNU-74654 for 2, 24, 48 and 72 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt)

was added to a final concentration of 0.5 mg/mL into the culture media and incubated for 3 h at 37 °C. Media was removed and 200 μL of isopropanol containing acetic acid 0.04M was added into the wells to solubilize the product reduced by MTT and absorbance was measured at 570 nm.

2.7. Proliferation assay

5-bromo-2′-deoxiuridin (BrdU) is a synthetic nucleoside analogous to thymidine that incorporates newly synthesized DNA strands from cells in replication processes, substituting thymidine. The highest cell proliferation index, higher incorporation of BrdU. We performed the assay using BrdU Cell Proliferation Assay Kit (2750, Millipore-Sigma, USA). 1.0×10^4 NCI–H295 cells were seeded in 96-well plates supplemented with 2% FBS and penicillin/streptomycin. Cells were incubated in atmosphere containing 5% CO_2 at 37 °C for 24 h, and thereafter treated with DMSO or 50 nM of AMG 900 or 50 μM of PNU-74654 or with a combination of AMG 900 and PNU-74654 for 24 h. BrdU was added to the cells 16 h before the final reading and BrdU incorporation was measured by fluorometric analysis at a wavelength of 450 nM.

2.8. Clonogenic survival assay

5×10^3 NCI–H295, 8×10^3 CU-ACC1 and 1×10^4 CU-ACC2 cells/well were seeded in six well plates for 24 h and treated with DMSO (control), AMG 900 50 nM, Alisertib 50 nM or PNU-74654 50 μM or the combination of AMG 900 and PNU-74654 or Alisertib and PNU-74654 for 48 h. After treatment, the culture medium was removed, and drug-free medium was added. Cells were incubated for 12 days. Colonies were fixed with a solution containing 0.05% w/v of crystal violet, 1% of formaldehyde and 1% methanol in PBS. Fixed colonies were photographed at a magnification of 4x and 1 mL of acetic acid 10% was added to each well. Plates were shaken for 15 min to discolor the plaque. 200 μL of the violet stain solution removed from the plate was transferred to a 96-well plate and read on a spectrophotometer at an absorbance of 590 nm. The intensity of staining is proportional to the number of colonies established after treatment.

2.9. Boyden chamber invasion assay

To evaluate the invasion potential of NCI–H295 cells after treatment with aurora kinase and beta-catenin inhibitors, a system of transverse plates or Boyden chamber in a 24-well was used (354 480, Corning-Costar, USA). The upper chamber contains a 8 μm diameter polycarbonate membrane covered by Matrigel. Cells were seeded at a concentration of 2×10^4/ml in serum-free culture medium containing DMSO or 50 nM of AMG 900 or 50 μM of PNU-74654 or with a combination of AMG 900 and PNU-74654 and 500 μL of the cell suspension with respective treatments were added to the wells (transwell). Cells were incubated for 96 h. Cells that did not cross the matrigel were removed using a cotton swab. Cells that crossed the matrigel barrier were fixed in 4% paraformaldehyde solution for 10 min and stained with 0.5% crystal violet solution for 20 min. Cells were counted in a microscope on a 4x objective.

2.10. Cortisol secretion

NCI–H295, 5×10^5 cells/well, were seeded in six well plates for 24 h. Cells were treated with DMSO (control) AMG 900 50 nM, Alisertib 50 nM or PNU-74654 50 μM or the combination of AMG 900 and PNU-74654 or Alisertib and PNU-74654 for 48 h. After treatment, the culture medium was collected, immediately frozen at −80 °C and total protein was extracted from cells. Cortisol levels were measured using Cortisol EIA Kit (K003H1W, Arbor Assays, USA) following the instructions of the manufacturer. Levels of cortisol secreted were normalized by the total protein extracted from the cells in the corresponding wells.

2.11. Drug synergism

A cell viability assay was performed to investigate drug synergism using the MTT method described above. NCI–H295 cells were treated with different doses ranging from 0 to 200 nM of AMG 900 or alisertib for 48 h. After incubation, medium was removed and cells were treated with PNU-74654 in concentrations ranging from 0 to 100 μM. Drug synergism of AMG 900 and PNU-74654 or Alisertib and PNU-74654 was quantified following the Chou-Talalay method using Combusyn software, in which the resulting combination index (CI) defines additive effect (CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combinations (Chou 2010).

2.12. RNAseq analysis

FASTQ RNAseq data files from The Cancer Genome Atlas (TCGAphs000178, http://cancergenome.nih.gov/) (n = 79 ACC) and Genotype-Tissue Expression project (GTEx-phs000424, https://www.gtexportal.org/home/) (n = 57 normal adrenals) were downloaded from dbGaP. Both RNAseq studies were performed on Illumina HiSeq platform. We analyzed raw FASTQ RNAseq reads concurrently and mapped to the human genome sequence (hg19) using GSNAP. The transcript expression was calculated by Cufflinks (Trapnell et al., 2010) and analyzed for differential gene expression by ANOVA in R (Kiseljak-Vassiliades et al., 2018; Roberts et al., 2011a; Roberts et al., 2011b; Trapnell et al., 2010). ACC tissues and ACC cell lines were compared with normal adrenal tissue.

2.13. Statistical analysis

Statistical analysis was performed using one-way ANOVA and Tukey test for multiple comparisons. Differences between mean values were considered significant when $p < 0.05$.

3. Results

3.1. Aurora kinase A and aurora kinase B mRNA expression is increased in ACC compared to normal adrenal

RNAseq was performed to investigate the expression levels of *Aurka, Aurkb* and *Aurkc* in normal adrenal versus ACC tissue and in normal adrenal versus ACC cell lines, NCI–H295, CU-ACC1 and CU-ACC2. In human tissue using TCGA and GTEX RNAseq data, expression of *Aurka* was 5.2 fold ($p < 0.001$) and *Aurkb* was 4-fold ($p < 0.001$) higher in ACC compared to normal adrenal, while mRNA expression of *Aurkc* was similar. Similarly, expression of *Aurka*vand *Aurkb* are increased in the three cell lines studied while no difference was observed in the mRNA expression of *Aurkc* (Fig. 1).

