UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Centro de Biotecnologia

Programa de Pós-Graduação em Biologia Celular e Molecular – PPGBCM

TESE DE DOUTORADO

ANÁLISE DE NOVOS AGENTES QUIMIOTERÁPICOS EM LINHAGENS CELULARES DE CÂNCER GÁSTRICO HUMANO

LARISSA SIQUEIRA PENNA

Orientadores:

Diego Bonatto

João Antonio Pêgas Henriques

Porto Alegre, 2017

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Centro de Biotecnologia

Programa de Pós-Graduação em Biologia Celular e Molecular – PPGBCM

ANÁLISE DE NOVOS AGENTES QUIMIOTERÁPICOS EM LINHAGENS CELULARES DE CÂNCER GÁSTRICO HUMANO

Tese de doutorado apresentada no Programa de Pós-Graduação em Biologia Celular e Molecular da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Doutora em Biologia Celular e Molecular.

LARISSA SIQUEIRA PENNA

Orientadores:

Diego Bonatto

João Antonio Pêgas Henriques

Porto Alegre, Abril de 2017

Dedicatória

Dedico este trabalho aos meus pais, meus grandes exemplos de vida, pelo amor incondicional e pelo constante incentivo ao meu crescimento, o que sempre me fez seguir adiante.

Agradecimentos

Aos meus orientadores, Dr. Diego Bonatto e Dr. José Antonio Pêgas Henriques, por me aceitarem como orientanda, acreditarem no meu potencial e pela participação na construção da minha carreira acadêmica. Muito obrigada por tudo!

Aos professores e funcionários do PPGBCM e aos membros da comissão de acompanhamento, professora Dra. Jenifer Saffi e Dr. José Cláudio Fonseca Moreira.

Aos membros da banca, professores Dr. Daniel Pens Gelain, Dra. Denise Pires de Carvalho, Dra. Márcia Rosângela Wink e Dr. Guido Lenz, por terem aceitado o convite e pelas contribuições feitas a este trabalho.

Ao CNPq e demais agências financiadoras que possibilitaram a realização desta tese.

À professora Dra. Sidia Maria Callegari-Jacques, por horas de análises estatísticas, essenciais à realização deste trabalho.

Aos meus amigos que me deram suporte e foram tolerantes com a minha ausência durante o doutorado, em especial à Flávia, Gabi, Pati e às primas Laís e Silvia.

À Miriana, a primeira pessoa que conheci ao iniciar essa jornada, que me incentivou e auxiliou quando precisei, além de sempre ter me contagiado com a sua alegria!

Ao professor Dr. Guido Lenz e aos que fazem ou já fizeram parte do seu laboratório, por terem me recebido e acolhido durante todos esses anos! Fran, Andrew, Eduardo, Marcos, Gio, Emilly e Karen, muito obrigada pela amizade e por todo auxílio que me deram!

À Eamim, pelas incansáveis horas no citômetro e que se tornou uma grande amiga que levarei para a vida.

Aos amigos Loulou e Dieguinho pelo essencial auxílio na execução dos experimentos e por quem sempre terei um grande carinho. Meus dias se tornaram muito mais divertidos ao lado de vocês!

Aos meus amigos do “Lab 219” que foram verdadeiros anjos que Deus colocou no meu caminho, pela paciência, parceria, diversão e carinho! Bia, Kendi, Gabino, Joice, Bruno, Chapola e Raquel, jamais terei palavras para agradecer tudo que fizeram por mim! Aprendi muito com vocês e os levarei sempre no meu coração!

À minha família, em especial aos meus pais e meus irmãos, pelo constante incentivo e suporte e sem os quais nada disso teria sido possível. Aos meus tios e às minhas cunhadas, que estiveram constantemente na minha torcida. Aos meus sobrinhos, que sempre trouxeram felicidade aos meus dias!

“Não haverá borboletas se a vida não passar por longas e silenciosas metamorfoses.”

(Rubem Alves)

Sumário

LISTA DE ABREVIATURAS

5-FU: 5-fluorouracil

AN/IP: anexina/iodeto de propídeo

APC/C: complexo promotor de anáfase

AURKA: aurora A

AURKB: aurora B

CPC: complexo passageiro cromossomal

CTGs: células tronco gástricas

CTTs: células tronco tumorais

CTTGs: células tronco tumorais gástricas

DHFU: dihidrofluorouracil

dTTP: trifosfato de timidina

DPD: dihidropirimidina-desidrogenase

EMT: transição epitélio-mesenquimal

FdUMP: monofosfato de fluorodesoxiuridina

FdUTP: trifosfato de fluorodesoxiuridina

FUdR: fluorodesoxiuridina

FUTP: trifosfato de fluorouridina

FUMP: monofosfato de fluorouridina

OMS: Organização Mundial da Saúde

OPRT: orotato fosforibosiltransferase

TP: timidina fosforilase

TS: timidilato sintase

VEB: vírus Epstein-Barr

LISTA DE FIGURAS

Introdução

Capítulo I - Artigo de Revisão

Capítulo II - Artigo de Dados

Capítulo III - Metodologia e Resultados Adicionais

Discussão Geral

Apêndice

LISTA DE TABELAS

Capítulo I – Artigo de Revisão

Capítulo II – Artigo de Dados

Capítulo III - Metodologia e Resultados Adicionais

Discussão Geral

RESUMO

O câncer gástrico é a terceira causa de morte por câncer no mundo e a quimioterapia combinatória é um dos principais tratamentos para esta doença. Contudo, a principal causa de falha do tratamento é a quimiorresistência. Considerando este obstáculo juntamente com a descoberta de que muitas proteínas envolvidas na mitose podem estar associadas à tumorigênese, vários agentes antimitóticos estão sendo desenvolvidos e testados. As proteínas mitóticas AURKB e NDC80 foram recentemente identificadas como proteínas potenciais a serem inibidas no câncer gástrico. Portanto, o objetivo deste estudo foi analisar os efeitos da inibição de AURKB e NDC80 pelos fármacos experimentais ZM447439 e INH1, respectivamente. Analisamos os efeitos em duas linhagens de adenocarcinoma gástrico avaliando expressão gênica, ciclo celular, morte celular, análise morfométrica nuclear, capacidade de migração e presença de células-tronco tumorais. É importante enfatizar que essas duas pequenas moléculas não foram testadas na quimioterapia do câncer gástrico até o momento. Além disso, após uma revisão da literatura, concluímos que os melhores resultados dos ensaios clínicos foram alcançados quando antimitóticos foram combinados com a terapia convencional. Dessa forma, avaliamos também os efeitos da associação de 5-FU com ZM447439. Nossos resultados demonstraram que a monoterapia com 5-FU, ZM447439 e INH1 induziu a expressão gênica diferencial nas linhagens de câncer gástrico (ACP02 e ACP03) de pelo menos 22 de um total de 82 genes envolvidos em várias vias, como apoptose, ciclo celular, senescência e transição epitélio-mesenquimal. Observamos também que ZM447439 e sua combinação com 5-FU induziram apoptose, catástrofe mitótica, senescência e ciclo celular alterado nas linhagens de câncer gástrico. Outro dado interessante foi a expressão dos marcadores de células-tronco tumorais gástricas, LGR5 e CD24, e a capacidade de formar esferas, indicando que ambas ACP02 e ACP03 podem conter células tronco tumorais. Além disso, a monoterapia com ZM447439 ou 5-FU foi capaz de inibir a formação de esferas. Por outro lado, o INH1 mostrou menor atividade antitumoral, uma vez que apresentou o maior valor de IC_{50} e foi capaz somente de induzir apoptose e reduzir a migração celular. Interessantemente, a combinação do 5-FU com ZM447439 permitiu o uso de doses menores dos compostos, além de ser capaz de induzir a apoptose num tempo mais curto quando comparado com o tratamento com os fármacos isolados. Em síntese, os resultados observados sugerem o inibidor de AURKB, ZM447439, e sua associação com o agente quimioterápico 5-FU, como tratamentos promissores do câncer gástrico com base em sua elevada atividade antitumoral *in vitro*.

Palavras-chave: antimitóticos, ZM447439, INH1, câncer gástrico.

ABSTRACT

Gastric cancer is the third cause of cancer death worldwide and combinatorial chemotherapy is one of the main treatments for this disease. However, the major cause of treatment failure is chemoresistance. Considering this obstacle together with the discovery that many proteins involved in mitosis might be associated to tumorigenesis, several new anti-mitotics are being developed and tested. The mitotic proteins AURKB and NDC80 were recently shown as potential proteins to be inhibited in gastric cancer. Therefore, the aim of this study was to analyze the effects of AURKB and NDC80 inhibition by the experimental drugs ZM447439 and INH1, respectively. We analyzed the effects in two gastric adenocarcinoma cell lines evaluating gene expression, cell cycle, cell death, nuclear morphometric analysis, migration ability and presence of gastric cancer stem cells. It is important to emphasize these two small molecules have not been tested in gastric cancer chemotherapy until now. Moreover, after reviewing the literature, we concluded the best results of clinical trials were achieved when anti-mitotics were combined with conventional therapy. Thus, we evaluated the effects of 5-FU and ZM447439 combination as well. Our results demonstrated that monotherapy with 5-FU, ZM447439 and INH1 induced differential gene expression in gastric cancer cell lines (ACP02 and ACP03) of at least 22 from 82 genes involved in several pathways such as apoptosis, cell cycle, senescence and epithelial mesenchymal transition. We also observed that ZM447439 and its combination with 5-FU induced apoptosis, mitotic catastrophe, senescence and altered cell cycle in gastric cancer cell lines. Another interesting data was the expression of the gastric cancer stem cell markers LGR5 and CD24, and the ability to form spheres, indicating that both ACP02 and ACP03 may be composed by cancer stem cells. Furthermore, monotherapy with either ZM447439 or 5-FU were capable of inhibiting the formation of spheres. On the other hand, INH1 have shown lower antitumoral activity since it presented the highest IC_{50} value and was only capable of inducing apoptosis and reducing cell migration. Interestingly, the combination of 5-FU with ZM447439 allowed the use of smaller doses of the compounds, in addition to being able to induce apoptosis in a shorter time when compared to the treatment with the isolated drugs. Taken together, our findings suggest that due to the high antitumoral activity shown by ZM447439 and mainly its association with 5-FU, these might be promising gastric cancer therapies.

Key words: anti-mitotics, ZM447439, INH1, gastric cancer.

APRESENTAÇÃO DA TESE

A presente tese foi estruturada da seguinte forma: Introdução, Objetivos, Capítulos I-III, Discussão Geral, Conclusões, Perspectivas, Referências, Apêndice e Anexo.

A Introdução descreve dados epidemiológicos do câncer gástrico, o papel das células tronco tumorais no processo tumorigênico, os tratamentos convencionais utilizados nesse tipo de câncer, bem como o direcionamento do tratamento quimioterápico pela utilização de antimitóticos, dentre eles as moléculas experimentais ZM447439 e INH1, que inibem as proteínas AURKB e NDC80, respectivamente.

O Capítulo I consiste no artigo de revisão "Anti-mitotic agents: Are they emerging molecules for cancer treatment?", aceito pela revista *Pharmacology & Therapeutics*. Este artigo descreve as principais proteínas envolvidas na mitose e dentre estas, quais tem sido alvo terapêutico contra o câncer em ensaios clínicos com diferentes antimitóticos.

O Capítulo II foi escrito na forma de artigo científico e contempla os resultados obtidos após utilização dos inibidores ZM447439 e INH1 e da combinação de ZM447439 com 5-FU, no tratamento das linhagens de adenocarcinoma gástrico humano ACP02 e ACP03, e da linhagem de epitélio gástrico não tumoral MN01. Neste capítulo, foram avaliados os efeitos dos diferentes tratamentos no ciclo celular, na indução de apoptose e na morfologia nuclear a fim de identificar a indução de senescência e catástrofe mitótica.

O Capítulo III ilustra resultados e metodologias adicionais obtidos neste trabalho após os diferentes tratamentos acima citados nas linhagens ACP02, ACP03 e MN01. Neste capítulo, foram verificados os efeitos dos tratamentos na expressão de genes envolvidos em diferentes vias associadas a processos de transformação e tumorigênese, além de efeitos na formação de esferas e migração celular.

Ao final, é apresentada uma Discussão Geral que contempla os três capítulos que compõe essa tese, seguida por Conclusões, Perspectivas geradas a partir desse estudo, Referências, Apêndice e Anexo.

INTRODUÇÃO

1. Câncer gástrico

O câncer é uma doença resultante de sucessivas alterações genéticas capazes de conferir diversas características que viabilizam a sobrevivência e proliferação de células durante o processo de tumorigênese (HANAHAN & WEINBERG, 2000). As características observadas nas células tumorais incluem (Figura 1): (i) a capacidade de manter a sinalização proliferativa; (ii) a evasão de sinais supressores de crescimento; (iii) a evasão da destruição imune; (iv) o potencial replicativo ilimitado; (v) a promoção de inflamação; (vi) a ativação da invasão e metástase; (vii) a indução da angiogênese; (viii) a instabilidade genômica e mutações; (ix) a resistência à morte celular e (x) a desregulação do metabolismo energético. Além disso, o microambiente tumoral propicia a progressão do câncer e é heterogêneo, pois além de sua composição por células tumorais, pode ser constituído por células tronco tumorais (CTTs), fibroblastos, pericitos, células endoteliais e do sistema imune (HANAHAN & WEINBERG, 2011).

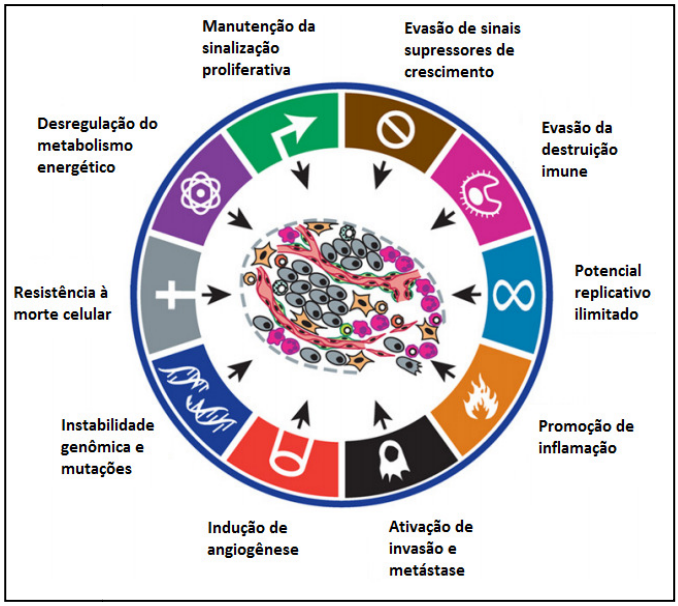

Figura 1. Caracterização das células tumorais. Figura adaptada de HANAHAN & WEINBERG (2011).

Segundo a Organização Mundial da Saúde (OMS), esta doença é uma das principais causas de mortalidade mundial e resultou em aproximadamente 8,2 milhões de óbitos em 2012. Dentre os tipos de câncer com maiores índices de mortalidade estão inclusos de pulmão, fígado, estômago e colorretal ("WHO | World Health Organization", 2017).

Apesar da diminuição nas taxas de incidência, o câncer gástrico é a terceira causa de morte por câncer no mundo e o quinto tipo mais incidente. Dados apresentados pelo Instituto Nacional do Câncer estimam que, em homens, esse é o segundo câncer mais frequente nas regiões Norte e Nordeste, quarto nas regiões Sul e Centro-Oeste e quinto na região Sudeste. Em mulheres é o quarto mais frequente na região Norte, quinto nas regiões Sul e Nordeste e sexto nas regiões Centro-Oeste e Sudeste. Estimou-se, para o Brasil, a ocorrência de aproximadamente 20.000 novos casos de câncer gástrico em 2016 ("Portal - Instituto Nacional de Câncer - INCA", 2017)

Diversos fatores predisponentes podem estar associados ao câncer gástrico sendo que um dos principais é a infecção pela bactéria *Helicobacter pylori*, que apresenta prevalência de aproximadamente 80% em pacientes com este tipo de câncer (BORNSCHEIN *et al*., 2010). Esta bactéria foi classificada como carcinógeno classe I em humanos pela OMS desde 1994. Acredita-se que, na gastrite inflamatória crônica associada à infecção por *H. pylori*, ocorra a formação de uma lesão inicial que induz mudanças histopatológicas progressivas na mucosa gástrica evoluindo de gastrite crônica a atrofia, metaplasia, displasia e por fim, ao carcinoma (CORREA, 1992; LEE *et al*., 2012). Estudos mostraram que a infecção por *H. pylori* pode estar associada a um risco de três a seis vezes maior na indução de câncer gástrico (ZALI *et al.*, 2011).

Outro patógeno que tem sido associado em aproximadamente 10% dos casos de carcinogênese gástrica é o vírus Epstein-Barr (VEB), que resulta em modificações na

expressão de genes relacionados a processos como apoptose, proliferação, migração e sinalização imune, favorecendo o desenvolvimento do carcinoma (SHINOZAKI-USHIKU *et al.*, 2015). Apesar de estar associado ao surgimento do câncer gástrico, estudos já mostraram que a taxa de sobrevida é mais elevada em pacientes positivos para o VEB em comparação com pacientes negativos para a presença deste vírus (FIGUEIREDO *et al.*, 2015). A melhor resposta desses pacientes positivos para o VEB é resultante, em parte, de uma menor heterogeneidade molecular e do recrutamento de células imunes para o tumor devido à infecção. Um estudo recente verificou que pacientes com carcinoma gástrico associado ao VEB apresentaram um maior grau de homogeneidade molecular e menos genes diferencialmente expressos, sendo a maioria destes responsáveis pela resposta imune, em comparação com tumores não infectados pelo VEB (KIM *et al.*, 2015).

Outros fatores são capazes de influenciar o desenvolvimento do câncer gástrico, tais como a história familiar, a obesidade, o histórico de anemia perniciosa, o tabagismo, a presença de pólipos gástricos adenomatosos maiores do que dois centímetros, condições socioeconômicas desfavoráveis, o alto consumo de carne vermelha, de álcool, de alimentos salgados e defumados (PLUMMER *et al.*, 2004; YANG *et al.*, 2009; ZALI; *et al.* 2011). A maioria dos casos de câncer gástrico ocorrem de forma esporádica, porém, 10% resultam de uma predisposição familiar e aproximadamente 1 a 3% são hereditários (FIGUEIREDO *et al.*, 2015).

Em muitos casos, a sintomatologia só é percebida em estágios mais avançados da doença. A maioria dos sintomas são inespecíficos, tais como disfagia, saciedade precoce, perda de peso, vômitos e dispepsia. O diagnóstico consiste na realização de gastroscopia e em uma análise histopatológica de biópsia (WADDELL *et al.*, 2014).

O tumor pode acometer as três regiões anatômicas do estômago conhecidas como cárdia, fundo/corpo e antro pilórico (Figura 2). O epitélio do estômago dessas diferentes regiões é constituído por unidades gástricas compostas por um epitélio plano, que possui uma invaginação conhecida como "fosseta" seguida por uma extensão tubular chamada de "glândula". Por sua vez, a glândula ainda poder ser subdividida em três regiões: istmo, colo e base. As glândulas são compostas principalmente por células mucosas do colo, parietais, zimogênicas, enteroendócrinas e células tronco gástricas (CTGs). De maneira geral, as fossetas possuem células responsáveis pela liberação de muco, enquanto que o istmo possui células imaturas altamente proliferativas e secretoras. Já o colo possui células produtoras de muco e a base contém, principalmente, células zimogênicas produtoras de enzimas digestivas. As CTGs estão localizadas na base glandular, sendo que as que expressam a proteína Lgr5 ou vilina estão geralmente localizadas em glândulas do antro pilórico, enquanto que as CTGs que expressam Troy ou Sox2 estão em sua maioria presentes em glândulas do corpo do estômago (Figura 2) (ZHAO *et al.*, 2015).

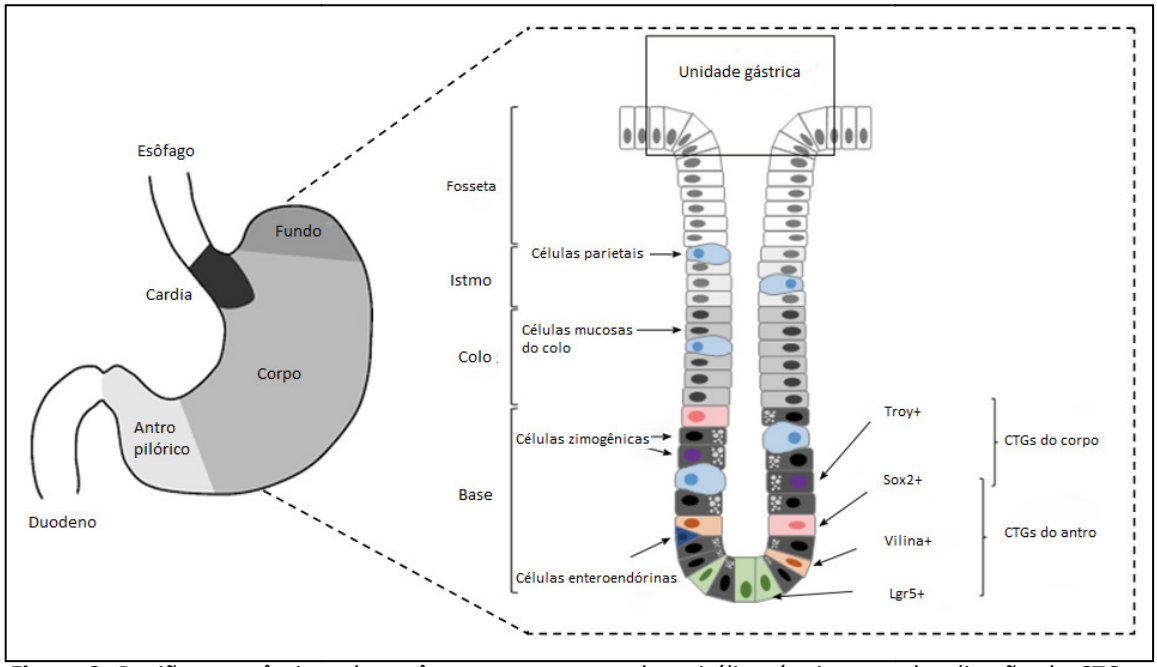

Figura 2. Regiões anatômicas do estômago e estrutura do epitélio gástrico com localização de CTGs. Figura adaptada de YANG *et al.* (2013) e ZHAO *et al.* (2015).

O câncer de estômago pode ser classificado como adenocarcinoma, linfoma ou leiomiossarcoma, sendo que adenocarcinoma é o mais frequentemente observado (aproximadamente 95% dos casos) (HWANG *et al.*, 1994). O adenocarcinoma gástrico pode ter duas classificações segundo a sua localização anatômica: cárdia/proximal ou distal sendo que nos últimos anos houve uma redução na incidência de tumores distais e aumento dos tumores da cárdia, geralmente associados a um pior prognóstico (ZALI *et al.*, 2011). Além disso, existem diferentes esquemas de classificação histopatológica sendo a de Lauren, uma das mais utilizadas. De acordo com essa classificação, o câncer gástrico pode ser categorizado em tipo difuso, que é pouco diferenciado e com ausência de formação glandular, ou tipo intestinal, que geralmente varia de moderadamente a bem diferenciado e, ao contrário do difuso, forma estruturas glandulares (VAN CUTSEM *et al.*, 2016). Recentemente, após avaliação molecular de diversas amostras, o projeto *The Cancer Genome Atlas* propôs uma outra classificação para adenocarcinomas gástricos em quatro subtipos: (i) tumores genomicamente estáveis, observado em 20% dos casos; (ii) tumores com instabilidade de microssatélites, observado em 22% dos casos; (iii) tumores com instabilidade cromossômica, observado em 50% dos casos; (iv) tumores positivos para VEB, observado em 9% dos casos (BASS *et al.*, 2014).

2. Células tronco tumorais gástricas (CTTGs)

O mecanismo envolvido no surgimento e progressão do câncer ainda não foi elucidado, contudo, alguns autores têm sugerido que as CTTs seriam as principais responsáveis pelo início, heterogeneidade, evolução, metástase e recidiva do tumor bem como pela resistência ao tratamento (RASSOULI *et al.*, 2016). Portanto, se o câncer é resultante da maioria de células que compõe o tumor ou de um pequeno grupo de CTTs,

ainda está em discussão. Nesse sentido, existem dois modelos principais utilizados para explicar o processo de carcinogênese. No modelo de evolução clonal, células com instabilidade genética podem apresentar vantagens seletivas que possibilitam a proliferação dessas novas populações com acúmulo de mutações. No modelo de CTTs, existe uma hierarquia onde as CTTs estão no topo e comandam a formação do tumor, gerando células idênticas ou, de maneira unidirecional, se diferenciando em células tumorais fenotipicamente heterogêneas que possuem potencial tumorigênico e metastático limitado. Esses dois modelos não são mutuamente exclusivos, já que os tumores que seguem o modelo de CTTs podem progredir também via modelo de evolução clonal.

Um novo modelo alternativo ao de CTTs tem sido sugerido, cuja diferença consiste na bidirecionalidade do processo tumoral, ou seja, as CTTs podem se diferenciar em células tumorais sem características tronco bem como essas mesmas, podem se dediferenciar, gerando continuamente populações de CTTs (BRUNGS *et al.*, 2016; MARJANOVIC *et al.*, 2013; SHACKLETON *et al.*, 2009; ZHAO *et al.*, 2015).

As CTTs foram identificadas a primeira vez em 1997 na leucemia mielóide aguda, onde células com os marcadores CD34+/CD38- foram capazes de gerar a doença quando transplantadas em camundongos imunodeficientes (BONNET & DICK, 1997). Algumas características observadas em células tronco normais também podem ser atribuídas às CTTs, tais como a capacidade de auto-renovação, o potencial de diferenciação e a alta capacidade de proliferação (BOHL *et al.*, 2011). Além disso, é possível se observar nas CTTs (Figura 3): (i) capacidade de formação de tumor que possui os mesmos padrões do tumor original após sucessivos transplantes das células tumorais em animais imunocomprometidos; (ii) capacidade de formação de colônias ou esferas *in vitro*, que indicam habilidade de auto-renovação; (iii) tendência à invasão e migração devido à expressão de proteínas relacionadas

ao processo de metástase como metaloproteinases de matriz; (iv) superexpressão de fatores de transcrição relacionados à pluripotência de células tronco como NANOG, SOX2 e OCT4; (v) resistência aos tratamentos devido à superexpressão de proteínas envolvidas no reparo de dano ao DNA ou bombas de efluxo de drogas e por fim, (v) presença de marcadores específicos de superfície ou intratumorais (RASSOULI *et al.*, 2016).

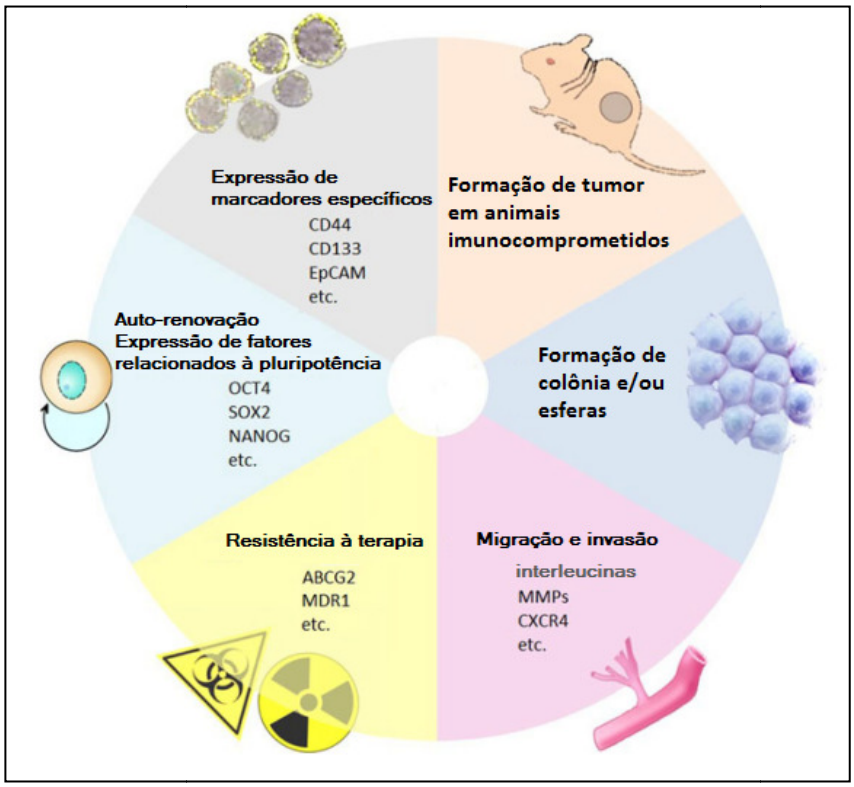

Figura 3. Características possíveis de serem observadas nas CTTs: capacidade de migração e invasão, resistência à terapia, capacidade de auto-renovação, expressão de marcadores específicos, formação de tumor *in vivo* e capacidade de formar colônias ou esferas. Figura adaptada de RASSOULI *et al.* (2016).

Em 2007, QIAO *et al.* identificaram a vilina como o primeiro biomarcador para CTGs, uma proteína específica de células epiteliais que, mediante regulação do cálcio, se liga à actina atuando na reorganização dos filamentos de actina das microvilosidades (ZHAO *et al.*, 2015). Desde então, diversos estudos identificaram outros marcadores de CTGs normais e

marcadores que podem estar presentes em populações de CTTGs tais como CD44, CD133, CD24, LGR5 e ALDH1.

O marcador CD44, uma proteína de superfície celular, resulta em características como alta proliferação, capacidade de invasão, resistência à terapia e tumorigenicidade. Células positivas para CD133, uma glicoproteína de superfície celular altamente conservada, possuem correlação com fatores clínico-patológicos como a taxa de sobrevivência, o tamanho do tumor e a profundidade infiltrativa (RASSOULI *et al.*, 2016). CD24 é uma molécula de adesão celular cuja expressão em CTTGs mostrou associação com a capacidade de migração e invasão além de estar relacionada com características clínico-patológicas agressivas (BRUNGS *et al.*, 2016). LGR5 é uma proteína transmembrana receptora de R-espondina que media a sinalização de Wnt, e quando superexpressa, promove proliferação, migração e resistência quimioterápica em células de câncer gástrico (GLINKA *et al.*, 2011; WANG *et al.*, 2016). Por sua vez, células que expressam o marcador ALDH1 foram descritas com alta tumorigenicidade bem como com elevada capacidade de auto-renovação e de geração de populações celulares heterogêneas (KATSUNO *et al.*, 2012).

Além desses marcadores, foram sugeridos para identificação de CTTGs os marcadores clássicos de células tronco embrionárias SOX2, OCT4 e NANOG (BRUNGS *et al.*, 2016). Um estudo que avaliou a expressão desses três fatores de transcrição em ressecções cirúrgicas de pacientes com câncer gástrico verificou positividade para Sox2 em 55% das amostras, para Oct3/4 em 44% das amostras e para Nanog em 10% das amostras. Adicionalmente, uma correlação entre a capacidade de invasão e um pior prognóstico foi observada em amostras positivas para a expressão de Sox2 e negativas para Oct3/4 (MATSUOKA *et al.*, 2012).

3. Tratamentos convencionais do câncer gástrico

O procedimento padrão para tratar o câncer gástrico consiste na linfadenectomia e gastrectomia (CATALANO *et al.*, 2009; CHOI *et al.*, 2015). Existe ampla discussão sobre qual a extensão da ressecção cirúrgica necessária para reduzir as taxas de complicações e morte a fim de propiciar um aumento na sobrevida dos pacientes com câncer gástrico. A gastrectomia subtotal com reconstrução, geralmente é aplicada a tumores localizados no corpo e antro pilórico do estômago enquanto que a gastrectomia total é realizada quando o tumor localiza-se no fundo do estômago. Contudo, não há um consenso sobre o tipo de ressecção a ser realizada em tumores na região da cárdia. Além disso, como o primeiro sítio de metástase do câncer gástrico geralmente são linfonodos localizados próximos ao local do tumor, a ressecção dos linfonodos e sua avaliação também são muito importantes nesse tipo de câncer (MISLEH *et al.*, 2013).

Apesar de cirurgia ser o tratamento de escolha para o câncer gástrico, a maioria dos pacientes não responde de maneira efetiva, resultando em altas taxas de recidiva e baixa sobrevida associados à cirurgia como único tratamento (AJANI *et al.*, 2013). Por esse motivo, é de grande relevância a aplicação de terapias adjuvantes como radioterapia e/ou quimioterapia.

Os quimioterápicos geralmente podem ser classificados em quatro grupos principais: (i) antimetabólitos, (ii) agentes alquilantes, (iii) inibidores de topoisomerases e (iv) agentes antimitóticos. Antimetabólitos são moléculas similares aos precursores necessários na síntese de DNA ou RNA que podem levar à inibição da produção de nucleotídeos ou à incorporação dos antimetabólitos ao ácido nucléico, resultando em limitações na síntese de RNA e DNA e podendo levar à apoptose. Os agentes alquilantes interagem com o DNA pela

adição de um grupo alquila à guanina e pela formação de ligações covalentes. Esse mecanismo resulta na fragmentação do DNA por enzimas de reparo ao tentar remover a base alquilada e na interferência na replicação e transcrição do DNA (WHITEBAY *et al.*, 2013). Os inibidores de topoisomerases interferem nas funções normais de quebra, relaxamento de torção das fitas e ligação ao DNA da enzima topoisomerase. Um dos mecanismos desses inibidores consiste na estabilização da enzima, impedindo que ela se ligue ao DNA. Consequentemente, existe um aumento de quebras no DNA, ativando vias que levam à apoptose (PERRY *et al.*, 2012; WHITEBAY *et al.*, 2013). Os agentes antimitóticos têm como alvo diferentes proteínas envolvidas no processo mitótico, como proteínas motoras, cinases e tubulinas (ver Capítulo I). Essas drogas podem desequilibrar e bloquear a mitose, resultando em morte celular (CHAN *et al.*, 2012).

As principais drogas utilizadas nos diferentes regimes de tratamentos quimioterápicos contra o câncer gástrico são 5-fluorouracil (5-FU) e capecitabina (antimetabólitos), oxaliplatina e cisplatina (agentes alquilantes ou relacionados à alquilantes), irinotecan (inibidor de topoisomerase) e docetaxel e paclitaxel (antimitóticos que afetam os microtúbulos). Além dessas drogas, epirrubicina, uma antraciclina, e trastuzumab, um anticorpo monoclonal, também fazem parte de alguns dos regimes quimioterápicos recomendados (AJANI *et al.*, 2013; PERRY *et al.*, 2012; ZANIBONI & MERIGGI, 2005).

Dentre essas drogas, a quimioterapia baseada em 5-FU é considerada o tratamento de primeira linha para o câncer gástrico (LI *et al.*, 2016). O 5-FU é um análogo de uracila, cuja diferença consiste em um átomo de flúor no carbono da posição 5. Até o momento, os mecanismos de ação descritos envolvem inibição da enzima timidilato sintase (TS), que está envolvida na síntese de timidina, e a incorporação errônea ao RNA e DNA. A maior parte da

droga administrada é catabolizada no fígado pela enzima dihidropirimidina-desidrogenase (DPD), sendo convertida para dihidrofluorouracil (DHFU), sua forma inativa. O restante das moléculas não metabolizadas no fígado são transportadas para dentro das células onde serão transformadas em três metabólitos citotóxicos pela via anabólica (Figura 4): (i) monofosfato de fluorodesoxiuridina (FdUMP) - metabólito resultante da conversão de 5-FU pela enzima timidina fosforilase (TP) em fluorodesoxiuridina (FUdR) que é então fosforilada pela timidina cinase em FdUMP. Essa molécula liga-se à TS formando um complexo ternário que inibe a atividade da enzima; (ii) trifosfato de fluorodesoxiuridina (FdUTP) - metabólito resultante da fosforilação de FdUDP que incorpora-se ao DNA no lugar de trifosfato de timidina (dTTP); e (iii) trifosfato de fluorouridina (FUTP) - metabólito resultante da conversão de 5-FU pela enzima orotato fosforibosiltransferase (OPRT) em monofosfato de fluorouridina (FUMP), que é finalmente convertido em FUTP. Essa molécula é incorporada ao RNA no lugar de UTP (LONGLEY *et al.*, 2003; SHIMOYAMA, 2009; TAMATANI *et al.*, 2012).

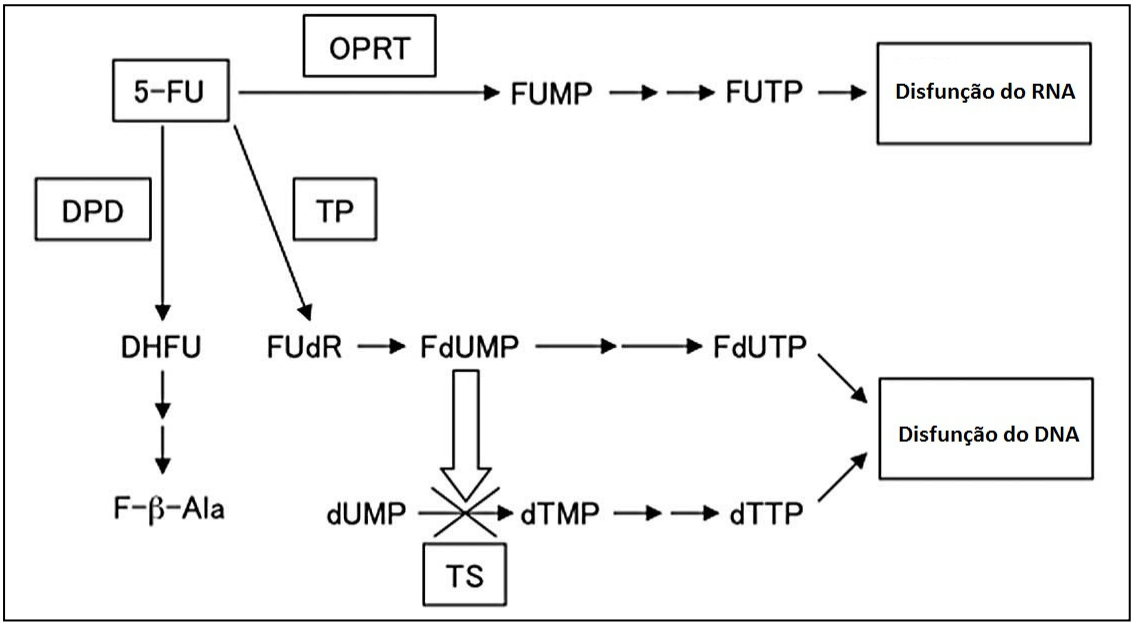

Figura 4. Via metabólica do 5-FU. Adaptado de TAMATANI *et al.* (2012).

A inibição da enzima TS, que causa um desequilíbrio de nucleotídeos, e a incorporação de FDUTP ao DNA, podem resultar em mutações pelo pareamento errôneo, falhas no reparo ao DNA, fragmentação do DNA por quebra simples ou dupla e inibição da síntese de DNA. A incorporação de FUTP ao RNA também pode ter diferentes efeitos tais como inibição do processamento do pré-rRNA em rRNA, inibição das modificações pós transcricionais de tRNAs, inibição de *splicing* no pré-mRNA e inibição da poliadenilação no mRNA (GHOSHAL & JACOB, 1997; LONGLEY *et al.*, 2003; MOJARDÍN *et al.*, 2013).

A administração do 5-FU é comumente realizada por infusão, contudo, o tratamento com fluoropirimidinas via oral, também vem sendo aplicado em casos de câncer gástrico. Estudos mostraram que houve uma mínima diferença na eficácia comparando-se a administração via oral com 5-FU infusional. A capecitabina é um exemplo de fluoropirimidina oral frequentemente utilizada para tratar esse tipo de tumor. Sua ativação ocorre através de dois processos iniciais no fígado e um processo final após atingir a célula tumoral através da enzima TP (MISLEH *et al.*, 2013).

Um aspecto relevante dos tratamentos quimioterápicos é o desenvolvimento de quimiorresistência. Atualmente, a quimioterapia é baseada principalmente na terapia combinatória, visando um tratamento mais efetivo na eliminação do tumor e limitando a toxicidade da droga (LARSSON *et al.*, 2009). Essa maximização no efeito do tratamento é resultante da sua atuação em vários alvos simultaneamente já que cada droga age sobre diferentes alvos moleculares (KITANO, 2007). Algumas das principais combinações recomendadas pelo guia do *National Comprehensive Cancer Network* no tratamento para o câncer gástrico consistem em oxaliplatina ou cisplatina combinada ao 5-FU infusional (ou oral - capecitabina), e em epirrubicina combinada com cisplatina e 5-FU (ECF) (AJANI *et al.*, 2013).

Além da resistência às drogas, outro obstáculo dos tratamentos quimioterápicos convencionais é sua baixa especificidade pelo tumor, pois como atuam em células proliferativas, atingem tanto células normais como tumorais resultando, clinicamente, em mais efeitos colaterais. Por esses motivos, diversos estudos com tratamentos direcionados a alvos específicos vêm sendo realizados considerando-se as anomalias de cada tumor. Essas anormalidades biológicas responsáveis pela evolução do câncer gástrico têm sido elucidadas e consistem basicamente em alterações genéticas e epigenéticas. O conhecimento sobre a complexidade e variabilidade molecular dos tumores permite a escolha de drogas que atuem nas vias responsáveis pela manutenção do câncer de acordo com cada paciente, evitando-se um regime quimioterápico desnecessário e obtendo-se uma resposta mais efetiva ao tratamento (CHOI *et al.*, 2015; WADDELL *et al.*, 2014).

Nesse sentido, poucos tratamentos direcionados foram aprovados pela FDA até o momento para tratar o câncer gástrico, como os anticorpos monoclonais trastuzumab e ramucirumab, que tem como alvo HER2 e VEGFR-2, respectivamente (MARQUÉS-LESPIER *et al.*, 2016). Contudo, outras terapias alvo têm sido estudadas e grande atenção tem sido destinada aos agentes antimitóticos.

4. Antimitóticos na terapia contra o câncer

Alterações genéticas ou epigenéticas são frequentemente observadas em genes envolvidos na progressão do ciclo celular, principalmente na fase G1 e no controle da transição da fase G1 para a fase S. Contudo, um grande de número de alterações em genes que possuem atividade na mitose, como manutenção do fuso mitótico e segregação cromossômica, já foram identificadas. Durante a mitose, os mecanismos que garantem a correta distribuição dos cromossomos para as células filhas são essenciais e qualquer falha

nesse processo pode resultar em instabilidade genômica, como aberrações cromossômicas, contribuindo para a transformação maligna (PÉREZ DE CASTRO *et al.*, 2007).

A maioria dos genes envolvidos na mitose que possuem alterações genéticas ou epigenéticas ou ainda, que possuem expressão alterada em tumores, atuam no ponto de checagem do fuso mitótico (38%). Genes com alterações, também estão frequentemente envolvidos nas funções do centrossomo e progressão de G2/M (26%) e na dinâmica do fuso (23%). Quanto à classificação da função molecular desses genes alterados, a maioria codifica proteínas cinases (26%), proteínas do cinetócoro (15%) e proteínas associadas aos microtúbulos, ao complexo passageiro cromossomal ou proteínas motoras (15%) (Figura 5)(PÉREZ DE CASTRO *et al.*, 2007).

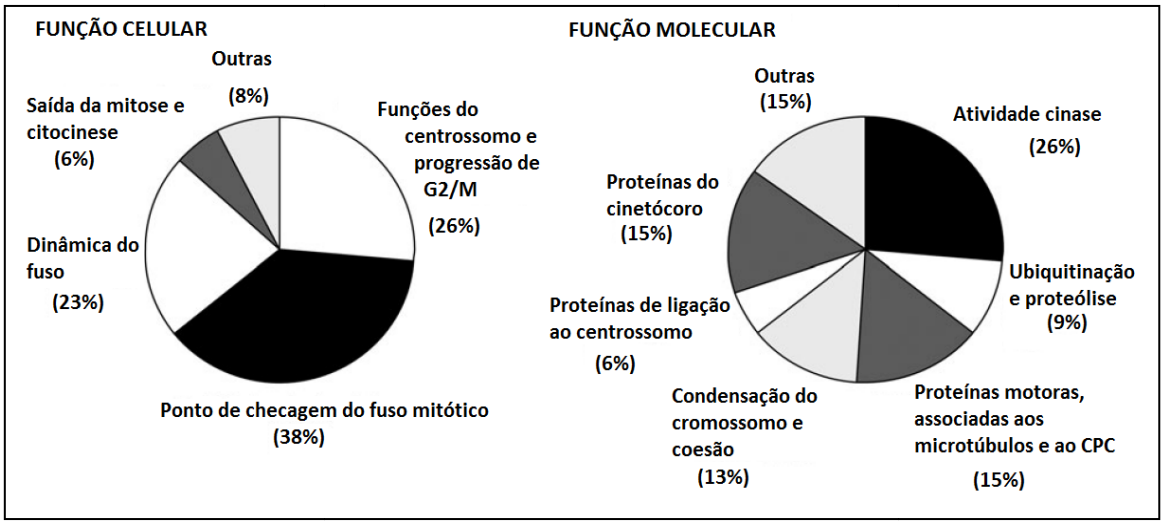

Figura 5. Principais funções celulares e moleculares de genes com alterações envolvidos na mitose. Adaptado de PÉREZ DE CASTRO *et al.* (2007).

Algumas dessas proteínas que resultam em anormalidades nas células tumorais têm sido consideradas como importantes alvos terapêuticos e por esse motivo, um grande número de agentes antimitóticos foi desenvolvido nos últimos anos. De maneira geral, o dano provocado por esse tipo de tratamento consiste em ativação do ponto de checagem do

fuso mitótico na tentativa de corrigir os erros, ou ainda no escape prematuro da mitose, podendo levar à morte celular (CHAN *et al.*, 2012).

Até o momento, os alvos da mitose mais estudados no tratamento contra o câncer incluem microtúbulos, cinases e proteínas motoras (ver Capítulo I). Os primeiros antimitóticos foram desenvolvidos há mais de 50 anos e possuem como alvo a tubulina. Sua ligação à tubulina pode estabilizar ou desestabilizar os microtúbulos, através do estímulo ou restrição da polimerização, respectivamente (JORDAN & WILSON, 2004). Os taxanos docetaxel e paclitaxel são exemplos de drogas estabilizadoras de microtúbulos que frequentemente são empregadas com sucesso no tratamento de tumores sólidos (STANTON *et al.*, 2011).

As principais drogas desenvolvidas que atuam nas cinases, possuem Plk1 ou aurora cinases como alvo. Plk1 faz parte de diversos processos da mitose e um deles consiste na maturação dos centrossomos (ver Capítulo I). Consequentemente, sua inibição *in vitro*, resultou em parada mitótica e fusos monopolares (SCHMIDT & BASTIANS, 2007). A inibição de aurora A (AURKA) possui efeitos semelhantes já que é uma proteína que também atua nos centrossomos (MALUMBRES & PÉREZ DE CASTRO, 2014). Contudo, a inibição de aurora B (AURKB), que possui atividade em diferentes pontos da mitose como alinhamento cromossômico (ver Capítulo I), resulta principalmente em células poliplóides devido à progressão do ciclo celular mesmo após bloqueio da divisão (SCHMIDT; BASTIANS, 2007). As proteínas motoras mais estudadas como alvos mitóticos são EG5 e CENP-E (ver Capítulo I). EG5 atua principalmente na separação dos centrossomos e na formação do fuso mitótico bipolar, sendo que o principal efeito resultante de sua inibição é a formação de fuso monopolar (JIANG *et al.*, 2006). Por sua vez, a inibição de CENP-E pode levar a um alinhamento incorreto dos cromossomos resultando em parada mitótica e morte celular ou

ainda levar a um enfraquecimento na interação cinetócoro-microtúbulo, resultando na ativação de ponto de checagem do fuso (CHAN *et al.*, 2012).

Considerando-se que com um maior direcionamento do tratamento pode-se obter uma resposta mais efetiva, é de suma importância a utilização técnicas que possam auxiliar no desenvolvimento de novas drogas para a terapia do câncer. O estudo de redes biológicas utilizando uma abordagem de biologia de sistemas tem sido aplicado com sucesso nas pesquisas sobre tumores (WIST *et al.*, 2009). A utilização de biologia de sistemas para analisar como compostos pequenos (incluindo quimioterápicos) interagem com proteínas ou outras macromoléculas levou ao desenvolvimento do campo da farmacologia de sistemas (BERGER & IYENGAR, 2009). Esta metodologia permite compreender como as drogas atuam em diferentes tecidos e tipos de células, bem como os efeitos de múltiplas ações dentro de um único tipo de célula que ocorrem devido à interação de diversas vias (BERGER & IYENGAR, 2009; WIST *et al.*, 2009).

Utilizando ferramentas de farmacologia de sistemas, um estudo recente feito pelo nosso grupo de pesquisas identificou como novos alvos potenciais para o tratamento quimioterápico de câncer gástrico, as proteínas AURKB e NDC80 (ou HEC1) (Figura 6) (ROSADO *et al.*, 2011). Estudos recentes observaram a superexpressão dessas proteínas no câncer gástrico, confirmando que podem representar uma nova estratégia terapêutica para esse tipo de tumor (HONMA *et al.*, 2014; QU *et al.*, 2014).

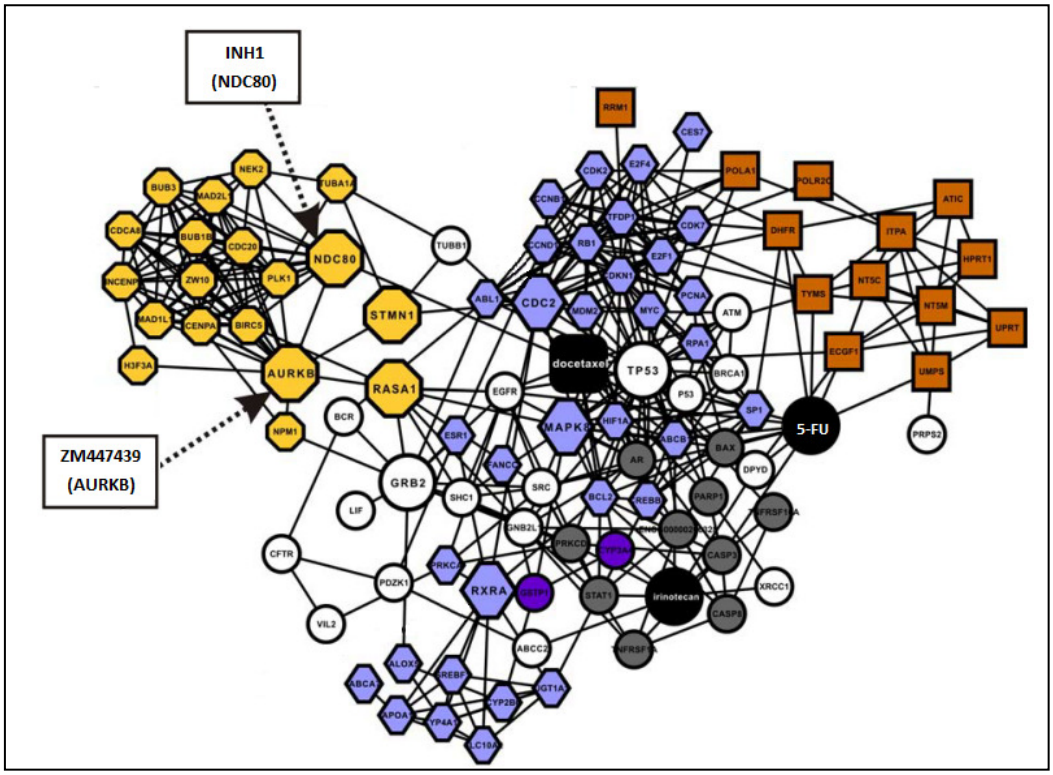

Figura 6. Identificação de novos alvos potenciais (AURKB e NDC80/HEC1) e os seus respectivos inibidores (dentro dos retângulos) para o tratamento quimioterápico do câncer gástrico a partir de dados de farmacologia de sistemas. As moléculas quimioterápicas comumente utilizadas no tratamento do câncer gástrico estão indicadas na rede por nós pretos. Adaptado de ROSADO *et al.* (2011).

5. As proteínas AURKB e NDC80 e seu papel na mitose

AURKB é uma serina treonina cinase que atua principalmente durante a mitose através da fosforilação da histona H3 e assegura o correto alinhamento e segregação dos cromossomos, bem como a execução da citocinese (CARMENA & EARNSHAW, 2003). AURKB faz parte do complexo passageiro cromossomal (CPC) que é composto também por borealina, survivina e INCENP (ver Capítulo I). Survivina e borealina se ligam à região N-terminal da proteína INCENP, enquanto AURKB se liga à região C-terminal. As subunidades não enzimáticas auxiliam na ativação de AURKB bem como no seu deslocamento durante a mitose, propiciando a fosforilação de seus diferentes substratos (CARMENA *et al.*, 2012; VAN DER HORST & LENS, 2014).

Durante a fase S do ciclo celular até o final da fase G2, a AURKB está presente nas regiões pericentroméricas e já apresenta atividade através da fosforilação da Serina 10 na histona H3. Quando a mitose inicia, a presença de AURKB e outras proteínas do CPC, pode ser verificada nos braços dos cromossomos. Contudo, ao final da prófase e durante a prometáfase e metáfase, o CPC desloca-se e concentra-se nos centrômeros (VAN DER HORST & LENS, 2014).

O centrômero de cada cromossomo possui dois complexos macromoleculares protéicos idênticos, chamados de cinetócoros, a partir dos quais são efetuadas ligações com os microtúbulos. Os cinetócoros são compostos por três camadas principais: uma placa externa, ou também, coroa fibrosa, uma camada intermediária e uma placa interna (MAIATO *et al.*, 2004; WAN *et al.*, 2009). Após a quebra do envelope nuclear, os cinetócoros são conectados ao fuso. Contudo, para que a transição metáfase/anáfase possa prosseguir com uma correta segregação cromossômica, é necessário que as cromátides irmãs estejam biorientadas, ou seja, os cinetócoros devem estar conectados a fusos de pólos opostos (ligação anfitélica). Caso a ligação seja monotélica, onde o cinetócoro está ligado ao microtúbulo de apenas um dos pólos, sintélica, quando os dois cinetócoros estão ligados a microtúbulos provenientes de um mesmo pólo, ou merotélica, onde o cinetócoro está conectado a microtúbulos de pólos diferentes, uma tensão incorreta é gerada e ocorre a ativação do ponto de checagem do fuso mitótico para que a ligação possa ser corrigida (Figura 7) (ANDREWS *et al.*, 2003).

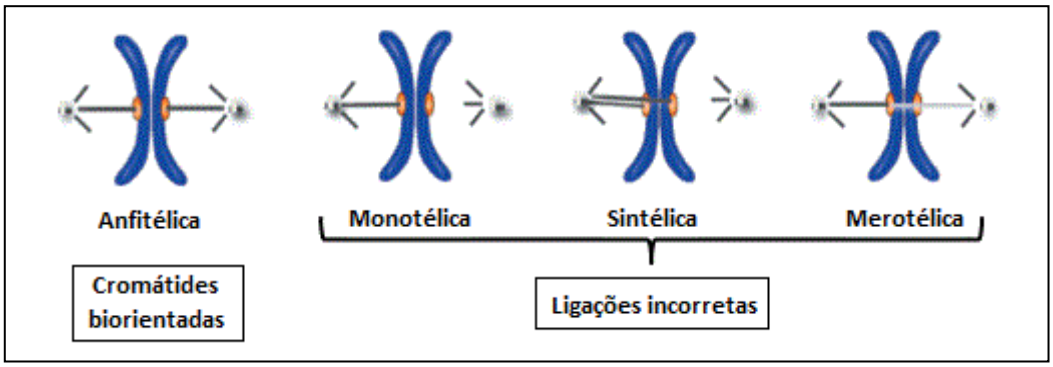

Figura 7. Diferentes tipos de ligações entre cinetócoros e microtúbulos que podem ocorrer no fuso mitótico. Adaptado de TANAKA & HIROTA (2009).

A AURKB possui papel essencial na estabilização de ligações incorretas, pois atua na interação dos microtúbulos com os cinetócoros. Um dos mecanismos de controle das ligações pela AURKB se dá através da fosforilação da proteína NDC80, reduzindo sua afinidade pelos microtúbulos (Figura 8) (CARMENA *et al.*, 2012; DELUCA *et al.*, 2006; VARMA & SALMON, 2012). NDC80 (ou HEC1) compõe juntamente com Nuf2, Spc24 e Spc25 o complexo NDC80 que faz parte de um complexo maior, chamado de KMN. O complexo KMN é composto ainda pela proteína Knl1 e pelo complexo Mis12, que contém as proteínas Mis12, Dsn1, Nnf1 e Nsl1 (WAN *et al.*, 2009). Estudos mostraram que esse complexo é essencial para a ligação do cinetócoro à extremidade positiva dos microtúbulos. A região N-terminal da proteína Hec1/NDC80 se liga aos microtúbulos, enquanto a região C-terminal de Spc24 e Spc25 se liga à proteína CENP-T ou ao complexo Mis12 para conectar o complexo NDC80 à placa interna do cinetócoro (VARMA & SALMON, 2012).

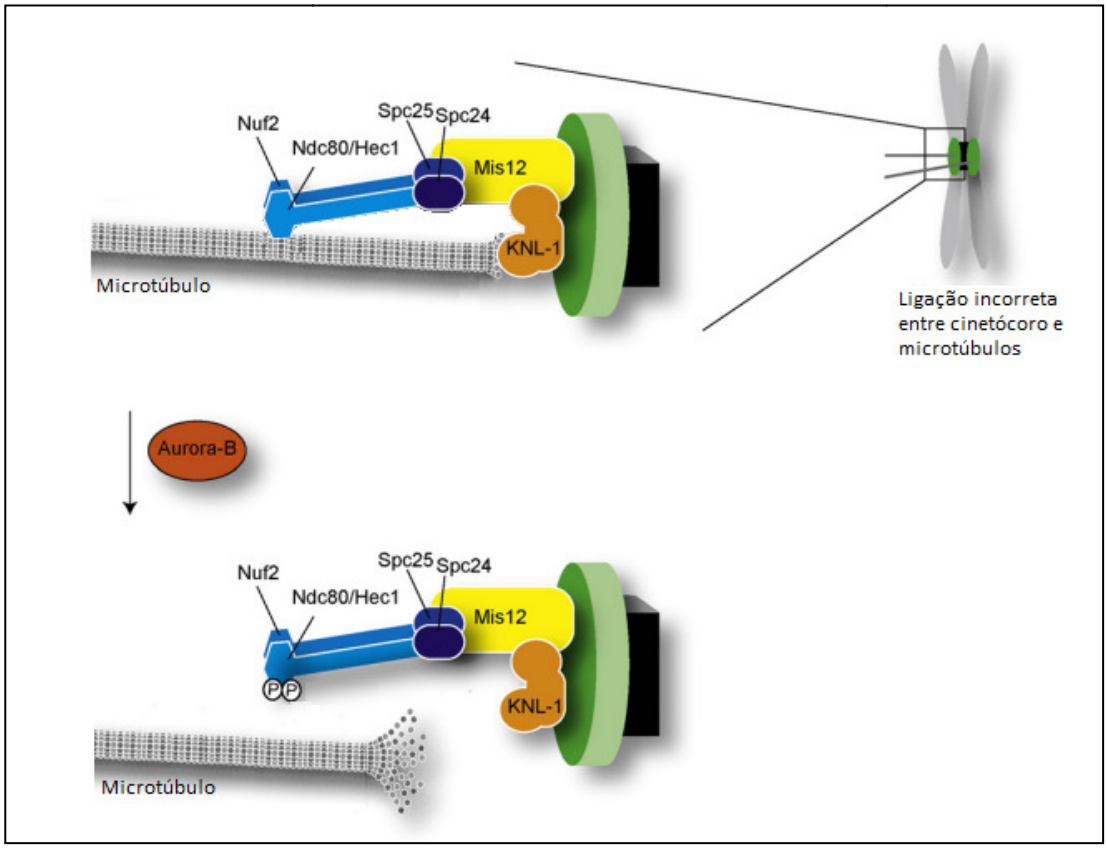

Figura 8. Estrutura do complexo NDC80 e fosforilação da proteína Hec1 pela AURKB, para correção da ligação incorreta do microtúbulo com o cinétocoro. Adaptado de VADER & LENS (2008).

A AURKB também fosforila a proteína MCAK, inibindo sua atividade de despolimerização dos microtúbulos. Sugere-se que essa capacidade de atuar quando existe uma ligação incorreta, deve-se a uma aproximação dos substratos, enquanto que na ligação anfitélica, a tensão gerada afasta os substratos da enzima AURKB impedindo sua atividade, já que não é mais necessária (WALCZAK *et al.*, 2010).

Quando o último cromossomo estiver alinhado no plano equatorial, a célula encontra-se na metáfase. O rompimento da coesão entre as cromátides irmãs permitindo seu deslocamento em direção aos pólos opostos, marca a transição para anáfase. Ao iniciar a anáfase, o CPC se desloca para o fuso central e sulco de clivagem (WALCZAK *et al.*, 2010). Durante a anáfase, o complexo auxilia na compactação das cromátides e estabilização do fuso e durante a citocinese, contribui para a maturação do anel contrátil e regulação da

dinâmica do citoesqueleto (CARMENA *et al.*, 2012). Ao final da mitose, o complexo promotor de anáfase (APC/C) reconhece AURKB que é ubiquitinada e degradada pelo proteassoma (PORTELLA *et al.*, 2011).

6. Os inibidores ZM447439 e INH1

Segundo ROSADO *et al.* (2011), as moléculas ZM447439 e INH1 mostraram-se inibidores potentes e seletivos das proteínas anteriormente abordadas AURKB e NDC80, respectivamente.

A molécula ZM447439, primeiro inibidor de aurora cinase desenvolvido, é um composto derivado de quinazolina cujo mecanismo de ação consiste em inibir a enzima AURKB por meio da competição com ATP pela ligação ao sítio ativo (Figura 9). Essa molécula também foi capaz de inibir AURKA. Contudo, a dose necessária foi vinte vezes maior do que para AURKB, e as células apresentaram o fenótipo característico de inibição de AURKB e não de AURKA ou de outras cinases (DITCHFIELD, 2003; GIRDLER *et al.*, 2006; YOON *et al.*, 2012). Além disso, GIRDLER *et al.* (2006) sugerem que em baixas concentrações micromolares, ZM447439 não é um inibidor significativo da atividade de AURKA nas células**.** Até o momento, somente estudos *in vitro* foram realizados com ZM447439 e os principais efeitos observados nas células foram o incorreto alinhamento e segregação dos cromossomos, ultrapassagem do ponto de checagem do fuso mitótico e a inibição da citocinese, resultando em células com conteúdo elevado de DNA devido à endoreduplicação (DITCHFIELD, 2003).

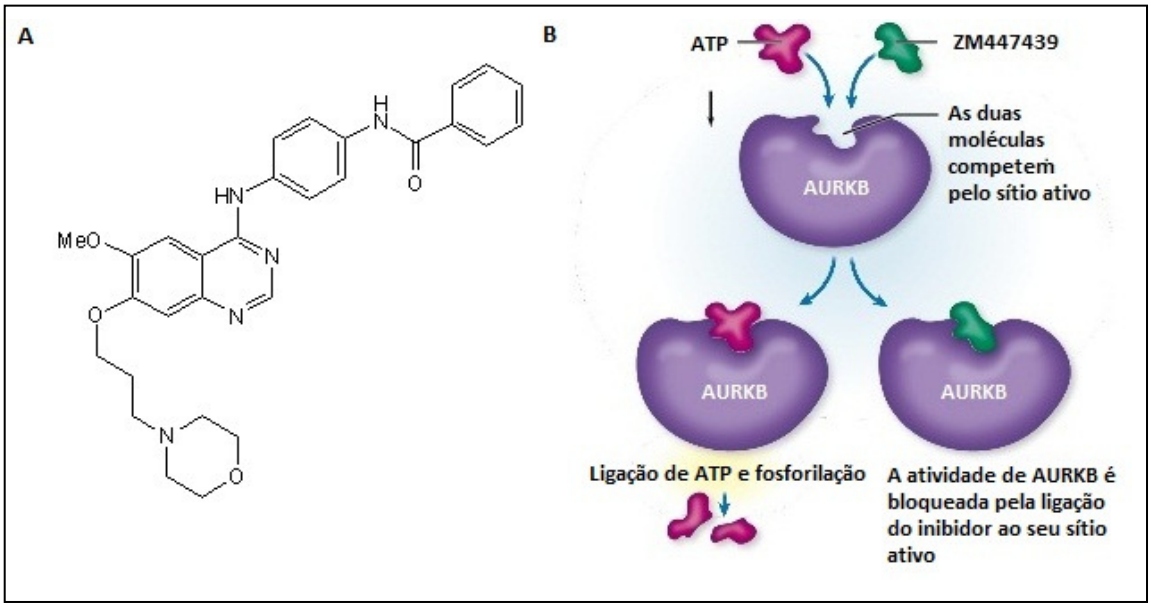

Figura 9. **A.** Estrutura molecular do inibidor ZM447439 **B.** Mecanismo de ação do inibidor consiste na competição pelo sítio de ligação de ATP. Adaptado de COWAN (2012) e "Tocris Bioscience" (2016).

O composto INH1, que também atua na mitose, se liga à proteína NDC80 impedindo sua ligação com a cinase NEK2 e consequentemente sua ativação (Figura 10). Essa droga já foi testada em camundongos imunodeficientes com xenoenxertos tumorais provenientes de células de câncer de mama. O tratamento resultou em mínimos efeitos colaterais e uma redução significativa do crescimento tumoral. Quando avaliada *in vitro*, a terapia com INH1 provocou uma localização anormal de NDC80 nos cinetócoros, redução significativa de NEK2 (efeito indireto da droga já que sua redução se mostrou independente de NDC80), além de anormalidades mitóticas, como desalinhamento cromossômico e irregularidades no fuso mitótico, ocasionando eventual morte celular (WU *et al.*, 2008).

Os compostos ZM447439 e INH1 já foram estudados em outros tipos de cânceres como o de mama (VIDARSDOTTIR *et al.*, 2012; WU *et al.*, 2008), cólon (KAESTNER *et al.*, 2009), cérvix (ZHANG & ZHANG, 2011), leucemia (WALSBY *et al.*, 2008) e glioblastoma

(BORGES *et al.*, 2012). Contudo, nenhuma destas moléculas foi utilizada isoladamente ou em combinação com quimioterápicos convencionais no tratamento de câncer gástrico.

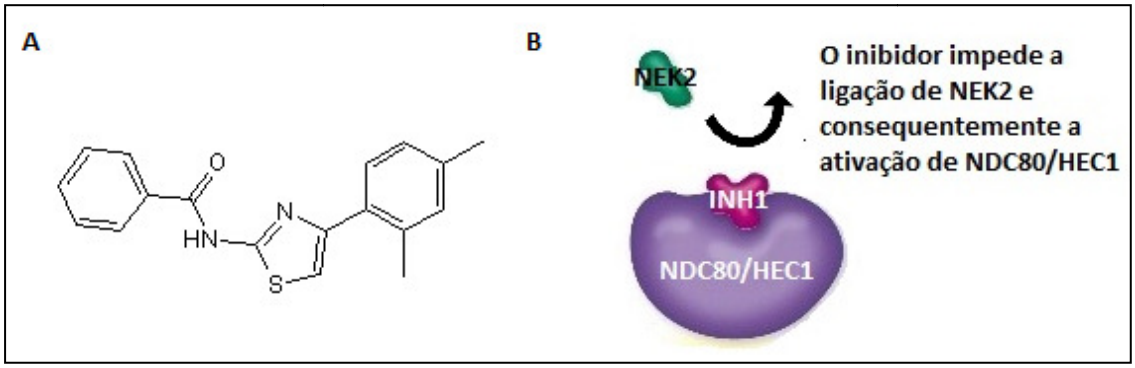

Figura 10. A. Estrutura molecular do inibidor INH1 **B.** Mecanismo de ação do inibidor consiste na ligação ao NDC80, impedindo sua ativação por NEK2. Adaptado de COWAN (2012) e "Tocris Bioscience" (2016).

Considerando-se a atual dificuldade no tratamento do câncer gástrico devido ao diagnóstico tardio e à resistência aos quimioterápicos convencionais, o desenvolvimento e estudo de novos compostos que atuem em alvos específicos para inclusão em regimes combinatórios a fim de permitir terapias mais direcionadas, é de extrema relevância. Por esse motivo, essa pesquisa consiste na avaliação do tratamento *in vitro* de linhagens de câncer gástrico com os compostos ZM447439 e INH1, inibidores de AURKB e NDC80, respectivamente.

OBJETIVOS

Objetivo gerail

Investigar os efeitos dos compostos INH1 e ZM447439 utilizados isoladamente e/ou em combinação com o agente quimioterápico clássico 5-FU, nas linhagens de adenocarcinoma gástrico ACP02 e ACP03.

Objetivos específicos

- Determinar o IC_{50} (Concentração Inibitória de 50 %) dos compostos 5-FU, ZM447439 e INH1 utilizados nas linhagens ACP02 e ACP03.
- Avaliar a expressão de genes frequentemente envolvidos em processos tumorais, nas linhagens ACP02 e ACP03 e na linhagem de epitélio gástrico não tumoral MN01, expostas e não expostas aos compostos.
- Avaliar o efeito dos inibidores ZM447439 e INH1 e do agente quimioterápico 5-FU sobre as linhagens ACP02, ACP03 e MN01 quanto à proliferação, ciclo celular, morfologia nuclear, morte e migração celular.
- Avaliar a presença de células tronco tumorais nas linhagens estudadas pelo ensaio de formação de esferas e expressão de marcadores tronco.

CAPÍTULO I – ARTIGO DE REVISÃO

ANTI-MITOTIC AGENTS: ARE THEY EMERGING MOLECULES FOR CANCER TREATMENT?

Artigo aceito pela revista *Pharmacology & Therapeutics*

ARTICLE IN PRESS
JPT-07015; No of Pages 16

Pharmacology & Therapeutics xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

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

Associate editor: Beverly Teicher

Anti-mitotic agents: Are they emerging molecules for cancer treatment?☆

Larissa Siqueira Penna, João Antonio Pêgas Henriques, Diego Bonatto *

Biotechnology Center of the Federal University of Rio Grande do Sul, Department of Molecular Biology and Biotechnology, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

ARTICLE INFO

Keywords:
Mitosis
Mitotic inhibitors
Cancer
Clinical trials
Cancer treatment

ABSTRACT

Mutations in cancer cells frequently result in cell cycle alterations that lead to unrestricted growth compared to normal cells. Considering this phenomenon, many drugs have been developed to inhibit different cell-cycle phases. Mitotic phase targeting disturbs mitosis in tumor cells, triggers the spindle assembly checkpoint and frequently results in cell death. The first anti-mitotics to enter clinical trials aimed to target tubulin. Although these drugs improved the treatment of certain cancers, and many anti-microtubule compounds are already approved for clinical use, severe adverse events such as neuropathies were observed. Since then, efforts have been focused on the development of drugs that also target kinases, motor proteins and multi-protein complexes involved in mitosis. In this review, we summarize the major proteins involved in the mitotic phase that can also be targeted for cancer treatment. Finally, we address the activity of anti-mitotic drugs tested in clinical trials in recent years.

Contents

1. Introduction

The mitotic phase is a crucial time period during which chromosomes must be segregated to opposite poles, eventually resulting in two identical daughter cells (Mogilner, Wollman, Civelekoglu-Scholey, & Scholey, 2006). It consists of several phases known as prophase, prometaphase, metaphase, anaphase and telophase (Pines, 2006). During prophase, the duplicated chromosomes from interphase condense, and the nuclear envelope breaks down. During prometaphase, kinetochores connect chromosomes to microtubules, and in metaphase, the chromosomes align at the equatorial plate between the spindle poles. Chromatids are separated and pulled to opposite poles during anaphase. Finally, the mitotic spindle disassembles, and the chromatids decondense and are surrounded by new nuclear envelopes in telophase. The cytokinesis process results in two daughter cells *via* division of the cytoplasm of the parent cell (Fig. 1) (Jongsma, Berlin, & Neefjes, 2014; Walczak, Cai, & Khodjakov, 2010). After taxanes and vinca alkaloids were shown to be clinically effective as anti-tumor compounds, many other drugs were developed to also target microtubules, kinases, motor proteins and multi-protein complexes, resulting in mitotic arrest

Abbreviations: AE, adverse event; AML, acute myeloid leukemia; APC, anaphase promoting complex; CLL, chronic lymphocytic leukemia; CPC, chromosomal passenger complex; CR, complete response; CRi, complete remission with incomplete blood count recovery; FDA, Food and Drug Administration; LDAC, low-dose cytosine arabinoside; MCC, mitotic checkpoint complex; NSCLC, non-small cell lung cancer; PD, progressive disease; P-gp, P-glycoprotein; PR, partial response; SAC, spindle assembly checkpoint; SD, stable disease; SCLC, small cell lung cancer.

☆ Contract/grant sponsor: CNPq, CAPES

* Corresponding author at: Centro de Biotecnologia da UFRGS, Sala 219, Departamento de Biologia Molecular e Biotecnologia, Universidade Federal do Rio Grande do Sul, UFRGS, Avenida Bento Gonçalves 9500, Prédio 43421, Caixa Postal 15005, Porto Alegre, Rio Grande do Sul 91509-900, Brazil.
E-mail addresses: diegobonatto@gmail.com, diego@cbiot.ufrgs.br (D. Bonatto).

http://dx.doi.org/10.1016/j.pharmthera.2017.02.007
0163-7258/

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

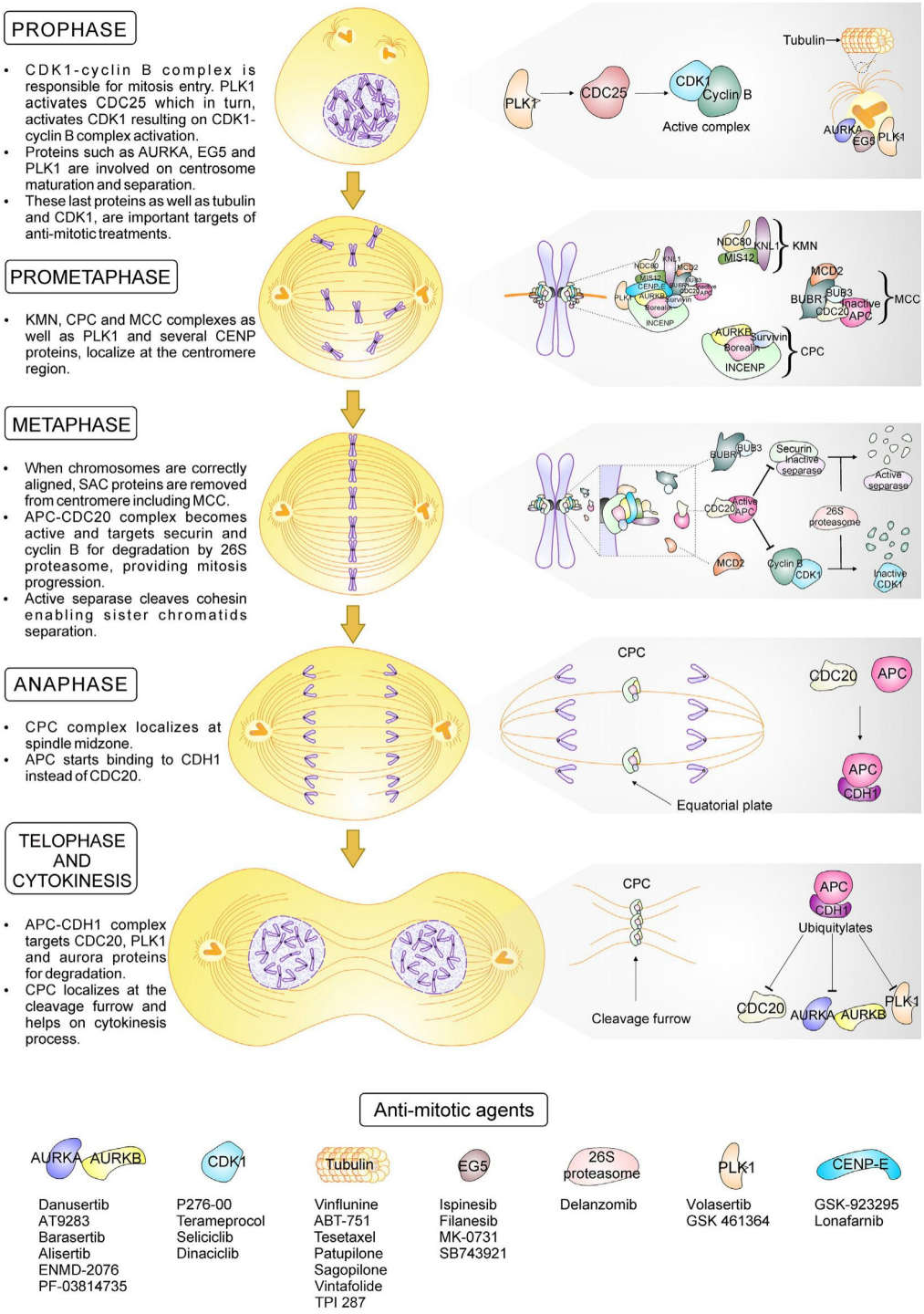

Fig. 1. Representation of the major proteins and complexes involved in mitosis as well as drugs that target them.

(triggered by spindle assembly checkpoint-SAC) and mitotic cell death (Chan, Koh, & Li, 2012). A number of these compounds did not achieve high response rates in clinical trials; therefore, the incorporation of new drug development into combination therapies with conventional chemotherapy remains a challenge. In this article, we will review clinical experimental anti-mitotic drugs for cancer therapy, and focus on drugs that are not yet approved by the Food and Drug Administration (FDA).

2. Overview of the major proteins involved in mitosis

The SAC is the most important mechanism of the mitotic phase, and it ensures that anaphase will not occur until the chromosomes are correctly aligned at the equatorial plate (Pérez de Castro, de Cárcer, & Malumbres, 2007). In this sense, cell cycle regulators such as the CDK1-cyclin B complex are important components of SAC (D'Angiolella, Mari, Nocera, Rametti, & Grieco, 2003). CDK1 is a crucial

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

protein involved in cell cycle regulation that binds to A and B cyclins, and during early mitosis, PLK1 aids in regulating this complex because it phosphorylates and activates CDC25, which in turn activates CDK1 by dephosphorylation (Fig. 1). PLK1 is also capable of negatively regulating Wee1 and Myt1, which are both involved in the inhibition of CDK1 (Gautier, Solomon, Booher, Bazan, & Kirschner, 1991; Kumagai & Dunphy, 1996; Malumbres & Barbacid, 2005; McGowan & Russell, 1993; Mueller, Coleman, Kumagai, & Dunphy, 1995; Nigg, Blangy, & Lane, 1996). The CDK1-cyclin B complex regulates mitosis entry and progression through phosphorylation of over 70 proteins, including the anaphase promoting complex (APC), which binds to CDC20 and is consequently activated (Malumbres & Barbacid, 2005; Pines, 2006). APC is a ubiquitin ligase that consists of 11 subunits in animal cells, and its function is to target proteins for degradation. The ubiquitination process is performed by E2 enzymes, and proteolysis of targeted proteins is conducted by the 26S proteasome (Hershko & Ciechanover, 1998; Peters, 2002; Pines, 2006; Wieser & Pines, 2015). The APC-CDC20 complex first targets cyclin A and NEK2A at the beginning of mitosis, before the spindle checkpoint (Geley et al., 2001; Hames, Wattam, Yamano, Bacchieri, & Fry, 2001). If any incorrect attachment occurs between kinetochores and microtubules during prophase, the APC is inactivated and SAC is activated (Rieder, Schultz, Cole, & Sluder, 1994). This checkpoint is performed by several proteins that localize to three different layers of the kinetochores, namely, the inner plate, the outer plate, and when microtubules are not attached, the fibrous corona (McEwen, Hsieh, Mattheyses, & Rieder, 1998; Musacchio & Salmon, 2007). CENP-A protein localizes at the inner plate and targets the centromere because it is a histone H3 homolog (Marshall, Marshall, & Choo, 2008).

The outer kinetochore consists primarily of KNL-1 protein, and Mis12 and NDC80 complexes (KMN network) (Fig. 1), which are responsible for regulating the kinetochore-microtubule attachment. This association with the outer kinetochore mainly serves as a base for other SAC proteins and cooperates in microtubule attachment to the centromeres (Cheeseman, Chappie, Wilson-Kubalek, & Desai, 2006; Varma & Salmon, 2012). KNL-1 is required for correct microtubule attachment and also acts on proteins responsible for SAC activation and inhibition (Caldas & DeLuca, 2014). The Mis12 complex was shown to be important for correct segregation of sister chromatids and interaction with ZWINT, a protein that binds to ZW10, which recruits MAD1 and MAD2 to the unattached kinetochores (Goshima, Kiyomitsu, Yoda, & Yanagida, 2003; Kops, 2005; Obuse et al., 2004). The NDC80 complex is composed of NDC80, NUF2, SPC24 and SPC25, and aids in the recruitment of MPS1, MAD1 and MAD2 to the kinetochores (Martin-Lluesma, Stucke, & Nigg, 2002). NDC80 and NUF2 bind directly to the microtubules, and these proteins bind to SPC24 and SPC25, which connect this complex to the kinetochores (Wan et al., 2009).

MAD2, BUBR1, BUB3 and CDC20 are essential SAC proteins that form the mitotic checkpoint complex (MCC) (Fig. 1). The main function of MCC is to prevent APC activity, which occurs through MCC binding to CDC20, thus inhibiting APC's activity since CDC20 is a cofactor of APC (Sudakin, Chan, & Yen, 2001). Another important protein is BUB1. This kinase is found in lower concentrations after attachment and is recruited when the tension at the kinetochores is reduced (Taylor, Hussein, Wang, Elderkin, & Morrow, 2001). BUB1 has an important role in the recruitment of MAD2, BUBR1, CENP-F, CENP-E and other SAC proteins to the kinetochores and centromeres (Johnson, Scott, Holt, Hussein, & Taylor, 2004; Musacchio & Salmon, 2007), and also aids in the association of MCC to APC (Morrow, 2005). CENP-E is an important kinesin-like motor protein that attaches to the kinetochores at the beginning of mitosis and moves to the spindle mid-zone during anaphase (Yen, Li, Schaar, Szilak, & Cleveland, 1992).

Some key proteins, such as the components of the chromosomal passenger complex – CPC (borealin, survivin, INCENP and AURKB), localize at the centromere until metaphase. During anaphase, CPC is found at the center of the spindle apparatus, and after telophase it supports the process of cytokinesis (Fig. 1) (Cooke, Heck, & Earnshaw, 1987; Gassmann et al., 2004; van der Horst & Lens, 2014). MCAK interacts with CPC, is controlled by phosphorylation by AURKB, and localizes at the centromeres as well. MCAK is involved in microtubule attachment to the kinetochores and chromosome alignment and segregation (Andrews et al., 2004; Kline-Smith, 2003; Knowlton, Lan, & Stukenberg, 2006; Wordeman & Mitchison, 1995). AURKB is an important SAC component that prevents the cell cycle from continuing if the correct tension does not exist between kinetochores. By interacting with the MCAK and NDC80 complex, AURKB can control the stabilization of kinetochores attachment to the microtubules. Phosphorylation of NDC80 drives the detachment of microtubules, enabling the correction of improper attachments (Andrews et al., 2004; DeLuca et al., 2006). AURKB is also a component of processes such as phosphorylation of H3 histone, chromosome alignment and cytokinesis (Crosio et al., 2002; Ditchfield, 2003). Survivin mediates binding of CPC to the centromere, and borealin assists in INCENP and survivin connection (Vader, Kauw, Medema, & Lens, 2006). INCENP is important for AURKB regulation, and its binding activates AURKB, whereas AURKB phosphorylates INCENP (Honda, 2003). INCENP is also phosphorylated by CDK1, aiding in its binding and recruitment of PLK1 (Goto et al., 2006).

Certain important proteins localize at the centrosome, such as AURKA, EG5 and PLK1 (Fig. 1). During prophase, AURKA primarily localizes at the centrosomes to support their maturation and separation (Hirota et al., 2003; Marumoto, Zhang, & Saya, 2005; Marumoto et al., 2003). The kinesin-related motor protein EG5 has a similar role in promoting centrosome separation, resulting in two spindle poles, and PLK1 is involved in centrosome maturation (Blangy et al., 1995; Lane & Nigg, 1996).

After the chromosomes are correctly positioned, SAC proteins are removed, and MCC is also disconnected from APC (Fig. 1). The end of SAC is marked by dynein, which is responsible for removing proteins from the kinetochores and redistributing them to the poles, and by proteolysis, which is triggered by APC that targets cyclin B and securin for degradation (Fig. 1) (Funabiki et al., 1996; Howell et al., 2001; Murray, 1995). Degradation of both proteins results in the progression of mitosis through anaphase since the targeting of cyclin B inactivates CDK1 activity, and securin negatively regulates the protease separase. Thus, APC activity results in separase activation, which cleaves cohesin to enable sister chromatid separation (Peters, 2006; Wieser & Pines, 2015). During anaphase, APC begins to bind to CDH1 instead of CDC20, and this new complex is responsible for targeting proteins such as PLK1, AURKA and finally AURKB (Fig. 1) (Lindon & Pines, 2004; Stewart & Fang, 2005). PLK1 is also a component of the group of proteins involved in mitotic exit since it activates CDC16 and CDC27 (members of APC) and phosphorylates cohesin, thus facilitating its cleavage by separase (Hauf et al., 2005; Jackson, Patrick, Dar, & Huang, 2007; Kraft et al., 2003).

3. Targets of anti-mitotic treatments and drugs tested in clinical trials

Several mitotic targets have been explored in recent years, including drugs that target microtubules, kinases, motor proteins and multi-protein complexes, such as the 26S proteasome (Chan et al., 2012). Clinical trials usually classify tumors according to the Response Evaluation Criteria in Solid Tumors. In summary, complete response (CR) is related to the disappearance of lesions, partial response (PR) refers to a minimum of 30% reduction in the tumor size, progressive disease (PD) indicates a minimum of 20% increase in the tumor size, and stable disease (SD) denotes a tumor size classified between PR and PD.

3.1. Targeting microtubules

Anti-microtubular drugs were the first treatment described for targeting mitosis and have been successfully used against different

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

Table 1
Summarized results of clinical trials with anti-mitotic drugs not yet approved by the FDA.