3.2. Treatment of NCI–H295 cells with AMG 900 increases CTNNB1 and MYC gene expression

Beta-catenin (*CTNNB1*) is known to be a genetic driver of ACC (Crona and Beuschlein 2019). Knowing that NCI–H295 ACC cells have the *CTNNB1* p. S45P mutation, we wanted to know if inhibition of aurora kinases would contribute to modulate this pathway. NCI–H295 cells were synchronized in mitosis and treated with the pan aurora kinase inhibitor AMG 900. We observed a 2-fold increase in *CTNNB1* expression after 6 h of AMG 900 treatment and a significant increase of the transcription factor *MYC* after 24 h of treatment (Fig. 2A), suggesting that AMG 900 might play a role in activating the Wnt-beta catenin pathway. Protein levels of c-Myc were also increased after 24 h of AMG 900 treatment; however, beta-catenin protein levels did not increase significantly (Fig. 2B). Validation of the cell synchronization protocol

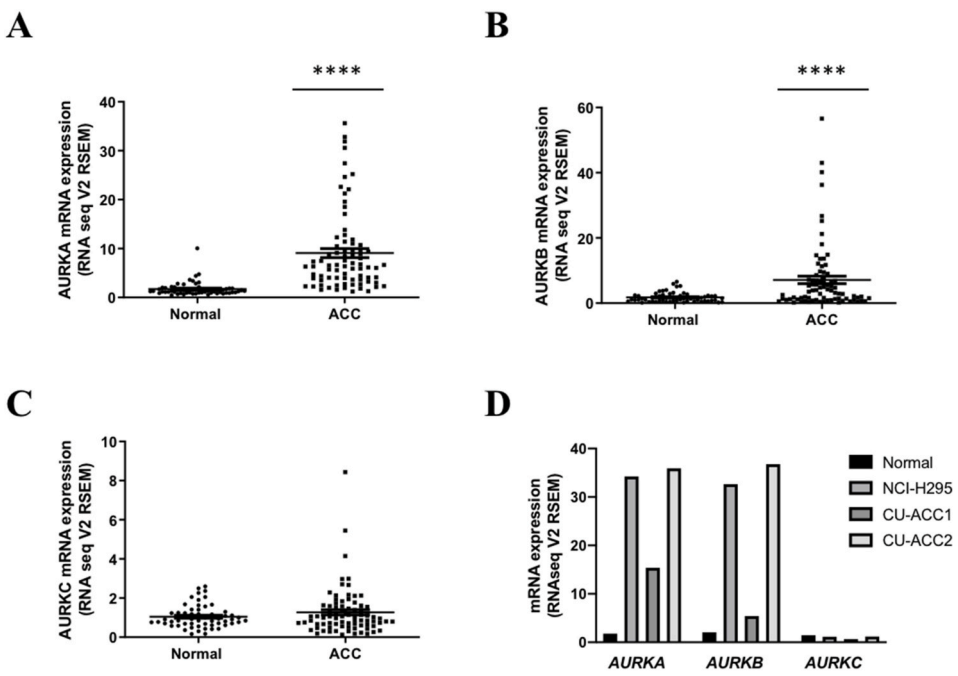


Fig. 1. mRNA expression of Aurora kinases in adrenocortical cancers and human adrenocortical cancer cell lines compared to normal adrenal. **A-C)** RNA sequencing analysis comparing expression of aurora kinase A, aurora kinase B and aurora kinase C in normal adrenal tissues against human adrenocortical cancer tissues. ****$p < 0.0001$ (normal tissue:$n = 57$, adrenocortical cancer tissue $n = 79$). **D)** RNA sequencing analysis comparing expression of aurora kinase A, aurora kinase B and aurora kinase C in normal adrenal tissue against human adrenocortical cancer ($n = 1$).

was performed using the mitotic markers MPM-2 and phospho-histone H3 (Supplementary Figure 1).

3.3. Inhibition of aurora kinases activity combined with Wnt-beta catenin pathway blockade decreased NCI–H295 cell proliferation

Proliferation is known to be a hallmark of cancer (Hanahan and Weinberg, 2000) and blockade of the Wnt-beta catenin pathway decreases cell proliferation and increases apoptosis in ACC cells (Gaujoux et al., 2013; Ferro-Leal et al., 2015). Thus, we investigated if the inhibition of aurora kinase activity, combined with the blockade of beta-catenin pathway, would alter rates of proliferation of NCI–H295 cells. We assessed cell viability by MTT and cell proliferation using BrDU assay. Either AMG 900 or PNU-74654 decreased cell viability and proliferation, individually. However, the combination of both inhibitors had a higher impact in cell viability and inhibition of proliferation (Fig. 3A–B), suggesting that the increased *CTNNB1* and c-Myc expression seen after AMG 900 treatment can be blocked by the PNU-74654, improving the anti-tumor effects of both drugs.

3.4. Inhibition of aurora kinases blocks cell invasion and colony formation of NCI–H295 cells

Invasion, the process in which malignant cells detach from the primary tumor and acquire the ability to invade other tissues, is the first step of tumor metastasis (Gerashchenko et al., 2019). Another important feature acquired by the cell to metastasize is the ability to resist cell death and establish colonies from a single cell in a distant site (Hanahan and Weinberg, 2000). Here we accessed these two features after treatment with AMG 900, PNU-74654 and combination. Inhibition of aurora kinases decreased the capacity of NCI–H295 cells to invade and establish colonies in 75 and 2.5 folds respectively when compared to non treated cells. PNU-74654 decreased the capacity of invasion and establish colonies in 48 folds 1.7 folds respectively compared to control. Combination of AMG 900 and PNU-74654 did not increase AMG 900's effectiveness (Fig. 3C–D).

3.5. AMG 900's effect on viability of ACC cells may be due to inhibition of aurora kinase A

The spectrum of actions of aurora kinases A, B and C are yet not well understood; nevertheless, the use of Alisertib, a specific aurora kinase A inhibitor has shown to be efficient in different types of tumors, while there are fewer studies focusing specifically in aurora B or aurora C inhibition (Borisa and Bhatt, 2017). Thus, we compared the effects of the pan aurora kinase activity inhibition with the specific inhibition of aurora kinase A activity, alone or in combination with the Wnt/beta-catenin pathway blocker, PNU-74654 in NCI–H295 cells. The combination of Alisertib and PNU-74654 was 20% more efficient than the combination of AMG 900 and PNU-74654 ($p = 0.0019$) in NCI–H295 cells (Fig. 4A). This data suggests that, when in combination with PNU-74654, AMG 900's effects were most likely due to inhibition of aurora kinase A activity.

In addition, we also investigated the drug effects in two other human ACC cell lines, CU-ACC1 and CU-ACC2, recently stablished by Kiseljak-Vassiliades et al. (2018). In CU-ACC1 cells, combination of AMG 900 or Alisertib with PNU-74654 was more effective in decreasing cell viability compared to each of the drugs alone (AMG 900 vs combination with PNU-74654: $p = 0.03$; Alisertib vs combination with PNU-74654: $p < 0.0001$; PNU-74654 vs combination with AMG 900: $p = 0.02$; PNU-74654 vs combination with alisertib: $p = 0.015$) (Fig. 4C). In CU-ACC2 cell lines, all drugs alone showed decreased cell viability compared to control, combination of AMG 900 with PNU-75654 did not enhance the effect of the drugs alone while combination of PNU-74654 with alisertib showed to be more effective compared to single treatment ($p = 0.0086$) (Fig. 4D). No significant differences in the combination between AMG 900 with PNU-74654 and Alisertib with PNU-74654 were found in CU-ACC1 and CU-ACC2 cell lines.