Targeted protein	Compound	Administration route	Range of doses tested (schedules were different across studies)	Cancer type treated	% of objective response (CR + PR) and clinical trial phase
Tubulin	Vinflunine	Oral and intravenous	30 mg/m^2–400 mg/m^2	Solid tumors	12% (phase I) (Bennouna et al., 2003), 0% (phase I) (Johnson et al., 2006), 3% (phase I) (Calvo et al., 2012), 0% (phase I)[a] (Sanoff et al., 2011a), 7.7% (phase I)[a] (Sanoff et al., 2011b), 3.8% (phase I) (Delord et al., 2013)
				Prostate adenocarcinoma	3% (phase II) (Hainsworth et al., 2010)
				Renal cell carcinoma	3% (phase II) (Goldstein et al., 2006)
				Bladder cancer	18% (phase II) (Culine et al., 2006)
				Urothelial carcinoma	14.6% (phase II) (Vaughn et al., 2009)
				NSCLC	10% (phase II) (Bennouna et al., 2006), 32.1% (phase I/II)[a] (Souquet et al., 2010), 37% (phase I)[a] (Tournoux-Facon et al., 2011), 36.8% (phase I)[a] (Tournoux-Facon et al., 2012), 8% (phase I)[a] (Krzakowski et al., 2014)
				SCLC	25% (phase II) (Spigel et al., 2010)
				Breast cancer	8.3% (phase II) (Blasinska-Morawiec et al., 2013), 14% (phase II) (Fumoleau et al., 2009), 30% (phase II) (Campone et al., 2006), 48% (phase I)[a] (Zaman et al., 2011), 73.3% (phase I)[a] (Paridaens et al., 2012), 48.6% (phase I)[a] (Chan et al., 2014), 9% (phase II) (Yardley et al., 2010), 33% (phase II)[a] (Yardley et al., 2010), 43.8% (phase I)[a] (Campone et al., 2012)
				Melanoma	3% (phase II) (Olver et al., 2007)
				Pleural mesothelioma	14.5% (phase II) (Talbot et al., 2007)
	EC145 (vintafolide)	Intravenous	1 mg/day–4 mg/day	Solid tumors	3.1% (phase I) (Lorusso et al., 2012)
				Ovarian cancer	5% (phase II) (Morris et al., 2014), 18% (phase II)[a] (Naumann et al., 2013)
				Lung adenocarcinoma	2.3% (phase II) (Edelman et al., 2012)
	ABT-751	Oral	100 mg/day–375 mg/day	Solid tumors	0% (phase I) (Fox et al., 2006, 2008; Hande et al., 2006)
				Solid tumors and/or neuroblastoma	0% (phase I) (Meany et al., 2010)
				Hematologic malignancies	17.2% (phase I) (Yee et al., 2005)
				NSCLC	2.9% (phase II) (Mauer et al., 2008), 11% (phase I)[a] (Ma et al., 2012)
				Prostate cancer	45% (phase I)[a] (Michels et al., 2010)
				Neuroblastoma	7% (phase II) (Fox et al., 2014)
	Tesetaxel	Oral	18 mg/m^2–35 mg/m^2	Solid tumors	0% (phase I)[a] (Saif et al., 2011)
				NSCLC	7.6% (phase I/II) (Baas et al., 2008)
	TPI 287	Intravenous	90 mg/m^2–125 mg/m^2	Neuroblastoma and medulloblastoma	0% (phase I), 12.5% (phase I)[a] (Mitchell et al., 2016)
	Patupilone (EPO906)	Intravenous	0.3 mg/m^2–11 mg/m^2	Solid tumors	5% (phase I) (Rubin et al., 2005), 7.5% (phase I) (Tsimberidou et al., 2011), 62% (phase I)[a] (Forster et al., 2007), 20% (phase I)[a] (Schelman et al., 2008)
				Brain metastases from breast cancer	13% (phase II) (Peereboom et al., 2014)
				Colorectal cancer	0% (phase II) (Moorcraft et al., 2013)
				Epithelial ovarian, primary fallopian tube or primary peritoneal cancer	15.5% (phase III) (Colombo et al., 2012), 19.5% (phase I) (Ten Bokkel Huinink et al., 2009)
				Colon cancer	7% (phase I) (Melichar et al., 2011)
				Prostate cancer	0% (phase II) (Hussain et al., 2009), 24% (phase II) (Chi et al., 2012)
				CNS malignancies	9% (phase I) (Fogh et al., 2010)
	Sagopilone (ZK-EPO)	Intravenous	0.6 mg/m^2–29 mg/m^2	Solid tumors	0% (phase I) (Arnold et al., 2009), 0% (phase I) (Araki et al., 2012), 6.7% (phase I) (Schmid et al., 2010)
				Glioma	0% (phase II) (Silvani et al., 2009; Stupp et al., 2011)
				Breast cancer/breast cancer brain metastases	4.6% (phase II) (Morrow et al., 2010)/13.3% (phase II) (Freedman et al., 2011)
				Ovarian cancer	17.7% (phase II) (Rustin et al., 2011), 63.5% (phase I/II)[a] (McMeekin et al., 2012)
				Prostate cancer	33.4% (phase II) (Beer et al., 2012)
				Melanoma	8.6% (phase II) (DeConti et al., 2010)
				SCLC	57.7% (phase I)[a] (Gauler et al., 2013)
				NSCLC	7% (phase III) (Heigener et al., 2013)
Auroras A, B and/or C	Alisertib (MLN-8237)	Oral	5 mg/day–200 mg/day	Solid tumors	0% (phase I) (Cervantes et al., 2012), 3% (phase I) (Mossé et al., 2012), 1% (phase I) (Dees et al., 2012), 13.2% (phase II) (Melichar et al., 2015)
				Epithelial ovarian, fallopian tube, primary peritoneal cancer	10% (phase II) (Matulonis et al., 2012)
				Chronic lymphocytic leukemia and/or B- and T-cell non-Hodgkin's lymphomas	13% (phase I) (Kelly et al., 2014), 27% (phase II) (Friedberg et al., 2014)

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

	Danusertib (PHA-739358)	Intravenous	45 mg/m^2–1000 mg/m^2	Prostate cancer	0% (phase II) (Meulenbeld et al., 2013)
				Solid tumors	0% (phase I) (Steeghs et al., 2009), 2.4% (phase I) (Cohen et al., 2009)
	AT9283	Intravenous	1.5 mg/m^2–162 mg/m^2	Solid tumors	0% (phase I) (Arkenau et al., 2012), 4.3% (phase I) (Moreno et al., 2015)
				Solid tumors and non-Hodgkin's lymphoma	3.1% (phase I) (Dent et al., 2013)
				Leukemias or myelofibrosis	0% (phase I) (Foran et al., 2014)
	Barasertib (AZD1152)	Intravenous	7.1 mg/day–650 mg/day	Solid tumors	0% (phase I) (Boss et al., 2011; Schwartz et al., 2013)
				AML	19% (phase I) (Tsuboi et al., 2011), 25% (phase I/II) (Löwenberg et al., 2011), 45% (phase I)[a] (Kantarjian et al., 2013b), 25% (phase I) (Dennis et al., 2012)
				Diffuse large B-cell lymphoma	20% (phase II) (Collins et al., 2015)
	ENMD-2076	Oral	30 mg/m^2–350 mg/m^2	Solid tumors	3% (phase I) (Diamond et al., 2011)
				Epithelial ovarian cancer (EOC), fallopian tube or peritoneal cancer	8% (phase II) (Matulonis et al., 2013)
	PF-03814735	Oral	5 mg/day–100 mg/day	Solid tumors	0% (phase I) (Schöffski et al., 2011)
PLK1	Volasertib (BI 6727)	Intravenous	12 mg/day–450 mg/day	Solid tumors	4.6% (phase I) (Schöffski et al., 2012), 3.4% (phase I) (Lin et al., 2014), 6.9% (phase I)[a] (Machiels et al., 2015)
				Urothelial cancer	14% (phase II) (Stadler et al., 2014)
				AML	31% (phase II)[a] (Döhner et al., 2014)
	GSK 461364	Intravenous	25 mg/day–300 mg/day	Solid tumors	0% (phase I) (Olmos et al., 2011)
CDK1	P276–00	Intravenous	185 mg/m^2	Mantle cell lymphoma	0% (phase II) (Cassaday et al., 2015)
	Terameprocol	Intravenous	750 mg/day–2200 mg/day	Glioma	0% (phase I) (Grossman et al., 2012)
				Leukemia	7% (phase I) (Tibes et al., 2015)
	Dinaciclib	Intravenous	0.33 mg/m^2–70 mg/m^2	Solid tumors	0% (phase I) (Nemunaitis et al., 2013)
				Acute leukemias	0% (phase II) (Gojo et al., 2013)
				CLL	54% (phase I) (Flynn et al., 2015), 20% (phase I)[a] (Fabre et al., 2014)
				Multiple myeloma	11.5% (phase I/II) (Kumar et al., 2014)
				NSCLC	0% (phase II) (Stephenson et al., 2014)
				Breast cancer	8.3% (phase II) (Mita et al., 2014)
	Seliciclib	Oral	200 mg/day–3600 mg/day	Solid tumors	0%(phase I) (Benson et al., 2007), 1.8% (phase I) (Le Tourneau et al., 2010)
EG5	Ispinesib (SB715992)	Intravenous	1 mg/m^2–18 mg/m^2	Solid tumors	0% (phase I) (Burris et al., 2011; Souid et al., 2010), 0% (phase I)[a] (Blagden et al., 2008)
				Renal cell cancer	0% (phase II) (Lee et al., 2008b)
				Prostate adenocarcinoma	0% (phase II) (Beer et al., 2008)
				Melanoma	0% (phase II) (Lee et al., 2008a)
				Squamous cell carcinoma of head and neck	0% (phase II) (Tang et al., 2008)
				Hepatocellular carcinoma	0% (phase II) (Knox et al., 2008)
				Breast cancer	6.7% (phase I) (Gomez et al., 2012)
	Filanesib (ARRY-520)	Intravenous	0.8 mg/m^2–5.6 mg/m^2	Myeloid leukemias	3% (phase I) (Khoury et al., 2012)
				Solid tumors	0% (phase I) (LoRusso et al., 2015)
	MK-0731	Intravenous	6 mg/m^2–40 mg/m^2	Solid tumors	0% (phase I) (Holen et al., 2012)
	SB743921	Intravenous	2 mg/m^2–8 mg/m^2	Solid tumors or lymphoma	2.4% (phase I) (Holen et al., 2011)
CENP-E	GSK923295	Intravenous	10 mg/m^2–250 mg/m^2	Solid tumors	3% (phase I) (Chung et al., 2012)
	Lonafarnib	Oral	50 mg/day–800 mg/day	Solid tumors	0% (phase I) (Awada et al., 2002; Castaneda et al., 2011; Eskens et al., 2001), 5% (phase I) (Adjei et al., 2000), 3.7% (phase I)[a] (Ready et al., 2007), 38% (phase I)[a] (Khuri et al., 2004), 8% (phase I)[a] (Wong et al., 2011), 9% (phase I)[a] (Chow et al., 2008)
				Colorectal cancer	0% (phase II) (Sharma et al., 2002)
				Urothelial cancer	0% (phase II) (Winquist et al., 2005), 32% (phase II)[a] (Theodore et al., 2005)
				NSCLC	10% (phase II)[a] (Kim et al., 2005)
				CML	35% (phase I)[a] (Cortes et al., 2007)
				Central nervous system tumors	2% (phase I) (Kieran et al., 2007)
				Chronic myelomonocytic leukemia	8.6% (phase II) (Feldman et al., 2008)
				Squamous cell carcinoma of the head and neck	0% (phase II) (Hanrahan et al., 2009)
				Glioma/glioblastoma	6% (phase I)[a] (Desjardins et al., 2011)/17.6% (phase I)[a] (Yust-Katz et al., 2013)
				Breast cancer	58% (phase I)[a] (Milojkovic Kerklaan et al., 2013)
26S		Intravenous	0.1 mg/m^2–1.8 mg/m^2	Solid tumors and multiple myeloma	0% (phase I) (Gallerani et al., 2013)

[a] In combination with radiotherapy or another chemotherapy.

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

types of cancers (Garber, 2005; Salmela & Kallio, 2013). These drugs modify microtubule dynamics either by inhibiting microtubule polymerization (thus destabilizing it) or by enabling polymerization (thus stabilizing it) (Jordan & Wilson, 2004). Both mechanisms occur through drug binding to vinca, taxane or colchicine sites, which results in the activation of SAC by unattached kinetochores (Chan et al., 2012; Jordan & Wilson, 2004; Kaur, Kaur, Gill, Soni, & Bariwal, 2014; Weaver & Cleveland, 2005). Consequently, mitotic cell death or mitotic slippage is observed (Chan et al., 2012; Salmela & Kallio, 2013). Although anti-microtubular targeting has been shown to be effective, important side effects were diagnosed, such as myelosuppression due to the arrest of mitosis (which prevents bone marrow cells from proliferating) and neuropathy because microtubules are essential in the transport processes inside neurons (Jordan & Wilson, 2004).

Taxanes and epothilones are microtubule stabilizers. In contrast, vinca alkaloids and colchicines act as microtubule destabilizers. These mechanisms of action are enabled by drug binding to the taxane site, vinca domain or colchicine site (Stanton, Gernert, Nettles, & Aneja, 2011). The FDA has already approved several anti-microtubular drugs for clinical use, and there are many other drugs being studied in clinical trials, including vinflunine, vintafolide (EC145), ABT-751 (E7010), tesetaxel, TPI 287, patupilone (EPO906) and sagopilone (ZK-EPO) (Table 1).

Although most trials with vinflunine monotherapy presented PRs lower than 15% for solid tumors (Bennouna et al., 2006, 2003; Blasinska-Morawiec, Tubiana-Mathieu, Fougeray, Pinel, & Bougnoux, 2013; Calvo et al., 2012; Fumoleau et al., 2009; Goldstein et al., 2006; Hainsworth et al., 2010; Johnson et al., 2006; Olver et al., 2007; Talbot et al., 2007; Vaughn et al., 2009), in another study, 49% of bladder cancer patients in which platinum treatment have failed exhibited SD while 18% exhibited PR with a median duration of approximately 9 months (Culine et al., 2006). A trial on small cell lung cancer (SCLC) observed that, out of the evaluable patients, 25% had PR and 30% had SD (Spigel et al., 2010). Another study in advanced breast cancer patients previously treated with anthracycline/taxane presented relevant results, with 30% of patients exhibiting PR and 35% exhibiting SD (Campone et al., 2006). A frequent hematologic adverse event (AE) observed in all trials using this drug as monotherapy was neutropenia, and common non-hematological AEs observed included constipation, fatigue and nausea. The combination of vinflunine with other drugs achieved higher activity against non-small cell lung cancer (NSCLC) and breast cancer. In the combined treatment with cisplatin, 32.1% of NSCLC patients presented PR and 47.2% presented SD (Souquet et al., 2010), while with gemcitabine 37% presented PR and 42% presented SD (Tournoux-Facon et al., 2011). Vinflunine combined with carboplatin resulted in 36.8% of patients presenting both PR and SD (Tournoux-Facon, Robinet, Pinel, Ferre, & Tourani, 2012). For breast cancer patients, vinflunine therapy combined with doxorubicin resulted in approximately 48% of patients exhibiting PR and 32% exhibiting SD (Zaman et al., 2011) while in the combination with capecitabine, 43.8% of patients presented both PR and SD. Combination with trastuzumab achieved higher response rates with 3.3% of patients presenting CR, 70% presented PR and 23.3% presented SD (Paridaens et al., 2012). When combining epirubicin with vinflunine, approximately 48.6% of patients had PR and 42.8% had SD (Chan et al., 2014). Other combinations have shown limited activity or were poorly tolerated in trials with drugs such as vinflunine with premetrexed or erlotinib on solid tumors (Sanoff et al., 2011a, 2011b, 2011b), with pazopanib on urothelial cancer (Gerullis et al., 2013) and with erlotinib on NSCLC (Krzakowski et al., 2014). Registered performed trials reached phase III, and additional studies will be started aiming to test the combination with other drugs or to treat other cancer types (NCT02665039, NCT01844947, NCT01953003 and NCT02057913).

Another drug included in clinical trials is the folic acid-desacetylvinblastine monohydrazide (vinca alkaloid molecule) conjugate, named EC145 (vintafolide). This compound acts in a different manner since it first targets the folate receptor, overexpressed in several cancers, and after being endocytosed, the vinca alkaloid molecule is released inside the cell (Vlahov et al., 2006). This drug was tested against different types of solid tumors, achieving the best results when treating ovarian cancer (Edelman et al., 2012; Lorusso et al., 2012; Morris et al., 2014; Naumann et al., 2013). A phase II trial with ovarian cancer patients showed that 5% presented PR and 37% SD, independently of folate receptor presence on lesions (Morris et al., 2014). A higher objective response (CR + PR) rate of 18% was observed in another phase II trial after treating the same type of cancer with EC145 combined to pegylated liposomal doxorubicin (Naumann et al., 2013). AEs commonly observed after vintafolide exposure included fatigue and constipation (Edelman et al., 2012; Li et al., 2009; Lorusso et al., 2012; Naumann et al., 2013). Complete trials were registered and reached phase II, and there is a phase II study ongoing with solid tumors (NCT01002924).

ABT-751 is a sulfonamide that acts by binding to the colchicine site of β-tubulin (Segaoula et al., 2016). Although usually well tolerated, except in a pediatric study (probably due to the treatment schedule) (Fox et al., 2008), ABT-751 presented a lack of activity against several types of cancers, with <7% of patients exhibiting PR (Fox et al., 2014; Hande et al., 2006; Mauer et al., 2008) or with no objective responses observed (Fox et al., 2006, 2008; Meany et al., 2010). A modest activity was observed in patients with refractory or relapsed hematologic malignancies, with approximately 3.4% exhibiting CR (one patient) and 13.8% PR (Yee et al., 2005). ABT-751 and docetaxel combination employed to treat patients with advanced castration-resistant prostate cancer, showed higher activity, reaching 45% of objective response and 50% of SD. Nevertheless, combination with carboplatin to treat NSCLC patients showed modest activity (Ma et al., 2012), and combination of pemetrexed with ABT-751 did not result in a better efficacy compared with pemetrexed monotherapy (Rudin et al., 2011). Registered trials achieved phase II, and there is one trial ongoing with patients with neuroblastoma (NCT00436852).

Tesetaxel, a semi-synthetic drug that acts similarly to taxanes, reached phase II in clinical trials. As a monotherapy in NSCLC patients, tesetaxel resulted in low objective response rates with 3.8% of evaluable patients presenting both CR and PR and 65% presenting SD. Although this treatment frequently resulted in AEs such as fatigue, nausea and neutropenia, four patients with cardiac problems died (Baas et al., 2008). When tesetaxel was combined with capecitabine for treatment of solid tumors, a higher level of SD was observed (82% with a median duration of 3.3 months), but no objective responses were noted (Saif et al., 2011).

TPI 287 is a new third-generation taxane capable of crossing the blood-brain barrier. *In vivo* experiments showed significantly reduced proliferation in brain metastases (Ferlini, Gallo, & Scambia, 2008; Fitzgerald et al., 2012). Recently, a study with children with either neuroblastoma or medulloblastoma observed that patients treated with TPI 287 alone exhibited SD as the best response. After that, patients presenting PD were treated with the combination of TPI 287 and temozolomide. PR was observed in only one patient (12.5%). However, it is not clear if the PR observed was an effect of the combined TPI 287-temozolomide therapy or just of temozolomide (Mitchell et al., 2016). Two trials will be started with cancer patients (NCT01933815 and NCT02187822) and complete trials have reached phase II.

Patupilone is an epothilone that has been tested against several types of tumors. Although this drug had no satisfactory effects in certain clinical trials such as a treatment for breast cancer brain metastasis (Peereboom et al., 2014), recurrent colorectal cancer (Moorcraft et al., 2013), epithelial ovarian/primary fallopian tube/primary peritoneal cancer (Colombo et al., 2012) and castration-resistant prostate cancer (Hussain et al., 2009), it showed good anti-tumor activity in other trials either as a monotherapy or combined therapy (Forster et al., 2007; Ten Bokkel Huinink et al., 2009). Another study treating castration-resistant prostate cancer resulted in 24% of patients with PR and 57% with SD. Other results as a monotherapy had lower objective responses and SD

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

rates, ranging from 5% to 19.5% and from 30.6% to 42%, respectively (Melichar et al., 2011; Rubin et al., 2005; Ten Bokkel Huinink et al., 2009; Tsimberidou et al., 2011). The best responses were observed in combination with patupilone or with other therapies such as gemcitabine against solid tumors and with carboplatin, achieving 5% CR, 57% PR and 14% SD (Forster et al., 2007). Furthermore, combination with radiotherapy has shown promising results as a treatment for glioblastoma multiforme and recurrent gliomas (Fogh et al., 2010). AEs frequently observed in these studies included diarrhea, fatigue, nausea and neuropathy. Clinical trials have reached phase III, and a phase I/II trial combining patupilone with celebrex against metastatic colorectal cancer is ongoing (NCT00159484). Sagopilone is another epothilone that did not present objective responses in trials that treated gliomas (Silvani et al., 2009; Stupp et al., 2011) and other types of solid tumors (Araki et al., 2012; Arnold et al., 2009). Some studies that also used this drug as a monotherapy resulted in objective responses with rates lower than 17.7% for ovarian cancer (Rustin et al., 2011), NSCLC (Heigener et al., 2013), metastatic breast cancer (Freedman et al., 2011; Morrow et al., 2010), metastatic melanoma (DeConti et al., 2010) and solid tumors in general (Schmid et al., 2010). Responses to treatment were more effective in combined therapies. Sagopilone combined with carboplatin for ovarian cancer treatment resulted in 9% of CR, 54.5% of PR and 33.33% of SD (McMeekin et al., 2012). Combination with prednisone against castration-resistant prostate cancer resulted in 2.8% of patients exhibiting CR and 30.6% exhibiting PR (Beer et al., 2012), while with cisplatin against SCLC, no CR was observed, and 57.7% of patients presented PR (Gauler et al., 2013). Clinical trials with sagopilone reached phase II, but there are no ongoing trials.

Although many of these clinical trials presented high response rates, some studies observed lack of response to anti-microtubular drugs. A few clinical trials and several preclinical studies verified factors that might account for resistance to these drugs. Two clinical trials observed that patients with low expression levels of folate receptor on tumors presented a worst response to vintafolide treatment (Edelman et al., 2012; Morris et al., 2014). Furthermore, an *in vitro* study using cancer cell lines observed that P-glycoprotein (P-gp) overexpression is related to innate and acquired resistance to vintafolide (Guertin et al., 2016). On the other hand, P-gp expression apparently does not account for resistance to vinflunine as much as to other *Vinca* alkaloids. Instead, a resistance to apoptosis was observed and related to Bcl-2 and Bfl-1/*A*1 ovexpression (Kavallaris, Annereau, & Barret, 2008; Kruczynski & Hill, 2001; Kruczynski et al., 2002). Regarding sagopilone, a study suggested that NSCLC tumors with higher expression of wild-type TP53 were more resistant to therapy (Hammer et al., 2010). Finally, several studies focused on possible explanations for resistance to patupilone. Overexpression of galectin-1 was suggested to be associated with resistance to patupilone (Albrethsen, Angeletti, Horwitz, & Yang, 2014) as well as MRP7 (Hopper-Borge et al., 2009) and Gli1 (Mozzetti et al., 2012). Another factor associated with resistance was the tubulin R282Q mutation in the drug binding pocket (Mozzetti et al., 2008).

3.2. Targeting mitotic kinases

Considering the obstacles caused by microtubule targeting such as neurotoxicity, other targets have been studied, *e.g.*, mitotic kinases. The main mitotic targeted kinases include CDK1, AURKA, AURKB, PLK1 and the NDC80-NEK2 complex (Chan et al., 2012; Salmela & Kallio, 2013). Certain kinases were found to be overexpressed in different types of cancer, making them likely to be attractive mitotic targets (Chang et al., 2005; Gritsko et al., 2003; Katayama et al., 1999; Li et al., 2003; Sen et al., 2002; Smith et al., 2005; Twu et al., 2009). Since AURKA and PLK1 act on centrosomes at the onset of mitosis, when these proteins are inhibited, spindle formation is affected, thus activating SAC and presumably resulting in cell death (Chan et al., 2012; Jiang et al., 2014; Spänkuch-Schmitt, Bereiter-Hahn, Kaufmann, & Strebhardt, 2002). AURKA inhibitors target the ATP binding domain, and PLK1 inhibitors aim to bind either the kinase domain, thus blocking catalytic activity, or the polo-box domain, which is responsible for binding to phosphopeptides (Elia, Cantley, & Yaffe, 2003; Gilmartin et al., 2009; Lavogina, Enkvist, Viht, & Uri, 2014; Salmela & Kallio, 2013; Steegmaier et al., 2007; Strebhardt & Ullrich, 2006; Yuan et al., 2011). This last type of PLK1 inhibitor results in incorrect placement of this protein, which affects alignment, activates SAC and leads to apoptosis. Inhibition of AURKB usually overcomes SAC, resulting in incorrect chromosome alignment and segregation, and since it is an important protein in cytokinesis, it inhibits this process by generating polyploid cells by endoreduplication (Hauf et al., 2003; Kaestner, Stolz, & Bastians, 2009). Some AURKB inhibitors also consist of ATP-competitive compounds (Ikezoe, 2008). NDC80-NEK2 attachment is another target of mitosis, and its inhibition is accomplished by a compound that binds to NDC80 to prevent its binding and activation by NEK2 (Wu et al., 2008). This inhibition usually results in defective mitosis and apoptosis (Gurzov & Izquierdo, 2006; Linton et al., 2014).

3.2.1. Aurora kinase inhibitors

Clinically tested aurora kinase inhibitors include MLN-8237 (alisertib), PHA-739358 (danusertib), AT9283, AZD1152 (barasertib), ENMD-2076 and PF-03814735 (Table 1).

Based on the AURKA ATP competitive inhibitor MLN-8054, another AURKA inhibitor known as MLN-8237 or alisertib was designed (Cervantes et al., 2012). Although this drug was not well tolerated in a pediatric phase I trial (Mossé et al., 2012), high percentages of SD were observed in adult patients with advanced solid tumors (Cervantes et al., 2012; Dees et al., 2012; Matulonis et al., 2012). MLN-8237 showed greater activity in Non-Hodgkin's lymphomas, multiple myeloma, and chronic lymphocytic leukemia with 13% of patients exhibiting PR and 28% exhibiting SD in a phase I trial (Kelly et al., 2014) and 27% exhibiting objective responses in a phase II study (Friedberg et al., 2014). The most frequent AEs were hematological disorders such as neutropenia (Cervantes et al., 2012; Dees et al., 2012; Friedberg et al., 2014; Kelly et al., 2014; Matulonis et al., 2012; Melichar et al., 2015). There are several phase I/II studies ongoing with this inhibitor, including combinations with other drugs such as irinotecan, temozolomide and paclitaxel (NCT01898078, NCT01799278, NCT00697346, NCT01091428, NCT01812005, NCT01094288, NCT01567709, NCT01639911, NCT01637961, NCT01923337, NCT02259010, NCT01779843, NCT02219789, NCT01154816, NCT01601535, NCT02367352, NCT01848067, NCT01540682, NCT02038647 and NCT02551055) as well as a phase III trial comparing MLN-8237 with the investigator's choice of treatment (NCT01482962).

Danusertib (PHA-739358) is a pan-aurora kinase inhibitor that produced SD as the best response in a phase I study of solid tumors (23.7%) (Steeghs et al., 2009) and a phase II study of metastatic castration-resistant prostate cancer (13.6%) (Meulenbeld et al., 2013). Despite these results, another phase I study observed better responses in patients with solid tumors, with one patient presenting PR and 45.2% of patients presenting SD, of whom 23.9% showed prolonged stabilization for >-13 months (Cohen et al., 2009). These three trials presented AEs such as neutropenia, fatigue and nausea, and the drug was usually well tolerated. No active trials are registered.

AT9283 is an AURKA, AURKB, c-ABL and JAK2 inhibitor (Foran et al., 2014). A previous trial did not report objective responses and presented a small percentage of SD in advanced solid tumors (Arkenau et al., 2012). Another study of leukemia and myelofibrosis showed no clinical responses, with cardiomyopathy reported in some patients and 4 drug-related deaths out of 48 patients (Foran et al., 2014). The best result observed was one patient with PR (4.3%) in a phase I study with solid tumor patients (children and adolescents) (Moreno et al., 2015).

Barasertib (AZD1152) is an ATP competitive AURKB inhibitor. Although this drug was shown to be well tolerated, approximately 25% of solid tumor patients presented SD as the best response in two trials

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

(Boss et al., 2011; Schwartz et al., 2013). Better results were observed in studies that treated patients with acute myeloid leukemia (AML). A phase I study showed that 19% of AML patients had a tumor response, including complete remission with incomplete recovery (CRi) of neutrophils, and 38% presented SD (Tsuboi et al., 2011). Similar results were observed in a phase I/II trial in which 25% of AML patients had a hematologic response being 14% either CR or CRi (Löwenberg et al., 2011). In a phase II trial, a comparison between AZD1152 and low-dose cytosine arabinoside (LDAC) treatments was performed, and the response was better for barasertib than for LDAC, a treatment commonly used against AML (Kantarjian et al., 2013a). Combination of both treatments in another study showed the highest tumor response compared with barasertib as a monotherapy (27% of CR, 9% of CRi and 9% of PR) (Kantarjian et al., 2013b). Treatment with barasertib was also tested against diffuse large B-cell lymphoma with 20% of patients presenting objective responses (Collins et al., 2015). A frequently reported AE in most studies with AZD1152 was febrile neutropenia. No registered trials are ongoing.

Studies with ENMD-2076, an AURKA, AURKB and angiogenic kinase inhibitor (such as VEGFR), have shown activity in ovarian cancer patients. In one of the studies that recruited patients with solid tumors, PR was only observed in ovarian cancers (3%), but a high SD rate of 85% was achieved, including other tumors (Diamond et al., 2011). In another trial, 58% of patients with platinum-resistant ovarian cancers exhibited SD and PR (Matulonis et al., 2013). For both studies, the most common AEs included hypertension, fatigue and gastrointestinal disorders. Phase II trials are scheduled to begin (NCT02234986, NCT01639248, NCT01914510).

The ATP competitive inhibitor of AURKA and AURKB known as PF-03814735, was tested in patients with advanced or metastatic solid tumors. Although this drug was well tolerated (with the most frequent AEs being diarrhea, fatigue and nausea), no objective responses were observed, and approximately 37% of patients presented SD (Schöffski et al., 2011). No additional trials were registered.

The possible reasons for the absence of clinical response to some aurora inhibitors might be explained by preclinical studies that identified drug resistance in cells presenting AURKA mutations (T217D and T217E) (Sloane et al., 2010). Moreover, in colon and pancreatic cancer cell lines, the resistance to barasertib was attributed to an overexpression of P-GP and ABCG2 (Guo et al., 2009). Similar results were observed in another study with AML cell lines (Grundy, Seedhouse, Russell, & Pallis, 2011). Additionally, a preclinical research verified that overexpression of GDF15 conferred resistance to danusertib in a colon cancer cell line (Bosotti et al., 2012).

3.2.2. PLK1 inhibitors

Clinically tested PLK1 inhibitors include BI6727 (volasertib) and GSK461364 (Table 1). Volasertib is an ATP competitive inhibitor of PLK proteins and has demonstrated a longer half-life and volume distribution than BI2536, a similar drug that had its development discontinued, being substituted by BI6727 (Schöffski et al., 2012). Hematologic toxicities and fatigue were frequent AEs observed in advanced solid tumor patients (Lin et al., 2014; Schöffski et al., 2012; Stadler et al., 2014). Two studies that evaluated different solid tumor types (Lin et al., 2014; Schöffski et al., 2012) presented low PRs but high SD percentages (ranging from 40% to 44.1%), while another study with only advanced or metastatic urothelial cancer showed a higher PR (14%) and lower SD percentage (26%) (Stadler et al., 2014). In a recent study combining volasertib with afatinib, only 6.9% of solid tumor patients presented PR (Machiels et al., 2015). Interestingly, a trial that tested LDAC with or without volasertib in AML patients showed an overall response (CR and CRi) that was much higher for combined treatment than just with LDAC, *i.e.*, 31% *vs.* 13.3% (Döhner et al., 2014). There is a phase III trial that tests this combination in AML patients and several other trials ongoing with most of them combining this compound with other drugs (NCT02003573, NCT01772563, NCT00804856, NCT01721876, NCT02273388 and NCT01971476).

GSK461364 is another ATP competitive PLK inhibitor with greater specificity for PLK1. One published trial with solid malignancies showed a lack of activity from this drug with no objective responses and only 15% of patients exhibiting SD for ≥4 months (Olmos et al., 2011). There are no registered trials ongoing with GSK461364.

It is difficult to determine the causes of treatment failure for some patients since only a few *in vivo* and *in vitro* studies have addressed the resistance mechanisms associated with PLK1 inhibitors. When testing BI2536 in hepatocellular carcinoma in nude mice, the lack of activity was considered as a consequence of low drug intratumoral levels (Haupenthal et al., 2012). Other preclinical studies with volasertib indicated that overexpression of P-gp conferred resistance to cancer cells (Wu et al., 2015) as well as AKT3 (Nonomiya et al., 2016). Additionally, overexpression of P-gp was also correlated with resistance to GSK 461364 (Wu et al., 2014) while another group observed that lack of RARA expression conferred resistance to cancer cells (Liu-Sullivan et al., 2011).

3.2.3. CDK1 inhibitors

CDK1 inhibitors include ATP-competitive compounds such as P276-00, terameprocol, dinaciclib and seliciclib (Table 1) (Gallorini, Cataldi, & di Giacomo, 2012; Mariaule & Belmont, 2014). Dinaciclib was recently shown to also interact with the bromodomain of CDKs (Martin, Olesen, Georg, & Schönbrunn, 2013). P276-00 was tested on mantle cell lymphoma patients, and SD was the best response observed (18.2%) (Cassaday et al., 2015). There are no ongoing clinical trials registered. Terameprocol has also no studies ongoing. This inhibitor was tested in leukemia patients with 7% presenting PR (one patient) and 33% presenting SD (Tibes et al., 2015), and also in glioma patients with 38% presenting SD as the best response (Grossman et al., 2012). Both P276-00 and terameprocol reached phase II in clinical trials.

Although dinaciclib had no objective responses against acute leukemias and several solid tumors (Gojo et al., 2013; Nemunaitis et al., 2013; Stephenson et al., 2014), certain objective responses were observed in a phase II study with breast cancer patients (8.3% PR) (Mita et al., 2014) and in a phase I/II study with multiple myeloma patients (11.5% PR) (Kumar et al., 2014). The best clinical activity of this inhibitor was against chronic lymphocytic leukemia (CLL), with 54% of patients exhibiting PR in a phase I trial (Flynn et al., 2015). In addition, despite the small sample (5 patients), 20% of CR was achieved when the treatment was combined with rituximab (Fabre et al., 2014). Clinical trials with dinaciclib have reached phase III, and there are several studies ongoing in combination with other drugs to treat leukemia, breast and pancreatic cancer (NCT01676753, NCT01783171, NCT01624441 and NCT01515176). Finally, seliciclib was tested against solid tumors in two phase I studies, and the best result observed was 1.8% of PR, whereas SD ranged from 10.7% to 38% (Benson et al., 2007; Le Tourneau et al., 2010). Trials with this inhibitor reached phase II, and there are no studies to be started in cancer patients.

There is a lack of evidence that explain the low response rates observed in many clinical trials with CDK1 inhibitors. A preclinical study evaluated mRNA levels and suggested that Notch and TGFβ pathways activation might be related to resistance to dinaciclib (Feldmann et al., 2011) while another study observed that overexpression of P-gp, ABCC1 and ABCG2 transporters were associated with resistance to dinaciclib (Cihalova, Ceckova, Kucera, Klimes, & Staud, 2015).

3.3. Targeting motor proteins

EG5 and CENP-E are the two main anti-motor proteins targeted thus far. Since EG5 acts on centrosome separation, inhibition of EG5 results in a monopolar mitotic apparatus, and SAC is consequently activated, which can lead to mitotic cell death (Kapoor, Mayer, Coughlin, & Mitchison, 2000). The majority of EG5 inhibitors are non-ATP-

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

competitive compounds, and inhibitors with this mechanism of action are preferentially applied in clinical trials because ATP-competitive inhibitors usually cannot cross cell membranes (Rickert et al., 2008; Salmela & Kallio, 2013). Inhibitors of CENP-E can be classified either as an ATPase antagonist or farnesyl transferase (Salmela & Kallio, 2013). Inhibition of ATPase activity results in chromosome mis-alignment and mitotic arrest leading to cell death (Wood et al., 2010). Farnesylation inhibition mislocalizes this protein from kinetochores and results in defective attachment of microtubules to kinetochores, incorrect tension between sister kinetochores, and activation of SAC (Schafer-Hales et al., 2007).

3.3.1. EG5 (KSP) inhibitors

Clinically tested KSP inhibitors include ispinesib (SB715992), ARRY-520 (filanesib), MK-0731 and SB743921 (Table 1). In almost all trials with ispinesib (which inhibits ATPase activity), SD was the best response observed, ranging from 12.5% in a pediatric study to 46% in patients with hepatocellular carcinoma (Burris et al., 2011; Knox et al., 2008; Lee et al., 2008a, 2008b; Souid et al., 2010; Tang et al., 2008). Although this drug was usually well tolerated, it was considered to have unsuccessful effects on tumor responses of melanoma (Lee et al., 2008a), hepatocellular carcinoma (Knox et al., 2008), renal cell cancer (Lee et al., 2008b) and prostate adenocarcinoma (Beer et al., 2008). Two deaths possibly occurred due to ispinesib in a phase II trial with squamous cell carcinoma of head and neck (Tang et al., 2008). Combination of ispinesib with docetaxel in a phase I trial for treatment of solid tumors produced no objective responses, and 29% of patients presented SD for ≥18 weeks (Blagden et al., 2008). The best results observed occurred in a study with advanced or metastatic breast cancer patients of which 6.7% had PR and 60% had SD (27% for ≥3 months) (Gomez et al., 2012). Registered studies with ispinesib achieved phase II but there are no trials in progress. Recently, another EG5 inhibitor named filanesib has been included in several trials. A study in multiple myeloma patients is ongoing (NCT01372540), and another registered study will begin (NCT02384083). Published data showed that treatment of advanced myeloid leukemia resulted in 3% of patients with PR and 29% with SD (Khoury et al., 2012) while 18% of solid tumors patients exhibited SD (LoRusso et al., 2015). Common AEs in both trials included myelosuppression.

Other drugs have been included in clinical trials in the last few years, but no registered studies are ongoing. One of these compounds is MK-0731, a non-ATP competitive inhibitor, which showed lack of activity against solid tumors, with no objective responses and only 10% of patients exhibiting SD for ≥5 months. Although well tolerated, hematological AEs were frequently reported (Holen et al., 2012). Additionally, SB 743921 does not have any registered ongoing trials and showed low activity against solid tumors in a phase I trial. Only one patient had PR, and approximately 14.6% had SD. One patient died of pulmonary embolism possibly as a treatment consequence (Holen et al., 2011).

Both CR and PR were absent in most of the clinical trials with EG5 inhibitors considered in this review. Some clinical studies evaluated EG5 expression and verified that there was a low expression of this protein in tumor samples (Beer et al., 2008; Knox et al., 2008). Considering the lack of the target protein expression, the authors stated that this may be one of the reasons why these inhibitors did not achieved clinical response. In addition, another important suggestion was that these drugs can only inhibit EG5 in cells undergoing mitotic division. Considering that many tumors have a low mitotic index, one might assume that this could be the reason for lack of treatment activity (Beer et al., 2008). In preclinical studies, D130V and A133D mutations on EG5 were identified as conferring resistance to ispinesib (Luo et al., 2007; Sheth et al., 2009). Another study verified that resistance to EG5 inhibitor is associated with Kif15 activity (Sturgill, Norris, Guo, & Ohi, 2016).

3.3.2. CENP-E inhibitors

Clinically tested CENP-E inhibitors include GSK923295 and lonafarnib (Table 1). GSK923295 (motor domain inhibitor) treatment resulted in 30% of patients with SD, and just one had PR when solid tumors were treated. Frequent AEs included fatigue, diarrhea and anemia (Chung et al., 2012). This study was the first and only clinical study published with this drug. The most studied drug capable of inhibiting CENP-E, a farnesylated mitotic protein, is lonafarnib (SCH66336). Lonafarnib is a farnesyl transferase inhibitor that competes for binding to this enzyme, responsible for post-translational modifications of proteins such as CENP-E, RAS, RAP2, RHOB and E (de Bono, Tolcher, & Rowinsky, 2003). Several clinical studies have shown lack of activity of lonafarnib as a monotherapy, with no objective responses in solid tumors (Awada et al., 2002; Castaneda et al., 2011; Eskens et al., 2001; Hanrahan et al., 2009; Sharma et al., 2002; Winquist et al., 2005). Two phase I studies, one that included different solid tumors (Adjei et al., 2000) and another with brain tumor patients (Kieran et al., 2007), observed only one patient with PR. The best response achieved with lonafarnib as a monotherapy occurred in one study that included 35 patients with chronic myelomonocytic leukemia in which two had CR and one had PR (Feldman et al., 2008). Higher response rates were observed when lonafarnib was combined with other drugs. Lonafarnib and paclitaxel treatment of solid tumor patients resulted in one patient with PR and approximately 61% with SD (Ready et al., 2007). In another study, 38% presented PR, mainly NSCLC patients, although all patients had PD after 3 months (Khuri et al., 2004). When this same combination was tested only in NSCLC patients, 10% had PR and 38% had SD (Kim et al., 2005). The combined therapy with lonafarnib and gemcitabine in advanced urothelial tract cancers resulted in one patient (3%) with CR and 29% with PR (Theodore et al., 2005), while another clinical trial on refractory solid tumor patients observed that only 8% achieved PR and 48%, SD (Wong et al., 2011). Two studies have shown that lonafarnib and temozolomide combination was well tolerated by patients with glioma, with 17.6% presenting PR in one study (Yust-Katz et al., 2013) and 64% presenting SD in another phase I trial (Desjardins et al., 2011). A high response rate of 35% was observed when treating CML patients with imatinib combined with lonafarnib (Cortes et al., 2007). Finally, therapy in solid tumor patients with docetaxel and lonafarnib resulted in one CR and 20.7% PR (Kauh et al., 2011). Overall frequent AEs included nausea, anorexia and fatigue. CENP-E inhibitor already reached phase III, and there is a trial ongoing with glioma patients (NCT00083096).

A possible explanation for resistance to CENP-E inhibitors may be through P-gp efflux pump mechanism as verified in a human sarcoma cell line treated with GSK923295 (Tcherniuk & Oleinikov, 2015). Other studies evaluated resistance to lonafarnib and observed that it could be associated with the overexpression of Akt and higher phosphorylation levels of mTOR and p70 S6 kinase (Bruzek, Poynter, Kaufmann, & Adjei, 2005) as well as to ABC transporter homolog ATP11a overexpression (Zhang, Groffen, & Heisterkamp, 2005). Resistance was also associated with activation of Erk and p38 pathways on acute lymphoblastic leukemia cells (Feldhahn et al., 2012). Another group analyzed samples of patients with leukemia and correlated W106R and Y361 mutations on FTPase enzyme to lonafarnib resistance (Raz, Nardi, Azam, Cortes, & Daley, 2007).

3.4. Targeting multiprotein complexes – 26S proteasome

An important multi-protein complex target in mitosis is the 26S proteasome, which is responsible for degrading proteins ubiquitinated by APC. Inhibition of this complex primarily results in mitotic arrest and apoptosis (Ling et al., 2003).

A clinically tested proteasome inhibitor is delanzomib (CEP-18770) (Table 1). This drug prevents 26S proteasome activity by binding reversibly to its β5 site (with chymotryptic activity), resulting in the accumulation of ubiquitinated proteins (Teicher & Tomaszewski, 2015). Delanzomib was tested against solid tumors and multiple myeloma,

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

with 37% of patients exhibiting SD as the best response and presenting skin rash as the most frequent AE (Gallerani et al., 2013). No registered trials are ongoing. Oprozomib, another drug that targets proteasome and was not approved by FDA, has no published results but several trials are ongoing mostly with multiple myeloma patients in phase I and II studies (NCT01881789, NCT01832727, NCT01416428, NCT01999335 and NCT02244112).

The possible explanations for the observed lack of response to delanzomib were not determined. As this drug has a mechanism of action similar to bortezomib (Lawasut et al., 2012), both drugs could display similar mechanisms of resistance. Bortezomib was the first proteasome inhibitor approved for human use; however, cancer resistance is commonly observed with this compound. The probable causes of inherent or acquired resistance to bortezomib are associated-mutations and/or overexpression of β5 subunit, downstream alterations in the activity of proteasome or even an imbalance on levels of proteins involved in apoptosis (Dou & Zonder, 2014; Ruschak, Slassi, Kay, & Schimmer, 2011).

4. Future perspectives

An important issue regarding these anti-mitotic drugs tested in clinical trials refers to the lack of response, mainly when these agents are employed in monotherapies. To our knowledge, there are no clinical trials that addressed the reasons for monotherapy treatment failure. However, several preclinical trials are focusing on the potential mechanisms of anti-mitotic drug resistance, such as mutations in the drug binding site of different targets and overexpression of proteins such as P-gp, involved in drug efflux (Guo et al., 2009; Mozzetti et al., 2008; Wu et al., 2014). Moreover, another possible mechanism of resistance that has been studied consists of cell exiting mitosis prematurely through mitotic slippage after being exposed to anti-mitotic agents (Chan et al., 2012; Rieder & Maiato, 2004). Accordingly, a recent preclinical study demonstrated an association between mitotic slippage and resistance to therapy after treating liver cancer cells with paclitaxel (He et al., 2016). The process of mitotic slippage consists of an active SAC that is bypassed mainly as a consequence of slowly cyclin B degradation triggered by APC. This depletion happens until a threshold is achieved before the beginning of mitotic cell death, allowing cells to progress through mitosis and resulting in a forced exit (Brito & Rieder, 2006).

Different cell mechanisms are positively and/or negatively modulated by anti-mitotic drugs targeting mitosis entry, spindle assembly and SAC, like mitotic cell death, interphase-associated cell death, cell cycle progression or cellular arrest in interphase (Gascoigne & Taylor, 2008; Salmela & Kallio, 2013). On the other hand, the outcome observed for cancer cells treated with drugs that target mitotic exit is cell death. The aim of mitotic exit inhibitors is to prevent cyclin B depletion, thus resulting in cells that will be arrested in mitosis and invariably will die (Manchado, Guillamot, & Malumbres, 2012). In this context, potential targets for mitotic exit inhibition include APC, which is essential for cyclin B depletion, and CDC20, as it acts by binding and activating this complex (Manchado et al., 2012; Salmela & Kallio, 2013). No clinical trials have targeted either APC or CDC20 but it has been shown that inhibiting proteins involved in mitotic exit was more effective against cancer cells than inhibiting proteins that will result in SAC activation (Manchado et al., 2010, 2012). Accordingly, a preclinical study verified that CDC20 knockdown was more effective in killing cells resistant to cell death when compared to either EG5 inhibition or treatment with paclitaxel (Huang, Shi, Orth, & Mitchison, 2009). Another study combined proTAME, an APC inhibitor, with paclitaxel or MLN-8054, both anti-mitotic drugs, which resulted in apoptosis activation in cancer cells (Giovinazzi, Bellapu, Morozov, & Ishov, 2013).

Another important consideration concerns the combined treatment. By reviewing clinical trials with anti-mitotic agents, it is clear that better responses were achieved when combined therapies were employed. Treatment strategies that seem to be interesting include combinations that act on different cellular processes as well as combined drugs that act at different steps of the same pathway. An example of combined therapy acting in different processes is barasertib and LDAC. Barasertib is an AURKB inhibitor while LDAC has a mechanism of action that is not well established (Montalban-Bravo & Garcia-Manero, 2015). This combination reached the highest percentage of objective response taking into account all aurora inhibitors that we reviewed (Kantarjian et al., 2013b). On the other hand, an example of effective combined therapy that affect the same pathway is the use of lonafarnib, which acts on CENP-E, with paclitaxel that targets microtubules (Khuri et al., 2004). At last, although mitotic exit inhibitors have been tested only in preclinical trials, combinations of these inhibitors with anti-mitotic drugs that activate SAC seem to be an efficient way to trigger mitotic cell death (Huang et al., 2009).

5. Conclusions

Cancer treatment is still a challenge. Hence, the development of new drugs aiming to increase survival rates of cancer patients is a crucial process. Since anti-microtubule drugs have shown clinical activity against several tumor types, many drugs have been developed to target other M-phase proteins such as aurora kinases, CDK1, PLK1, EG5, CENP-E and the 26S proteasome complex.

From aurora inhibitors, treatment with barasertib and alisertib presented the highest objective response rates. The best response was achieved with a combination of barasertib with LDAC for AML and diffuse large B-cell lymphoma treatment. In contrast, anti-EG5 drugs have shown lack of activity. From PLK1 inhibitors, trials on AML patients with volasertib combined with LDAC showed better results than GSK 461364. From CENP-E inhibitors, lonafarnib had the highest objective response rates when combined with paclitaxel for solid tumor treatment, with gemcitabine for urothelial cancer, with imatinib mesylate for CML, with temozolomide for glioma, and with trastuzumab and paclitaxel for breast cancer.

Regarding 26S proteasome inhibitors, ixazomib presented the best activity for treatment of lymphoma and multiple myeloma while for anti-CDK1 drugs, the best results were observed with dinaciclib on CLL patients. Finally, new anti-microtubular drugs such as vinflunine, ABT-751, patupilone and sagopilone have shown good clinical activity in combination with classical drugs (carboplatin, docetaxel, doxorubicin, trastuzumab, epirubucin or capecitabine).

The results observed have shown that targeting other mitotic proteins than tubulin, in fact, avoided the neurotoxicity issue. On the other hand, these new approaches were not as effective as already-approved anti-microtubular drugs. Most anti-mitotics presented limited effectiveness as single agents. As observed in preclinical trials, this might be due to SAC activation, which can result in mitotic slippage, or even a consequence of mutations in the targeted protein as well as overexpression of specific proteins, such as P-gp. Targeting mitotic exit seems an attractive therapeutic strategy rather than activating SAC since it is not susceptible to mitotic slippage. The highest objective response rates observed in the conducted trials were achieved with combinations of the new inhibitors with conventional chemotherapy. In summary, further studies are required to establish new combined therapies with effective synergism, as well as novel inhibitors and anti-mitotic targets aiming to provide a better response to treatments.