3.6. Blockade of Wnt-beta catenin pathway has higher effect in decreasing cortisol secretion in NCI–H295 compared to aurora kinases inhibition

We investigated whether AMG 900, Alisertib or PNU-74654 would have an effect in the cortisol secretion levels of NCI–H295 cells and our results show that inhibition of aurora kinases significantly decreased

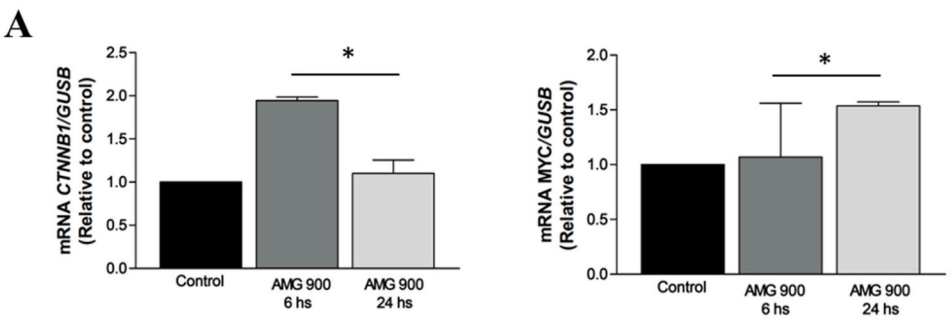
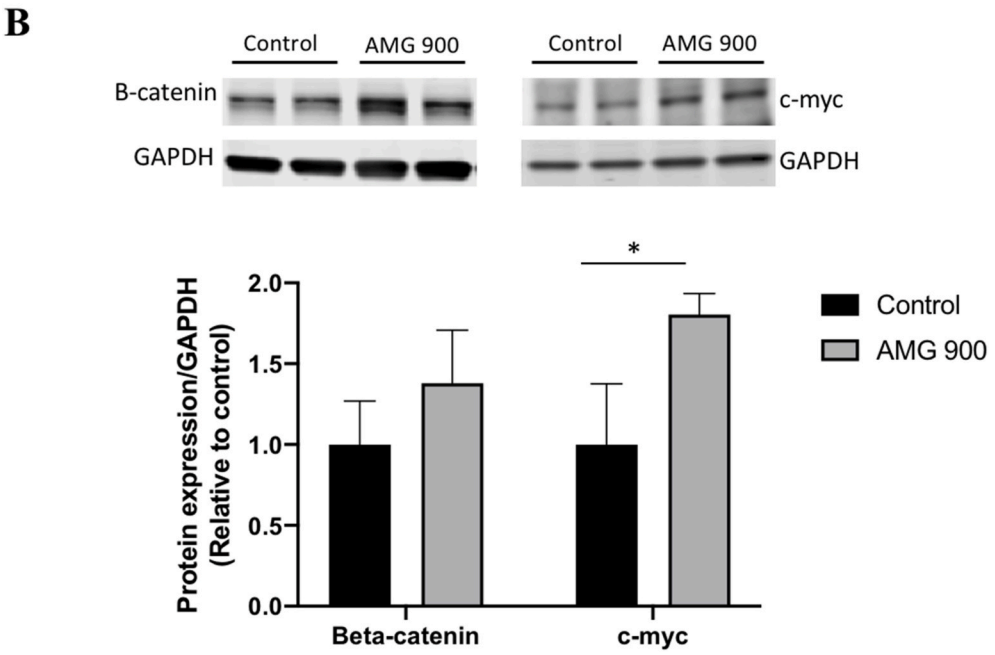


Fig. 2. AMG 900 treatment increases beta-catenin and *MYC* expression. mRNA expression of **A)** beta-catenin and **B)** *MYC* in NCI–H295 cells treated with 50 nM of AMG 900 for 6 and 24 h. Experiments were performed by quantitative real-time PCR. Data was obtained as fold of change from relative expression of target gene/*GUSB* ± SEM; n = 6. *CTNNB1*: p = 0.02; *MYC*: p = 0.031. **C)** Protein expression of beta-catenin and c-Myc in NCI–H295 cells after treatment with 50 nM of AMG 900 for 24 h. Data are expressed as fold of change from relative expression of the target protein/GAPDH ± SEM; n = 3. *p = 0.019.



levels of cortisol in 1.2 folds, while, PNU-74654 showed a greater effect decreasing cortisol secretion in 4.6 folds. PNU-74654 in combination with AMG 900 or alisertib showed a decrease of 3.5 folds after 48 h of treatment (Fig. 4B).

3.7. AMG 900 shows greater effects in the inhibition of clonogenic capacity than alisertib or PNU-74654 in NCI–H295, CU-ACC1 and CU-ACC2 cell lines

We further wanted to know wheter the inhibition of aurora kinases A, B or C, or specific aurora kinase A inhibition or blockade of beta-catenin would impact the three different ACC cells lines: NCI–H295, CU-ACC1 and CU-ACC2. We treated the cells with 50 nM of AMG 900 or alisertib or 50 μM of PNU-74654 or combination. All drugs or combination showed to decrease the clonogenic capacity of the ACC cells, however, AMG 900, the pan aurora kinase inhibitor, showed to be the most effective drug to act decreasing clonogenic capacity (Fig. 5A–E). In NCI–H295 AMG 900 decreased the clonogenic capacity in 5 folds and combination with PNU-74654 did not potentialize the AMG 900 effects (Fig. 5A). In CU-ACC1 cells, combination of aurora kinase inhibitors with Wnt-beta catenin pathway blockade was more effective in deceasing clonogenic capacity (AMG 900 vs combination with PNU-74654: p = 0.03; Alisertib vs combination with PNU-74654: p < 0.0001; PNU-74654 vs combination with AMG 900: p < 0.0001; PNU-74654 vs combination with alisertib: p < 0.0001) (Fig. 5B). In CU-ACC2 cells, AMG 900 alone presented better response against clonogenic capacity and combination with PNU-74654 did not improve this impairment (Fig. 5C). These results suggest that despite the different ACC humam cell lines have variantion in the response against AMG 900, alisertib and PNU-74654, overall, AMG 900 is the most effective drug against the hability of the cells to establish clones.