Conflict of interest statement

The authors have no conflict of interest, and nothing to disclose.

References

Adjei, A. A., Erlichman, C., Davis, J. N., Cutler, D. L., Sloan, J. A., Marks, R. S., et al. (2000). A phase I trial of the farnesyl transferase inhibitor SCH66336: Evidence for biological and clinical activity. *Cancer Research 60*, 1871–1877.

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

Albrethsen, J., Angeletti, R. H., Horwitz, S. B., & Yang, C. -P. H. (2014). Proteomics of cancer cell lines resistant to microtubule-stabilizing agents. *Molecular Cancer Therapeutics 13*, 260–269.

Andrews, P. D., Ovechkina, Y., Morrice, N., Wagenbach, M., Duncan, K., Wordeman, L., et al. (2004). Aurora B regulates MCAK at the mitotic centromere. *Developmental Cell 6*, 253–268.

Araki, K., Kitagawa, K., Mukai, H., Mukohara, T., Kodama, K., Ando, Y., et al. (2012). First clinical pharmacokinetic dose-escalation study of sagopilone, a novel, fully synthetic epothilone, in Japanese patients with refractory solid tumors. *Investigational New Drugs 30*, 2327–2333.

Arkenau, H. -T., Plummer, R., Molife, L. R., Olmos, D., Yap, T. A., Squires, M., et al. (2012). A phase I dose escalation study of AT9283, a small molecule inhibitor of aurora kinases, in patients with advanced solid malignancies. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 23*, 1307–1313.

Arnold, D., Voigt, W., Kiewe, P., Behrmann, C., Lindemann, S., Reif, S., et al. (2009). Weekly administration of sagopilone (ZK-EPO), a fully synthetic epothilone, in patients with refractory solid tumours: Results of a phase I trial. *British Journal of Cancer 101*, 1241–1247.

Awada, A., Eskens, F. A. L. M., Piccart, M., Cutler, D. L., van der Gaast, A., Bleiberg, H., et al. (2002). Phase I and pharmacological study of the oral farnesyltransferase inhibitor SCH 66336 given once daily to patients with advanced solid tumours. *European Journal of Cancer (Oxford, England: 1990) 38*, 2272–2278.

Baas, P., Szczesna, A., Albert, I., Milanowski, J., Juhász, E., Sztancsik, Z., et al. (2008). Phase I/II study of a 3 weekly oral taxane (DJ-927) in patients with recurrent, advanced non-small cell lung cancer. *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer 3*, 745–750.

Beer, T. M., Goldman, B., Synold, T. W., Ryan, C. W., Vasist, L. S., Van Veldhuizen, P. J., et al. (2008). Southwest Oncology Group phase II study of ispinesib in androgen-independent prostate cancer previously treated with taxanes. *Clinical Genitourinary Cancer 6*, 103–109.

Beer, T. M., Smith, D. C., Hussain, A., Alonso, M., Wang, J., Giurescu, M., et al. (2012). Phase II study of first-line sagopilone plus prednisone in patients with castration-resistant prostate cancer: A phase II study of the Department of Defense Prostate Cancer Clinical Trials Consortium. *British Journal of Cancer 107*, 808–813.

Bennouna, J., Fumoleau, P., Armand, J. -P., Raymond, E., Campone, M., Delgado, F. -M., et al. (2003). Phase I and pharmacokinetic study of the new vinca alkaloid vinflunine administered as a 10-min infusion every 3 weeks in patients with advanced solid tumours. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 14*, 630–637.

Bennouna, J., Breton, J. -L., Tourani, J. -M., Ottensmeier, C., O'Brien, M., Kosmidis, P., et al. (2006). Vinflunine — An active chemotherapy for treatment of advanced non-small-cell lung cancer previously treated with a platinum-based regimen: Results of a phase II study. *British Journal of Cancer 94*, 1383–1388.

Benson, C., White, J., De Bono, J., O'Donnell, A., Raynaud, F., Cruickshank, C., et al. (2007). A phase I trial of the selective oral cyclin-dependent kinase inhibitor seliciclib (CYC202; R-Roscovitine), administered twice daily for 7 days every 21 days. *British Journal of Cancer 96*, 29–37.

Blagden, S. P., Molife, L. R., Seebaran, A., Payne, M., Reid, A. H. M., Protheroe, A. S., et al. (2008). A phase I trial of ispinesib, a kinesin spindle protein inhibitor, with docetaxel in patients with advanced solid tumours. *British Journal of Cancer 98*, 894–899.

Blangy, A., Lane, H. A., d'Hérin, P., Harper, M., Kress, M., & Nigg, E. A. (1995). Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell 83*, 1159–1169.

Blasinska-Morawiec, M., Tubiana-Mathieu, N., Fougeray, R., Pinel, M. -C., & Bougnoux, P. (2013). Phase II study of intravenous vinflunine after failure of first-line vinorelbine based regimen for advanced breast cancer. *Breast (Edinburgh, Scotland) 22*, 58–63.

Bosotti, R., Carpinelli, P., Healy, S., Locatelli, G., Cappella, P., Lanfrancone, L., et al. (2012). Transcriptional analysis of the Aurora inhibitor Danusertib leading to biomarker identification in TP53 wild type cells. *Gene 494*, 202–208.

Boss, D. S., Witteveen, P. O., van der Sar, J., Lolkema, M. P., Voest, E. E., Stockman, P. K., et al. (2011). Clinical evaluation of AZD1152, an i.v. inhibitor of Aurora B kinase, in patients with solid malignant tumors. *Annals of Oncology 22*, 431–437.

Brito, D. A., & Rieder, C. L. (2006). Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. *Current Biology 16*, 1194–1200.

Bruzek, L. M., Poynter, J. N., Kaufmann, S. H., & Adjei, A. A. (2005). Characterization of a human carcinoma cell line selected for resistance to the farnesyl transferase inhibitor 4-(2-(4-(8-chloro-3,10-dibromo-6,11-dihydro-5H-benzo-(5,6)-cyclohepta(1,2-b)-pyridin-11(*R*)-yl)-1-piperidinyl)-2-oxo-ethyl)-1-piperidinecarboxamide (SCH66336). *Molecular Pharmacology 68*, 477–486.

Burris, H. A., Jones, S. F., Williams, D. D., Kathman, S. J., Hodge, J. P., Pandite, L., et al. (2011). A phase I study of ispinesib, a kinesin spindle protein inhibitor, administered weekly for three consecutive weeks of a 28-day cycle in patients with solid tumors. *Investigational New Drugs 29*, 467–472.

Caldas, G. V., & DeLuca, J. G. (2014). KNL1: Bringing order to the kinetochore. *Chromosoma 123*, 169–181.

Calvo, E., Vermorken, J. B., Hiret, S., Rodon, J., Cortes, J., Senellart, H., et al. (2012). Phase I dose-escalation study of vinflunine hard capsules administered twice a day for 2 consecutive days every week in patients with advanced/metastatic solid tumors. *Cancer Chemotherapy and Pharmacology 69*, 1467–1475.

Campone, M., Cortes-Funes, H., Vorobiof, D., Martin, M., Slabber, C. F., Ciruelos, E., et al. (2006). Vinflunine: A new active drug for second-line treatment of advanced breast cancer. Results of a phase II and pharmacokinetic study in patients progressing after first-line anthracycline/taxane-based chemotherapy. *British Journal of Cancer 95*, 1161–1166.

Campone, M., Isambert, N., Bourbouloux, E., Roché, H., Bonneterre, J., Milano, G., et al. (2012). A phase I study of vinflunine in combination with capecitabine in patients with metastatic breast cancer previously treated with anthracyclines and taxanes. *Cancer Chemotherapy and Pharmacology 69*, 871–879.

Cassaday, R. D., Goy, A., Advani, S., Chawla, P., Nachankar, R., Gandhi, M., et al. (2015). A phase II, single-arm, open-label, multicenter study to evaluate the efficacy and safety of P276-00, a cyclin-dependent kinase inhibitor, in patients with relapsed or refractory mantle cell lymphoma. *Clinical Lymphoma, Myeloma & Leukemia 15*, 392–397.

Castaneda, C., Meadows, K. L., Truax, R., Morse, M. A., Kaufmann, S. H., Petros, W. P., et al. (2011). Phase I and pharmacokinetic study of lonafarnib, SCH 66336, using a 2-week on, 2-week off schedule in patients with advanced solid tumors. *Cancer Chemotherapy and Pharmacology 67*, 455–463.

Cervantes, A., Elez, E., Roda, D., Ecsedy, J., Macarulla, T., Venkatakrishnan, K., et al. (2012). Phase I pharmacokinetic/pharmacodynamic study of MLN8237, an investigational, oral, selective aurora a kinase inhibitor, in patients with advanced solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 18*, 4764–4774.

Chan, K. -S., Koh, C. -G., & Li, H. -Y. (2012). Mitosis-targeted anti-cancer therapies: Where they stand. *Cell Death & Disease 3*, e411.

Chan, S., Campone, M., Santoro, A., Conte, P. F., Bostnavaron, M., & Nguyen, L. (2014). A phase I clinical and pharmacokinetic study evaluating vinflunine in combination with epirubicin as first-line treatment in metastatic breast cancer. *Cancer Chemotherapy and Pharmacology 73*, 903–910.

Chang, J. T., Wang, H. -M., Chang, K. -W., Chen, W. -H., Wen, M. -C., Hsu, Y. -M., et al. (2005). Identification of differentially expressed genes in oral squamous cell carcinoma (OSCC): Overexpression of NPM, CDK1 and NDRG1 and underexpression of CHES1. *International Journal of CancerJournal International Du Cancer 114*, 942–949.

Cheeseman, I. M., Chappie, J. S., Wilson-Kubalek, E. M., & Desai, A. (2006). The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell 127*, 983–997.

Chi, K. N., Beardsley, E., Eigl, B. J., Venner, P., Hotte, S. J., Winquist, E., et al. (2012). A phase 2 study of patupilone in patients with metastatic castration-resistant prostate cancer previously treated with docetaxel: Canadian Urologic Oncology Group study P07a. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 23*, 53–58.

Chow, L. Q. M., Eckhardt, S. G., O'Bryant, C. L., Schultz, M. K., Morrow, M., Grolnic, S., et al. (2008). A phase I safety, pharmacological, and biological study of the farnesyl protein transferase inhibitor, lonafarnib (SCH 663366), in combination with cisplatin and gemcitabine in patients with advanced solid tumors. *Cancer Chemotherapy and Pharmacology 62*, 631–646.

Chung, V., Heath, E. I., Schelman, W. R., Johnson, B. M., Kirby, L. C., Lynch, K. M., et al. (2012). First-time-in-human study of GSK923295, a novel antimitotic inhibitor of centromere-associated protein e (CENP-E), in patients with refractory cancer. *Cancer Chemotherapy and Pharmacology 69*, 733–741.

Cihalova, D., Ceckova, M., Kucera, R., Klimes, J., & Staud, F. (2015). Dinaciclib, a cyclin-dependent kinase inhibitor, is a substrate of human ABCB1 and ABCG2 and an inhibitor of human ABCC1 in vitro. *Biochemical Pharmacology 98*, 465–472.

Cohen, R. B., Jones, S. F., Aggarwal, C., von Mehren, M., Cheng, J., Spigel, D. R., et al. (2009). A phase I dose-escalation study of danusertib (PHA-739358) administered as a 24-hour infusion with and without granulocyte colony-stimulating factor in a 14-day cycle in patients with advanced solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 15*, 6694–6701.

Collins, G. P., Eyre, T. A., Linton, K. M., Radford, J., Vallance, G. D., Soilleux, E., et al. (2015). A phase II trial of AZD1152 in relapsed/refractory diffuse large B-cell lymphoma. *British Journal of Haematology 170*, 886–890.

Colombo, N., Kutarska, E., Dimopoulos, M., Bae, D. -S., Rzepka-Gorska, I., Bidzinski, M., et al. (2012). Randomized, open-label, phase III study comparing patupilone (EPO906) with pegylated liposomal doxorubicin in platinum-refractory or -resistant patients with recurrent epithelial ovarian, primary fallopian tube, or primary peritoneal cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 30*, 3841–3847.

Cooke, C. A., Heck, M. M., & Earnshaw, W. C. (1987). The inner centromere protein (INCENP) antigens: Movement from inner centromere to midbody during mitosis. *The Journal of Cell Biology 105*, 2053–2067.

Cortes, J., Jabbour, E., Daley, G. Q., O'Brien, S., Verstovsek, S., Ferrajoli, A., et al. (2007). Phase 1 study of lonafarnib (SCH 66336) and imatinib mesylate in patients with chronic myeloid leukemia who have failed prior single-agent therapy with imatinib. *Cancer 110*, 1295–1302.

Crosio, C., Fimia, G. M., Loury, R., Kimura, M., Okano, Y., Zhou, H., et al. (2002). Mitotic phosphorylation of histone H3: Spatio-temporal regulation by mammalian Aurora kinases. *Molecular and Cellular Biology 22*, 874–885.

Culine, S., Theodore, C., De Santis, M., Bui, B., Demkow, T., Lorenz, J., et al. (2006). A phase II study of vinflunine in bladder cancer patients progressing after first-line platinum-containing regimen. *British Journal of Cancer 94*, 1395–1401.

D'Angiolella, V., Mari, C., Nocera, D., Rametti, L., & Grieco, D. (2003). The spindle checkpoint requires cyclin-dependent kinase activity. *Genes & Development 17*, 2520–2525.

de Bono, J. S., Tolcher, A. W., & Rowinsky, E. K. (2003). Farnesyltransferase inhibitors and their potential in the treatment of breast carcinoma. *Seminars in Oncology 30*, 79–92.

DeConti, R. C., Algazi, A. P., Andrews, S., Urbas, P., Born, O., Stoeckigt, D., et al. (2010). Phase II trial of sagopilone, a novel epothilone analog in metastatic melanoma. *British Journal of Cancer 103*, 1548–1553.

Dees, E. C., Cohen, R. B., von Mehren, M., Stinchcombe, T. E., Liu, H., Venkatakrishnan, K., et al. (2012). Phase I study of aurora A kinase inhibitor MLN8237 in advanced solid tumors: Safety, pharmacokinetics, pharmacodynamics, and bioavailability of two oral formulations. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 18*, 4775–4784.

Delord, J. P., Tourani, J. M., Lefresne, F., Pétain, A., Pouget, J. -C., & Ravaud, A. (2013). Phase I dose-escalation study of oral vinflunine administered once daily for 6 weeks every

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

8 weeks in patients with advanced/metastatic solid tumours. *Cancer Chemotherapy and Pharmacology 71*, 647–656.
DeLuca, J. G., Gall, W. E., Ciferri, C., Cimini, D., Musacchio, A., & Salmon, E. D. (2006). Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. *Cell 127*, 969–982.
Dennis, M., Davies, M., Oliver, S., D'Souza, R., Pike, L., & Stockman, P. (2012). Phase I study of the Aurora B kinase inhibitor barasertib (AZD1152) to assess the pharmacokinetics, metabolism and excretion in patients with acute myeloid leukemia. *Cancer Chemotherapy and Pharmacology 70*, 461–469.
Dent, S. F., Gelmon, K. A., Chi, K. N., Jonker, D. J., Wainman, N., Capier, C. A., et al. (2013). NCIC CTG IND.181: Phase I study of AT9283 given as a weekly 24 hour infusion in advanced malignancies. *Investigational New Drugs 31*, 1522–1529.
Desjardins, A., Reardon, D. A., Peters, K. B., Threatt, S., Coan, A. D., Herndon, J. E., et al. (2011). A phase I trial of the farnesyl transferase inhibitor, SCH 66336, with temozolomide for patients with malignant glioma. *Journal of Neuro-Oncology 105*, 601–606.
Diamond, J. R., Bastos, B. R., Hansen, R. J., Gustafson, D. L., Eckhardt, S. G., Kwak, E. L., et al. (2011). Phase I safety, pharmacokinetic, and pharmacodynamic study of ENMD-2076, a novel angiogenic and Aurora kinase inhibitor, in patients with advanced solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 17*, 849–860.
Ditchfield, C. (2003). Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *The Journal of Cell Biology 161*, 267–280.
Döhner, H., Lübbert, M., Fiedler, W., Fouillard, L., Haaland, A., Brandwein, J. M., et al. (2014). Randomized, phase 2 trial of low-dose cytarabine with or without volasertib in AML patients not suitable for induction therapy. *Blood 124*, 1426–1433.
Dou, Q. P., & Zonder, J. A. (2014). Overview of proteasome inhibitor-based anti-cancer therapies: Perspective on bortezomib and second generation proteasome inhibitors versus future generation inhibitors of ubiquitin-proteasome system. *Current Cancer Drug Targets 14*, 517–536.
Edelman, M. J., Harb, W. A., Pal, S. E., Boccia, R. V., Kraut, M. J., Bonomi, P., et al. (2012). Multicenter trial of EC145 in advanced, folate-receptor positive adenocarcinoma of the lung. *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer 7*, 1618–1621.
Elia, A. E. H., Cantley, L. C., & Yaffe, M. B. (2003). Proteomic screen finds pSer/pThr-binding domain localizing Plk1 to mitotic substrates. *Science (New York, N.Y.) 299*, 1228–1231.
Eskens, F. A., Awada, A., Cutler, D. L., de Jonge, M. J., Luyten, G. P., Faber, M. N., et al. (2001). Phase I and pharmacokinetic study of the oral farnesyl transferase inhibitor SCH 66336 given twice daily to patients with advanced solid tumors. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 19*, 1167–1175.
Fabre, C., Gobbi, M., Ezzili, C., Zoubir, M., Sablin, M. -P., Small, K., et al. (2014). Clinical study of the novel cyclin-dependent kinase inhibitor dinaciclib in combination with rituximab in relapsed/refractory chronic lymphocytic leukemia patients. *Cancer Chemotherapy and Pharmacology 74*, 1057–1064.
Feldhahn, N., Arutyunyan, A., Stoddart, S., Zhang, B., Schmidhuber, S., Yi, S. -J., et al. (2012). Environment-mediated drug resistance in Bcr/Abl-positive acute lymphoblastic leukemia. *OncoImmunology 1*, 618–629.
Feldman, E. J., Cortes, J., DeAngelo, D. J., Holyoake, T., Simonsson, B., O'Brien, S. G., et al. (2008). On the use of lonafarnib in myelodysplastic syndrome and chronic myelomonocytic leukemia. *Leukemia 22*, 1707–1711.
Feldmann, G., Mishra, A., Bisht, S., Karikari, C., Garrido-Laguna, I., Rasheed, Z., et al. (2011). Cyclin-dependent kinase inhibitor Dinaciclib (SCH727965) inhibits pancreatic cancer growth and progression in murine xenograft models. *Cancer Biology & Therapy 12*, 598–609.
Ferlini, C., Gallo, D., & Scambia, G. (2008). New taxanes in development. *Expert Opinion on Investigational Drugs 17*, 335–347.
Fitzgerald, D. P., Emerson, D. L., Qian, Y., Anwar, T., Liewehr, D. J., Steinberg, S. M., et al. (2012). TPI-287, a new taxane family member, reduces the brain metastatic colonization of breast cancer cells. *Molecular Cancer Therapeutics 11*, 1959–1967.
Flynn, J., Jones, J., Johnson, A. J., Andritsos, L., Maddocks, K., Jaglowski, S., et al. (2015). Dinaciclib is a novel cyclin-dependent kinase inhibitor with significant clinical activity in relapsed and refractory chronic lymphocytic leukemia. *Leukemia 29*, 1524–1529.
Fogh, S., Machtay, M., Werner-Wasik, M., Curran, W. J., Bonanni, R., Axelrod, R., et al. (2010). Phase I trial using patupilone (epothilone B) and concurrent radiotherapy for central nervous system malignancies. *International Journal of Radiation Oncology, Biology, Physics 77*, 1009–1016.
Foran, J., Ravandi, F., Wierda, W., Garcia-Manero, G., Verstovsek, S., Kadia, T., et al. (2014). A phase I and pharmacodynamic study of AT9283, a small-molecule inhibitor of aurora kinases in patients with relapsed/refractory leukemia or myelofibrosis. *Clinical Lymphoma, Myeloma & Leukemia 14*, 223–230.
Forster, M., Kaye, S., Oza, A., Sklenar, I., Johri, A., Cheung, W., et al. (2007). A phase Ib and pharmacokinetic trial of patupilone combined with carboplatin in patients with advanced cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 13*, 4178–4184.
Fox, E., Maris, J. M., Widemann, B. C., Meek, K., Goodwin, A., Goodspeed, W., et al. (2006). A phase 1 study of ABT-751, an orally bioavailable tubulin inhibitor, administered daily for 7 days every 21 days in pediatric patients with solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 12*, 4882–4887.
Fox, E., Maris, J. M., Widemann, B. C., Goodspeed, W., Goodwin, A., Kromplewski, M., et al. (2008). A phase I study of ABT-751, an orally bioavailable tubulin inhibitor, administered daily for 21 days every 28 days in pediatric patients with solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 14*, 1111–1115.
Fox, E., Mosse', Y. P., Meany, H. M., Gurney, J. G., Khanna, G., Jackson, H. A., et al. (2014). Time to disease progression in children with relapsed or refractory neuroblastoma treated with ABT-751: A report from the Children's Oncology Group (ANBL0621). *Pediatric Blood & Cancer 61*, 990–996.
Freedman, R. A., Bullitt, E., Sun, L., Gelman, R., Harris, G., Ligibel, J. A., et al. (2011). A phase II study of sagopilone (ZK 219477; ZK-EPO) in patients with breast cancer and brain metastases. *Clinical Breast Cancer 11*, 376–383.
Friedberg, J. W., Mahadevan, D., Cebula, E., Persky, D., Lossos, I., Agarwal, A. B., et al. (2014). Phase II study of alisertib, a selective Aurora A kinase inhibitor, in relapsed and refractory aggressive B- and T-cell non-Hodgkin lymphomas. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 32*, 44–50.
Fumoleau, P., Cortés-Funes, H., Taleb, A. B., Chan, S., Campone, M., Pouget, J. -C., et al. (2009). Phase 2 study of single-agent IV vinflunine as third-line treatment of metastatic breast cancer after failure of anthracycline−/taxane-based chemotherapy. *American Journal of Clinical Oncology 32*, 375–380.
Funabiki, H., Yamano, H., Kumada, K., Nagao, K., Hunt, T., & Yanagida, M. (1996). Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature 381*, 438–441.
Gallerani, E., Zucchetti, M., Brunelli, D., Marangon, E., Noberasco, C., Hess, D., et al. (2013). A first in human phase I study of the proteasome inhibitor CEP-18770 in patients with advanced solid tumours and multiple myeloma. *European Journal of Cancer (Oxford, England: 1990) 49*, 290–296.
Gallorini, M., Cataldi, A., & di Giacomo, V. (2012). Cyclin-dependent kinase modulators and cancer therapy. *BioDrugs: Clinical Immunotherapeutics, Biopharmaceuticals and Gene Therapy 26*, 377–391.
Garber, K. (2005). Divide and conquer: New generation of drugs targets mitosis. *Journal of the National Cancer Institute 97*, 874–876.
Gascoigne, K. E., & Taylor, S. S. (2008). Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. *Cancer Cell 14*, 111–122.
Gassmann, R., Carvalho, A., Henzing, A. J., Ruchaud, S., Hudson, D. F., Honda, R., et al. (2004). Borealin: A novel chromosomal passenger required for stability of the bipolar mitotic spindle. *The Journal of Cell Biology 166*, 179–191.
Gauler, T. C., Christoph, D. C., Fischer, J., Frickhofen, N., Huber, R., Gonschorek, C., et al. (2013). Phase-I study of sagopilone in combination with cisplatin in chemotherapy-naive patients with metastasised small-cell lung cancer. *European Journal of Cancer (Oxford, England: 1990) 49*, 2461–2468.
Gautier, J., Solomon, M. J., Booher, R. N., Bazan, J. F., & Kirschner, M. W. (1991). cdc25 is a specific tyrosine phosphatase that directly activates p34cdc2. *Cell 67*, 197–211.
Geley, S., Kramer, E., Gieffers, C., Gannon, J., Peters, J. M., & Hunt, T. (2001). Anaphase-promoting complex/cyclosome-dependent proteolysis of human cyclin A starts at the beginning of mitosis and is not subject to the spindle assembly checkpoint. *The Journal of Cell Biology 153*, 137–148.
Gerullis, H., Eimer, C., Ecke, T. H., Georgas, E., Arndt, C., & Otto, T. (2013). Combined treatment with pazopanib and vinflunine in patients with advanced urothelial carcinoma refractory after first-line therapy. *Anti-Cancer Drugs 24*, 422–425.
Gilmartin, A. G., Bleam, M. R., Richter, M. C., Erskine, S. G., Kruger, R. G., Madden, L., et al. (2009). Distinct concentration-dependent effects of the polo-like kinase 1-specific inhibitor GSK461364A, including differential effect on apoptosis. *Cancer Research 69*, 6969–6977.
Giovinazzi, S., Bellapu, D., Morozov, V. M., & Ishov, A. M. (2013). Targeting mitotic exit with hyperthermia or APC/C inhibition to increase paclitaxel efficacy. *Cell Cycle 12*, 2598–2607.
Gojo, I., Sadowska, M., Walker, A., Feldman, E. J., Iyer, S. P., Baer, M. R., et al. (2013). Clinical and laboratory studies of the novel cyclin-dependent kinase inhibitor dinaciclib (SCH 727965) in acute leukemias. *Cancer Chemotherapy and Pharmacology 72*, 897–908.
Goldstein, D., Ackland, S. P., Bell, D. R., Olver, I. N., Davis, I. D., Rosenthal, M. A., et al. (2006). Phase II study of vinflunine in patients with metastatic renal cell carcinoma. *Investigational New Drugs 24*, 429–434.
Gomez, H. L., Philco, M., Pimentel, P., Kiyan, M., Monsalvo, M. L., Conlan, M. G., et al. (2012). Phase I dose-escalation and pharmacokinetic study of ispinesib, a kinesin spindle protein inhibitor, administered on days 1 and 15 of a 28-day schedule in patients with no prior treatment for advanced breast cancer. *Anti-Cancer Drugs 23*, 335–341.
Goshima, G., Kiyomitsu, T., Yoda, K., & Yanagida, M. (2003). Human centromere chromatin protein hMis12, essential for equal segregation, is independent of CENP-A loading pathway. *The Journal of Cell Biology 160*, 25–39.
Goto, H., Kiyono, T., Tomono, Y., Kawajiri, A., Urano, T., Furukawa, K., et al. (2006). Complex formation of Plk1 and INCENP required for metaphase-anaphase transition. *Nature Cell Biology 8*, 180–187.
Gritsko, T. M., Coppola, D., Paciga, J. E., Yang, L., Sun, M., Shelley, S. A., et al. (2003). Activation and overexpression of centrosome kinase BTAK/Aurora-A in human ovarian cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 9*, 1420–1426.
Grossman, S. A., Ye, X., Peereboom, D., Rosenfeld, M. R., Mikkelsen, T., Supko, J. G., et al. (2012). Phase I study of terameprocol in patients with recurrent high-grade glioma. *Neuro-Oncology 14*, 511–517.
Grundy, M., Seedhouse, C., Russell, N. H., & Pallis, M. (2011). P-glycoprotein and breast cancer resistance protein in acute myeloid leukaemia cells treated with the Aurora-B Kinase Inhibitor barasertib-hQPA. *BMC Cancer 11*, 254.
Guertin, A. D., O'Neil, J., Stoeck, A., Reddy, J. A., Cristescu, R., Haines, B. B., et al. (2016). High levels of expression of P-glycoprotein/multidrug resistance protein result in resistance to vintafolide. *Molecular Cancer Therapeutics 15*, 1998–2008.
Guo, J., Anderson, M. G., Tapang, P., Palma, J. P., Rodriguez, L. E., Niquette, A., et al. (2009). Identification of genes that confer tumor cell resistance to the Aurora B kinase inhibitor, AZD1152. *The Pharmacogenomics Journal 9*, 90–102.

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

Gurzov, E. N., & Izquierdo, M. (2006). RNA interference against Hec1 inhibits tumor growth in vivo. *Gene Therapy 13*, 1–7.
Hainsworth, J. D., Meluch, A. A., Lane, C. M., Spigel, D. R., Burris, H. A., Gandhi, J. G., et al. (2010). Single agent vinflunine in the salvage treatment of patients with castration-resistant prostate cancer: A phase II trial of the Sarah Cannon research consortium. *Cancer Investigation 28*, 275–279.
Hames, R. S., Wattam, S. L., Yamano, H., Bacchieri, R., & Fry, A. M. (2001). APC/C-mediated destruction of the centrosomal kinase Nek2A occurs in early mitosis and depends upon a cyclin A-type D-box. *The EMBO Journal 20*, 7117–7127.
Hammer, S., Sommer, A., Fichtner, I., Becker, M., Rolff, J., Merk, J., et al. (2010). Comparative profiling of the novel epothilone, Sagopilone, in xenografts derived from primary non-small cell lung cancer. *Clinical Cancer Research 16*, 1452–1465.
Hande, K. R., Hagey, A., Berlin, J., Cai, Y., Meek, K., Kobayashi, H., et al. (2006). The pharmacokinetics and safety of ABT-751, a novel, orally bioavailable sulfonamide antimitotic agent: Results of a phase 1 study. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 12*, 2834–2840.
Hanrahan, E. O., Kies, M. S., Glisson, B. S., Khuri, F. R., Feng, L., Tran, H. T., et al. (2009). A phase II study of Lonafarnib (SCH66336) in patients with chemorefractory, advanced squamous cell carcinoma of the head and neck. *American Journal of Clinical Oncology 32*, 274–279.
Hauf, S., Cole, R. W., LaTerra, S., Zimmer, C., Schnapp, G., Walter, R., et al. (2003). The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *The Journal of Cell Biology 161*, 281–294.
Hauf, S., Roitinger, E., Koch, B., Dittrich, C. M., Mechtler, K., & Peters, J. -M. (2005). Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. *PLoS Biology 3*, e69.
Haupenthal, J., Bihrer, V., Korkusuz, H., Kollmar, O., Schmithals, C., Kriener, S., et al. (2012). Reduced efficacy of the Plk1 inhibitor BI 2536 on the progression of hepatocellular carcinoma due to low intratumoral drug levels. *Neoplasia (New York, N.Y.) 14*, 410–419.
He, Y., Yan, D., Zheng, D., Hu, Z., Li, H., & Li, J. (2016). Cell division cycle 6 promotes mitotic slippage and contributes to drug resistance in paclitaxel-treated cancer cells. *PloS One 11*, e0162633.
Heigener, D. F., von Pawel, J., Eschbach, C., Brune, A., Schmittel, A., Schmelter, T., et al. (2013). Prospective, multicenter, randomized, independent-group, open-label phase II study to investigate the efficacy and safety of three regimens with two doses of sagopilone as second-line therapy in patients with stage IIIB or IV non-small-cell lung cancer. *Lung Cancer (Amsterdam, Netherlands) 80*, 319–325.
Hershko, A., & Ciechanover, A. (1998). The ubiquitin system. *Annual Review of Biochemistry 67*, 425–479.
Hirota, T., Kunitoku, N., Sasayama, T., Marumoto, T., Zhang, D., Nitta, M., et al. (2003). Aurora-A and an interacting activator, the LIM protein Ajuba, are required for mitotic commitment in human cells. *Cell 114*, 585–598.
Holen, K. D., Belani, C. P., Wilding, G., Ramalingam, S., Volkman, J. L., Ramanathan, R. K., et al. (2011). A first in human study of SB-743921, a kinesin spindle protein inhibitor, to determine pharmacokinetics, biologic effects and establish a recommended phase II dose. *Cancer Chemotherapy and Pharmacology 67*, 447–454.
Holen, K., DiPaola, R., Liu, G., Tan, A. R., Wilding, G., Hsu, K., et al. (2012). A phase I trial of MK-0731, a kinesin spindle protein (KSP) inhibitor, in patients with solid tumors. *Investigational New Drugs 30*, 1088–1095.
Honda, R. (2003). Exploring the functional interactions between aurora B, INCENP, and survivin in mitosis. *Molecular Biology of the Cell 14*, 3325–3341.
Hopper-Borge, E., Xu, X., Shen, T., Shi, Z., Chen, Z. -S., & Kruh, G. D. (2009). Human multidrug resistance protein 7 (ABCC10) is a resistance factor for nucleoside analogues and epothilone B. *Cancer Research 69*, 178–184.
Howell, B. J., McEwen, B. F., Canman, J. C., Hoffman, D. B., Farrar, E. M., Rieder, C. L., et al. (2001). Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *The Journal of Cell Biology 155*, 1159–1172.
Huang, H. -C., Shi, J., Orth, J. D., & Mitchison, T. J. (2009). Evidence that mitotic exit is a better cancer therapeutic target than spindle assembly. *Cancer Cell 16*, 347–358.
Hussain, A., DiPaola, R. S., Baron, A. D., Higano, C. S., Tchekmedyian, N. S., & Johri, A. R. (2009). Phase II trial of weekly patupilone in patients with castration-resistant prostate cancer. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 20*, 492–497.
Ikezoe, T. (2008). Aurora kinases as an anti-cancer target. *Cancer Letters 262*, 1–9.
Jackson, J. R., Patrick, D. R., Dar, M. M., & Huang, P. S. (2007). Targeted anti-mitotic therapies: Can we improve on tubulin agents? *Nature Reviews. Cancer 7*, 107–117.
Jiang, Z., Jiang, J., Yang, H., Ge, Z., Wang, Q., Zhang, L., et al. (2014). Silencing of Aurora kinase A by RNA interference inhibits tumor growth in human osteosarcoma cells by inducing apoptosis and G2/M cell cycle arrest. *Oncology Reports 31*, 1249–1254.
Johnson, V. L., Scott, M. I. F., Holt, S. V., Hussein, D., & Taylor, S. S. (2004). Bub1 is required for kinetochore localization of BubR1, Cenp-E, Cenp-F and Mad2, and chromosome congression. *Journal of Cell Science 117*, 1577–1589.
Johnson, P., Geldart, T., Fumoleau, P., Pinel, M. -C., Nguyen, L., & Judson, I. (2006). Phase I study of vinflunine administered as a 10-minute infusion on days 1 and 8 every 3 weeks. *Investigational New Drugs 24*, 223–231.
Jongsma, M. L. M., Berlin, I., & Neefjes, J. (2014). On the move: Organelle dynamics during mitosis. *Trends in Cell Biology 25*, 112–124.
Jordan, M. A., & Wilson, L. (2004). Microtubules as a target for anticancer drugs. *Nature Reviews. Cancer 4*, 253–265.
Kaestner, P., Stolz, A., & Bastians, H. (2009). Determinants for the efficiency of anticancer drugs targeting either Aurora-A or Aurora-B kinases in human colon carcinoma cells. *Molecular Cancer Therapeutics 8*, 2046–2056.
Kantarjian, H. M., Martinelli, G., Jabbour, E. J., Quintás-Cardama, A., Ando, K., Bay, J. -O., et al. (2013a). Stage I of a phase 2 study assessing the efficacy, safety, and tolerability of barasertib (AZD1152) versus low-dose cytosine arabinoside in elderly patients with acute myeloid leukemia. *Cancer 119*, 2611–2619.
Kantarjian, H. M., Sekeres, M. A., Ribrag, V., Rousselot, P., Garcia-Manero, G., Jabbour, E. J., et al. (2013b). Phase I study assessing the safety and tolerability of barasertib (AZD1152) with low-dose cytosine arabinoside in elderly patients with AML. *Clinical Lymphoma, Myeloma & Leukemia 13*, 559–567.
Kapoor, T. M., Mayer, T. U., Coughlin, M. L., & Mitchison, T. J. (2000). Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. *The Journal of Cell Biology 150*, 975–988.
Katayama, H., Ota, T., Jisaki, F., Ueda, Y., Tanaka, T., Odashima, S., et al. (1999). Mitotic kinase expression and colorectal cancer progression. *Journal of the National Cancer Institute 91*, 1160–1162.
Kauh, J., Chanel-Vos, C., Escuin, D., Fanucchi, M. P., Harvey, R. D., Saba, N., et al. (2011). Farnesyl transferase expression determines clinical response to the docetaxel-lonafarnib combination in patients with advanced malignancies. *Cancer 117*, 4049–4059.
Kaur, R., Kaur, G., Gill, R. K., Soni, R., & Bariwal, J. (2014). Recent developments in tubulin polymerization inhibitors: An overview. *European Journal of Medicinal Chemistry 87*, 89–124.
Kavallaris, M., Annereau, J. -P., & Barret, J. -M. (2008). Potential mechanisms of resistance to microtubule inhibitors. *Seminars in Oncology 35*, S22–S27.
Kelly, K. R., Shea, T. C., Goy, A., Berdeja, J. G., Reeder, C. B., McDonagh, K. T., et al. (2014). Phase I study of MLN8237—Investigational Aurora A kinase inhibitor—In relapsed/refractory multiple myeloma, non-Hodgkin lymphoma and chronic lymphocytic leukemia. *Investigational New Drugs 32*, 489–499.
Khoury, H. J., Garcia-Manero, G., Borthakur, G., Kadia, T., Foudray, M. C., Arellano, M., et al. (2012). A phase 1 dose-escalation study of ARRY-520, a kinesin spindle protein inhibitor, in patients with advanced myeloid leukemias. *Cancer 118*, 3556–3564.
Khuri, F. R., Glisson, B. S., Kim, E. S., Statkevich, P., Thall, P. F., Meyers, M. L., et al. (2004). Phase I study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 10*, 2968–2976.
Kieran, M. W., Packer, R. J., Onar, A., Blaney, S. M., Phillips, P., Pollack, I. F., et al. (2007). Phase I and pharmacokinetic study of the oral farnesyltransferase inhibitor lonafarnib administered twice daily to pediatric patients with advanced central nervous system tumors using a modified continuous reassessment method: A pediatric brain tumor cons. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 25*, 3137–3143.
Kim, E. S., Kies, M. S., Fossella, F. V., Glisson, B. S., Zaknoen, S., Statkevich, P., et al. (2005). Phase II study of the farnesyltransferase inhibitor lonafarnib with paclitaxel in patients with taxane-refractory/resistant nonsmall cell lung carcinoma. *Cancer 104*, 561–569.
Kline-Smith, S. L. (2003). Depletion of centromeric MCAK leads to chromosome congression and segregation defects due to improper kinetochore attachments. *Molecular Biology of the Cell 15*, 1146–1159.
Knowlton, A. L., Lan, W., & Stukenberg, P. T. (2006). Aurora B is enriched at merotelic attachment sites, where it regulates MCAK. *Current Biology: CB 16*, 1705–1710.
Knox, J. J., Gill, S., Synold, T. W., Biagi, J. J., Major, P., Feld, R., et al. (2008). A phase II and pharmacokinetic study of SB-715992, in patients with metastatic hepatocellular carcinoma: A study of the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG IND.168). *Investigational New Drugs 26*(3), 265–272.
Kops, G. J. P. L. (2005). ZW10 links mitotic checkpoint signaling to the structural kinetochore. *The Journal of Cell Biology 169*, 49–60.
Kraft, C., Herzog, F., Gieffers, C., Mechtler, K., Hagting, A., Pines, J., et al. (2003). Mitotic regulation of the human anaphase-promoting complex by phosphorylation. *The EMBO Journal 22*, 6598–6609.
Kruczynski, A., & Hill, B. T. (2001). Vinflunine, the latest Vinca alkaloid in clinical development. A review of its preclinical anticancer properties. *Critical Reviews in Oncology/Hematology 40*, 159–173.
Kruczynski, A., Etiévant, C., Perrin, D., Chansard, N., Duflos, A., & Hill, B. T. (2002). Characterization of cell death induced by vinflunine, the most recent Vinca alkaloid in clinical development. *British Journal of Cancer 86*, 143–150.
Krzakowski, M., Bennouna, J., Dansin, E., Kowalski, D., Hiret, S., Penel, N., et al. (2014). Phase I dose-escalation study of oral vinflunine in combination with erlotinib in pre-treated and unselected EGFR patients with locally advanced or metastatic non-small-cell lung cancer. *Cancer Chemotherapy and Pharmacology 73*, 231–236.
Kumagai, A., & Dunphy, W. G. (1996). Purification and molecular cloning of Plx1, a Cdc25-regulatory kinase from Xenopus egg extracts. *Science (New York, N.Y.) 273*, 1377–1380.
Kumar, S. K., LaPlant, B., Chng, W. J., Zonder, J., Callander, N., Fonseca, R., et al. (2014). Dinaciclib, a novel CDK inhibitor, demonstrates encouraging single-agent activity in patients with relapsed multiple myeloma. *Blood 125*, 443–448.
Lane, H. A., & Nigg, E. A. (1996). Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes. *The Journal of Cell Biology 135*, 1701–1713.
Lavogina, D., Enkvist, E., Viht, K., & Uri, A. (2014). Long residence times revealed by Aurora A kinase-targeting fluorescent probes derived from inhibitors MLN8237 and VX-689. *Chembiochem: A European Journal of Chemical Biology 15*, 443–450.
Lawasut, P., Chauhan, D., Laubach, J., Hayes, C., Fabre, C., Maglio, M., et al. (2012). New proteasome inhibitors in myeloma. *Current Hematologic Malignancy Reports 7*, 258–266.
Le Tourneau, C., Faivre, S., Laurence, V., Delbaldo, C., Vera, K., Girre, V., et al. (2010). Phase I evaluation of seliciclib (R-roscovitine), a novel oral cyclin-dependent kinase inhibitor, in patients with advanced malignancies. *European Journal of Cancer (Oxford, England: 1990) 46*, 3243–3250.

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

Lee, C. W., Belanger, K., Rao, S. C., Petrella, T. M., Tozer, R. G., Wood, L., et al. (2008a). A phase II study of ispinesib (SB-715992) in patients with metastatic or recurrent malignant melanoma: A National Cancer Institute of Canada Clinical Trials Group trial. *Investigational New Drugs 26*, 249–255.

Lee, R. T., Beekman, K. E., Hussain, M., Davis, N. B., Clark, J. I., Thomas, S. P., et al. (2008b). A University of Chicago consortium phase II trial of SB-715992 in advanced renal cell cancer. *Clinical Genitourinary Cancer 6*, 21–24.

Li, D., Zhu, J., Firozi, P. F., Abbruzzese, J. L., Evans, D. B., Cleary, K., et al. (2003). Overexpression of oncogenic STK15/BTAK/Aurora A kinase in human pancreatic cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 9*, 991–997.

Li, J., Sausville, E. A., Klein, P. J., Morgenstern, D., Leamon, C. P., Messmann, R. A., & LoRusso, P. (2009). Clinical pharmacokinetics and exposure-toxicity relationship of a folate-Vinca alkaloid conjugate EC145 in cancer patients. *Journal of Clinical Pharmacology 49*, 1467–1476.

Lin, C. -C., Su, W. -C., Yen, C. -J., Hsu, C. -H., Su, W. -P., Yeh, K. -H., et al. (2014). A phase I study of two dosing schedules of volasertib (BI 6727), an intravenous polo-like kinase inhibitor, in patients with advanced solid malignancies. *British Journal of Cancer 110*, 2434–2440.

Lindon, C., & Pines, J. (2004). Ordered proteolysis in anaphase inactivates Plk1 to contribute to proper mitotic exit in human cells. *The Journal of Cell Biology 164*, 233–241.

Ling, Y. -H., Liebes, L., Jiang, J. -D., Holland, J. F., Elliott, P. J., Adams, J., et al. (2003). Mechanisms of proteasome inhibitor PS-341-induced G(2)-M-phase arrest and apoptosis in human non-small cell lung cancer cell lines. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 9*, 1145–1154.

Linton, A., Cheng, Y. Y., Griggs, K., Kirschner, M. B., Gattani, S., Srikaran, S., et al. (2014). An RNAi-based screen reveals PLK1, CDK1 and NDC80 as potential therapeutic targets in malignant pleural mesothelioma. *British Journal of Cancer 110*, 510–519.

Liu-Sullivan, N., Zhang, J., Bakleh, A., Marchica, J., Li, J., Siolas, D., et al. (2011). Pooled shRNA screen for sensitizers to inhibition of the mitotic regulator polo-like kinase (PLK1). *Oncotarget 2*, 1254–1264.

Lorusso, P. M., Edelman, M. J., Bever, S. L., Forman, K. M., Pilat, M., Quinn, M. F., et al. (2012). Phase I study of folate conjugate EC145 (Vintafolide) in patients with refractory solid tumors. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 30*, 4011–4016.

LoRusso, P. M., Goncalves, P. H., Casetta, L., Carter, J. A., Litwiler, K., Roseberry, D., et al. (2015). First-in-human phase 1 study of filanesib (ARRY-520), a kinesin spindle protein inhibitor, in patients with advanced solid tumors. *Investigational New Drugs 33*, 440–449.

Löwenberg, B., Muus, P., Ossenkoppele, G., Rousselot, P., Cahn, J. -Y., Ifrah, N., et al. (2011). Phase I/II study to assess the safety, efficacy, and pharmacokinetics of barasertib (AZD1152) in patients with advanced acute myeloid leukemia. *Blood*, 6030–6036.

Luo, L., Parrish, C. A., Nevins, N., McNulty, D. E., Chaudhari, A. M., Carson, J. D., et al. (2007). ATP-competitive inhibitors of the mitotic kinesin KSP that function via an allosteric mechanism. *Nature Chemical Biology 3*, 722–726.

Ma, T., Fuld, A. D., Rigas, J. R., Hagey, A. E., Gordon, G. B., Dmitrovsky, E., et al. (2012). A phase I trial and in vitro studies combining ABT-751 with carboplatin in previously treated non-small cell lung cancer patients. *Chemotherapy 58*, 321–329.

Machiels, J. -P., Peeters, M., Herremans, C., Surmont, V., Specenier, P., De Smet, M., et al. (2015). A phase I study of volasertib combined with afatinib, in advanced solid tumors. *Cancer Chemotherapy and Pharmacology 76*, 843–851.

Malumbres, M., & Barbacid, M. (2005). Mammalian cyclin-dependent kinases. *Trends in Biochemical Sciences 30*, 630–641.

Manchado, E., Guillamot, M., de Cárcer, G., Eguren, M., Trickey, M., García-Higuera, I., et al. (2010). Targeting mitotic exit leads to tumor regression in vivo: Modulation by Cdk1, Mastl, and the PP2A/B55α,δ phosphatase. *Cancer Cell 18*, 641–654.

Manchado, E., Guillamot, M., & Malumbres, M. (2012). Killing cells by targeting mitosis. *Cell Death and Differentiation 19*, 369–377.

Mariaule, G., & Belmont, P. (2014). Cyclin-dependent kinase inhibitors as marketed anticancer drugs: Where are we now? A short survey. *Molecules (Basel, Switzerland) 19*, 14366–14382.

Marshall, O. J., Marshall, A. T., & Choo, K. H. A. (2008). Three-dimensional localization of CENP-A suggests a complex higher order structure of centromeric chromatin. *The Journal of Cell Biology 183*, 1193–1202.

Martin, M. P., Olesen, S. H., Georg, G. I., & Schönbrunn, E. (2013). Cyclin-dependent kinase inhibitor dinaciclib interacts with the acetyl-lysine recognition site of bromodomains. *ACS Chemical Biology 8*, 2360–2365.

Martin-Lluesma, S., Stucke, V. M., & Nigg, E. A. (2002). Role of Hec1 in spindle checkpoint signaling and kinetochore recruitment of Mad1/Mad2. *Science (New York, N.Y.) 297*, 2267–2270.

Marumoto, T., Honda, S., Hara, T., Nitta, M., Hirota, T., Kohmura, E., et al. (2003). Aurora-A kinase maintains the fidelity of early and late mitotic events in HeLa cells. *The Journal of Biological Chemistry 278*, 51786–51795.

Marumoto, T., Zhang, D., & Saya, H. (2005). Aurora-A - a guardian of poles. *Nature Reviews. Cancer 5*, 42–50.

Matulonis, U. A., Sharma, S., Ghamande, S., Gordon, M. S., Del Prete, S. A., Ray-Coquard, I., et al. (2012). Phase II study of MLN8237 (alisertib), an investigational Aurora A kinase inhibitor, in patients with platinum-resistant or -refractory epithelial ovarian, fallopian tube, or primary peritoneal carcinoma. *Gynecologic Oncology 127*, 63–69.

Matulonis, U. A., Lee, J., Lasonde, B., Tew, W. P., Yehwalashet, A., Matei, D., et al. (2013). ENMD-2076, an oral inhibitor of angiogenic and proliferation kinases, has activity in recurrent, platinum resistant ovarian cancer. *European Journal of Cancer 49*, 121–131.

Mauer, A. M., Cohen, E. E. W., Ma, P. C., Kozloff, M. F., Schwartzberg, L., Coates, A. I., et al. (2008). A phase II study of ABT-751 in patients with advanced non-small cell lung cancer. *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer 3*, 631–636.

McEwen, B. F., Hsieh, C. E., Mattheyses, A. L., & Rieder, C. L. (1998). A new look at kinetochore structure in vertebrate somatic cells using high-pressure freezing and freeze substitution. *Chromosoma 107*, 366–375.

McGowan, C. H., & Russell, P. (1993). Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. *The EMBO Journal 12*, 75–85.

McMeekin, S., Patel, R., Verschraegen, C., Celano, P., Burke, J., Plaxe, S., et al. (2012). Phase I/II study of sagopilone (ZK-EPO) plus carboplatin in women with recurrent platinum-sensitive ovarian cancer. *British Journal of Cancer 106*, 70–76.

Meany, H. J., Sackett, D. L., Maris, J. M., Ward, Y., Krivoshik, A., Cohn, S. L., et al. (2010). Clinical outcome in children with recurrent neuroblastoma treated with ABT-751 and effect of ABT-751 on proliferation of neuroblastoma cell lines and on tubulin polymerization in vitro. *Pediatric Blood & Cancer 54*, 47–54.

Melichar, B., Casado, E., Bridgewater, J., Bennouna, J., Campone, M., Vitek, P., et al. (2011). Clinical activity of patupilone in patients with pretreated advanced/metastatic colon cancer: Results of a phase I dose escalation trial. *British Journal of Cancer 105*, 1646–1653.

Melichar, B., Adenis, A., Lockhart, A. C., Bennouna, J., Dees, E. C., Kayaleh, O., et al. (2015). Safety and activity of alisertib, an investigational aurora kinase A inhibitor, in patients with breast cancer, small-cell lung cancer, non-small-cell lung cancer, head and neck squamous-cell carcinoma, and gastro-oesophageal adenocarcinoma: A five-arm ph. *The Lancet. Oncology 16*, 395–405.

Meulenbeld, H. J., Bleuse, J. P., Vinci, E. M., Raymond, E., Vitali, G., Santoro, A., et al. (2013). Randomized phase II study of danusertib in patients with metastatic castration-resistant prostate cancer after docetaxel failure. *BJU International 111*, 44–52.

Michels, J., Ellard, S. L., Le, L., Kollmannsberger, C., Murray, N., Tomlinson Guns, E. S., et al. (2010). A phase IB study of ABT-751 in combination with docetaxel in patients with advanced castration-resistant prostate cancer. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 21*, 305–311.

Milojkovic Kerklaan, B., Kerklaan, B. M., Diéras, V., Le Tourneau, C., Mergui-Roelvink, M., Huitema, A. D. R., et al. (2013). Phase I study of lonafarnib (SCH66336) in combination with trastuzumab plus paclitaxel in Her2/neu overexpressing breast cancer: EORTC study 16023. *Cancer Chemotherapy and Pharmacology 71*, 53–62.

Mita, M. M., Joy, A. A., Mita, A., Sankhala, K., Jou, Y. -M., Zhang, D., et al. (2014). Randomized phase II trial of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus capecitabine in patients with advanced breast cancer. *Clinical Breast Cancer 14*, 169–176.

Mitchell, D., Bergendahl, G., Ferguson, W., Roberts, W., Higgins, T., Ashikaga, T., et al. (2016). A phase 1 trial of TPI 287 as a single agent and in combination with temozolomide in patients with refractory or recurrent neuroblastoma or medulloblastoma. *Pediatric Blood & Cancer 63*, 39–46.

Mogilner, A., Wollman, R., Civelekoglu-Scholey, G., & Scholey, J. (2006). Modeling mitosis. *Trends in Cell Biology 16*, 88–96.

Montalban-Bravo, G., & Garcia-Manero, G. (2015). Novel drugs for older patients with acute myeloid leukemia. *Leukemia 29*, 760–769.

Moorcraft, S. Y., Chau, I., Peckitt, C., Cunningham, D., Rao, S., Yim, K. L., et al. (2013). Patupilone in patients with pretreated metastatic/locally recurrent colorectal cancer: Results of the phase II CINATRA trial. *Investigational New Drugs 31*, 1339–1344.

Moreno, L., Marshall, L. V., Pearson, A. D. J., Morland, B., Elliott, M., Campbell-Hewson, Q., et al. (2015). A phase I trial of AT9283 (a selective inhibitor of aurora kinases) in children and adolescents with solid tumors: A Cancer Research UK study. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 21*, 267–273.

Morris, R. T., Joyrich, R. N., Naumann, R. W., Shah, N. P., Maurer, A. H., Strauss, H. W., et al. (2014). Phase II study of treatment of advanced ovarian cancer with folate-receptor-targeted therapeutic (vintafolide) and companion SPECT-based imaging agent (99mTc-etarfolatide). *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 25*, 852–858.

Morrow, C. J. (2005). Bub1 and aurora B cooperate to maintain BubR1-mediated inhibition of APC/CCdc20. *Journal of Cell Science 118*, 3639–3652.

Morrow, P. K., Divers, S., Provencher, L., Luoh, S. -W., Petrella, T. M., Giurescu, M., et al. (2010). Phase II study evaluating the efficacy and safety of sagopilone (ZK-EPO) in patients with metastatic breast cancer that has progressed following chemotherapy. *Breast Cancer Research and Treatment 123*, 837–842.

Mossé, Y. P., Lipsitz, E., Fox, E., Teachey, D. T., Maris, J. M., Weigel, B., et al. (2012). Pediatric phase I trial and pharmacokinetic study of MLN8237, an investigational oral selective small-molecule inhibitor of Aurora kinase: A Children's Oncology Group Phase I Consortium study. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 18*, 6058–6064.

Mozzetti, S., Iantomasi, R., De Maria, I., Prislei, S., Mariani, M., Camperchioli, A., et al. (2008). Molecular mechanisms of patupilone resistance. *Cancer Research 68*, 10197–10204.

Mozzetti, S., Martinelli, E., Raspaglio, G., Prislei, S., De Donato, M., Filippetti, F., et al. (2012). Gli family transcription factors are drivers of patupilone resistance in ovarian cancer. *Biochemical Pharmacology 84*, 1409–1418.

Mueller, P. R., Coleman, T. R., Kumagai, A., & Dunphy, W. G. (1995). Myt1: A membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15. *Science (New York, N.Y.) 270*, 86–90.

Murray, A. (1995). Cyclin ubiquitination: The destructive end of mitosis. *Cell 81*, 149–152.

Musacchio, A., & Salmon, E. D. (2007). The spindle-assembly checkpoint in space and time. *Nature Reviews. Molecular Cell Biology 8*, 379–393.

Naumann, R. W., Coleman, R. L., Burger, R. A., Sausville, E. A., Kutarska, E., Ghamande, S. A., et al. (2013). PRECEDENT: A randomized phase II trial comparing vintafolide (EC145) and pegylated liposomal doxorubicin (PLD) in combination versus PLD alone in patients with platinum-resistant ovarian cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 31*, 4400–4406.

Nemunaitis, J. J., Small, K. A., Kirschmeier, P., Zhang, D., Zhu, Y., Jou, Y. -M., et al. (2013). A first-in-human, phase 1, dose-escalation study of dinaciclib, a novel cyclin-dependent

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

kinase inhibitor, administered weekly in subjects with advanced malignancies. *Journal of Translational Medicine 11*, 259.
Nigg, E. A., Blangy, A., & Lane, H. A. (1996). Dynamic changes in nuclear architecture during mitosis: On the role of protein phosphorylation in spindle assembly and chromosome segregation. *Experimental Cell Research 229*, 174–180.
Nonomiya, Y., Noguchi, K., Tanaka, N., Kasagaki, T., Katayama, K., & Sugimoto, Y. (2016). Effect of AKT3 expression on MYC- and caspase-8-dependent apoptosis caused by polo-like kinase inhibitors in HCT 116 cells. *Cancer Science* (Epub ahead of print).
Obuse, C., Iwasaki, O., Kiyomitsu, T., Goshima, G., Toyoda, Y., & Yanagida, M. (2004). A conserved Mis12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1. *Nature Cell Biology 6*, 1135–1141.
Olmos, D., Barker, D., Sharma, R., Brunetto, A. T., Yap, T. A., Taegtmeyer, A. B., et al. (2011). Phase I study of GSK461364, a specific and competitive polo-like kinase 1 inhibitor, in patients with advanced solid malignancies. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 17*, 3420–3430.
Olver, I. N., Byrne, M. J., Walpole, E., Vorobiof, D., Jacobs, C., Maart, K., et al. (2007). Phase II study of IV vinflunine in patients with chemotherapy naive metastatic malignant melanoma. *European Journal of Cancer (Oxford, England: 1990) 43*, 1829–1832.
Paridaens, R., Rixe, O., Pinel, M. C., Wildiers, H., Zorza, G., Ferré, P., et al. (2012). A phase 1 study of vinflunine in combination with trastuzumab for the treatment for HER2-positive metastatic breast cancer. *Cancer Chemotherapy and Pharmacology 70*, 503–511.
Peereboom, D. M., Murphy, C., Ahluwalia, M. S., Conlin, A., Eichler, A., Van Poznak, C., et al. (2014). Phase II trial of patupilone in patients with brain metastases from breast cancer. *Neuro-Oncology 16*, 579–583.
Pérez de Castro, I., de Cárcer, G., & Malumbres, M. (2007). A census of mitotic cancer genes: New insights into tumor cell biology and cancer therapy. *Carcinogenesis 28*, 899–912.
Peters, J. -M. (2002). The anaphase-promoting complex: Proteolysis in mitosis and beyond. *Molecular Cell 9*, 931–943.
Peters, J. -M. (2006). The anaphase promoting complex/cyclosome: A machine designed to destroy. *Nature Reviews. Molecular Cell Biology 7*, 644–656.
Pines, J. (2006). Mitosis: A matter of getting rid of the right protein at the right time. *Trends in Cell Biology 16*, 55–63.
Raz, T., Nardi, V., Azam, M., Cortes, J., & Daley, G. Q. (2007). Farnesyl transferase inhibitor resistance probed by target mutagenesis. *Blood 110*, 2102–2109.
Ready, N. E., Lipton, A., Zhu, Y., Statkevich, P., Frank, E., Curtis, D., et al. (2007). Phase I study of the farnesyltransferase inhibitor lonafarnib with weekly paclitaxel in patients with solid tumors. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 13*, 576–583.
Rickert, K. W., Schaber, M., Torrent, M., Neilson, L. A., Tasber, E. S., Garbaccio, R., et al. (2008). Discovery and biochemical characterization of selective ATP competitive inhibitors of the human mitotic kinesin KSP. *Archives of Biochemistry and Biophysics 469*, 220–231.
Rieder, C. L., & Maiato, H. (2004). Stuck in division or passing through: What happens when cells cannot satisfy the spindle assembly checkpoint. *Developmental Cell 7*, 637–651.
Rieder, C. L., Schultz, A., Cole, R., & Sluder, G. (1994). Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *The Journal of Cell Biology 127*, 1301–1310.
Rubin, E. H., Rothermel, J., Tesfaye, F., Chen, T., Hubert, M., Ho, Y. -Y., et al. (2005). Phase I dose-finding study of weekly single-agent patupilone in patients with advanced solid tumors. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 23*, 9120–9129.
Rudin, C. M., Mauer, A., Smakal, M., Juergens, R., Spelda, S., Wertheim, M., et al. (2011). Phase I/II study of pemetrexed with or without ABT-751 in advanced or metastatic non-small-cell lung cancer. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 29*, 1075–1082.
Ruschak, A. M., Slassi, M., Kay, L. E., & Schimmer, A. D. (2011). Novel proteasome inhibitors to overcome bortezomib resistance. *JNCI Journal of the National Cancer Institute 103*, 1007–1017.
Rustin, G., Reed, N., Jayson, G. C., Ledermann, J. A., Adams, M., Perren, T., et al. (2011). A phase II trial evaluating two schedules of sagopilone (ZK-EPO), a novel epothilone, in patients with platinum-resistant ovarian cancer. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 22*, 2411–2416.
Saif, M. W., Sarantopoulos, J., Patnaik, A., Tolcher, A. W., Takimoto, C., & Beeram, M. (2011). Tesetaxel, a new oral taxane, in combination with capecitabine: A phase I, dose-escalation study in patients with advanced solid tumors. *Cancer Chemotherapy and Pharmacology 68*, 1565–1573.
Salmela, A. -L., & Kallio, M. J. (2013). Mitosis as an anti-cancer drug target. *Chromosoma 122*, 431–449.
Sanoff, H. K., Davies, J. M., Walko, C., Irvin, W., Buie, L., Keller, K., et al. (2011a). A phase I evaluation of the combination of vinflunine and erlotinib in patients with refractory solid tumors. *Investigational New Drugs 29*, 978–983.
Sanoff, H. K., Davies, J., Walko, C., Buie, L., Chiu, W. -K., Ivanova, A., et al. (2011b). Phase I trial of vinflunine and pemetrexed in refractory solid tumors. *Investigational New Drugs 29*, 131–136.
Schafer-Hales, K., Iaconelli, J., Snyder, J. P., Prussia, A., Nettles, J. H., El-Naggar, A., et al. (2007). Farnesyl transferase inhibitors impair chromosomal maintenance in cell lines and human tumors by compromising CENP-E and CENP-F function. *Molecular Cancer Therapeutics 6*, 1317–1328.
Schelman, W., Morgan-Meadows, S., Bailey, H., Holen, K., Thomas, J. P., Eickhoff, J., et al. (2008). A phase I trial of gemcitabine in combination with patupilone in patients with advanced solid tumors. *Cancer Chemotherapy and Pharmacology 62*, 727–733.
Schmid, P., Kiewe, P., Possinger, K., Korfel, A., Lindemann, S., Giurescu, M., et al. (2010). Phase I study of the novel, fully synthetic epothilone sagopilone (ZK-EPO) in patients with solid tumors. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 21*, 633–639.
Schöffski, P., Jones, S. F., Dumez, H., Infante, J. R., Van Mieghem, E., Fowst, C., et al. (2011). Phase I, open-label, multicentre, dose-escalation, pharmacokinetic and pharmacodynamic trial of the oral aurora kinase inhibitor PF-03814735 in advanced solid tumours. *European Journal of Cancer (Oxford, England: 1990) 47*, 2256–2264.
Schöffski, P., Awada, A., Dumez, H., Gil, T., Bartholomeus, S., Wolter, P., et al. (2012). A phase I, dose-escalation study of the novel polo-like kinase inhibitor volasertib (BI 6727) in patients with advanced solid tumours. *European Journal of Cancer (Oxford, England: 1990) 48*, 179–186.
Schwartz, G. K., Carvajal, R. D., Midgley, R., Rodig, S. J., Stockman, P. K., Ataman, O., et al. (2013). Phase i study of barasertib (AZD1152), a selective inhibitor of Aurora B kinase, in patients with advanced solid tumors. *Investigational New Drugs 31*, 370–380.
Segaoula, Z., Leclercq, J., Verones, V., Flouquet, N., Lecoeur, M., Ach, L., et al. (2016). Synthesis and biological evaluation of N-[2-(4-Hydroxyphenylamino)-pyridin-3-yl]-4-methoxy-benzenesulfonamide (ABT-751) tricyclic analogues as antimitotic and antivascular agents with potent in vivo antitumor activity. *Journal of Medicinal Chemistry 59*, 8422–8440.
Sen, S., Zhou, H., Zhang, R. -D., Yoon, D. S., Vakar-Lopez, F., Ito, S., et al. (2002). Amplification/overexpression of a mitotic kinase gene in human bladder cancer. *Journal of the National Cancer Institute 94*, 1320–1329.
Sharma, S., Kemeny, N., Kelsen, D. P., Ilson, D., O'Reilly, E., Zaknoen, S., et al. (2002). A phase II trial of farnesyl protein transferase inhibitor SCH 66336, given by twice-daily oral administration, in patients with metastatic colorectal cancer refractory to 5-fluorouracil and irinotecan. *Annals of Oncology: Official Journal of the European Society for Medical Oncology / ESMO 13*, 1067–1071.
Sheth, P. R., Basso, A., Duca, J. S., Lesburg, C. A., Ogas, P., Gray, K., et al. (2009). Thermodynamics of nucleotide and inhibitor binding to wild-type and ispinesib-resistant forms of human kinesin spindle protein. *Biochemistry 48*, 11045–11055.
Silvani, A., Gaviani, P., Fiumani, A., Scaioli, V., Lamperti, E., Eoli, M., et al. (2009). Systemic sagopilone (ZK-EPO) treatment of patients with recurrent malignant gliomas. *Journal of Neuro-Oncology 95*, 61–64.
Sloane, D. A., Trikic, M. Z., Chu, M. L. H., Lamers, M. B. A. C., Mason, C. S., Mueller, I., ... Eyers, P. A. (2010). Drug-resistant aurora a mutants for cellular target validation of the small molecule kinase inhibitors MLN8054 and MLN8237. *ACS Chemical Biology 5*(6), 563–576. http://dx.doi.org/10.1021/cb100053q.
Smith, S. L., Bowers, N. L., Betticher, D. C., Gautschi, O., Ratschiller, D., Hoban, P. R., et al. (2005). Overexpression of aurora B kinase (AURKB) in primary non-small cell lung carcinoma is frequent, generally driven from one allele, and correlates with the level of genetic instability. *British Journal of Cancer 93*, 719–729.
Souid, A. -K., Dubowy, R. L., Ingle, A. M., Conlan, M. G., Sun, J., Blaney, S. M., et al. (2010). A pediatric phase I trial and pharmacokinetic study of ispinesib: A Children's Oncology Group phase I consortium study. *Pediatric Blood & Cancer 55*, 1323–1328.
Souquet, P. -J., Krzakowski, M., Ramlau, R., Sun, X. -S., Lopez-Vivanco, G., Puozzo, C., et al. (2010). Phase I/II and pharmacokinetic study of intravenous vinflunine in combination with cisplatin for the treatment of chemonaive patients with advanced non-small-cell lung cancer. *Clinical Lung Cancer 11*, 105–113.
Spänkuch-Schmitt, B., Bereiter-Hahn, J., Kaufmann, M., & Strebhardt, K. (2002). Effect of RNA silencing of polo-like kinase-1 (PLK1) on apoptosis and spindle formation in human cancer cells. *Journal of the National Cancer Institute 94*, 1863–1877.
Spigel, D. R., Hainsworth, J. D., Lane, C. M., Clark, B., Burris, H. A., & Greco, F. A. (2010). Phase II trial of vinflunine in relapsed small cell lung cancer. *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer 5*, 874–878.
Stadler, W. M., Vaughn, D. J., Sonpavde, G., Vogelzang, N. J., Tagawa, S. T., Petrylak, D. P., et al. (2014). An open-label, single-arm, phase 2 trial of the polo-like kinase inhibitor volasertib (BI 6727) in patients with locally advanced or metastatic urothelial cancer. *Cancer 120*, 976–982.
Stanton, R. A., Gernert, K. M., Nettles, J. H., & Aneja, R. (2011). Drugs that target dynamic microtubules: A new molecular perspective. *Medicinal Research Reviews 31*, 443–481.
Steeghs, N., Eskens, F. A. L. M., Gelderblom, H., Verweij, J., Nortier, J. W. R., Ouwerkerk, J., et al. (2009). Phase I pharmacokinetic and pharmacodynamic study of the aurora kinase inhibitor danusertib in patients with advanced or metastatic solid tumors. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 27*, 5094–5101.
Steegmaier, M., Hoffmann, M., Baum, A., Lénárt, P., Petronczki, M., Krssák, M., et al. (2007). BI 2536, a potent and selective inhibitor of polo-like kinase 1, inhibits tumor growth in vivo. *Current Biology: CB 17*, 316–322.
Stephenson, J. J., Nemunaitis, J., Joy, A. A., Martin, J. C., Jou, Y. -M., Zhang, D., et al. (2014). Randomized phase 2 study of the cyclin-dependent kinase inhibitor dinaciclib (MK-7965) versus erlotinib in patients with non-small cell lung cancer. *Lung Cancer (Amsterdam, Netherlands) 83*, 219–223.
Stewart, S., & Fang, G. (2005). Destruction box-dependent degradation of aurora B is mediated by the anaphase-promoting complex/cyclosome and Cdh1. *Cancer Research 65*, 8730–8735.
Strebhardt, K., & Ullrich, A. (2006). Targeting polo-like kinase 1 for cancer therapy. *Nature Reviews. Cancer 6*, 321–330.
Stupp, R., Tosoni, A., Bromberg, J. E. C., Hau, P., Campone, M., Gijtenbeek, J., et al. (2011). Sagopilone (ZK-EPO, ZK 219477) for recurrent glioblastoma. A phase II multicenter trial by the European Organisation for Research and Treatment of Cancer (EORTC) Brain Tumor Group. *Annals of Oncology: Official Journal of the European Society for Medical Oncology/ESMO 22*, 2144–2149.
Sturgill, E. G., Norris, S. R., Guo, Y., & Ohi, R. (2016). Kinesin-5 inhibitor resistance is driven by kinesin-12. *The Journal of Cell Biology 213*, 213–227.

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

Sudakin, V., Chan, G. K., & Yen, T. J. (2001). Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, and MAD2. *The Journal of Cell Biology 154*, 925–936.

Talbot, D. C., Margery, J., Dabouis, G., Dark, G., Taylor, H., Boussemart, H., et al. (2007). Phase II study of vinflunine in malignant pleural mesothelioma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 25*, 4751–4756.

Tang, P. A., Siu, L. L., Chen, E. X., Hotte, S. J., Chia, S., Schwarz, J. K., et al. (2008). Phase II study of ispinesib in recurrent or metastatic squamous cell carcinoma of the head and neck. *Investigational New Drugs 26*, 257–264.

Taylor, S. S., Hussein, D., Wang, Y., Elderkin, S., & Morrow, C. J. (2001). Kinetochore localisation and phosphorylation of the mitotic checkpoint components Bub1 and BubR1 are differentially regulated by spindle events in human cells. *Journal of Cell Science 114*, 4385–4395.

Tcherniuk, S. O., & Oleinikov, A. V. (2015). Pgp efflux pump decreases the cytostatic effect of CENP-E inhibitor GSK923295. *Cancer Letters 361*, 97–103.

Teicher, B. A., & Tomaszewski, J. E. (2015). Proteasome inhibitors. *Biochemical Pharmacology 96*, 1–9.

Ten Bokkel Huinink, W. W., Sufliarsky, J., Smit, W. M., Spanik, S., Wagnerova, M., Hirte, H. W., et al. (2009). Safety and efficacy of patupilone in patients with advanced ovarian, primary fallopian, or primary peritoneal cancer: A phase I, open-label, dose-escalation study. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 27*, 3097–3103.

Theodore, C., Geoffrois, L., Vermorken, J. B., Caponigro, F., Fiedler, W., Chollet, P., et al. (2005). Multicentre EORTC study 16997: Feasibility and phase II trial of farnesyl transferase inhibitor & gemcitabine combination in salvage treatment of advanced urothelial tract cancers. *European Journal of Cancer (Oxford, England: 1990) 41*, 1150–1157.

Tibes, R., McDonagh, K. T., Lekakis, L., Bogenberger, J. M., Kim, S., Frazer, N., et al. (2015). Phase I study of the novel Cdc2/CDK1 and AKT inhibitor terameprocol in patients with advanced leukemias. *Investigational New Drugs 33*, 389–396.

Tournoux-Facon, C., Senellart, H., Lemarie, E., Tourani, J. M., Favrel, S., Pouget, J. C., et al. (2011). Phase I and pharmacokinetic study of IV vinflunine in combination with gemcitabine for treatment of advanced non-small cell lung cancer in Chemonaive patients. *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer 6*, 1247–1253.

Tournoux-Facon, C., Robinet, G., Pinel, M. -C., Ferre, P., & Tourani, J. -M. (2012). Phase I and pharmacokinetic study of IV vinflunine in combination with carboplatin in chemonaive patients with advanced non-small cell lung cancer. *American Journal of Clinical Oncology 35*, 378–385.

Tsimberidou, A. -M., Takimoto, C. H. M., Moulder, S., Uehara, C., Mita, M., Mita, A., et al. (2011). Effects of patupilone on the pharmacokinetics and pharmacodynamics of warfarin in patients with advanced malignancies: A phase I clinical trial. *Molecular Cancer Therapeutics 10*, 209–217.

Tsuboi, K., Yokozawa, T., Sakura, T., Watanabe, T., Fujisawa, S., Yamauchi, T., et al. (2011). A phase I study to assess the safety, pharmacokinetics and efficacy of barasertib (AZD1152), an Aurora B kinase inhibitor, in Japanese patients with advanced acute myeloid leukemia. *Leukemia Research 35*, 1384–1389.

Twu, N. -F., Yuan, C. -C., Yen, M. -S., Lai, C. -R., Chao, K. -C., Wang, P. -H., et al. (2009). Expression of Aurora kinase A and B in normal and malignant cervical tissue: High Aurora A kinase expression in squamous cervical cancer. *European Journal of Obstetrics, Gynecology, and Reproductive Biology 142*, 57–63.

Vader, G., Kauw, J. J. W., Medema, R. H., & Lens, S. M. A. (2006). Survivin mediates targeting of the chromosomal passenger complex to the centromere and midbody. *EMBO Reports 7*, 85–92.

van der Horst, A., & Lens, S. M. A. (2014). Cell division: Control of the chromosomal passenger complex in time and space. *Chromosoma 123*, 25–42.

Varma, D., & Salmon, E. D. (2012). The KMN protein network–chief conductors of the kinetochore orchestra. *Journal of Cell Science 125*, 5927–5936.

Vaughn, D. J., Srinivas, S., Stadler, W. M., Pili, R., Petrylak, D., Sternberg, C. N., et al. (2009). Vinflunine in platinum-pretreated patients with locally advanced or metastatic urothelial carcinoma: Results of a large phase 2 study. *Cancer 115*, 4110–4117.

Vlahov, I. R., Santhapuram, H. K. R., Kleindl, P. J., Howard, S. J., Stanford, K. M., & Leamon, C. P. (2006). Design and regioselective synthesis of a new generation of targeted chemotherapeutics. Part 1: EC145, a folic acid conjugate of desacetylvinblastine monohydrazide. *Bioorganic & Medicinal Chemistry Letters 16*, 5093–5096.

Walczak, C. E., Cai, S., & Khodjakov, A. (2010). Mechanisms of chromosome behaviour during mitosis. *Nature Reviews. Molecular Cell Biology 11*, 91–102.

Wan, X., O'Quinn, R. P., Pierce, H. L., Joglekar, A. P., Gall, W. E., DeLuca, J. G., et al. (2009). Protein architecture of the human kinetochore microtubule attachment site. *Cell 137*, 672–684.

Weaver, B. A. A., & Cleveland, D. W. (2005). Decoding the links between mitosis, cancer, and chemotherapy: The mitotic checkpoint, adaptation, and cell death. *Cancer Cell 8*, 7–12.

Wieser, S., & Pines, J. (2015). The biochemistry of mitosis. *Cold Spring Harbor Perspectives in Biology 7*, a015776.

Winquist, E., Moore, M. J., Chi, K. N., Ernst, D. S., Hirte, H., North, S., et al. (2005). A multinomial phase II study of lonafarnib (SCH 66336) in patients with refractory urothelial cancer. *Urologic Oncology 23*, 143–149.

Wong, N. S., Meadows, K. L., Rosen, L. S., Adjei, A. A., Kaufmann, S. H., Morse, M. A., et al. (2011). A phase I multicenter study of continuous oral administration of lonafarnib (SCH 66336) and intravenous gemcitabine in patients with advanced cancer. *Cancer Investigation 29*, 617–625.

Wood, K. W., Lad, L., Luo, L., Qian, X., Knight, S. D., Nevins, N., et al. (2010). Antitumor activity of an allosteric inhibitor of centromere-associated protein-E. *Proceedings of the National Academy of Sciences of the United States of America 107*, 5839–5844.

Wordeman, L., & Mitchison, T. J. (1995). Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *The Journal of Cell Biology 128*, 95–104.

Wu, G., Qiu, X. -L., Zhou, L., Zhu, J., Chamberlin, R., Lau, J., et al. (2008). Small molecule targeting the Hec1/Nek2 mitotic pathway suppresses tumor cell growth in culture and in animal. *Cancer Research 68*, 8393–8399.

Wu, C. -P., Hsiao, S. -H., Luo, S. -Y., Tuo, W. -C., Su, C. -Y., Li, Y. -Q., et al. (2014). Overexpression of human ABCB1 in cancer cells leads to reduced activity of GSK461364, a specific inhibitor of polo-like kinase 1. *Molecular Pharmaceutics 11*, 3727–3736.

Wu, C. -P., Hsieh, C. -H., Hsiao, S. -H., Luo, S. -Y., Su, C. -Y., Li, Y. -Q., et al. (2015). Human ATP-binding cassette transporter ABCB1 confers resistance to Volasertib (BI 6727), a selective inhibitor of polo-like kinase 1. *Molecular Pharmaceutics 12*, 3885–3895.

Yardley, D. A., McCleod, M., Schreiber, F., Murphy, P., Patton, J., Thompson, D. S., et al. (2010). A phase II trial of vinflunine as monotherapy or in combination with trastuzumab as first-line treatment of metastatic breast cancer. *Cancer Investigation 28*, 925–931.

Yee, K. W. L., Hagey, A., Verstovsek, S., Cortes, J., Garcia-Manero, G., O'Brien, S. M., et al. (2005). Phase 1 study of ABT-751, a novel microtubule inhibitor, in patients with refractory hematologic malignancies. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research 11*, 6615–6624.

Yen, T. J., Li, G., Schaar, B. T., Szilak, I., & Cleveland, D. W. (1992). CENP-E is a putative kinetochore motor that accumulates just before mitosis. *Nature 359*, 536–539.

Yuan, J., Sanhaji, M., Krämer, A., Reindl, W., Hofmann, M., Kreis, N. -N., et al. (2011). Polo-box domain inhibitor poloxin activates the spindle assembly checkpoint and inhibits tumor growth in vivo. *The American Journal of Pathology 179*, 2091–2099.

Yust-Katz, S., Liu, D., Yuan, Y., Liu, V., Kang, S., Groves, M., et al. (2013). Phase 1/1b study of lonafarnib and temozolomide in patients with recurrent or temozolomide refractory glioblastoma. *Cancer 119*, 2747–2753.

Zaman, K., Durando, X., Baurain, J. -F., Humblet, Y., Mazzeo, F., Bostnavaron, M., et al. (2011). A phase I clinical and pharmacological study evaluating vinflunine in combination with doxorubicin as first line treatment in metastatic breast cancer. *Breast Cancer Research and Treatment 127*, 689–696.

Zhang, B., Groffen, J., & Heisterkamp, N. (2005). Resistance to farnesyltransferase inhibitors in Bcr/Abl-positive lymphoblastic leukemia by increased expression of a novel ABC transporter homolog ATP11a. *Blood 106*, 1355–1361.

Please cite this article as: Penna, L.S., et al., Anti-mitotic agents: Are they emerging molecules for cancer treatment?, *Pharmacology & Therapeutics* (2017), http://dx.doi.org/10.1016/j.pharmthera.2017.02.007

CAPÍTULO II – ARTIGO DE DADOS

EFFECTS OF ANTIMITOTIC COMPOUNDS IN GASTRIC CANCER CELL LINES

Artigo a ser submetido em periódico internacional da área

EFFECTS OF ANTIMITOTIC COMPOUNDS IN GASTRIC CANCER CELL LINES

Larissa Siqueira Penna, João Antonio Pêgas Henriques and Diego Bonatto*

Biotechnology Center of the Federal University of Rio Grande do Sul, Department of Molecular Biology and Biotechnology, Federal University of Rio Grande do Sul, Porto Alegre, RS – Brazil.

* **To whom correspondence should be sent:**

Diego Bonatto
Centro de Biotecnologia da UFRGS - Sala 219
Departamento de Biologia Molecular e Biotecnologia
Universidade Federal do Rio Grande do Sul – UFRGS
Avenida Bento Gonçalves 9500 - Prédio 43421
Caixa Postal 15005
Porto Alegre – Rio Grande do Sul
BRAZIL
91509-900
Phone: (+55 51) 3308-6080
Fax: (+55 51) 3308-7309

Contract/grant sponsor: CNPq, CAPES

Abstract

Gastric cancer has high mortality rates worldwide. Despite chemotherapy is one of the main treatments for this disease, failure commonly occurs and is usually a consequence of chemoresistance. In this study we evaluated the effects of ZM447439 and INH1, two anti-mitotic compounds that inhibit the activity of AURKB and NDC80, respectively, in two gastric adenocarcinoma cell lines. We evaluated nuclear morphometry, cell cycle and cell death. Furthermore, we analyzed the combination of ZM447439 with the standard antitumoral drug 5-fluorouracil (5-FU). The data indicated that ZM447439 alone and in combination with 5-FU were effective against the two gastric adenocarcinoma cell lines, resulting in nuclei with characteristics of mitotic catastrophe and senescence. An increase in polyploidization (>4N DNA content) was also observed, probably a consequence of endoreduplication, as reported by other studies with cancer cells. Despite drugs alone and combination of 5-FU with ZM447439 induced apoptosis after 72 and 120 hours, the combined treatment was the only regimen capable of inducing apoptosis already after 24 hours in both gastric adenocarcinoma cell lines, indicating that the combined therapy targeting different biological processes may improve treatment of gastric cancer.

Key words: gastric cancer, AURKB inhibitor, NDC80 inhibitor.

Introduction

Gastric cancer is the third cause of cancer death worldwide being the highest mortality rates observed in Eastern Asia, Central and Eastern Europe and South America.[1] Increased risk of developing stomach cancer is associated with *Helicobacter pylori* infection, tobacco use, family history, inappropriate diet and overweight, among other factors. The conventional treatment for gastric cancer involves surgery, chemotherapy and/or radiotherapy, but the combined chemotherapy is one of the main treatment options for this disease.[2] Despite tumor resection is the leading treatment of choice, 5-year survival rates of these patients range from just 10% to 30%.[3]

Combined therapies usually consist of different regimens containing two or three drugs, such as docetaxel, cisplatin, 5-fluorouracil (5-FU), epirubicin, oxaliplatin, capecitabine and irinotecan.[2,4] However, lack of response is frequently observed and is mainly caused by chemoresistance.[5] Targeting different biological processes by a combined therapy may be an option to avoid chemoresistance and to enhance the efficiency against the tumor.

Through the use of systems pharmacology tools and evaluation of biological networks of chemo-protein and protein-protein interactions in gastric cancer, a previous study identified AURKB and NDC80 as potential proteins to be inhibited by the experimental drugs ZM447439 and INH1, respectively.[6] AURKB is an essential mitosis-associated protein and one of the components of chromosomal passenger complex (CPC) that is also composed by INCENP, borealin and survivin. AURKB mainly localizes at centromeres until metaphase and, by controlling kinetochore-microtubule attachment, it promotes a correct chromosome alignment and segregation, while from anaphase until the end of mitosis it localizes at the spindle midzone and cleavage furrow acting on cytokinesis.[7–9] ZM447439 inhibits this kinase by competing for its ATP binding site.[10]

NDC80 protein is one of AURKB substrates. Coupled with NUF2, SPC24 and SPC25, NDC80 is a component of NDC80 complex that localizes at the outer kinetochore plate.[11] NDC80 is involved on kinetochore-microtubule attachment and plays an important role on chromosome segregation, as well as on spindle assembly checkpoint.[12] Besides being regulated by AURKB, NDC80 is also phosphorylated by NEK2.[13] By binding to NDC80, INH1 prevents NEK2 from activating this protein.[14]

These two mitotic inhibitors, ZM447439 and INH1, have been tested *in vitro* in different tumors such as glioblastoma[15,16], leukemia[17–22], breast cancer[14,16,23,24] and cervical cancer[25–28] but they still have not been tested for gastric cancer chemotherapy. Although INH1 has not been tested in as many cancer cell lines as ZM447439, it was already used as an *in vivo* treatment of a breast cancer xenograft, showing satisfactory antitumor activity.[14]

The aim of this study was to analyze the effects of the anti-mitotic compounds INH1 and ZM447439, as well as the combination of 5-FU with ZM447439, in two gastric adenocarcinoma cell lines evaluating cell cycle, cell death and nuclear morphometric analysis.

Material and Methods

Cell lines and drugs

The gastric adenocarcinoma cell lines ACP02 and ACP03 were obtained from João de Barros Barreto University Hospital, Brazil.[29] MN01, a normal gastric epithelium cell line, was kindly provided by Dr. Rommel Mario Rodríguez Burbano (Federal University of Pará) and was included as a non-tumoral control. Cells were grown in RPMI 1640 medium (Sigma-Aldrich) with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco) at 37 ºC in humidified 5% CO_2 incubator.

The anti-mitotic compounds, ZM447439 and INH1, were purchased from Tocris Bioscience and 5-FU from Sigma-Aldrich. Stock solutions of all drugs were prepared in DMSO (Sigma-Aldrich), stored at -80 ºC. Working solutions were prepared just before use in fresh medium to its final concentrations. In this sense, IC_{50} determination was made by cell counting using the Guava EasyCyte flow cytometer considering a minimum of six points. The dose response curve varied from 0.05 µM to 4 µM for 5-FU, from 1 µM to 3.5 µM for ZM447439 and from 10 µM to 35 µM for INH1. For the 5-FU and ZM447439 combination, the values varied from 0.6 µM to 2.4 µM and from 1 µM to 4 µM, respectively. IC_{50} values were calculated after 120 hours of treatment as a mean of IC_{50} ± s.e.m. of at least two independent experiments using GraphPad Prism 5 Software (https://www.graphpad.com/). Negative control samples were cultured in a medium containing DMSO with final concentrations that never exceeded 0.05% (v/v) for all experiments.

RT-PCR

Total RNA was isolated with Ilustra RNAspin Mini^{TM} Kit (GE Healthcare), according to manufacturer's instruction. The cDNA synthesis was obtained with Phusion^{TM} RT-PCR kit (Finnzymes). RNA and cDNA were quantified using NanoDrop spectrophotometer (Thermo Fisher Scientific). PCR amplification was performed with a 25 µL mixture containing 100 ng of cDNA, 0.5 µL of dNTP, 0.25 µL of Taq DNA polymerase, 5 µL of 5× buffer and 0.5 µL of each primer (forward and reverse). The PCR program applied consisted of an initial denaturation of 30 seconds at 98 ºC, followed by 35 cycles of 10 seconds at 98 ºC, annealing temperature ranging from 54 ºC to 64 ºC for 10 seconds and 40 seconds at 72 ºC, with a final extension step at 72 ºC for 5 minutes. Primers (Supplemental Table S1) were designed based on GenBank data and with Primer3Plus software (http://primer3plus.com/). PCR products were

stained with SYBR green (Life Technologies) and detected using a 2% agarose gel electrophoresis conducted with 1× TAE buffer.

Nuclear Morphometric Analysis (NMA)

Apoptosis, mitotic catastrophe (MC) and senescence were evaluated after 72 and 120 hours of drug treatment using Nuclear Morphometric Analysis (NMA).[30] For this analysis, adhered cells were washed three times with PBS, fixed with 2% paraformaldehyde for 20 minutes and then incubated for 30 minutes in the dark with 300nM DAPI. Images of marked nuclei were evaluated with Image Pro Plus 6.0 software (Media Cybernetics) considering nuclear size and regularity parameters. With these data, a Nuclear Irregularity Index (NII) was obtained and finally the results were distributed in a plot of area versus NII.

Cell cycle and DNA content analysis

Cells were treated for 120 hours, trypsinized and centrifuged for 7 minutes at 1800 rpm. The supernatant was discarded, the pellet was resuspended in 1 mL of PBS and cells were centrifuged again. Then, the pellet was resuspended in 150 µL of PBS and cells were fixed with 1 mL of cold ethanol (70% in PBS). After incubation at -20ºC, cells were centrifuged, resuspended in 1 mL of PBS and recentrifuged. Finally, the pellet was incubated for 30 minutes in the dark in 200 µL of a solution containing propidium iodide (PI), Triton X-100 and RNAse. The samples were analyzed using the Guava EasyCyte flow cytometer and guavaSoft 2.7 software (Millipore/Merck).

Caspase 3 activity

Measurement of caspase 3 activity was obtained using the fluorimetric Caspase 3 Assay kit from Sigma-Aldrich. The procedure was performed according to manufacturer's

instructions. Briefly, cells were treated for 24 hours, lysed with 1x lysis buffer and sonicated for 30 seconds (up to three times). Protein quantification was performed using Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). The reaction solution (200 µL per sample) containing 1x assay buffer and caspase 3 substrate was mixed with 50 µg of proteins. The fluorescence intensity (in relative fluorescence units – RFU) was measured during 90 minutes at room temperature using SpectraMax i3x.

Annexin-V/IP double-staining assay

To evaluate cell death of MN01 and ACP02, Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich) was used. After 120 hours of treatment, cells were trypsinized and centrifuged for 5 minutes at 1200 rpm. Samples were washed twice with PBS before staining. The pellet was resuspended in 1x binding buffer containing annexin V-FITC conjugate and PI and was incubated for 10 minutes in the dark. The analysis was performed using Guava EasyCyte flow cytometer and guavaSoft 2.7 software (Millipore/Merck). Cells stained just with annexin were considered in early apoptosis, with both annexin and PI in late apoptosis, just with PI in necrosis and without being stained as viable.

Statistical analysis

Results were expressed as mean ± s.e.m. of at least two experiments. ANOVA and Dunnett tests were used for multiple comparisons among treatments and control, while for single comparisons, Student's *t*-test was employed. When data did not satisfy the assumptions for these tests, Kruskal-Walllis and Wilcoxon-Mann-Whitney with correction for multiple comparisons were used instead. The caspase data were log transformed due to asymmetry. P values lower than 0.05 were considered as statistically significant.