3.8. AMG 900 or alisertib in combination with PNU-74654 show synergistic effect in NCI–H295 cells viability in different concentrations

To investigate if combination of AMG 900 or Alisertib with PNU-74654 have a synergistic effect in NCI–H295 cells, we studied, using the Chou-Talalay method (Chou 2010), the effect of drug combination in cell viability with concentrations ranging from 0 to 200 nM of AMG 900 or Alisertib combined with different concentrations of PNU-74654 ranging from 0 to 100 μM. Our results showed synergistic effects when AMG 900 50 nM was combined with PNU-74654 in a concentration of 50 μM, cells decreased viability in 36% compared to control (Fa = 0.522; CI = 0.898) and when Alisertib 100 nM was combined with PNU-74654 100 μM cells decreased viability in 17% (Fa = 0725; CI = 0.691). No synergistic effects were observed when NCI–H295 cells were treated with lower concentrations.

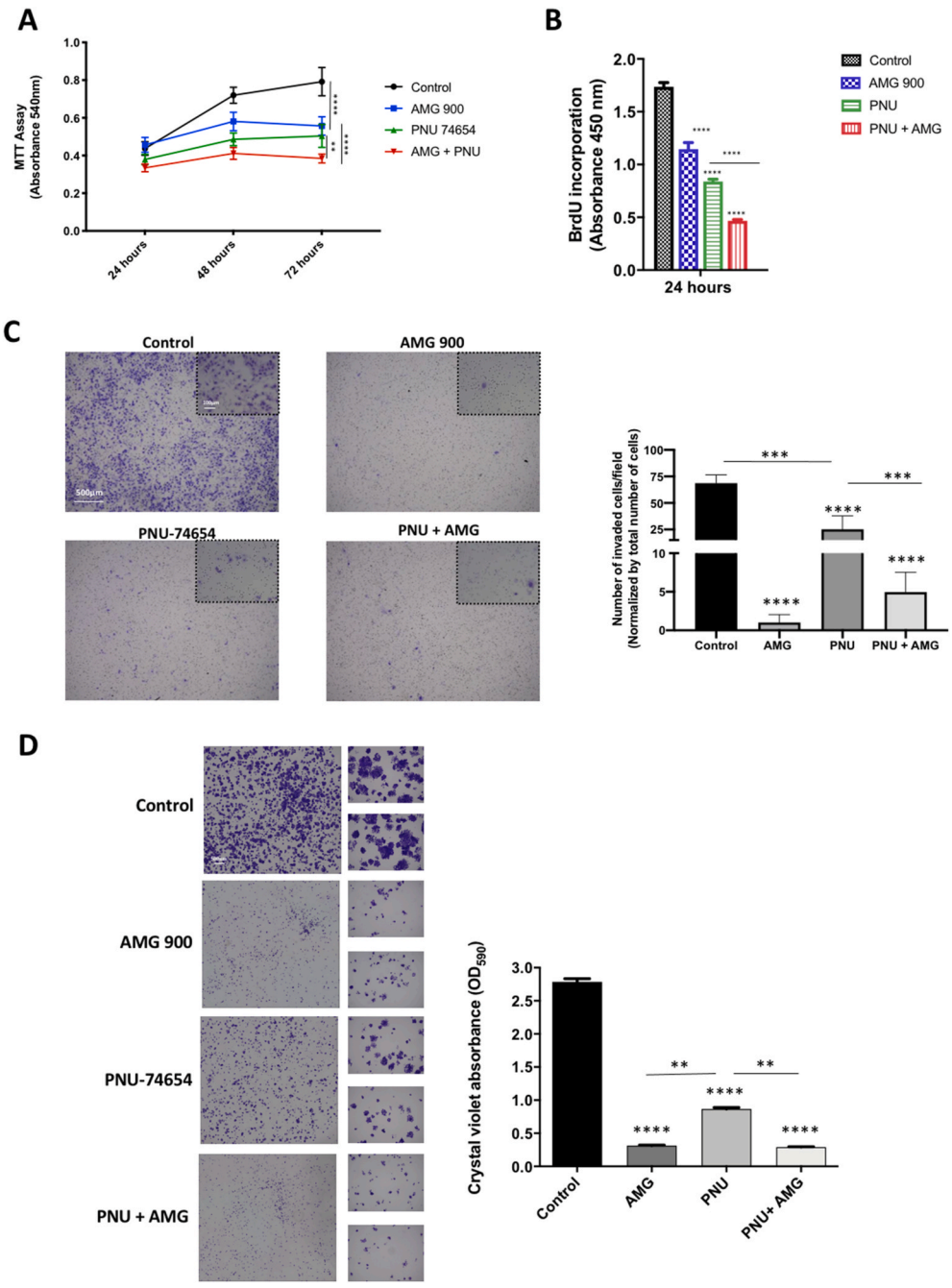


Fig. 3. Effects of AMG 900 and PNU-74654 in cell proliferation, invasion and clonogenic capacity. A) Cell viability accessed by MTT assay in NCI–H295 cells treated with AMG 900 50 nM or PNU-74654 50 μM or combination of AMG 900 and PNU-74654 for 24, 48 and 72 h. ****p < 0.0001; **p = 0.0024. **B)** Cell proliferation accessed BrDU incorporation in NCI–H295 cells treated with AMG 900 50 nM or PNU-74654 50 μM or combination of AMG 900 and PNU-74654 for 24 h ****p < 0.0001. C) Left panel: representative images of invasion assay performed using matrigel invasion chamber. NCI–H295 cells were treated with AMG 900 50 nM or PNU-74654 50 μM or combination of AMG 900 and PNU-74654. Invaded cells were stained with Crystal Violet. Right panel: plot of invaded cells counted per field. ****p < 0.0001; **p < 0.01. Experiment was done in 2 independent experiments, n = 4. D) Left panel: representative images of clonogenic assay to access the capacity of NCI–H295 cells treated with AMG 900 50 nM or PNU-74654 50 μM or combination of AMG 900 and PNU-74654 to establish colonies. Cells were stained with Crystal Violet after 12 days of treatment. Right panel: plot of the absorbance related to the colony staining. ****p < 0.0001; **p < 0.01. Studies were performed in 2 independent experiments, n = 6.