Results and Discussion

Determination of IC_{50} values

The dose of choice of 5-FU for experiments was 1.2 µM, since IC_{50} value was 1.3 µM ± 0.12 for ACP02 and 1.25 µM ± 0.04 for ACP03. Both gastric cancer cell lines included in this study seem to be more sensitive compared to other gastric cancer cell lines that presented higher IC_{50} values for 5-FU, ranging from 4.2 µM to 8. µM[31]. For ZM447439, the selected dose was 2 µM, since IC_{50} value was 2.1 µM ± 0.18 for ACP02 and 2.1 µM ± 0.36 for ACP03. These IC_{50} values were similar to other cancer cell lines, such as breast and pancreatic cancer[32] and osteosarcoma[33] despite other studies described much higher values for anaplastic thyroid carcinoma[34] (over 500 µM). The dose of choice of INH1 was 30 µM being the IC_{50} values of 24 µM ± 3.8 for ACP02 and 32.6 µM ± 0.76 for ACP03. These values were similar to the IC_{50} dose observed for malignant pleural mesothelioma cells[35]. Since both cell lines were more sensitive to ZM447439 than INH1 regarding IC_{50} values and did not show major alterations on cell cycle and NMA after treatment with INH1, we decided to test the combination of ZM447439 with 5-FU. Therefore, for the combined treatment of 5-FU and ZM447439 the selected values were 0.8 µM and 1.3 µM, respectively. The IC_{50} values when combining these drugs were 0.8 µM ± 0.03 (5-FU) and 1.3 µM ± 0.05 (ZM447439) for ACP02 while for ACP03 the values were 0.8 µM ± 0.05 (5-FU) and 1.3 µM ± 0.08 µM (ZM447439). On the other hand, the same combination was tested in a colorectal carcinoma cell line by another group but they observed that these drugs acted as antagonists[36].

Both gastric cancer cell lines presented AURKB and NDC80 transcripts while TP53 expression was absent

The presence of AURKB and NDC80 transcripts in both ACP02 and ACP03 cell lines was analyzed by RT-PCR. Other genes that encode proteins frequently involved on tumoral processes were also evaluated. The proteins that we aimed to target in this study, AURKB and NDC80, were shown to be overexpressed in gastric cancer by other groups[37,38]. Accordingly, we detected the presence of their transcripts on both adenocarcinoma cell lines (Fig. 1). Expression of CDC2, Bcl-2, BAX, cyclin D1, VEGF, C-Myc and RAS was reported in different gastric cancer cell lines by other studies[39–45] and we verified the presence of these gene expressions in both ACP02 and ACP03 as well (Fig. 1). On the other hand, RXRA and MMP-9 transcripts were not detected on either ACP02 and ACP03, corroborating with other groups that did not detect MMP-9 at normal culture conditions[46] or detected significantly lower expression levels of RXRA on gastric cancers[47,48]. Furthermore, the tumor suppressor gene p53 was not detected on both adenocarcinoma cell lines. Absence of p53 was also reported on the gastric cancer cell line Kato-III, but most gastric cancer cell lines present either wild type or mutated p53[49].

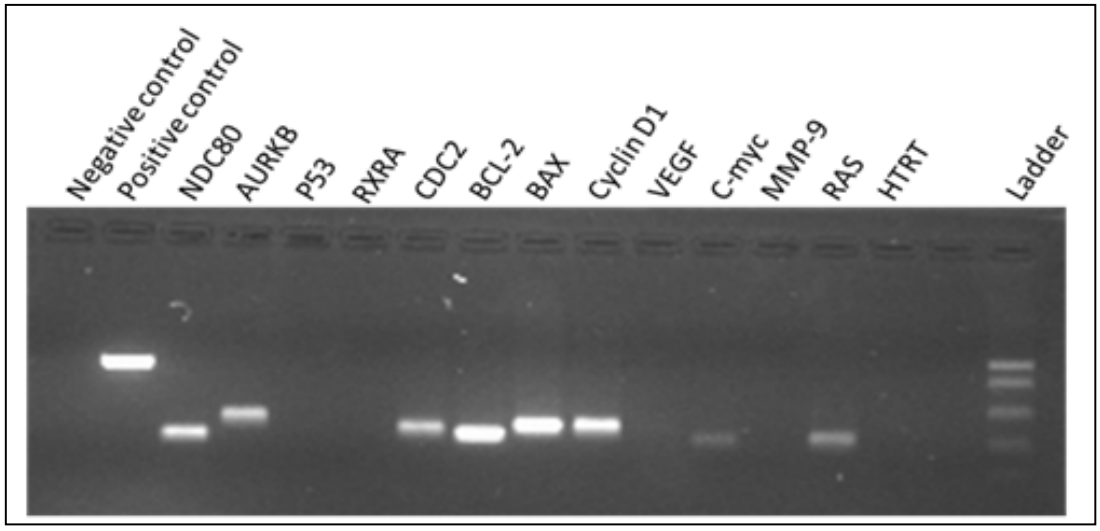

Fig. 1 Representative RT-PCR analysis of ACP03 in a 2% agarose gel of genes that encode proteins frequently involved in the tumoral process.

The combined treatment induced senescence and mitotic catastrophe-ike nuclear phenotype

To assess the morphological effects of treatments on nuclei, cells were stained with DAPI and observed by fluorescent microscopy. The normal gastric cell line, GES-1, presented a significant increase of large regular (LR) nuclei after 72 hours and 120 hours of combined treatment ($p<0.001$). This morphological alteration was also induced after treatment with ZM447439 ($P<0.01$) and 5-FU ($P<0.001$) alone (Fig. 2). In addition to presenting LR nuclei, a typical feature of senescence, tumoral cell lines also presented irregular (I) and large irregular nuclei (LI), indicating MC. Both ACP02 and ACP03 had a significant increase of I and LI nuclei after combined treatment ($p<0.05$) as well as nuclei indicative of senescence after 120 hours ($p<0.001$) (Fig. 3 and 4). ACP02 treated with ZM447439, presented higher levels of LI, I and LR nuclei after 72 hours ($p<0.001$). Similar results were observed after 120 hours, with an increased number of LR nuclei ($p<0.01$) and I and LI nuclei ($p<0.001$). ACP03 only presented significantly higher levels of LR nuclei when treated with ZM447439 for 72 hours ($p<0.05$). On the other hand, INH1 did not induce any nuclear morphometric changes. A representative result of an experiment with ACP02 is shown in NMA graphics of control and treated cells as well as some nuclei pictures corresponding to the different classifications of this analysis (Fig. 5).

MC was recently suggested not to be an actual type of cell death, but a pathway that begins on M phase, as a consequence of mitosis disturbance, triggering cell death or senescence[50]. Multinucleation due to cytokinesis failure is a common morphological characteristic observed in MC[51], and it was frequently observed in ACP02 (Fig. 5) after treatment with ZM447439. Similarly to our results, other studies previously observed MC as

a consequence of AURKB inhibition in hepatoma[16] and colorectal carcinoma[52] cell lines. Increase on nuclear size, a typical phenotype of senescence[53], was frequently observed after the combined treatment or treatment with ZM447439 alone. Senescent cells were also observed in osteosarcoma cells after 72 hours of treatment with 1µM ZM447439[54].

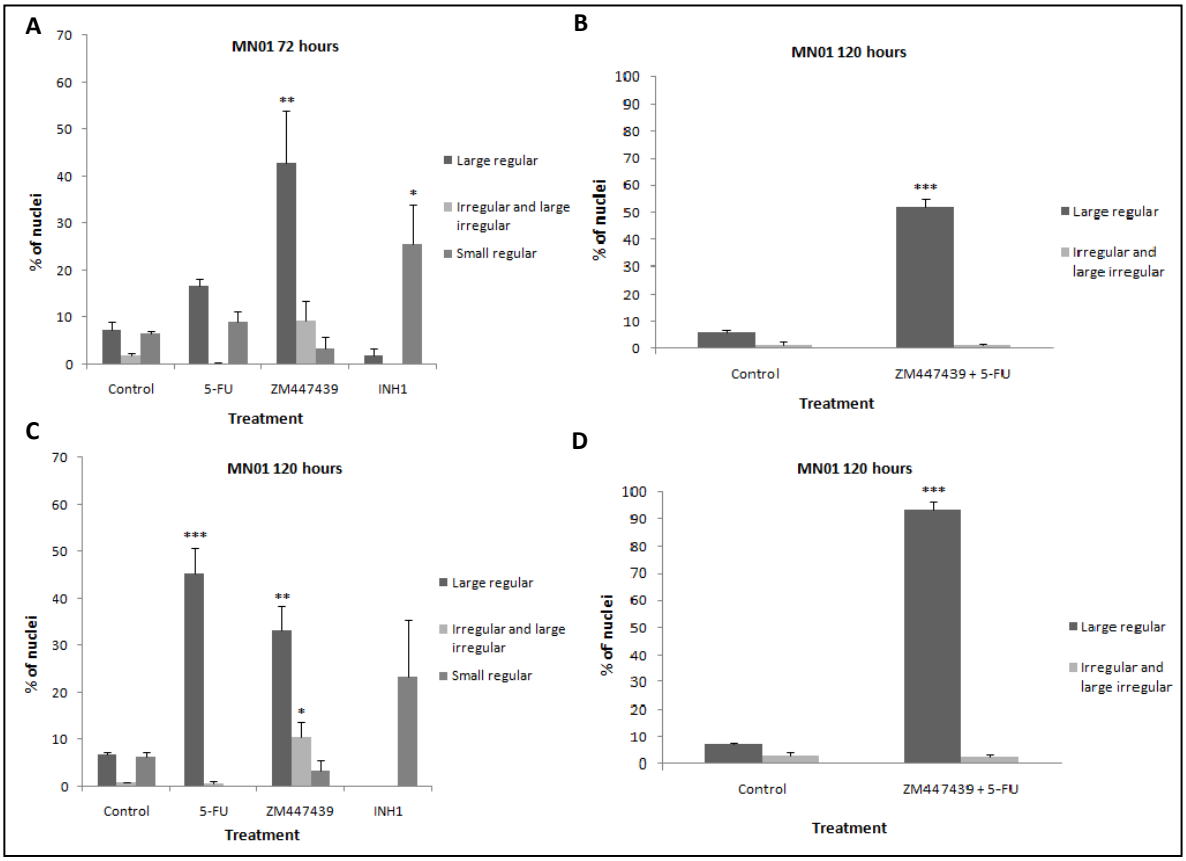

Fig. 2 Results of different NMA populations according to nuclear morphological appearances from MN01 cells. (A) 72 hours of treatment with 5-FU, ZM447439 and INH1; (B) 72 hours of 5-FU and ZM447439 combined treatment; (C) 120 hours of 5-FU, ZM447439 or INH1 treatment and (D) 120 hours of 5-FU and ZM447439 combined treatment. Large regular indicates senescence, irregular and large irregular indicates mitotic catastrophe and small regular indicates apoptosis. *p<0.05,**p<0.01, ***p<0.001

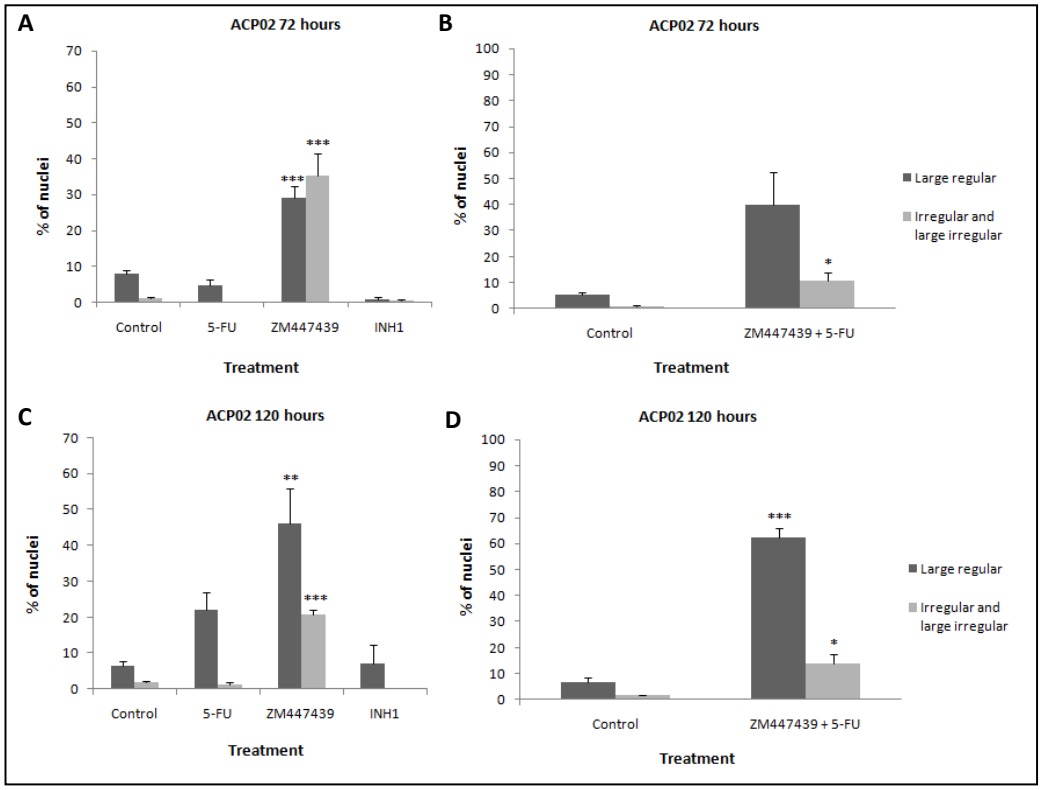

Fig. 3 Results of different NMA populations according to nuclear morphological appearances from ACP02 cells. (A) 72 hours of treatment with 5-FU, ZM447439 and INH1; (B) 72 hours of 5-FU and ZM447439 combined treatment; (C) 120 hours of 5-FU, ZM447439 or INH1 treatment and (D) 120 hours of 5-FU and ZM447439 combined treatment. Large regular indicates senescence while irregular and large irregular indicates mitotic catastrophe. *p<0.05,**p<0.01, ***p<0.001

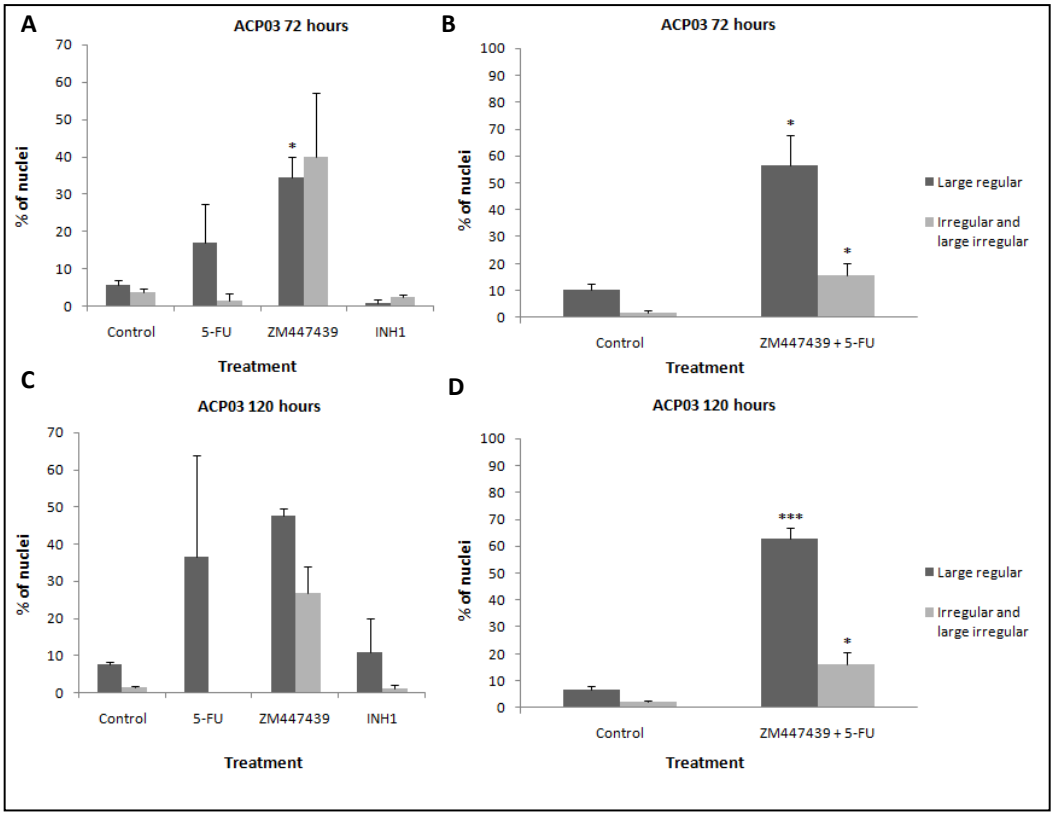

Fig. 4 Results of different NMA populations according to nuclear morphological appearances from ACP03 cells. (A) 72 hours of treatment with 5-FU, ZM447439 and INH1; (B) 72 hours of 5-FU and ZM447439 combined treatment; (C) 120 hours of 5-FU, ZM447439 or INH1 treatment and (D) 120 hours of 5-FU and ZM447439 combined treatment. Large regular indicates senescence while irregular and large irregular indicates mitotic catastrophe.*p<0.05,**p<0.01, ***p<0.001

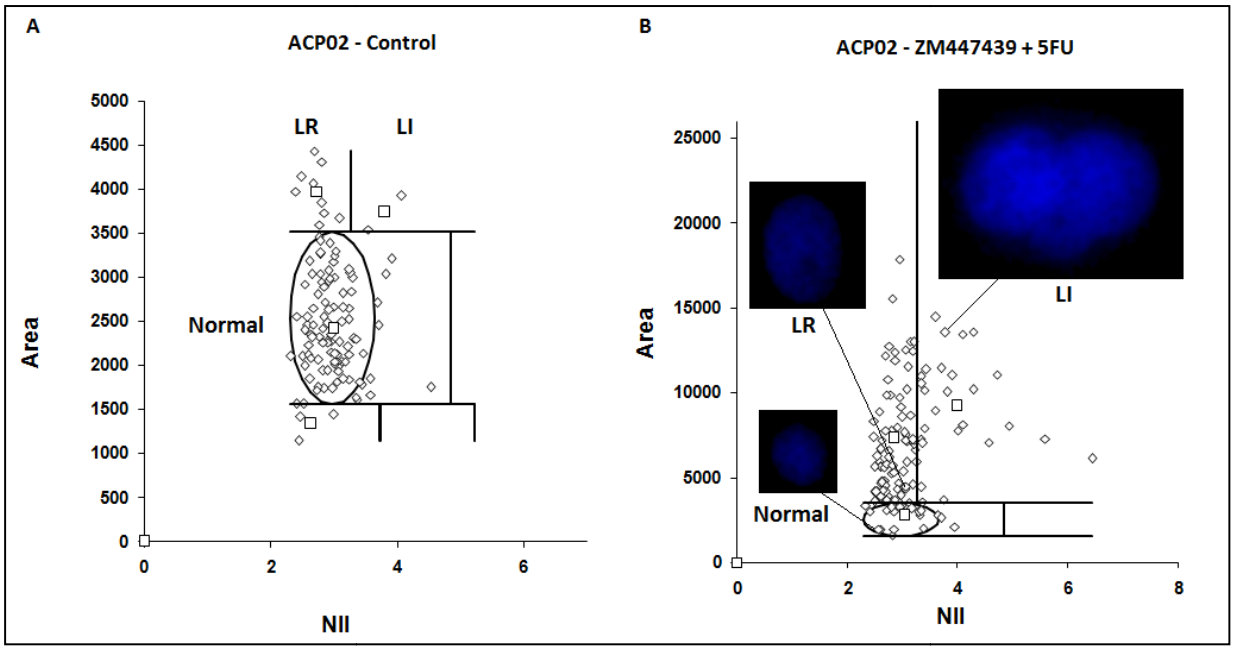

Fig. 5 Representative result of a 120 hours NMA experiment of ACP02 cells. (A) NMA graphic of ACP02 cells not treated and (B) NMA graphic of ACP02 cells treated with 5-FU combined with ZM447439. Examples of nuclei observed in each population are shown. LR: large regular; LI: large irregular.

Cell cycle analysis have shown a significant increase of cells with DNA content of 4N and >4N after treatment with ZM447439

Cell cycle alterations after treatment with 5-FU consisted of a higher number of cells on S phase ($p<0.001$ for ACP02 and ACP03 and $p<0.05$ for MN01), on G2/M phase ($p<0.001$ for MN01 and ACP03) and cells with >4N ($p<0.001$ for ACP03). Since we did not observe TP53 transcripts, a possible explanation might be that lack of p53 results in an inefficient G1 checkpoint, depending on S and G2/M checkpoints to arrest cells after DNA damage in an attempt to repair this effect caused by the drug[55]. S and/or G2/M arrest was also reported by other groups in colorectal carcinoma cells[55,56].

Treatment with ZM447439 alone induced a significant increase of cells in S and G2/M phase as well as cells with DNA content greater than 4N for all cell lines (Fig. 6, 7 and 8). Several studies have also observed an increased number of polyploid cells (with >4N DNA content) after treatment with ZM447439, including breast cancer cells[23,24], leukemia cells[17,19–21], mesothelioma cell lines[57], hepatoma cells[16], osteosarcoma and corectal

carcinoma cells[54]. We observed, as well as other authors, an increase of cells with 4N and >4N DNA content because inhibiting AURKB results in cytokinesis failure, so cells may endoreduplicate and become polyploid[58]. Interestingly, the combined treatment of ZM447439 with 5-FU resulted in a significant cell cycle arrest on S phase ($p<0.001$ for ACP02 and ACP03) and in an increased number of cells with >4N ($p<0.05$ for ACP02 and $p<0.001$ for ACP03). A similar result was reported after combining cisplatin with ZM447439 for 72 hours, which remarkably induced a significant increase of cervical cancer cells on S phase, as well as increased cells on G2/M phase, but to a less extent[27].

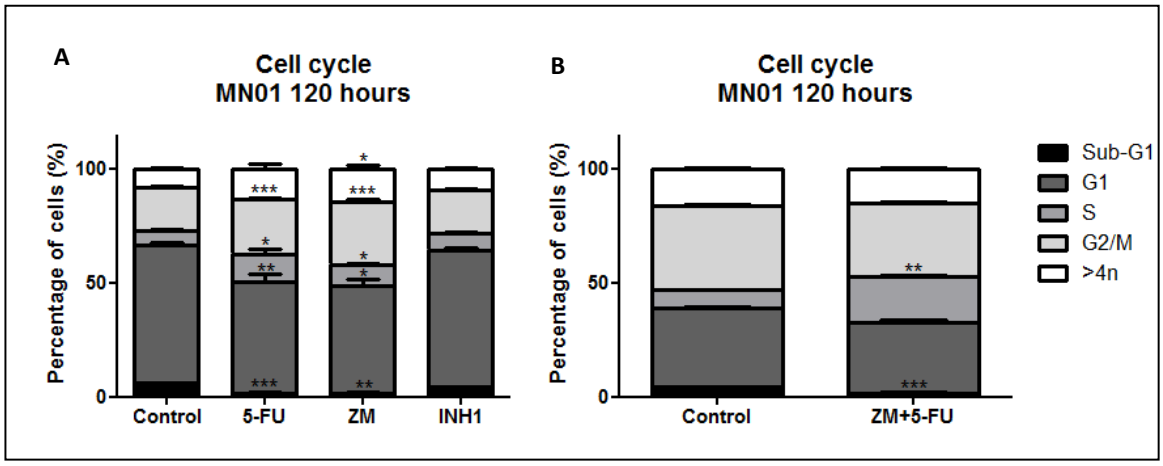

Fig. 6 MN01 cell cycle analysis after 120 hours of treatment with (A) 5-FU, ZM447439 or INH1 and (B) 5-FU combined with ZM447439. *p<0.05,**p<0.01, ***p<0.001

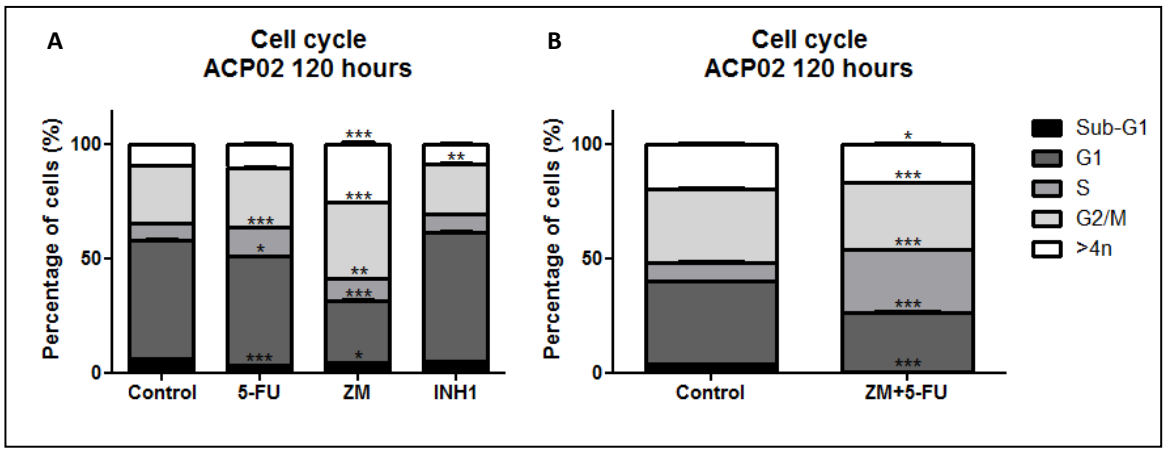

Fig. 7 ACP02 cell cycle analysis after 120 hours of treatment with (A) 5-FU, ZM447439 or INH1 and (B) 5-FU combined with ZM447439. *p<0.05,**p<0.01, ***p<0.001

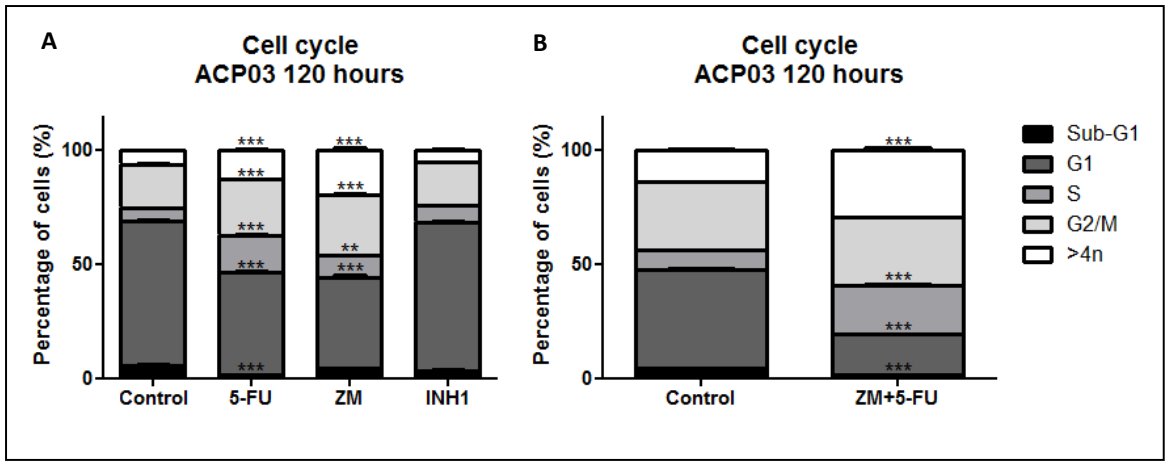

Fig. 8 ACP03 cell cycle analysis after 120 hours of treatment with (A) 5-FU, ZM447439 or INH1 and (B) 5-FU combined with ZM447439. *p<0.05,**p<0.01, ***p<0.001

As stated before, we verified a lack of TP53 expression in basal conditions in both ACP02 and ACP03 cell lines. This might an explanation for the significant increase of polyploid cells since a previous study have shown that lack of p53 enhances endoreduplication/polyploidization of cells treated with ZM447439, resulting in colon carcinoma cells with DNA content greater than 4N[59]. Another cell cycle analysis of colorectal tumor cells treated with ZM447439 presented increased polyploidy levels when cells were p53- as well[60]. Another study observed that a p53- cell line exposed to ZM447439 presented polyploidy, but afterwards cell cycle progression continued and viability decreased while other cell lines with active p53, presented most cells arrested with a DNA content of 4N or 8N. They concluded that lack of p53 increases endoreduplication levels[58]. Furthermore, a previous study has shown that tetraploid hepatoma cells were more sensitive to AURKB inhibition than diploid cells, mainly because of failure on cytokinesis resulting in reduplication of genome. They suggested that since tetraploid cells reach an excessive DNA content faster than diploid cells, apoptosis is triggered earlier[26]. Finally, still concerning ZM447439, another study verified that the efficacy of this drug relies on endoreduplication, which induces apoptosis independently of p53 status in colon carcinoma cells[61].

None of the gastric cell lines studied by our group presented major cell cycle changes when treated with INH1, similarly to a study with breast cancer cells[14]. The only alteration reported by this other study, and that we did not observe, was an increase of sub-G1 cells, indicating apoptosis.

Combination of ZM44739 and 5-FU presented a higher caspase 3 activity than drugs alone after 24 hours

Treatment of all cell lines with isolated drugs for 24 hours did not result in a higher caspase 3 activity when compared to control samples. Interestingly, the combination of ZM447439 with 5-FU exhibited a significant increase of caspase 3 activity, indicating apoptosis ($p<0.001$ for ACP02 and ACP03 and $p<0.05$ for MN01) (Fig. 9). The lack of caspase activity when treating with isolated drugs (Supplemental Figure S1), may be due to a short time of exposure (24 hours), not allowing cells to endoreduplicate and consequently, trigger cell death, in the case of ZM447439. Probably, the combination enhanced the apoptosis process earlier. Similarly to our results, another study with pancreatic cancer cells also observed a significant increase of caspase activity when combining ZM447439 with imatinib (through PDGFR inhibition)[62].

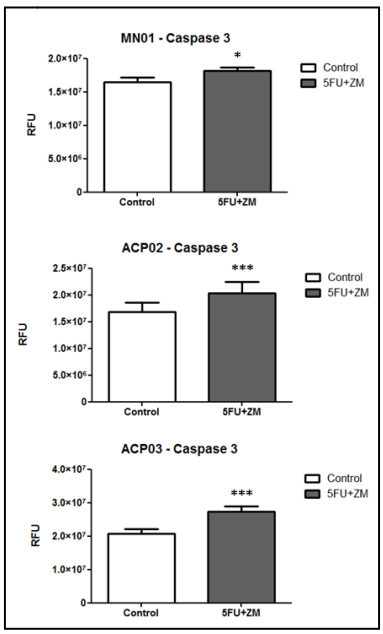

Fig. 9 Caspase 3 activity of MN01, ACP02 and ACP03, after 24 hours of 5-FU and ZM447439 combined treatment. *p<0.05, ***p<0.001

Treatment with drugs alone and the combination of 5-FU with ZM447439 induced apoptosis after 72 and 120 hours

After 72 hours of treatment of both MN01 and ACP02, 5-FU induced early apoptosis ($p<0.001$) and ZM447439 resulted in an increase of early ($p<0.001$ for ACP02 and $p<0.05$ for MN01) and late apoptotic cells ($p<0.01$ for ACP02 and $p<0.001$ for MN01) (Fig. 10 and 11). An increase in late apoptotic ACP02 cells was verified after 120 hours of combined treatment ($p<0.001$) (Fig. 11). Regarding the treatment for 120 hours with drugs alone, 5-FU induced early apoptosis ($p<0.001$) in both MN01 and ACP02 and late apoptosis just in ACP02 ($p<0.001$). ZM447439 induced early apoptosis in MN01 ($p<0.05$) as well as early and late apoptosis in ACP02 ($p<0.001$). On the other hand, INH1 induced early apoptosis just in ACP02 ($p<0.001$). Apoptosis induction after treatment with ZM447439 was reported on several cell lines such as leukemia cells[63], Hep2 cells[28], colorectal carcinoma cells[60], glioblastoma cell lines[15] and breast cell lines[24]. A commonly observed result was the significant reduction of necrotic cells after 120 hours of treatment compared to controls. We hypothesized that this can be attributed to a high confluence of control cells at the end of the experiment leading to cell death.

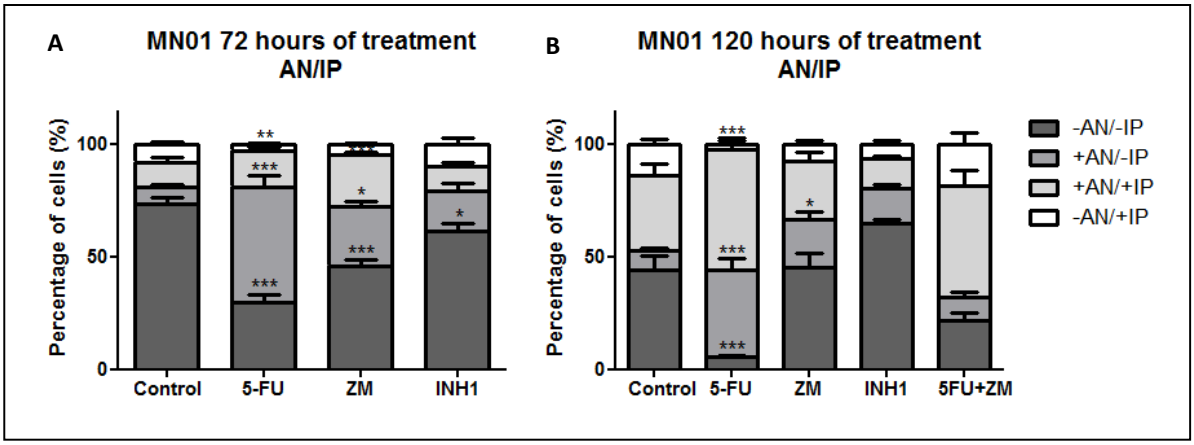

Fig. 10 Results of MN01 AN/PI staining (A) after 72 hours of treatment with 5-FU, ZM447439 or INH1 and (B) after 120 hours of treatment with 5-FU, ZM447439, INH1 or the combined therapy (5-FU + ZM447439).*p<0.05,**p<0.01, ***p<0.001

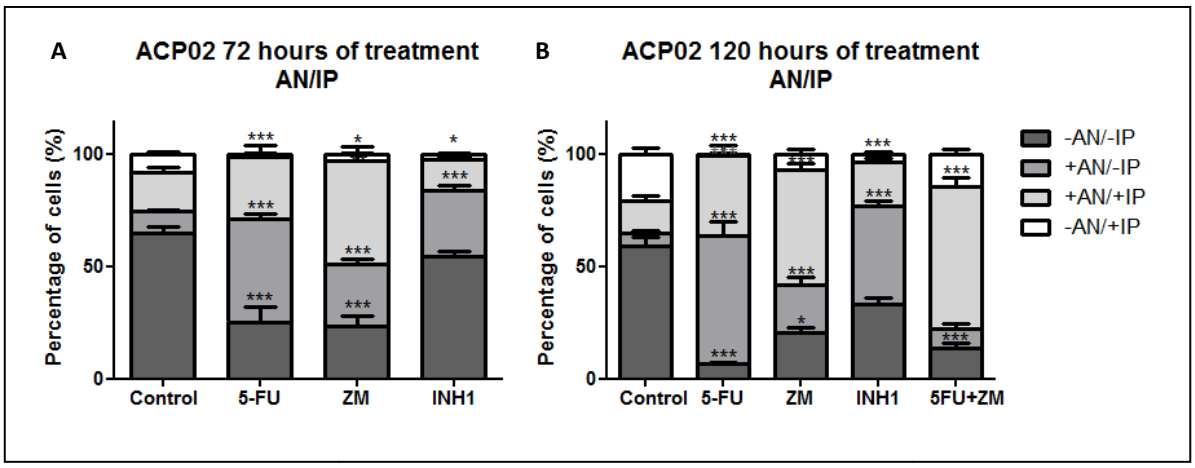

Fig. 11 Results of ACP02 AN/PI staining (A) after 72 hours of treatment with 5-FU, ZM447439 or INH1 and (B) after 120 hours of treatment with 5-FU, ZM447439, INH1 or the combined therapy (5-FU + ZM447439). *p<0.05,**p<0.01, ***p<0.001

Conclusions

In summary, this study shows that either ZM447439 alone or combined with 5-FU induced a significant increase of large irregular and large regular nuclei, indicating the occurrence of mitotic catastrophe and senescence, respectively. This is probably a consequence of endoreduplication, since we also verified an increase of cells with DNA content greater than 4N. Furthermore, we verified that all treatments tested resulted in a significant increase of apoptosis after 72 and/or 120 hours of treatment, as shown by annexin and PI staining. Interestingly, when cells were treated for just 24 hours, the only treatment capable of inducing apoptosis (significant increase in caspase activity) was the combination of 5-FU and ZM447439. Altogether, our results indicate that ZM447439 and mainly its combination with 5-FU, are potential therapy agents against gastric cancer cells, thus, further preclinical studies are required to evaluate this promising targeted anticancer therapy.

Supplemental material

Table S1 Primers used for cDNA detection by RT-PCR

Target gene	Primers (5'-3')
NDC80	F: caaggacccgagaccactta R: tgtgaggccatgtatgagga
AURKB	F: gggagagctgaagattgctg R: gacagattgaagggcagagg
P53	F: gcgcacagaggaagagaatc R: cagtctgagtcaggcccttc
RXRA	F: cctttctcggtcatcagctc R: tgtcaatcaggcagtccttg
CDC2	F: caggatgtgcttatgcagga R: gctgaccccagcaatacttc
BCL2	F: ccctgcacaacaacaacaac R: gagagctatggtccctgctg
BAX	F: tacgctgttggaagctctga R: accgtaacaggtgcaggaat
Cyclin D1	F: cgtggcctctaagatgaagg R: tgaggcggtagtaggacagg
VEGF	F: aaggaggagggcagaatcat R: aaatgctttctccgctctga
C-myc	F: attacaaagccgccgactc R: ctattcagcacgcttctcctc
MMP-9	F: gagaccggtgagctggatag R: agggaccacaactcgtcatc
RAS	F: ccatccagttcatccagtcc R: ctccagatctgccttgttcc

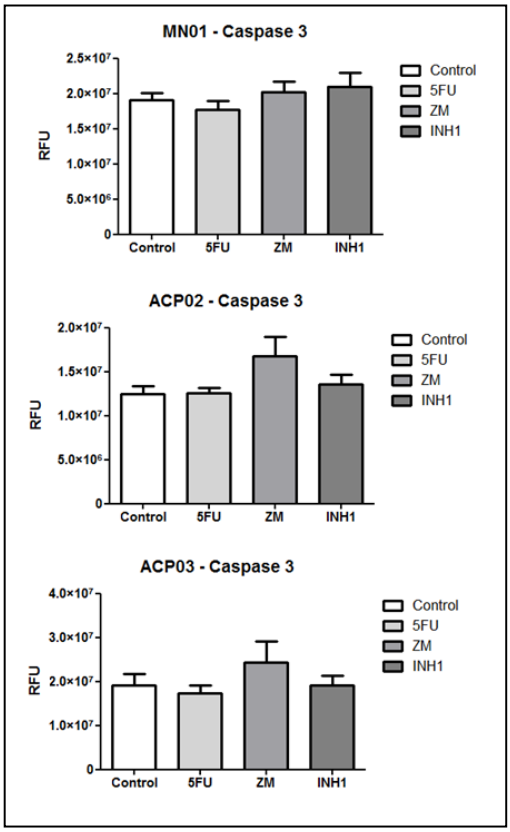

Fig. S1 Caspase 3 activity verified in MN01, ACP02 and ACP03 after 24 hours of treatment with either 5-FU, ZM447439 or INH1.

References

1. Globocan 2012 - Home. Available at: http://globocan.iarc.fr/Default.aspx.
2. Catalano, V. *et al.* Gastric cancer. *Crit. Rev. Oncol. Hematol.* **71,** 127–64 (2009).
3. Dicken, B. J. *et al.* Gastric adenocarcinoma: review and considerations for future directions. *Ann. Surg.* **241,** 27–39 (2005).
4. Smyth, E. C. *et al.* Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **27,** v38–v49 (2016).
5. Larsen, A. K., Escargueil, A. E. & Skladanowski, A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. *Pharmacol. Ther.* **85,** 217–29 (2000).
6. Rosado, J. O., Henriques, J. P. & Bonatto, D. A systems pharmacology analysis of major chemotherapy combination regimens used in gastric cancer treatment: predicting potential new protein targets and drugs. *Curr. Cancer Drug Targets* **11,** 849–69 (2011).
7. Hindriksen, S., Meppelink, A. & Lens, S. M. A. Functionality of the chromosomal passenger complex in cancer. *Biochem. Soc. Trans.* **43,** 23–32 (2015).
8. van der Horst, A. & Lens, S. M. A. Cell division: control of the chromosomal passenger complex in time and space. *Chromosoma* **123,** 25–42 (2014).
9. Lens, S. M. A., Voest, E. E. & Medema, R. H. Shared and separate functions of polo-like kinases and aurora kinases in cancer. *Nat. Rev. Cancer* **10,** 825–41 (2010).
10. Ditchfield, C. *et al.* Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J. Cell Biol.* **161,** 267–80 (2003).
11. Ciferri, C. *et al.* Architecture of the human ndc80-hec1 complex, a critical constituent of the outer kinetochore. *J. Biol. Chem.* **280,** 29088–95 (2005).
12. Lin, Y.-T., Chen, Y., Wu, G. & Lee, W.-H. Hec1 sequentially recruits Zwint-1 and ZW10 to kinetochores for faithful chromosome segregation and spindle checkpoint control. *Oncogene* **25,** 6901–14 (2006).
13. Chen, Y., Riley, D. J., Zheng, L., Chen, P.-L. & Lee, W.-H. Phosphorylation of the mitotic regulator protein Hec1 by Nek2 kinase is essential for faithful chromosome segregation. *J. Biol. Chem.* **277,** 49408–16 (2002).
14. Wu, G. *et al.* Small molecule targeting the Hec1/Nek2 mitotic pathway suppresses

tumor cell growth in culture and in animal. *Cancer Res.* **68,** 8393–9 (2008).

15. Borges, K. S. *et al.* Inhibition of Aurora kinases enhances chemosensitivity to temozolomide and causes radiosensitization in glioblastoma cells. *J. Cancer Res. Clin. Oncol.* **138,** 405–14 (2012).
16. Yoon, M. J. *et al.* Aurora B confers cancer cell resistance to TRAIL-induced apoptosis via phosphorylation of survivin. *Carcinogenesis* **33,** 492–500 (2012).
17. Failes, T. W. *et al.* Evolution of resistance to Aurora kinase B inhibitors in leukaemia cells. *PLoS One* **7,** e30734 (2012).
18. Ikezoe, T. *et al.* A novel treatment strategy targeting Aurora kinases in acute myelogenous leukemia. *Mol. Cancer Ther.* **6,** 1851–7 (2007).
19. Liu, L.-L. *et al.* Inhibition of mTOR pathway sensitizes acute myeloid leukemia cells to aurora inhibitors by suppression of glycolytic metabolism. *Mol. Cancer Res.* **11,** 1326–36 (2013).
20. Walsby, E., Walsh, V., Pepper, C., Burnett, A. & Mills, K. Effects of the aurora kinase inhibitors AZD1152-HQPA and ZM447439 on growth arrest and polyploidy in acute myeloid leukemia cell lines and primary blasts. *Haematologica* **93,** 662–9 (2008).
21. Zhou, W. *et al.* Inhibition of Bcl-xL overcomes polyploidy resistance and leads to apoptotic cell death in acute myeloid leukemia cells. *Oncotarget* **6,** 21557–71 (2015).
22. Cai, J. *et al.* Discovery of 4-aminoquinazoline--urea derivatives as Aurora kinase inhibitors with antiproliferative activity. *Bioorg. Med. Chem.* **22,** 5813–23 (2014).
23. Sánchez-Bailón, M. P. *et al.* Src kinases catalytic activity regulates proliferation, migration and invasiveness of MDA-MB-231 breast cancer cells. *Cell. Signal.* **24,** 1276–86 (2012).
24. Vidarsdottir, L., Steingrimsdottir, G., Bodvarsdottir, S. K., Ogmundsdottir, H. M. & Eyfjord, J. E. Sensitivity of BRCA2 mutated human cell lines to Aurora kinase inhibition. *Invest. New Drugs* **30,** 425–34 (2012).
25. Marxer, M., Ma, H. T., Man, W. Y. & Poon, R. Y. C. p53 deficiency enhances mitotic arrest and slippage induced by pharmacological inhibition of Aurora kinases. *Oncogene* **33,** 3550–60 (2014).
26. Marxer, M. *et al.* Tetraploidization increases sensitivity to Aurora B kinase inhibition. *Cell Cycle* **11,** 2567–77 (2012).

27. Zhang, L. & Zhang, S. ZM447439, the Aurora kinase B inhibitor, suppresses the growth of cervical cancer SiHa cells and enhances the chemosensitivity to cisplatin. *J. Obstet. Gynaecol. Res.* **37,** 591–600 (2011).
28. Long, Z.-J. *et al.* ZM 447439 inhibition of aurora kinase induces Hep2 cancer cell apoptosis in three-dimensional culture. *Cell Cycle* **7,** 1473–9 (2008).
29. Leal, M. F. *et al.* Establishment and conventional cytogenetic characterization of three gastric cancer cell lines. *Cancer Genet. Cytogenet.* **195,** 85–91 (2009).
30. Filippi-Chiela, E. C. *et al.* Nuclear morphometric analysis (NMA): screening of senescence, apoptosis and nuclear irregularities. *PLoS One* **7,** e42522 (2012).
31. Wada, Y. *et al.* Synergistic effects of docetaxel and S-1 by modulating the expression of metabolic enzymes of 5-fluorouracil in human gastric cancer cell lines. *Int. J. Cancer* **119,** 783–791 (2006).
32. Vidarsdottir, L., Steingrimsdottir, G., Bodvarsdottir, S. K., Ogmundsdottir, H. M. & Eyfjord, J. E. Sensitivity of BRCA2 mutated human cell lines to Aurora kinase inhibition. *Invest. New Drugs* **30,** 425–434 (2012).
33. Tavanti, E. *et al.* Preclinical validation of Aurora kinases-targeting drugs in osteosarcoma. *Br. J. Cancer* **109,** 2607–2618 (2013).
34. Baldini, E. *et al.* The dual Aurora kinase inhibitor ZM447439 prevents anaplastic thyroid cancer cell growth and tumorigenicity. *J. Biol. Regul. Homeost. Agents* **27,** 705–15
35. Linton, A. *et al.* An RNAi-based screen reveals PLK1, CDK1 and NDC80 as potential therapeutic targets in malignant pleural mesothelioma. *Br. J. Cancer* **110,** 510–519 (2014).
36. Georgieva, I. *et al.* ZM447439, a novel promising aurora kinase inhibitor, provokes antiproliferative and proapoptotic effects alone and in combination with bio- and chemotherapeutic agents in gastroenteropancreatic neuroendocrine tumor cell lines. *Neuroendocrinology* **91,** 121–30 (2010).
37. Honma, K. *et al.* Contribution of Aurora-A and -B expression to DNA aneuploidy in gastric cancers. *Surg. Today* **44,** 454–461 (2014).
38. Qu, Y., Li, J., Cai, Q. & Liu, B. Hec1/Ndc80 is overexpressed in human gastric cancer and regulates cell growth. *J. Gastroenterol.* **49,** 408–18 (2014).

39. Korbakis, D. & Scorilas, A. Quantitative expression analysis of the apoptosis-related genes BCL2, BAX and BCL2L12 in gastric adenocarcinoma cells following treatment with the anticancer drugs cisplatin, etoposide and taxol. *Tumor Biol.* **33,** 865–875 (2012).

40. Mita, H. *et al.* A novel method, digital genome scanning detects KRAS gene amplification in gastric cancers: involvement of overexpressed wild-type KRAS in downstream signaling and cancer cell growth. *BMC Cancer* **9,** 198 (2009).

41. Son, T. *et al.* Novel anti-cancer role of naphthazarin in human gastric cancer cells. *Int. J. Oncol.* **40,** 157–62 (2011).

42. Vollmers, H. P. *et al.* Characterization of four new gastric cancer cell lines. *Virchows Arch. B Cell Pathol. Incl. Mol. Pathol.* **63,** 335–343 (1993).

43. Yang, M. *et al.* Overexpression of nuclear apoptosis-inducing factor 1 altered the proteomic profile of human gastric cancer cell MKN45 and induced cell cycle arrest at G1/S phase. *PLoS One* **9,** e100216 (2014).

44. Zhang, X.-M., Zhou, C., Gu, H., Yan, L. & Zhang, G.-Y. Correlation of RKIP, STAT3 and cyclin D1 expression in pathogenesis of gastric cancer. *Int. J. Clin. Exp. Pathol.* **7,** 5902–8 (2014).

45. Zhang, H., Wu, J., Meng, L. & Shou, C.-C. Expression of vascular endothelial growth factor and its receptors KDR and Flt-1 in gastric cancer cells. *World J. Gastroenterol.* **8,** 994–8 (2002).

46. Gencer, S., Cebeci, A. & Irmak-Yazicioglu, M. B. Matrix metalloproteinase gene expressions might be oxidative stress targets in gastric cancer cell lines. *Chin. J. Cancer Res.* **25,** 322–33 (2013).

47. Liu, S., Wu, Q., Chen, Z. M. & Su, W. J. The effect pathway of retinoic acid through regulation of retinoic acid receptor alpha in gastric cancer cells. *World J. Gastroenterol.* **7,** 662–6 (2001).

48. Hu, K.-W., Chen, F.-H., Ge, J.-F., Cao, L.-Y. & Li, H. Retinoid receptors in gastric cancer: expression and influence on prognosis. *Asian Pac. J. Cancer Prev.* **13,** 1809–17 (2012).

49. Yokozaki, H. Molecular characteristics of eight gastric cancer cell lines established in Japan. *Pathol. Int.* **50,** 767–77 (2000).

50. Galluzzi, L. *et al.* Molecular definitions of cell death subroutines: recommendations of

the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* **19,** 107–120 (2012).

51. Kroemer, G. *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* **16,** 3–11 (2009).
52. Tao, Y. *et al.* The Aurora B kinase inhibitor AZD1152 sensitizes cancer cells to fractionated irradiation and induces mitotic catastrophe. *Cell Cycle* **8,** 3172–3181 (2009).
53. Bemiller, P. M. & Lee, L.-H. Nucleolar changes in senescing WI-38 cells. *Mech. Ageing Dev.* **8,** 417–427 (1978).
54. Kumari, G., Ulrich, T. & Gaubatz, S. A role for p38 in transcriptional elongation of p21 (CIP1) in response to Aurora B inhibition. *Cell Cycle* **12,** 2051–60 (2013).
55. Akasaka, T. *et al.* 5‐FU resistance abrogates the amplified cytotoxic effects induced by inhibiting checkpoint kinaseï¿½1 in p53‐mutated colon cancer cells. *Int. J. Oncol.* (2014). doi:10.3892/ijo.2014.2693
56. Abaza, M.-S.-I. Roscovitine synergizes with conventional chemo-therapeutic drugs to induce efficient apoptosis of human colorectal cancer cells. *World J. Gastroenterol.* **14,** 5162 (2008).
57. Crispi, S. *et al.* Antiproliferative effect of Aurora kinase targeting in mesothelioma. *Lung Cancer* **70,** 271–9 (2010).
58. Ditchfield, C. *et al.* Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J. Cell Biol.* **161,** 267–280 (2003).
59. Dreier, M. R., Grabovich, A. Z., Katusin, J. D. & Taylor, W. R. Short and long-term tumor cell responses to Aurora kinase inhibitors. *Exp. Cell Res.* **315,** 1085–99 (2009).
60. Li, M. *et al.* Aurora kinase inhibitor ZM447439 induces apoptosis via mitochondrial pathways. *Biochem. Pharmacol.* **79,** 122–9 (2010).
61. Kaestner, P., Stolz, A. & Bastians, H. Determinants for the efficiency of anticancer drugs targeting either Aurora-A or Aurora-B kinases in human colon carcinoma cells. *Mol. Cancer Ther.* **8,** 2046–56 (2009).
62. Xie, L. *et al.* Kinome-wide siRNA screening identifies molecular targets mediating the sensitivity of pancreatic cancer cells to Aurora kinase inhibitors. *Biochem. Pharmacol.* **83,** 452–61 (2012).

63. Ikezoe, T. *et al.* A novel treatment strategy targeting Aurora kinases in acute myelogenous leukemia. *Mol. Cancer Ther.* **6,** 1851–1857 (2007).

CAPÍTULO III

METODOLOGIA E RESULTADOS ADICIONAIS

METODOLOGIA E RESULTADOS ADICIONAIS

Morfologia celular

Materiais e Métodos

As linhagens de adenocarcinoma gástrico ACP02 e ACP03 provenientes das regiões da cárdia e do antro pilórico do estômago, respectivamente, são originárias de pacientes do Hospital Universitário João de Barros Barreto. A linhagem de epitélio gástrico normal, MN01, consiste em um *pool* de células obtidas em biópsias e foram cedidas gentilmente pelo Dr. Rommel Mario Rodríguez Burbano (Instituto de Ciências Biológicas, Universidade Federal do Pará). As linhagens foram cultivadas com e sem os tratamentos a 37 ºC em meio RPMI 1640 (Sigma-Aldrich) contendo 10% de soro fetal bovino (Gibco) e 1% de penicilina/estreptomicina. O microscópio invertido Zeiss Axiovert 200 equipado com uma câmera Axiocam MRc (Carl Zeiss) foi utilizado para visualização das células e as imagens foram obtidas pelo Software AxioVision Rel 4.8.

Resultados

As linhagens MN01, ACP02 e ACP03 se proliferam em monocamada e apresentam morfologia semelhante a fibroblastos, com extremidades afiladas. O fenótipo apresentado para cada tratamento foi semelhante entre as linhagens estudadas, sendo que o tratamento com ZM447439 induziu alteração na morfologia com grupos de células dismórficas e de tamanho aumentado. O fenótipo das células tratadas com 5-FU e INH1 se manteve semelhante ao controle, com a presença de algumas células de maior tamanho no tratamento com 5-FU. As Figuras 1, 2 e 3 apresentam a morfologia de MN01, ACP02 e ACP03

expostas por 120 horas aos tratamentos isolados. A Figura 4 apresenta a morfologia das três linhagens expostas ao tratamento combinado de 5-FU e ZM447439 por 24 horas.

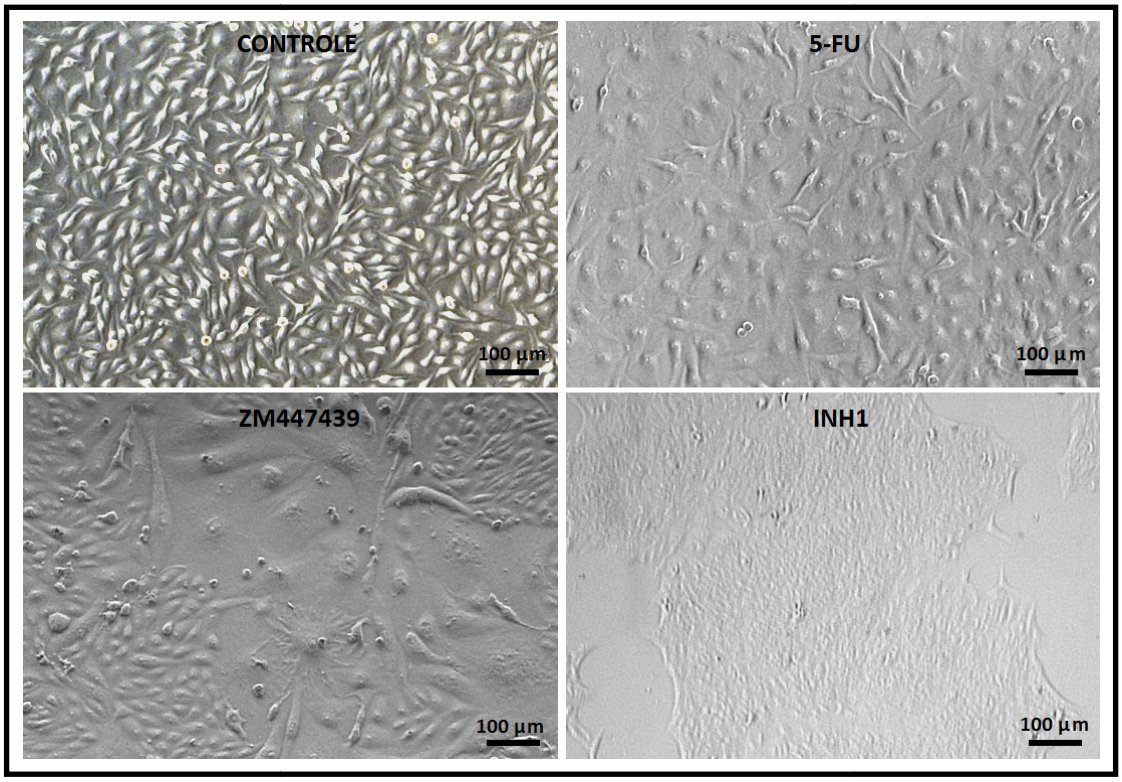

Figura 1. Imagem representativa da morfologia das células MN01 nos diferentes tratamentos.

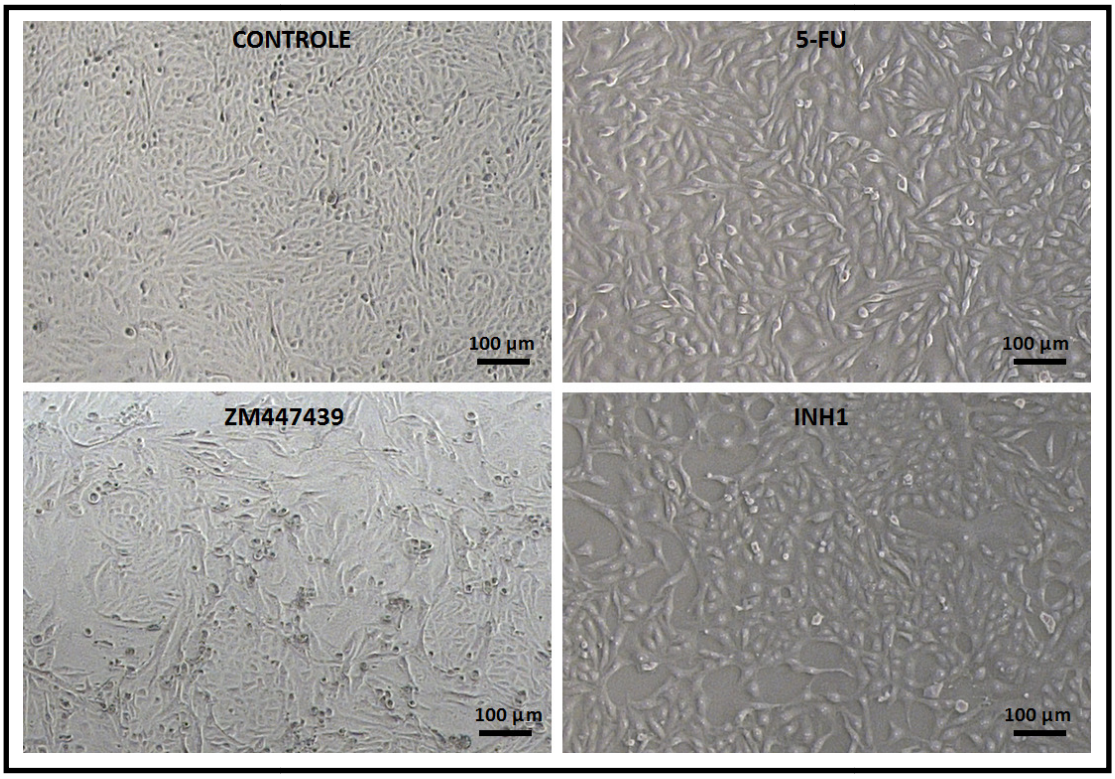

Figura 2. Imagem representativa da morfologia das células ACP02 nos diferentes tratamentos.

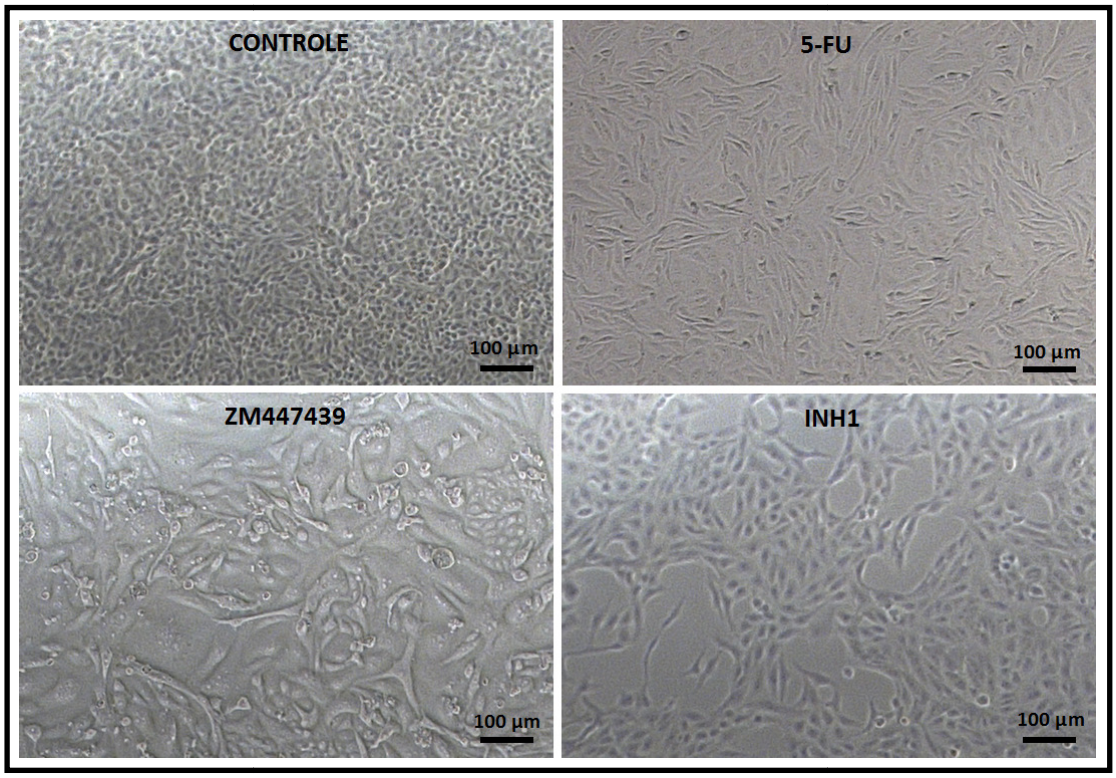

Figura 3. Imagem representativa da morfologia das células ACP03 nos diferentes tratamentos.

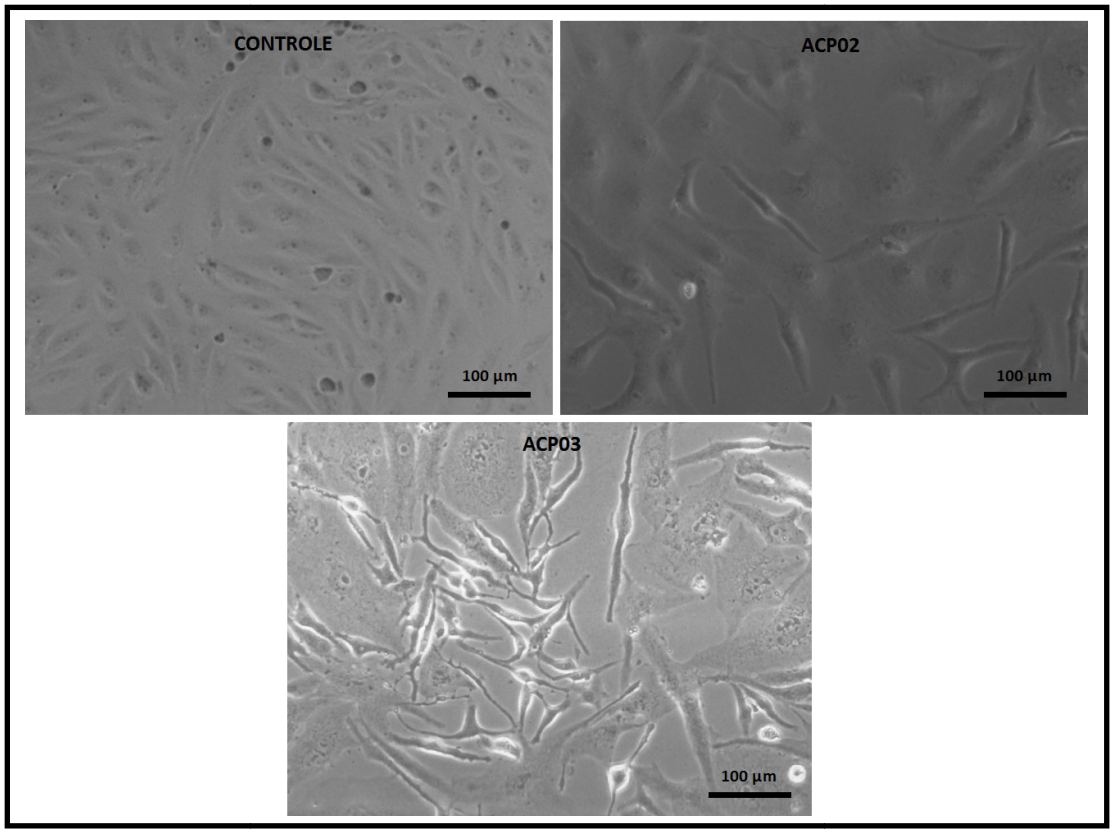

Figura 4. Imagem representativa da morfologia das células MN01, ACP02 e ACP03 expostas ao tratamento combinado 5-FU + ZM447439.

PCR em tempo real quantitativo

Materiais e Métodos

O RNA total foi isolado com o kit *Ilustra RNAspin Mini*^{TM} (GE Healthcare) após cinco dias de tratamento com 5-FU, ZM447439 ou INH1 nas três linhagens estudadas. A síntese de cDNA foi realizada com o kit RT^2 First Strand da Qiagen. A amplificação foi realizada com *RT*^{2} *SYBR Green ROX qPCR Mastermix* e com *RT*^{2} *Profiler PCR Array Human Cancer PathwayFinder* da Qiagen no equipamento *StepOnePlus* (Applied Biosystems). Os valores da expressão relativa de genes envolvidos em diferentes processos biológicos (Tabela 1) foi determinada pela aplicação do modelo matemático $2^{-\Delta\Delta CT}$ e os resultados estão representados em $Log_2(2^{-\Delta\Delta CT})$. A normalização foi realizada com base no gene *housekeeping* RPLP0. Os genes com valor de $2^{-\Delta\Delta CT}$ maior ou igual a 2 foram considerados superexpressos e com valor menor ou igual a 0,5 subexpressos.

Tabela 1. Genes avaliados por qRT-PCR e os principais processos biológicos em que atuam.

Processo Biológico	**Genes**
Angiogênese	ANGPT1, ANGPT2, CCL2, FGF2, FLT1, SERPINF1, TEK, VEGFC.
Apoptose	APAF1, BCL2L11, BIRC3, CASP2, CASP7, CASP9, CFLAR, FASLG, NOL3, XIAP.
Ciclo celular	AURKA, CCND2, CCND3, CDC20, E2F4, MCM2, MKI67, SKP2, STMN1, WEE1.
Senescência	BMI1, ETS2, IGFBP3, IGFBP5, IGFBP7, MAP2K1, MAP2K3, MAPK14, SERPINB2, SOD1, TBX2.
Reparo de dano ao DNA	DDB2, DDIT3, ERCC3, ERCC5, GADD45G, LIG4, POLB, PPP1R15A.
Transição epitélio-mesenquimal	CDH2, DSP, FOXC2, GSC, KRT14, OCLN, SNAI1, SNAI2, SNAI3, SOX10.
Sinalização de hipóxia	ADM, ARNT, CA9, EPO, HMOX1, LDHA, SLC2A1.
Metabolismo	ACLY, ACSL4, ATP5A1, COX5A, CPT2, G6PD, GPD2, LPL, PFKL, UQCRFS1.
Telômeros e telomerase	DKC1, PINX1, TEP1, TERF1, TERF2IP, TINF2, TNKS, TNKS2.

Resultados

Após o tratamento com 5-FU, o total de genes diferencialmente expressos foi 31 para MN01, 34 para ACP02 e 26 para ACP03. Já no tratamento com ZM447439 o total foi 32 genes para MN01, 29 genes para ACP02 e 22 genes para ACP03. Considerando-se o tratamento com INH1, verificamos que o total foi 27 para MN01, 33 para ACP02 e 23 para ACP03. De maneira geral, foi possível observar genes diferencialmente expressos em todas as vias analisadas. Entretanto, a linhagem MN01 exposta ao 5-FU, ACP02 exposta ao ZM447439 e ACP03 exposta aos três tratamentos, não apresentaram alterações quanto a processos associados a telômeros e telomerases (Tabela 2). As Figuras 5-13 apresentam os genes diferencialmente expressos de acordo com a linhagem e tratamento avaliados.

Tabela 2. Número de genes diferencialmente expressos de acordo com os processos biológicos avaliados.

Processo Biológico	Número de genes diferencialmente expressos								
	MN01			ACP02			ACP03		
	5-FU	ZM447439	INH1	5-FU	ZM447439	INH1	5-FU	ZM447439	INH1
Angiogênese	4	3	1	3	4	3	2	2	4
Apoptose	3	6	2	3	5	2	4	4	3
Ciclo celular	6	6	6	7	2	8	2	3	3
Senescência	4	3	2	4	5	4	3	3	2
Reparo de dano ao DNA	4	4	4	5	3	4	5	3	3
Transição epitélio-mesenquimal	3	4	3	3	4	3	3	4	1
Sinalização de hipóxia	4	1	4	3	3	3	4	1	5
Metabolismo	3	3	3	4	3	4	3	2	2
Telômeros e telomerase		2	2	2		2			
Total	31	32	27	34	29	33	26	22	23

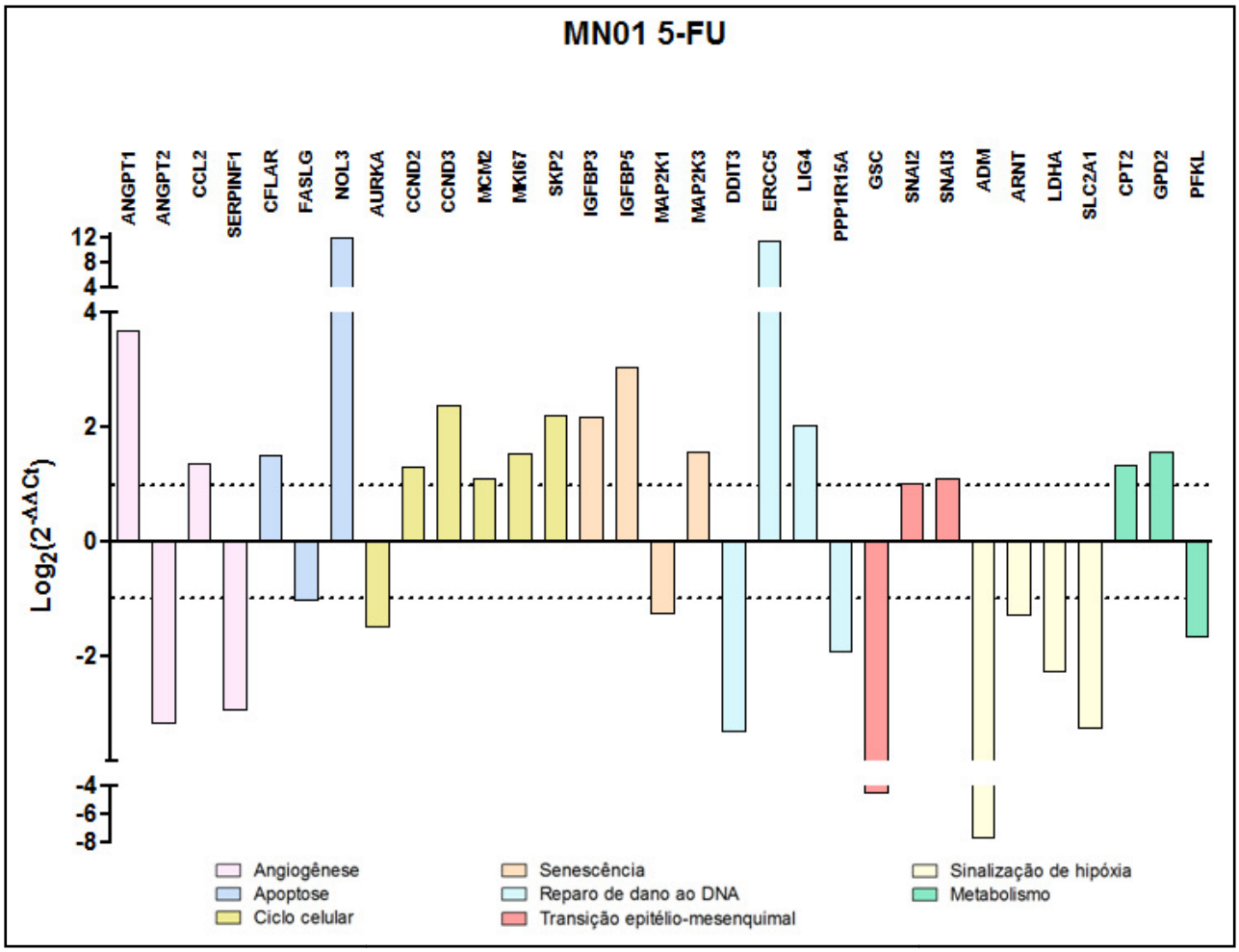

Figura 5. Genes diferencialmente expressos em MN01 após tratamento com 5-FU.

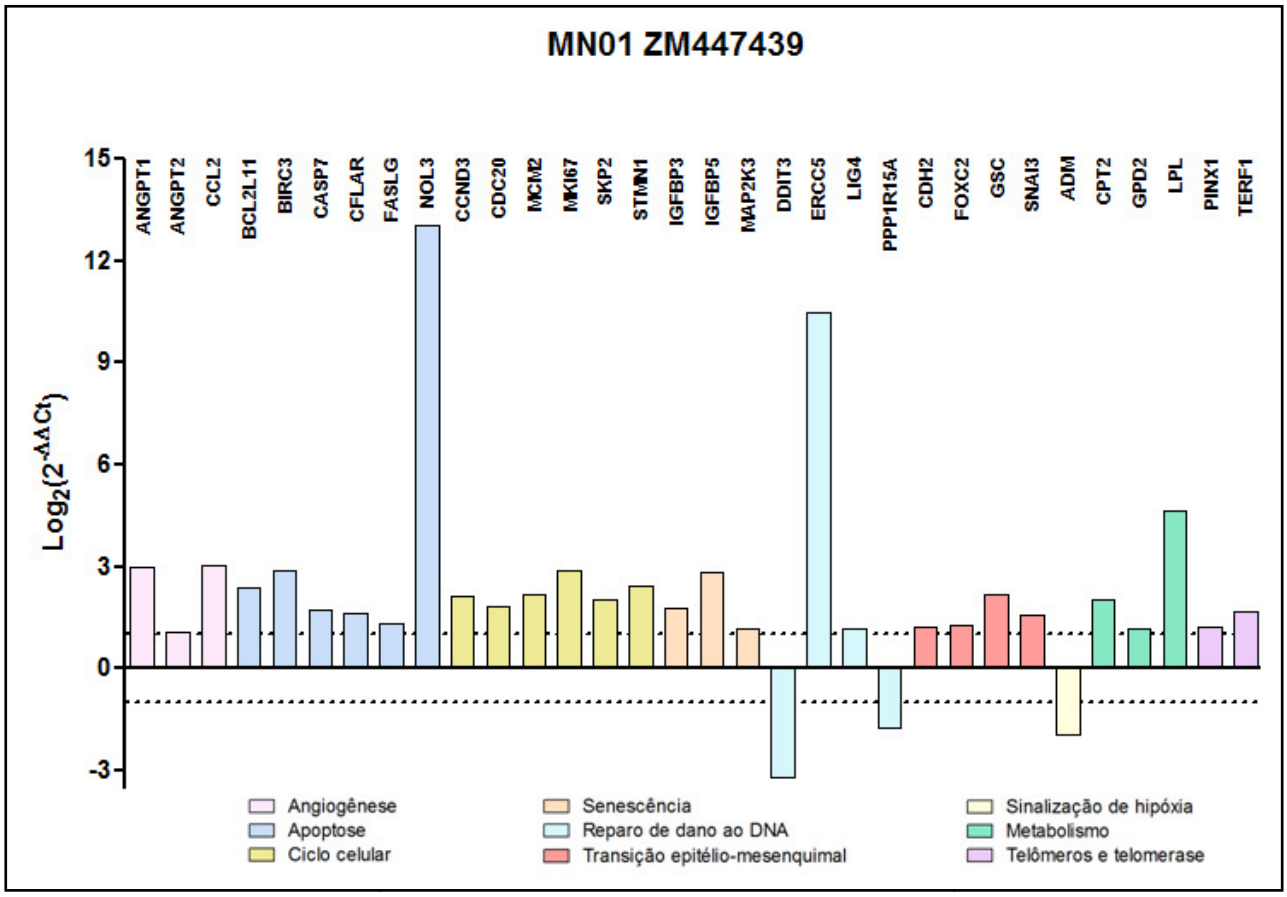

Figura 6. Genes diferencialmente expressos em MN01 após tratamento com ZM447439.

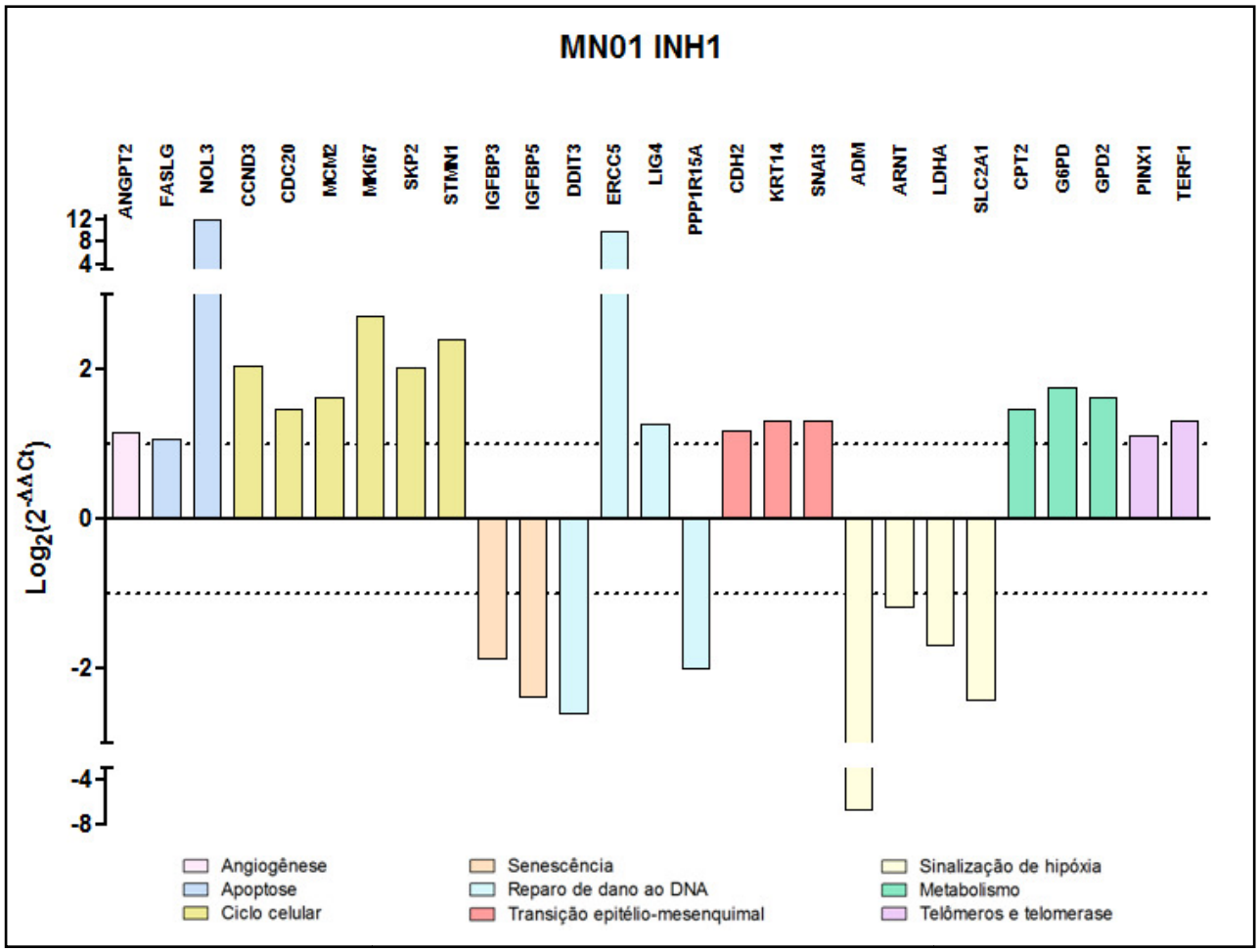

Figura 7. Genes diferencialmente expressos em MN01 após tratamento com inh1.

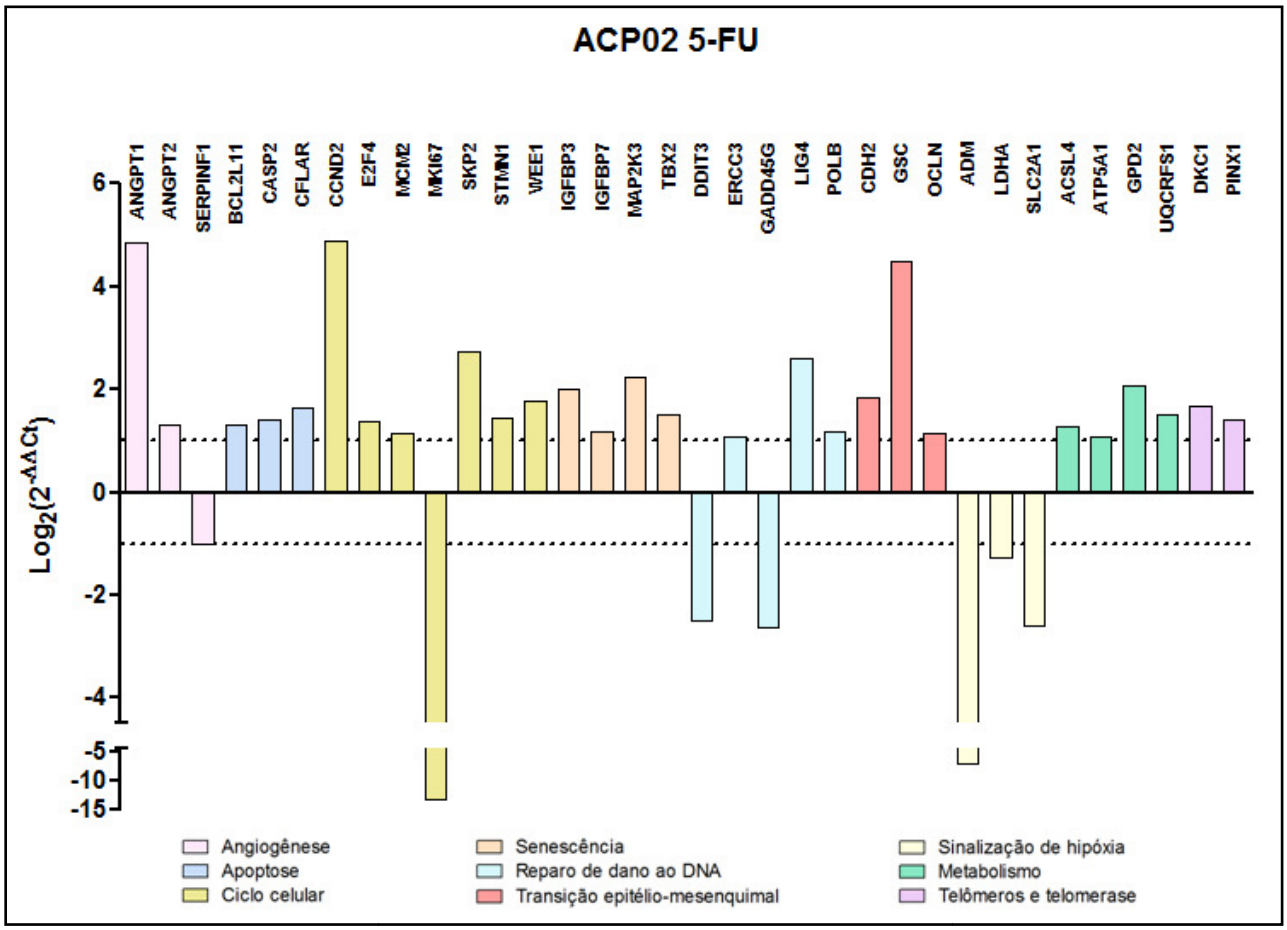

Figura 8. Genes diferencialmente expressos em ACP02 após tratamento com 5-FU.

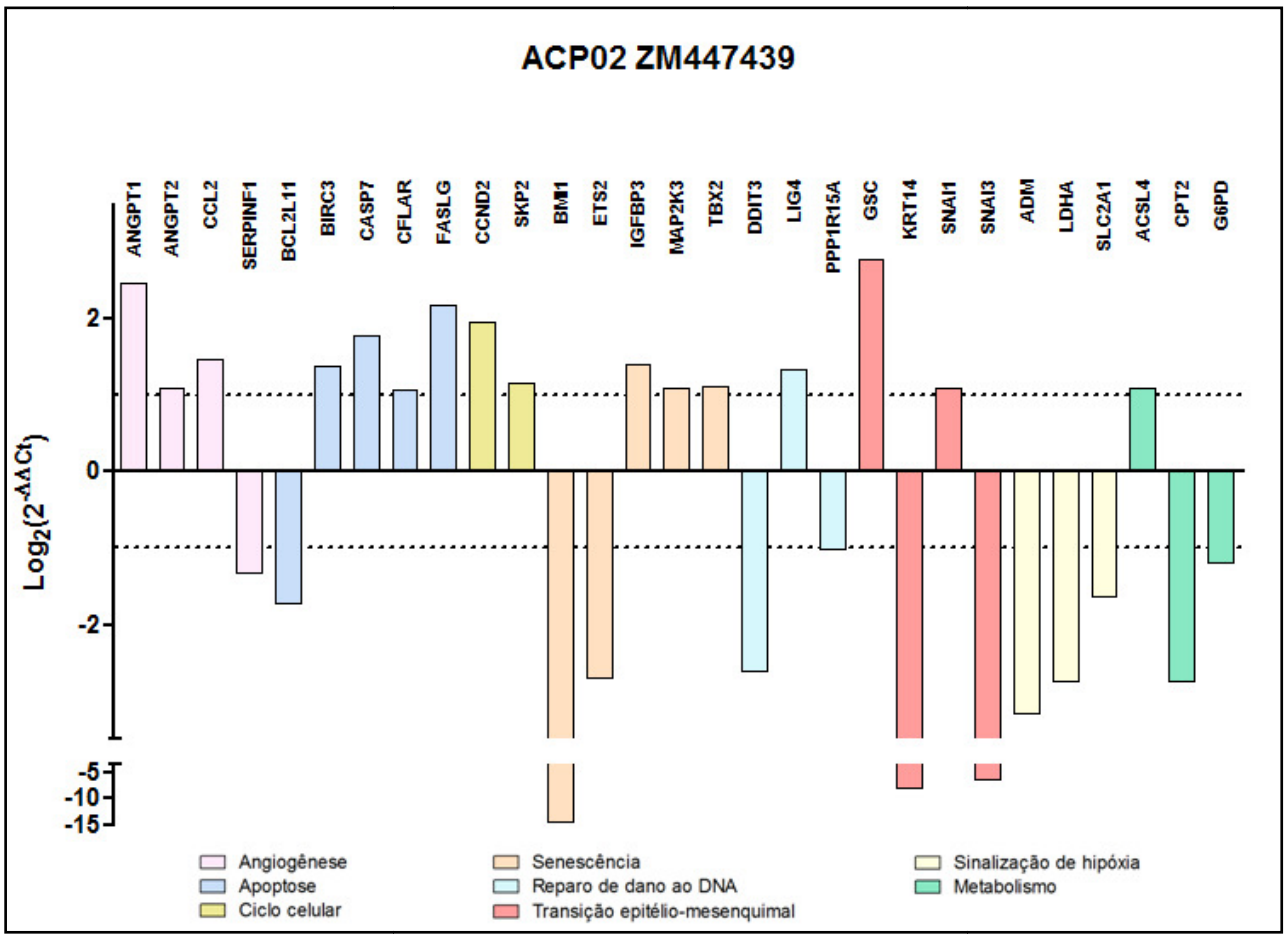

Figura 9. Genes diferencialmente expressos em ACP02 após tratamento com ZM447439.

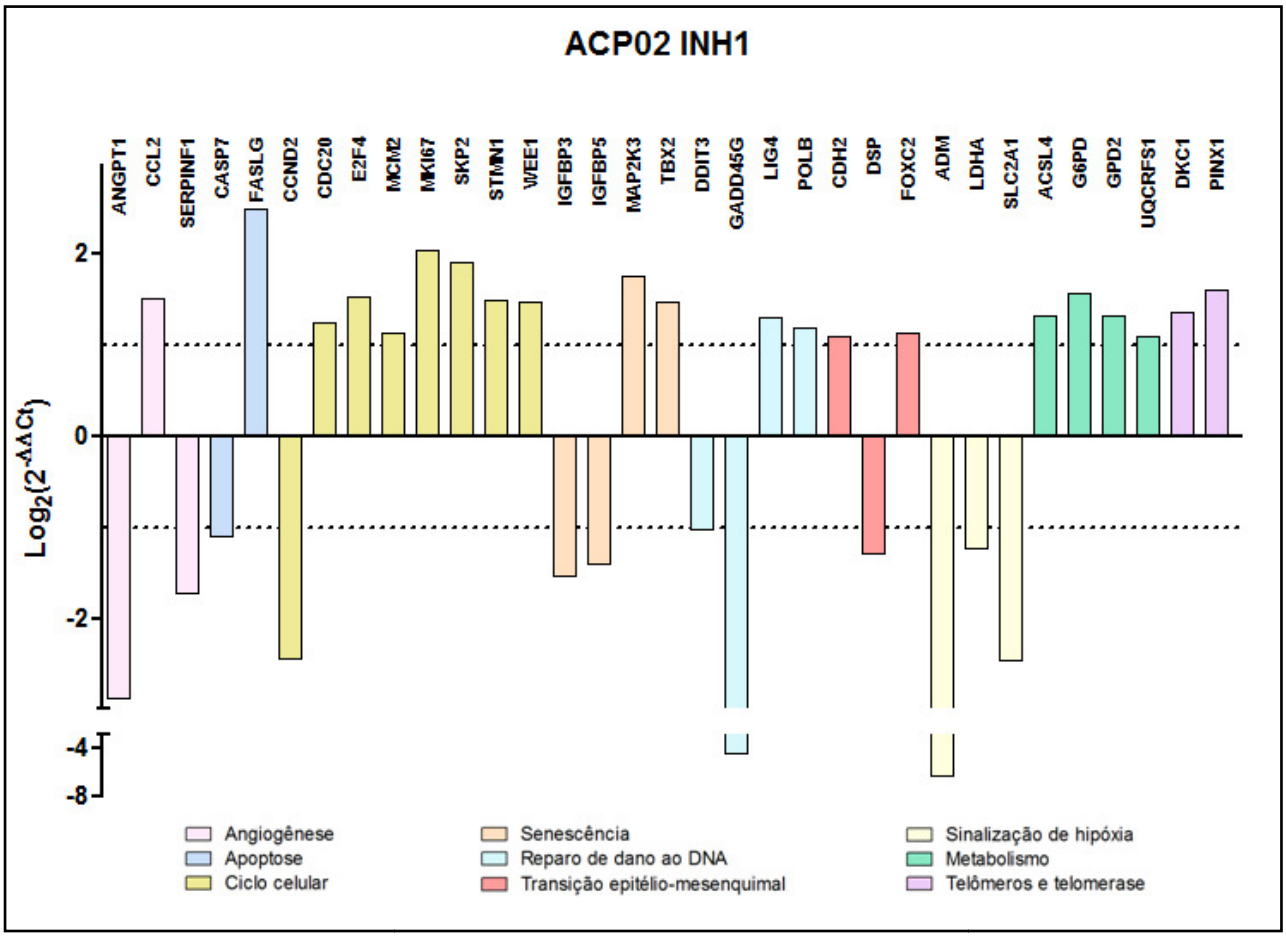

Figura 10. Genes diferencialmente expressos em ACP02 após tratamento com INH1.