4. Discussion

ACC is a rare and aggressive type of cancer with high risk of recurrence and metastasis (Mohan et al., 2018). Options of treatment remain limited; mitotane which has low efficiency in advanced stages of the disease and is associated with high toxicity and side effects is perhaps the only drug that is widely used in both early and late phases of the disease (Lalli and Luconi, 2018; Crona and Beuschlein 2019; Fassnacht et al., 2018; Kerkhofs et al., 2013). The common molecular dysregulation of ACC are overexpression of the insulin-like growth factor-2 pathway, abnormal p53/Rb and dysregulation of the Wnt/beta-catenin signaling (Crona and Beuschlein 2019; Rahane et al., 2019). Inhibition of the Wnt/beta-catenin pathway leads to decrease in tumor growth and impairs adrenocortical cancer cell viability and steroidogenesis *in vitro* (Gaujoux et al., 2013; Ferro-Leal et al., 2015). Aurora kinases are proteins that act by regulating the cell cycle and, in turn, control cell growth (Willems et al., 2018). Expression of aurora kinases is increased in ACCs and inhibition of this class of kinases decreases cell proliferation and increases the apoptotic effects of mitotane (Borges et al., 2017). In our study, RNA sequencing analysis showed increased expression of aurora kinase A and aurora kinase B in ACC tissues or ACC cell lines when compared to normal tissue (Fig. 1). We evaluated the effects of the aurora kinases activity inhibitor, AMG 900, combined with the beta-catenin pathway blocker, PNU-74654. We show that AMG 900 combined with PNU-74654 enhanced the anti-proliferative response in NCI–H295 cells. Also, inhibition of aurora kinases produced better responses against cell invasion and colony establishment compared to blockade of Wnt/beta-catenin pathway only, while PNU-74654 showed to be more effective in decreasing cortisol secretion than AMG 900. Furthermore, we show that aurora kinase A is

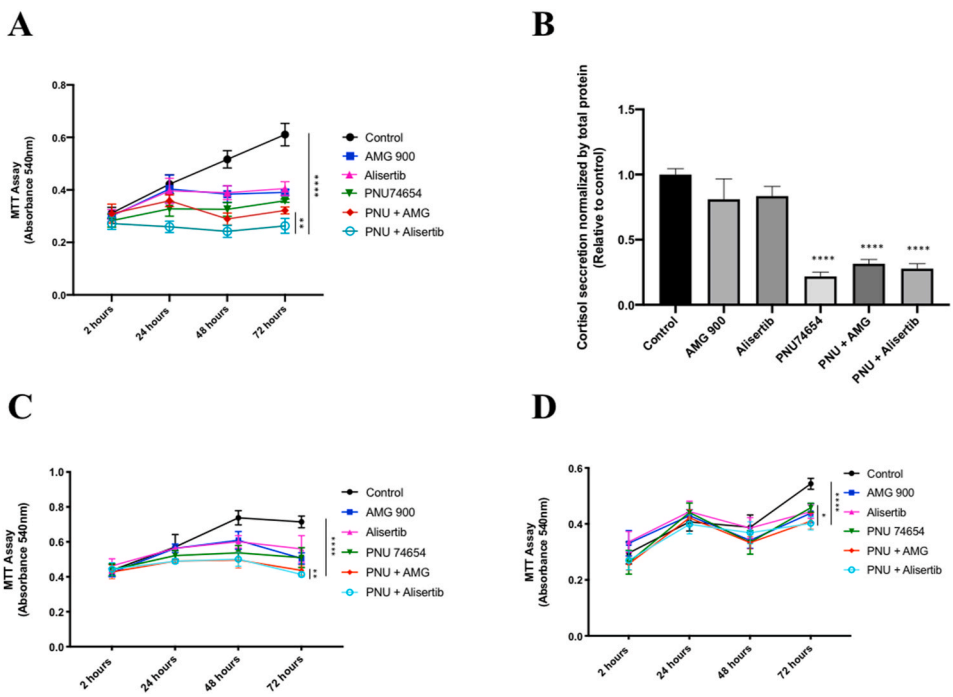


Fig. 4. Effects of AMG 900, Alisertib and PNU-74654 in cell viability of NCI–H295, CU-ACC1 and CU-ACC2 cell lines and cortisol secretion in NCI–H295 cells A) Cell viability accessed by MTT assay in NCI–H295 cells treated with AMG 900 50 nM or Alisertib 50 nM or PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654 for 2, 24, 48 and 72 h ****p < 0.0001; **p = 0.0019 **B)** Cortisol secretion after treatment of NCI–H295 cells with AMG 900 50 nM, Alisertib 50 nM, PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654. ****p < 0.0001. **C)** Cell viability accessed by MTT assay in CU-ACC1 cells treated with AMG 900 50 nM or Alisertib 50 nM or PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654 for 2, 24, 48 and 72. ****p < 0.0001; **p < 0.015. **D)** Cell viability accessed by MTT assay in CU-ACC2 cells treated with AMG 900 50 nM or Alisertib 50 nM or PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654 for 2, 24, 48 and 72. ****p < 0.0001, *0.04. Studies were performed in 2 independent experiments, n = 6.

more likely to be responsible for the decreased viability effects seen with the AMG 900 treatment and might play a role in controlling colony establishment of ACC cells.

Abnormal activation of Wnt/beta-catenin pathway leads beta-catenin translocation to the nucleus promoting the transcription of oncogenes such as *MYC* and *CCND1* (Shang et al., 2017). Due to the lack of available treatment and the fact that the Wnt/beta-catenin pathway is frequently altered in ACCs, studies are focused on finding strategies that can block the activation of beta-catenin pathway and are safe and effective against ACCs (Abduch et al., 2016; He et al., 2018; Rubin et al., 2020). AMG 900, an oral bioavailable and highly selective pan-aurora kinase inhibitor, showed to be effective in decreasing cell proliferation and active in multi-drug resistant cell lineages including NCI–H295 cells (Kalous et al., 2013; Borges et al., 2017). Here, we first wanted to know if AMG 900 would have an effect in the beta-catenin pathway in NCI–H295 cells which has the beta-catenin p. S45P mutation. Surprisingly, we observed that the treatment of NCI–H295 cells with AMG 900 increased beta-catenin mRNA, c-Myc mRNA (Fig. 1A) and c-Myc protein levels (Fig. 1B), suggesting that combination of AMG 900 with the beta-catenin pathway blocker, PNU-74654, could potentiate the anti-proliferative effects of the drugs alone. In fact, either cell viability and cell proliferation showed significantly decrease in the combinatorial treatment when compared to single treatment of AMG 900 or PNU-74654.

The delay to diagnose ACCs is one of the main reasons that leads the disease to progress to metastatic levels. The capacity of cancer cells to migrate, invade and establish themselves in another environment are crucial to the event of metastasis (Hanahan and Weinberg, 2011). Beta-catenin is one of the main proteins involved in the epithelial-mesenchymal transition (EMT) which requires ability of the cells to migrate and invade (Syn et al., 2016). We investigated the capacity of invasion of NCI–H295 cells after treatment. AMG 900 conferred higher efficiency in inhibiting cell invasion and colony establishment when compared to PNU-74654, despite both showed significant decrease compared to control. The effects of aurora kinases inhibition in cell invasion have also been showed in breast cancer cells (Zhao et al., 2017). Furthermore, in a (3D) microfluidic cell invasion model, the leading cells in the collective movement exhibited increased expression of aurora kinase A (Xia et al., 2017).

Alisertib is a specific aurora kinase A inhibitor and has showed efficacy in the treatment of hematologic malignancies and solid tumors (Liewer and Huddleston, 2018). We wanted to know if specific inhibition of aurora A would show antitumor effects as seen in the inhibition of aurora A, B and C. We evaluate cell viability, colony establishment and cortisol secretion as it is linked to tumorigenesis (Zheng et al., 2016). Alisertib treatment had similar effect on cell viability and colony establishment compared to the pan aurora kinase inhibitor, AMG 900, suggesting that the antitumor effects here observed in NCI–H295 cells after AMG 900 treatment might be due to the inhibition of aurora kinase A. Although both AMG 900 and Alisertib combined with PNU-74654 showed synergistic effects in different concentrations, it is important to highlight that combination of Alisertib and PNU-74654 showed a statistically higher effect on the inhibition of cell viability. Treatment with PNU-74654 showed to be significantly more efficient in decreasing cortisol secretion compared to AMG 900 or Alisertib treatment. Furthermore, studying the effects of AMG 900, Alisertib, PNU-7465 or combination in the cell viability and clonogenic capacity of another two ACC cell lines, we observed that cell viability and clonogenic capacity was decreased when aurora kinase inhibitors were combined with PNU-74654 in CU-ACC1cells. For CU-ACC2 cells, a similar scenario seen in NCI–H295 cells was observed: AMG 900 showed to be the most effective drug to impair clonogenic capacity and combination with PNU-74654 did not improve this impairment. Taken together, these results suggest that for long term treatment, AMG 900 might be more effective while combination of aurora kinase inhibitors and beta-catenin blockers may be acting as fast responders against ACCs.

In conclusion, our *in vitro* study suggests that aurora kinase A might be a major contributor for the antiproliferative effects observed when aurora A, B and C activity are inhibited in ACC cell lines. Inhibition of Aurora kinases A, B and C showed better effects in decelerate colony establishment in NCI–H295, CU-ACC1 and CU-ACC2 cells while PNU-74654 showed to decrease cortisol secretion of NCI–H295. Thus, we suggest that inhibition of aurora kinase A activity in combination with the inhibition of beta-catenin pathway impaired tumor progression by decreasing cell proliferation. Alisertib has been tested in Phase I/II/III clinical trials for cancer treatment and has demonstrated promising efficacy, despite side effects (Tayyar et al., 2017), supporting the idea that it could also be an approach for ACC treatment. Further studies are

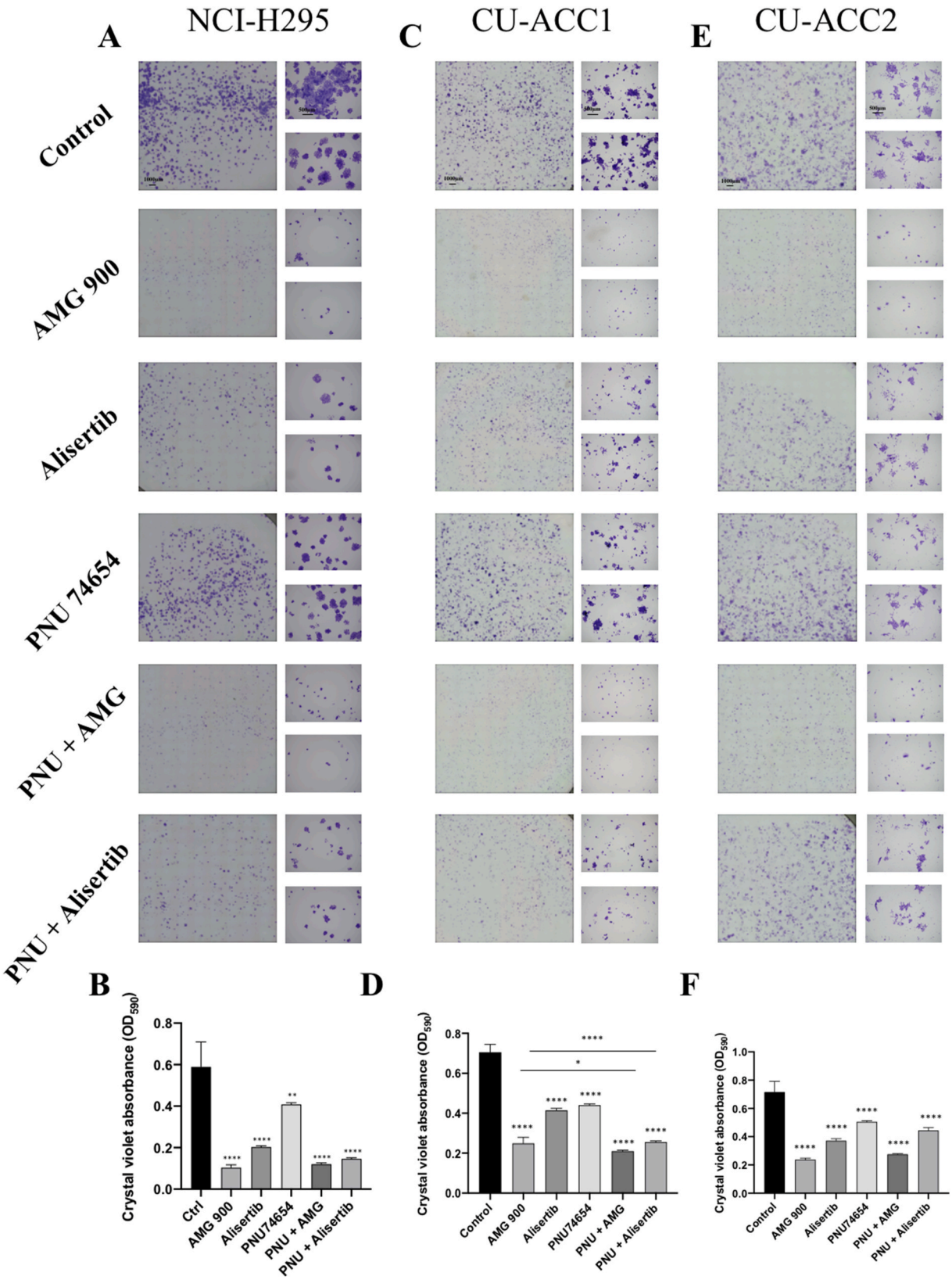


Fig. 5. Effects of AMG 900, Alisertib and PNU-74654 in the clonogenic capacity of NCI–H295, CU-ACC1 and CU-ACC2 cell lines
A) Representative images of clonogenic assay of NCI–H295 cells treated with AMG 900 50 nM, Alisertib 50 nM, PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654. **B)** plot of the absorbance related to the colony staining. ****$p < 0.0001$; **$p = 0.0028$. **C)** Representative images of clonogenic assay of CU-ACC1 cells treated with AMG 900 50 nM, Alisertib 50 nM, PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654. **D)** Plot of the absorbance related to the colony staining. ****$p < 0.0001$; *$p = 0.03$. **E)** Representative images of clonogenic assay of CU-ACC2 cells treated with AMG 900 50 nM, Alisertib 50 nM, PNU-74654 50 μM or combination of AMG 900/Alisertib with PNU-74654. **F)** Plot of the absorbance related to the colony staining. ****$p < 0.0001$. Cells were stained with Crystal Violet after 12 days of treatment. Studies were performed in 2 independent experiments, $n = 6$.

needed to increase the evidences of the antitumor effects of Alisertib and PNU-74654 combination in ACC cells and the side effects of this drugs, however, our results suggest the rationale for a possible clinical application of both inhibitors to treat this deadly disease.

CRediT authorship contribution statement

Andrea Gutierrez Maria: Conceptualization, conception and design of the work, Data curation, data collection, Formal analysis, data analysis and interpretation, Writing – original draft, drafting the article. **Kleiton Silva Borges:** Conceptualization, conception and design of the work, Formal analysis, data analysis and interpretation critical revision of the article. **R.C.P. Lira:** Data curation, data collection, critical revision of the article. **Carolina Hassib Thomé:** Data curation, data collection. **Annabel Berthon:** Formal analysis, data analysis and interpretation. **Ludivine Drougat:** Formal analysis, data analysis and interpretation. **Katja Kiseljak-Vassiliades:** Data curation, provided RNAseq data, critical revision of the article. **Margaret E. Wierman:** critical revision of the article. **Fabio R. Faucz:** Formal analysis, data analysis and interpretation, critical revision of the article. **Vitor Marcel Faça:** Conceptualization, conception and design of the work. **Luiz Gonzaga Tone:** Conceptualization, conception and design of the work, critical revision of the article. **Constantine A. Stratakis:** Conceptualization, conception and design of the work, critical revision of the article, final approval of the version to be published.

Declaration of competing interest

C.A.S. holds patents on the function of the *PRKAR1A*, *PDE11A*, and *GPR101* genes and related issues; his laboratory has also received research funding on the GPR101 gene, abnormal growth hormone secretion and its treatment by Pfizer, Inc; F.R.F. holds patent on the GPR101 gene and/or its function. The other authors have nothing to disclose.

Acknowledgements

This work was funded by the NIH Intramural Grant of the *Eunice Kennedy Shriver* National Institute for Child Health & Human Development Division of Intramural Research (DIR) to Dr. Constantine A. Stratakis (grant number: Z01-HD008920-01) and by the Sao Paulo Research Foundation (FAPESP) Grant to Dr. Luiz Gonzaga Tone, process number: 2014/20341–0. Andrea Gutierrez Maria received fellowship from FAPESP, processes number: 2015/23713–8 and 2014/16839–2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2021.111243.

References

Abduch, R.H., Carolina Bueno, A., Leal, L.F., et al., 2016. Unraveling the expression of the oncogene YAP1, a Wnt/beta-catenin target, in adrenocortical tumors and its association with poor outcome in pediatric patients. Oncotarget 7 (51), 84634–84644.

Assié, G., Letouzé, E., Fassnacht, M., et al., 2014. Integrated genomic characterization of adrenocortical carcinoma. Nat. Genet. 46 (6), 607–612.

Berthon, A., Sahut-Barnola, I., Lambert-Langlais, S., et al., 2010. Constitutive beta-catenin activation induces adrenal hyperplasia and promotes adrenal cancer development. Hum. Mol. Genet. 19 (8), 1561–1576.

Bertolin, G., Tramier, M., 2020. Insights into the non-mitotic functions of Aurora kinase A: more than just cell division. Cell. Mol. Life Sci. 77 (6), 1031–1047.

Borges, K.S., Moreno, D.A., Martinelli Jr., C.E., et al., 2013. Spindle assembly checkpoint gene expression in childhood adrenocortical tumors (ACT): overexpression of Aurora kinases A and B is associated with a poor prognosis. Pediatr. Blood Canc. 60 (11), 1809–1816.

Borges, K.S., Andrade, A.F., Silveira, V.S., et al., 2017. The aurora kinase inhibitor AMG 900 increases apoptosis and induces chemosensitivity to anticancer drugs in the NCI-H295 adrenocortical carcinoma cell line. Anti Canc. Drugs 28 (6), 634–644.

Borges, K.S., Pignatti, E., Leng, S., et al., 2020. Wnt/β-catenin activation cooperates with loss of p53 to cause adrenocortical carcinoma in mice. Oncogene 39 (30), 5282–5291.

Borisa, A.C., Bhatt, H.G., 2017. A comprehensive review on Aurora kinase: small molecule inhibitors and clinical trial studies. Eur. J. Med. Chem. 140, 1–19.

Carmena, M., Earnshaw, W.C., 2003. The cellular geography of aurora kinases. Nat. Rev. Mol. Cell Biol. 4 (11), 842–854.

Chandrasekar, T., Goldberg, H., Klaassen, Z., et al., 2019. The who, when, and why of primary adrenal malignancies: insights into the epidemiology of a rare clinical entity. Cancer 125 (7), 1050–1059.

Chou, T.C., 2010. Drug combination studies and their synergy quantification using the Chou-Talalay method. Canc. Res. 70 (2), 440–446.

Crona, J., Beuschlein, F., 2019. Adrenocortical carcinoma - towards genomics guided clinical care. Nat. Rev. Endocrinol. 15 (9), 548–560.

Damodaran, A.P., Vaufrey, L., Gavard, O., Prigent, C., 2017. Aurora A kinase is a priority pharmaceutical target for the treatment of cancers. Trends Pharmacol. Sci. 38 (8), 687–700.

de Reyniès, A., Assié, G., Rickman, D.S., et al., 2009. Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. J. Clin. Oncol. 27 (7), 1108–1115.

Doghman, M., Cazareth, J., Lalli, E., 2008. The T cell factor/beta-catenin antagonist PKF115-584 inhibits proliferation of adrenocortical carcinoma cells. J. Clin. Endocrinol. Metab. 93 (8), 3222–3225.

Fassnacht, M., Dekkers, O.M., Else, T., et al., 2018. European society of Endocrinology clinical practice guidelines on the management of adrenocortical carcinoma in adults, in collaboration with the European network for the study of adrenal tumors. Eur. J. Endocrinol. 179 (4), G1–G46.

Gaujoux, S., Hantel, C., Launay, P., et al., 2013. Silencing mutated β-catenin inhibits cell proliferation and stimulates apoptosis in the adrenocortical cancer cell line H295R. PloS One 8 (2), e55743.

Gerashchenko, T.S., Novikov, N.M., Krakhmal, N.V., et al., 2019. Markers of cancer cell invasion: are they good enough? J. Clin. Med. 8 (8), 1092. Published 2019 Jul 24.

Glover, D.M., 2005. Polo kinase and progression through M phase in Drosophila: a perspective from the spindle poles. Oncogene 24 (2), 230–237.

Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. Cell 100 (1), 57–70.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. Cell 144 (5), 646–674.

He, H., Dai, J., Yang, X., Wang, X., Sun, F., Zhu, Y., 2018. Silencing of MED27 inhibits adrenal cortical carcinogenesis by targeting the Wnt/β-catenin signaling pathway and the epithelial-mesenchymal transition process. Biol. Chem. 399 (6), 593–602.

Kalous, O., Conklin, D., Desai, A.J., et al., 2013. AMG 900, pan-Aurora kinase inhibitor, preferentially inhibits the proliferation of breast cancer cell lines with dysfunctional p53. Breast Canc. Res. Treat. 141 (3), 397–408.

Kerkhofs, T.M., Baudin, E., Terzolo, M., et al., 2013. Comparison of two mitotane starting dose regimens in patients with advanced adrenocortical carcinoma. J. Clin. Endocrinol. Metab. 98 (12), 4759–4767.

Kiseljak-Vassiliades, K., Zhang, Y., Bagby, S.M., et al., 2018. Development of new preclinical models to advance adrenocortical carcinoma research. Endocr. Relat. Canc. 25 (4), 437–451.

Lalli, E., Luconi, M., 2018. The next step: mechanisms driving adrenocortical carcinoma metastasis. Endocr. Relat. Canc. 25 (2), R31–R48.

Leal, L.F., Bueno, A.C., Gomes, D.C., Abduch, R., de Castro, M., Antonini, S.R., 2015. Inhibition of the Tcf/beta-catenin complex increases apoptosis and impairs adrenocortical tumor cell proliferation and adrenal steroidogenesis. Oncotarget 6 (40), 43016–43032. https://doi.org/10.18632/oncotarget.5513.

Liewer, S., Huddleston, A., 2018. Alisertib: a review of pharmacokinetics, efficacy and toxicity in patients with hematologic malignancies and solid tumors. Expet Opin. Invest. Drugs 27 (1), 105–112.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (4), 402–408.

Malumbres, M., Pérez de Castro, I., 2014. Aurora kinase A inhibitors: promising agents in antitumoral therapy. Expert Opin. Ther. Targets 18 (12), 1377–1393.

Mohan, D.R., Lerario, A.M., Hammer, G.D., 2018. Therapeutic targets for adrenocortical carcinoma in the genomics era. J Endocr Soc 2 (11), 1259–1274. Published 2018 Sep. 26.

Paragliola, R.M., Torino, F., Papi, G., Locantore, P., Pontecorvi, A., Corsello, S.M., 2018. Role of mitotane in adrenocortical carcinoma - review and state of the art. Eur. Endocrinol. 14 (2), 62–66.

Pereira, S.S., Monteiro, M.P., Bourdeau, I., Lacroix, A., Pignatelli, D., 2018. Mechanisms OF endocrinology: cell cycle regulation in adrenocortical carcinoma. Eur. J. Endocrinol. 179 (2), R95–R110.

Pignatti, E., Leng, S., Yuchi, Y., et al., 2020. Beta-catenin causes adrenal hyperplasia by blocking zonal transdifferentiation. Cell Rep. 31 (3), 107524.

Pittaway, J.F.H., Guasti, L., 2019. Pathobiology and genetics of adrenocortical carcinoma. J. Mol. Endocrinol. 62 (2), R105–R119.

Rahane, C.S., Kutzner, A., Heese, K., 2019. Establishing a human adrenocortical carcinoma (ACC)-specific gene mutation signature. Cancer Genet 230, 1-12.

Roberts, A., Pimentel, H., Trapnell, C., Pachter, L., 2011. Identification of novel transcripts in annotated genomes using RNA-Seq. Bioinformatics 27, 2325–2329.

Roberts, A., Trapnell, C., Donaghey, J., Rinn, J.L., Pachter, L., 2011b. Improving RNA-Seq expression estimates by correcting for fragment bias. Genome Biol 12 (3). https://doi.org/10.1186/gb-2011-12-3-r22. Epub 2011 Mar 16. PMID: 21410973; PMCID: PMC3129672.

Rubin, B., Pilon, C., Pezzani, R., Rebellato, A., Fallo, F., 2020. The effects of mitotane and 1α,25-dihydroxyvitamin D_3 on Wnt/beta-catenin signaling in human adrenocortical carcinoma cells. J. Endocrinol. Invest. 43 (3), 357–367.
Salomon, A., Keramidas, M., Maisin, C., Thomas, M., 2015. Loss of β-catenin in adrenocortical cancer cells causes growth inhibition and reversal of epithelial-to-mesenchymal transition. Oncotarget 6 (13), 11421–11433.
Shang, S., Hua, F., Hu, Z.W., 2017. The regulation of β-catenin activity and function in cancer: therapeutic opportunities. Oncotarget 8 (20), 33972–33989.
Subramanian, C., Cohen, M.S., 2019. Over expression of DNA damage and cell cycle dependent proteins are associated with poor survival in patients with adrenocortical carcinoma. Surgery 165 (1), 202–210.
Syn, N., Wang, L., Sethi, G., Thiery, J.P., Goh, B.C., 2016. Exosome-mediated metastasis: from epithelial-mesenchymal transition to escape from immunosurveillance. Trends Pharmacol. Sci. 37 (7), 606–617.
Tayyar, Y., Jubair, L., Fallaha, S., McMillan, N.A.J., 2017. Critical risk-benefit assessment of the novel anti-cancer aurora a kinase inhibitor alisertib (MLN8237): a comprehensive review of the clinical data. Crit. Rev. Oncol. Hematol. 119, 59–65.
Trapnell, C., Williams, B.A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M.J., Salzberg, S.L., Wold, B.J., Pachter, L., 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat. Biotechnol. 28, 511–515.
Willems, E., Dedobbeleer, M., Digregorio, M., Lombard, A., Lumapat, P.N., Rogister, B., 2018. The functional diversity of Aurora kinases: a comprehensive review. Cell Div. 13 (7). Published 2018 Sep. 19.
Xia, J.L., Fan, W.J., Zheng, F.M., et al., 2017. Inhibition of AURKA kinase activity suppresses collective invasion in a microfluidic cell culture platform. Sci. Rep. 7 (1), 2973. Published 2017 Jun 7.
Zhao, H., Owen, S., Davies, E.L., Jiang, W.G., Martin, T.A., 2017. The effect of aurora kinase inhibitor on adhesion and migration in human breast cancer cells and clinical implications. World J. Oncol. 8 (5), 151-161.
Zheng, S., Cherniack, A.D., Dewal, N., et al., 2016. Comprehensive pan-genomic characterization of adrenocortical carcinoma [published correction appears in cancer cell. 2016 Aug 8;30(2):363]. Canc. Cell 29 (5), 723–736.