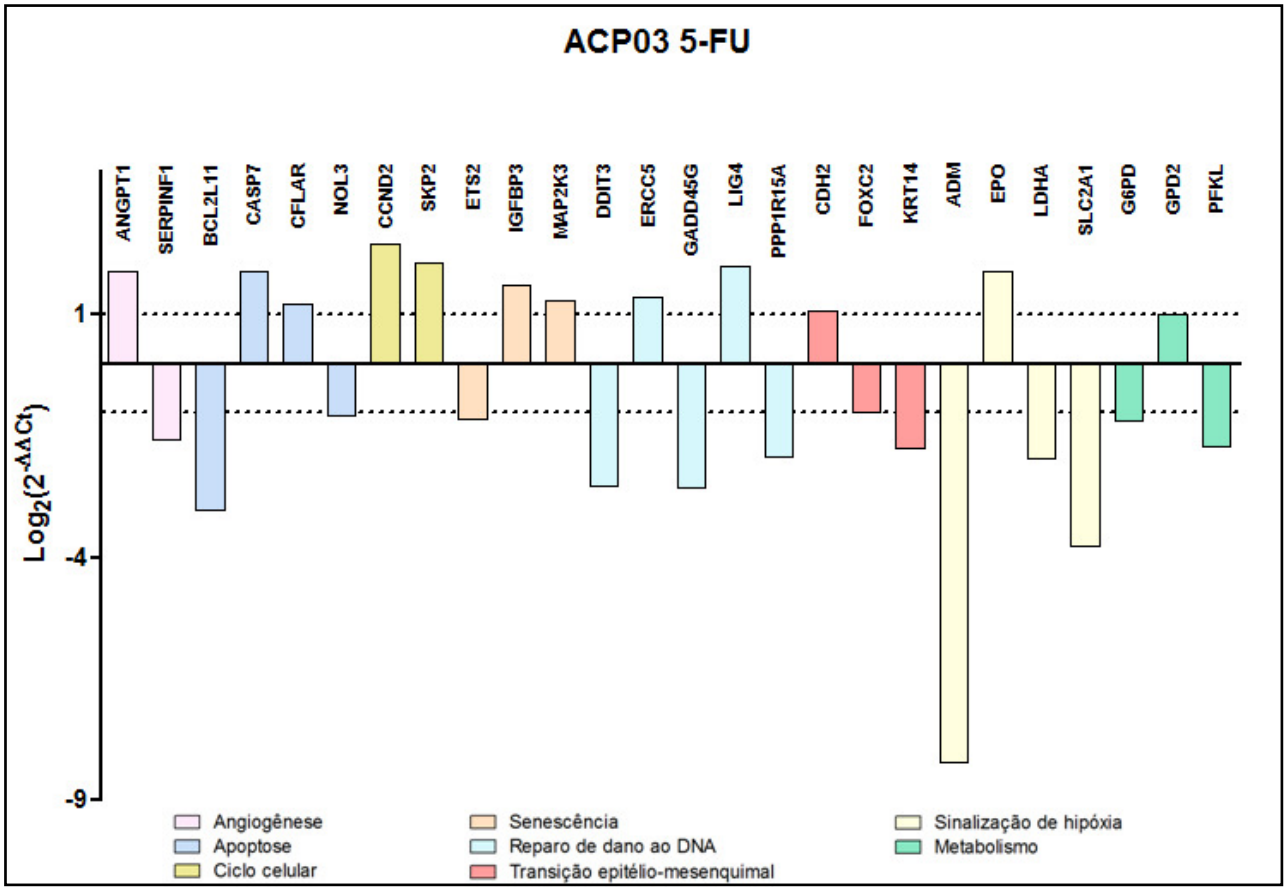

Figura 11. Genes diferencialmente expressos em ACP03 após tratamento com 5-FU.

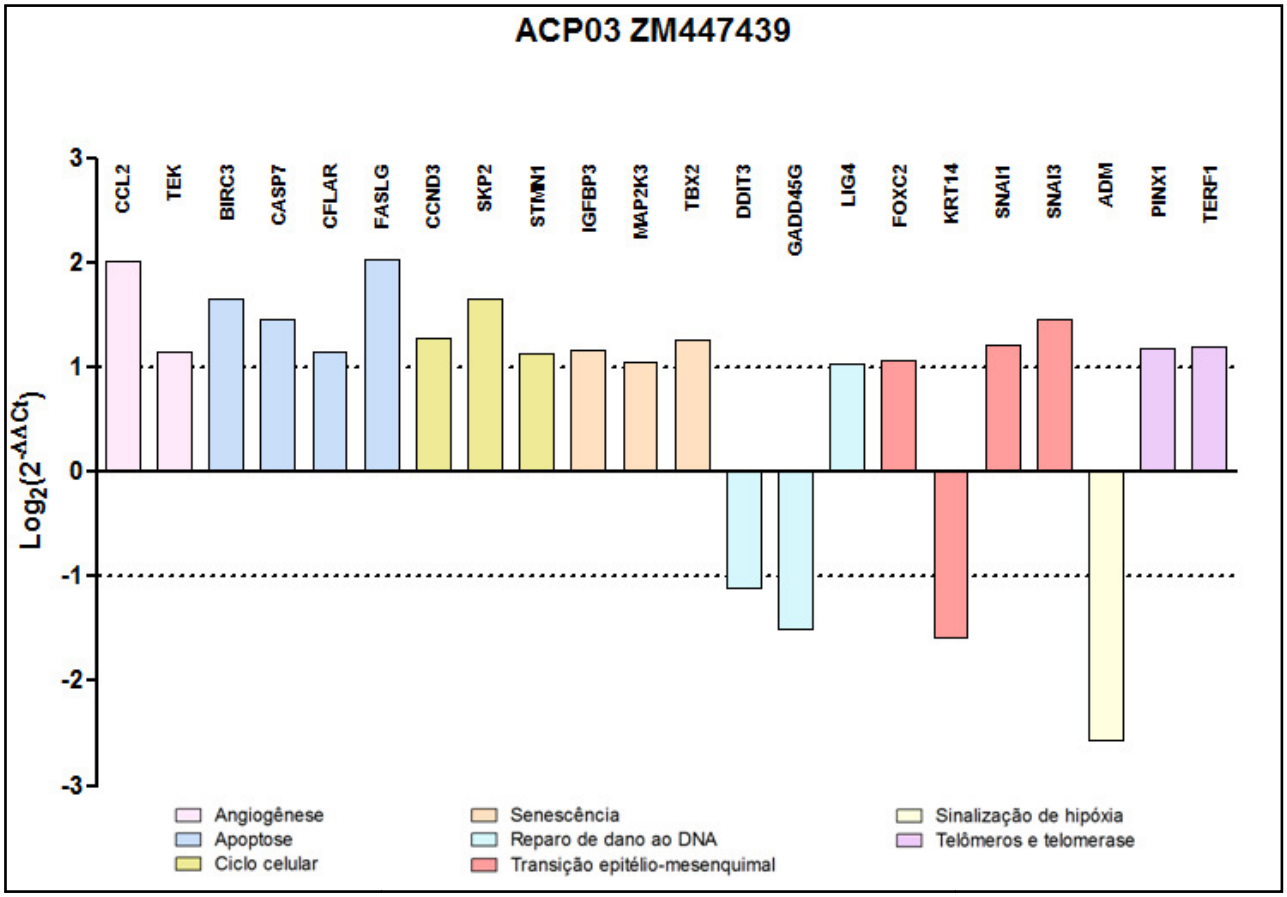

Figura 12. Genes diferencialmente expressos em ACP03 após tratamento com ZM447439.

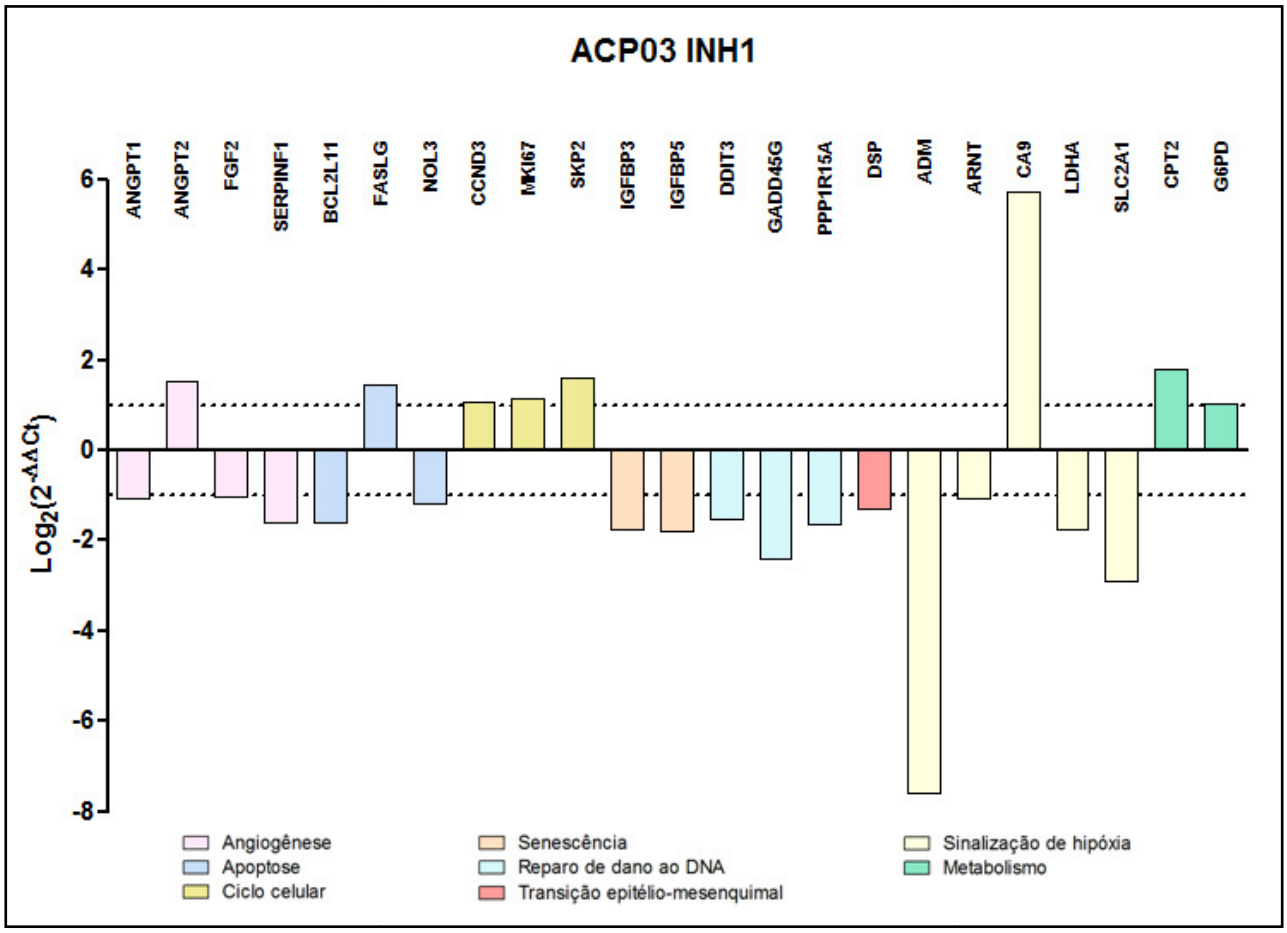

Figura 13. Genes diferencialmente expressos em ACP03 após tratamento com INH1.

Ensaios para verificação da presença de células tronco tumorais

Materiais e Métodos

Foram utilizadas duas metodologias para avaliação da presença de células tronco tumorais: ensaio de formação de esferas e RT-PCR para verificar a expressão de marcadores tronco característicos de câncer gástrico.

O ensaio de formação de esferas foi realizado com as três linhagens em tratamento com 5-FU e ZM447439. O teste foi realizado nas placas Nunc® Microwell de 96 poços tratadas para não aderência da Sigma-Aldrich, utilizando-se RPMI sem soro com estreptomicina durante 15 dias. Nos primeiros ensaios, verificamos que o inibidor INH1 não

se dilui em meio sem soro e por esse motivo não foi dada a continuidade para os experimentos subsequentes. O número total de esferas observadas em oito replicatas do experimento foi comparado entre o controle e cada tratamento, usando como teste estatístico o teste z para variáveis com distribuição de Poisson. Os valores-p obtidos foram ajustados por Bonferroni.

No ensaio de RT-PCR foi avaliada a expressão dos marcadores LGR5, CD24, CD44 e CD133 conforme metodologia descrita no Capítulo II. Os *primers* utilizados para LGR5 foram F-5'-ATGTTGCTCAGGGTGGACTG-3' e R-5'-TTGGGGGCACATAGCTGATG-3', para CD24 foram F-5'-ATGGGCAGAGCAATGGTG-3' e R-5'-GTGAGACCACGAAGAGACTGG-3', para CD44 foram F-5'-CTGGGGACTCTGCCTCGT-3' e R-5'-GGTCTCAAATCCGATGCTCA-3' e para CD133 foram F-5'-AGGCGTTGGAGAACATGAAC-3' e R-5'- TGGCGTTGTACTCTGTCAGG-3'.

Resultados

No ensaio de formação de esferas, a linhagem MN01 só apresentou uma esfera entre todas as replicatas do controle. Diferentemente, as replicatas do controle das linhagens de câncer gástrico, apresentaram um total de 17 esferas para ACP02 e de 18 esferas para ACP03. Esse número de esferas foi reduzido significativamente após tratamento com 5-FU ou ZM447439 ($p<0,001$) (Figura 14). A média de esferas observadas nos experimentos está apresentada na Figura 15. A Figura 16 mostra esferas observadas em controle de ACP02 e ACP03.

Na análise de expressão gênica de marcadores de CTTGs, foi verificada a expressão de LGR5 e CD24 em ambas as linhagens de adenocarcinoma gástrico (Figura 17).

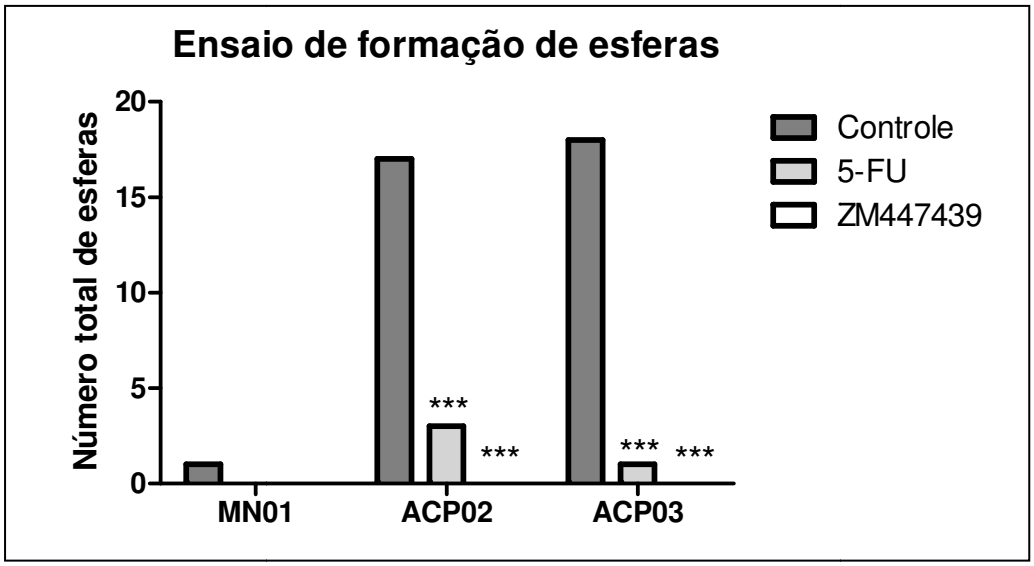

Figura 14. Número total de esferas observadas em oito replicatas do controle e após tratamento com 5-FU e ZM447439. ***p<0,001

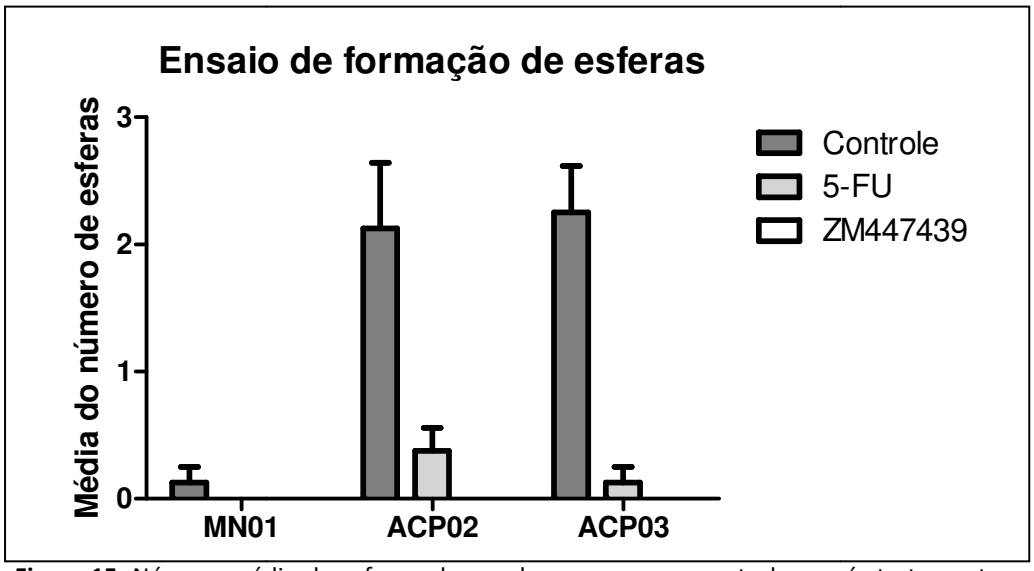

Figura 15. Número médio de esferas observadas por poço no controle e após tratamento com 5-FU e ZM447439.

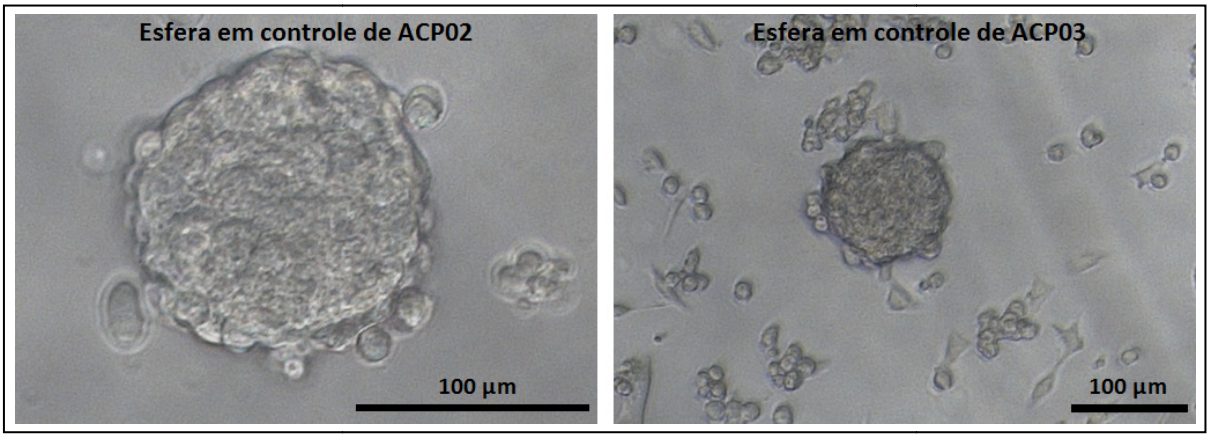

Figura 16. Imagem representativa de esferas observadas em ACP02 e ACP03 não tratadas.

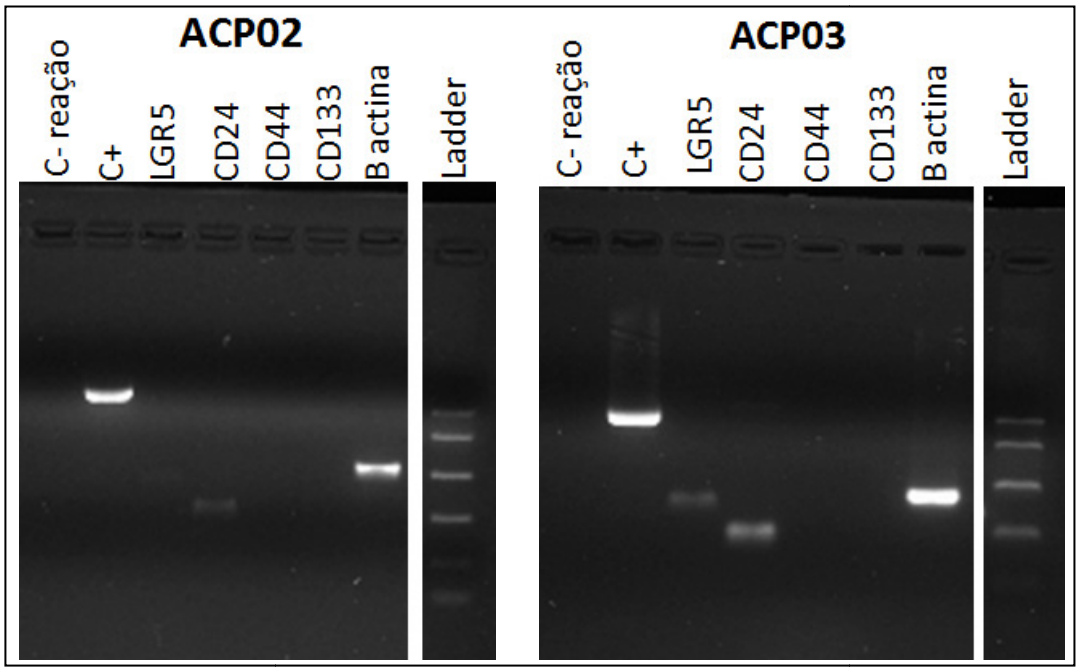

Figura 17. Análise da expressão gênica de marcadores de CTTGs em ACP02 e ACP03.

Ensaio de migração celular

Materiais e métodos

A capacidade de migração das células tumorais está associada com processos de invasão e metástase no câncer, sendo assim, para análise da eficácia dos diferentes tratamentos realizamos ensaios de migração celular. Para realização deste ensaio, foram plaqueadas 5×10^4 células por poço, em placas de 12 poços tratadas com 7,6 µg de fibronectina (Sigma-Aldrich). Após 24 horas de cultivo, uma área livre de células foi gerada com ponteiras de 200 µL na monocamada de células, e o meio de cultura foi retirado para remoção dos restos celulares. Logo após, foi adicionado meio de cultura contendo as concentrações de IC_{50} previamente determinadas (Capítulo II) para 5-FU, ZM447439, INH1 e a combinação de 5-FU com ZM447439. As imagens das áreas geradas foram obtidas em microscópio invertido Zeiss Axiovert 200 equipado com uma câmera Axiocam MRc (Carl Zeiss) pela utilização do Software AxioVision Rel 4.8 no tempo 0 e de 24 horas de tratamento. A área livre de células foi medida com o software ImageJ (National Institutes of

Health) e a área de migração foi obtida subtraindo-se a área final (24 horas) da área inicial (tempo 0). As análises estatísticas foram realizadas com resultados de pelo menos dois experimentos pelo teste ANOVA e Dunnet no caso de múltiplas comparações e por teste t de Student no caso de comparações entre dois grupos. Os resultados estão representados como média ± erro padrão. Valores-p menores que 0,05 foram considerados significativos.

Resultados

Ao comparar a área de migração celular após 24 horas, verificamos que as duas linhagens tumorais migraram significativamente quando comparadas com a linhagem de epitélio gástrico normal (Figura 18). Além disso, ACP03 mostrou maior capacidade de migração do que ACP02 ($p<0,05$). Considerando esses resultados, realizamos novas análises para verificar se os tratamentos seriam capazes de reduzir a migração das células tumorais (Figuras 19 e 20). Observamos que só houve redução significativa na migração de ACP03 exposta à droga INH1 ($p<0,001$). Para exemplificar esse resultado, a Figura 21 apresenta imagens de um experimento de migração de ACP03 exposta e não exposta ao tratamento com INH1.

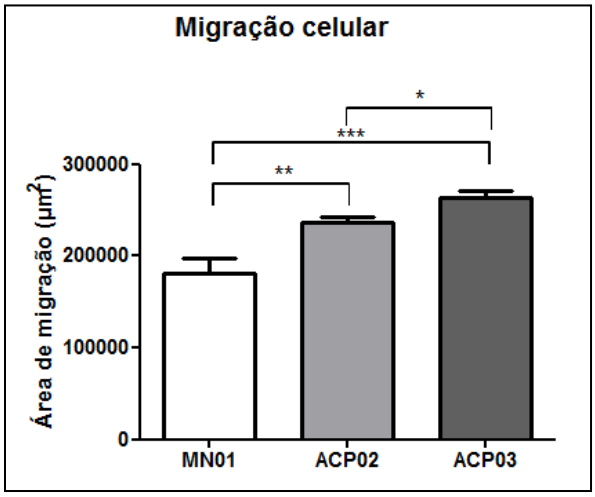

Figura 18. Capacidade de migração celular de MN01, ACP02 e ACP03. *p<0,05 **p<0,01 ***p<0,001.

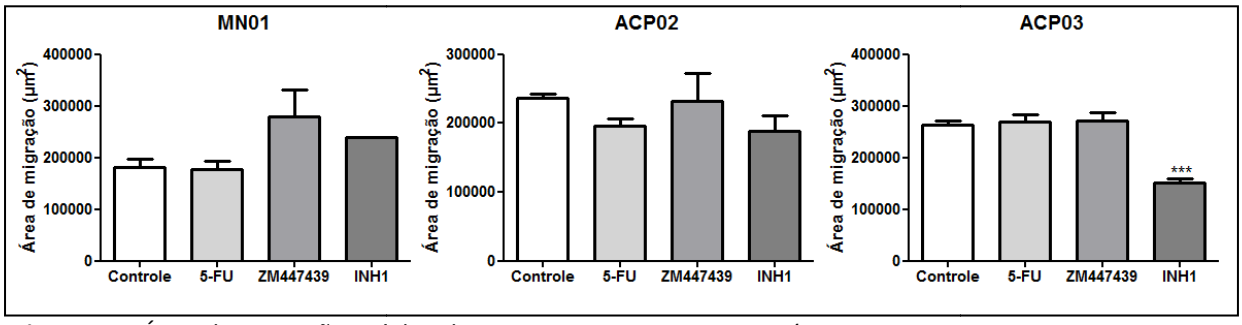

Figura 19. Área de migração celular de MN01, ACP02 e ACP03 após tratamento com 5-FU, ZM447439 e INH1.***p<0,001.

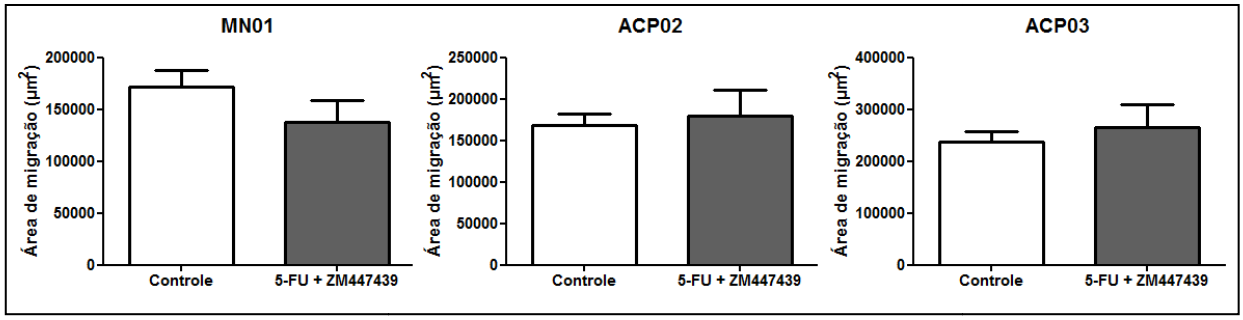

Figura 20. Área de migração celular de MN01, ACP02 e ACP03 após tratamento combinado de 5-FU e ZM447439.

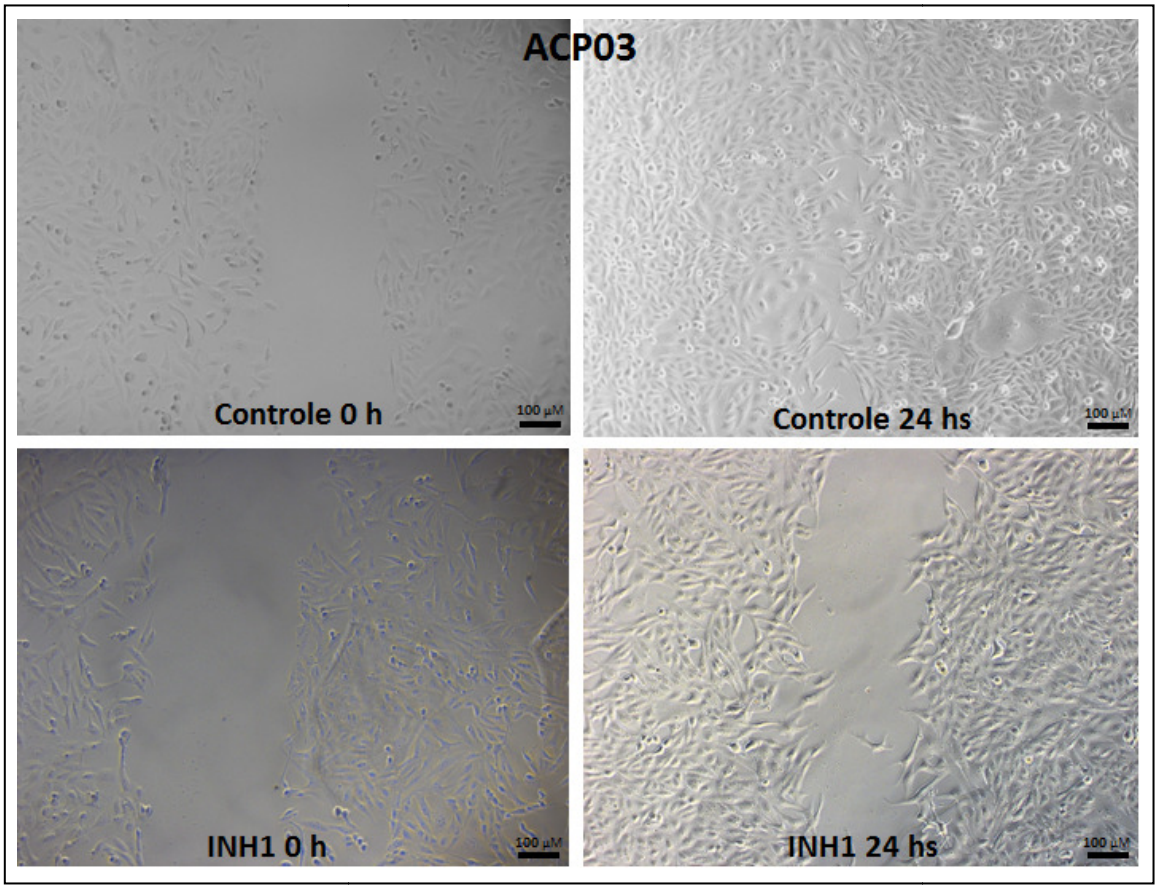

Figura 21. Imagem representativa de experimento de migração celular com a linhagem ACP03 controle e tratada com INH1.

DISCUSSÃO GERAL

O câncer gástrico é considerado um dos tipos de câncer com maiores índices de mortalidade na população mundial ("WHO | World Health Organization", 2017). Apesar do número crescente de novas drogas para o tratamento dessa doença, a taxa de sobrevida em cinco anos ainda é muito baixa no Ocidente, variando de 20 a 25% (MEYER & WILKE, 2011). Portanto, existe uma grande necessidade de desenvolvimento de novos agentes que possam aprimorar as terapias convencionalmente utilizadas. A aplicação de tratamentos mais direcionados vem ganhando espaço nos últimos anos. As proteínas AURKB e NDC80 são exemplos de novos alvos terapêuticos, pois se apresentam superexpressas em diversos tipos de tumores, incluindo o câncer gástrico, e possuem papel importante durante a mitose (HONMA *et al.*, 2014; QU *et al.*, 2014; ROSADO *et al.*, 2011). Por esse motivo, o principal objetivo deste estudo foi avaliar a atividade antitumoral de ZM447439, inibidor de AURKB, e de INH1, inibidor de NDC80 em linhagens de adenocarcinoma gástrico humano.

Primeiramente, ao analisarmos a expressão gênica após os tratamentos com 5-FU, ZM447439 e INH1 das três linhagens estudadas por qRT-PCR, verificamos diversos genes diferencialmente expressos. Esses perfis de expressão que observamos podem ter sido induzidos pelas drogas. Contudo, deve-se considerar a possibilidade de que os elevados índices de morte celular que verificamos sejam indicativos de uma seleção de células que já possuíam esse perfil, mas que não foi observado anteriormente devido à composição heterogênea do tumor.

Muitos desses genes diferencialmente expressos estão relacionados a processos como apoptose, senescência, ciclo celular e transição epitélio-mesenquimal (EMT) (Figuras 5-13, Capítulo III). É importante ressaltar que os mecanismos antiproliferativos induzidos

por terapias como a senescência, a catástrofe mitótica e a apoptose, são determinantes no sucesso do tratamento contra o câncer a longo prazo (SHAY & RONINSON, 2004). Nesse sentido, realizamos ensaios a fim de avaliar os efeitos das drogas citadas previamente, nos diferentes processos celulares.

A catástrofe mitótica é considerada um mecanismo oncosupressor que se inicia na fase M em decorrência de perturbações no aparato mitótico, levando à morte celular ou senescência (GALLUZZI *et al.*, 2012). A presença de núcleos com grandes alterações como, por exemplo, multinucleação, vem sendo utilizada como um marcador morfológico para identificação desse mecanismo (GALLUZZI *et al.*, 2012). Por avaliação da morfologia nuclear verificamos que somente ZM447439 ou sua combinação com 5-FU foi capaz de induzir catástrofe mitótica (Figuras 2-4, Capítulo II). Observamos também que um grande número de células apresentou tamanho aumentado (Figuras 1-3, Capítulo III) e multinucleação, provavelmente como consequência da falha na citocinese devido à inibição de AURKB, corroborando com outros estudos que também utilizaram inibidores desta proteína (GHANIZADEH-VESALI *et al.*, 2016; TAO *et al.*, 2009; YOON *et al.*, 2012; ZEKRI *et al.*, 2016).

A senescência, considerada também um mecanismo antiproliferativo, consiste na parada irreversível do ciclo celular, acompanhada por alterações específicas no fenótipo das células (SHAY & RONINSON, 2004). Em nossos experimentos, o tratamento com INH1 não induziu senescência em nenhuma linhagem estudada, corroborando com a análise por qRT-PCR em que verificamos uma subexpressão gênica de IGFBP3 e IGFBP5 nas três linhagens, possivelmente resultando em uma inibição da senescência (KIM *et al.*, 2007; YANG *et al.*, 2011). Em contrapartida, o tratamento com ZM447439 ou sua combinação com 5-FU aumentou significativamente, nas três linhagens estudadas, o número de células com morfologia nuclear característica de senescência (Figuras 2-4, Capítulo II). Além disso, ao

analisarmos a expressão de genes relacionados à senescência, verificamos que tanto as células tratadas com 5-FU quanto com ZM447439, apresentaram a superexpressão de MAP2K3 e IGFBP3 (Figura 1), genes cuja expressão está associada à indução de senescência (JIA *et al.*, 2010; WANG *et al.*, 2002). Poucos estudos avaliaram a indução desse mecanismo após utilização de inibidores de AURKB. Contudo, um estudo recente também verificou senescência após tratamento com ZM447439, sugerindo que sua indução é tardia e ocorre progressivamente nas células tetraplóides ou poliplóides (SADAIE *et al.*, 2015).

A indução de morte celular por apoptose é outro efeito importante do tratamento antitumoral. Nas análises realizadas por qRT-PCR, verificamos o maior número de genes diferencialmente expressos relacionados à apoptose, após tratamento com ZM447439, sendo que a superexpressão dos genes CASP7, FASLG, CFLAR e BIRC3 foi comum às três linhagens estudadas (Figura 1). CASP7 e FASLG estão associados à indução de apoptose, uma vez que CASP7 pertence ao subgrupo de caspases efetoras e FASLG é uma proteína transmembrana capaz de se ligar ao receptor FAS, ativando o processo de apoptose (LAMKANFI & KANNEGANTI, 2010; NAGATA, 1999). A superexpressão do gene CFLAR também está associada à indução de apoptose, contudo, ainda existem divergências quanto ao seu papel, pois outros estudos mostraram sua capacidade de inibição da apoptose (INOHARA *et al.*, 1997; SCAFFIDI *et al.*, 1999). BIRC3 pertence à família de proteínas IAP, que são inibidoras da apoptose. A proteína BIRC3 é capaz de se ligar às caspases, mas não é capaz de inibi-las diretamente em condições fisiológicas, diferentemente de XIAP, que faz parte da mesma família e é considerado o mais potente inibidor de morte celular *in vitro* (CALLUS & VAUX, 2007; KUMAR, 2007). O gene XIAP, por sua vez, não teve seus níveis de expressão alterados com as drogas testadas em nosso estudo.

Dentre os três tratamentos, as linhagens expostas ao INH1 apresentaram um menor número de genes diferencialmente expressos da via apoptótica, porém todas apresentaram superexpressão do indutor de apoptose FASLG (Figura 1).

Cabe ressaltar, que principalmente no tratamento com ZM447439, observamos a superexpressão de genes indutores e inibidores de apoptose. Esse comportamento é frequentemente observado quando há propagação da cascata de sinalização pró-apoptótica, pois mecanismos antiapoptóticos também são ativados em uma tentativa de permitir que a célula lide com o estresse (GALLUZZI *et al.*, 2012). Mesmo com a superexpressão de alguns genes antiapoptóticos, verificamos que todos os tratamentos induziram apoptose ou por ensaio de atividade de caspase ou por marcação com anexina e iodeto de propídeo (AN/IP). Inicialmente, avaliamos a atividade de caspase após 24 horas de tratamento e verificamos aumento significativo de apoptose somente na combinação de 5-FU e ZM447439 (Figura 9, Capítulo II). Com a finalidade de verificar se as drogas utilizadas isoladamente estavam induzindo apoptose após as 24 horas, analisamos a marcação com AN/IP após 72 e 120 horas de tratamento. As três drogas induziram apoptose nos dois tempos em ACP02 (Figura 11, Capítulo II). Também avaliamos se a combinação das drogas 5-FU e ZM447439 ainda estava induzindo apoptose com 120 horas de tratamento e verificamos uma quantidade significativa de células apoptóticas em ACP02 e ACP03 (Figura 11, Capítulo II e Apêndice). Nossos resultados corroboram com estudos que também observaram indução de apoptose por ZM447439 e INH1 em outras linhagens tumorais (LI *et al.*, 2010; VIDARSDOTTIR *et al.*, 2012; WU *et al.*, 2008) e por 5-FU em células de câncer gástrico (LI & WANG, 2016). Contudo, os mecanismos envolvidos na indução de apoptose por ZM447439 e INH1 ainda não foram elucidados.

.

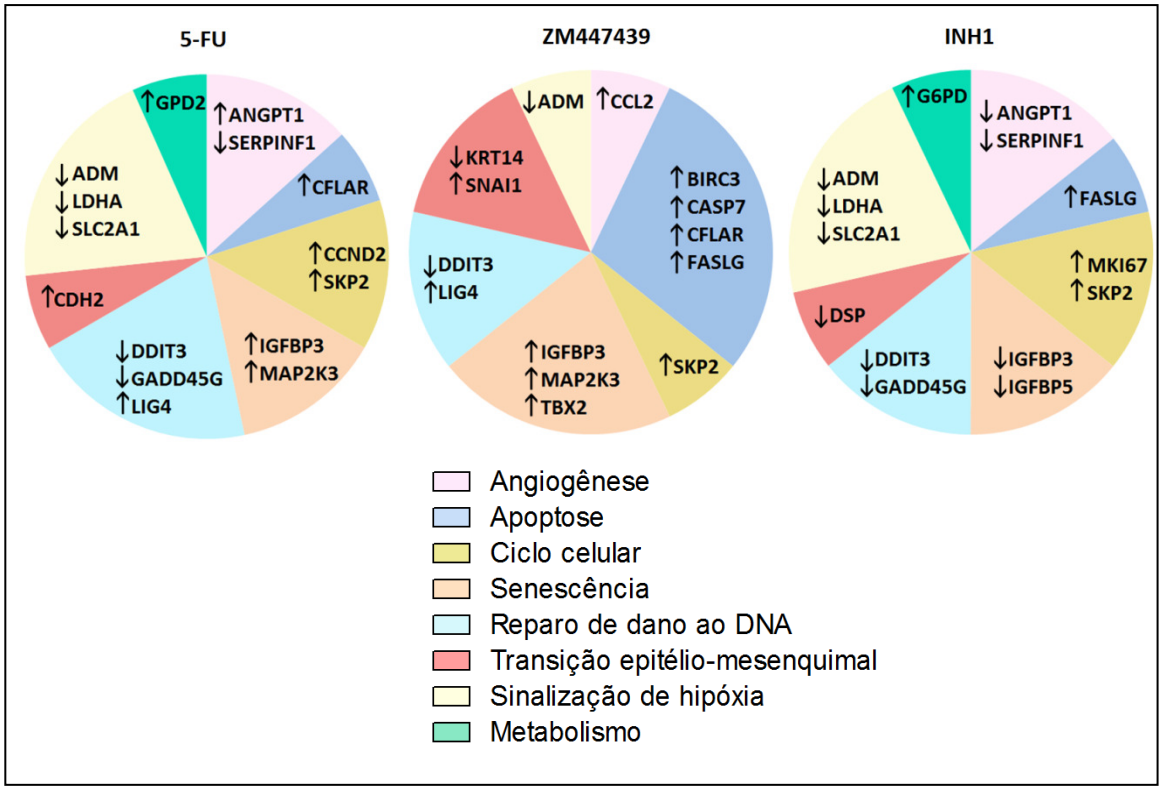

Figura 1 Genes subexpressos ou superexpressos tanto em ACP02 quanto em ACP03 após tratamento com 5-FU, ZM447439 ou INH1.

A capacidade de EMT está associada à capacidade de invasão e migração em diversos tipos de tumores, características comumente observadas em mestástases. Por esse motivo, células tumorais em estado mesenquimal, estão associadas a um pior prognóstico. Além disso, a aquisição de propriedades tronco também está relacionada à EMT. Alguns dos genes cuja superexpressão caracteriza estado epitelial como GSC, OCLN e SNAI3 e cuja superexpressão caracteriza estado EMT parcial ou estado mesenquimal como CDH2, DSP, ETS2, FOXC2, KRT14, SNAI1, SNAI2 (CHANG *et al.*, 2016) foram avaliados por qRT-PCR. De maneira geral, as três linhagens expostas aos tratamentos apresentaram no mínimo três genes diferencialmente expressos associados ao estado epitelial ou mesenquimal com exceção de ACP03 exposta ao agente INH1, onde somente um gene envolvido nessa via se mostrou subexpresso. Ao verificarmos o efeito das drogas na capacidade de migração

celular, INH1 foi o único tratamento que alterou significativamente a migração da linhagem ACP03, reduzindo sua capacidade de migração em comparação com células não tratadas (Figura 19, Capítulo III). Considerando-se o tratamento com ZM447439, nossos resultados corroboram com pesquisa conduzida por TAVANTI *et al.* (2013), onde também não se verificou redução na migração celular de linhagens tumorais. Ao comparar a capacidade de migração de ACP02 e ACP03, verificamos que ACP03 migrou significativamente mais em comparação com ACP02 (Figura 18, Capítulo III), confirmando estudo anterior onde foi observada uma capacidade de migração maior de ACP03 (CALCAGNO *et al.*, 2013). Contudo, esse mesmo estudo, realizou ensaio em matrigel e constatou que ACP02 possui maior potencial de invasão do que ACP03, provavelmente uma consequência da ausência de proteases ativas em ACP03, necessárias para degradar o substrato (CALCAGNO *et al.*, 2013).

A presença de CTTs tem sido relacionada ao desenvolvimento do tumor, proliferação celular, metástase e resistência ao tratamento (CHANG, 2016). A fim de verificar a presença de CTTs em ACP02 e ACP03, realizamos RT-PCR para avaliar a expressão de marcadores tronco. A expressão de dois marcadores, LGR5 e CD24, estava presente (Figura 17, Capítulo III). Nesse sentido, realizamos ensaio de formação de esferas para confirmação da presença de CTTs. As duas linhagens de adenocarcinoma gástrico apresentaram formação de esferas, sendo que o tratamento com 5-FU ou ZM447439 foi capaz de inibir significativamente a formação das mesmas. A presença de CTTGs com expressão dos marcadores LGR5 ou CD24 tem sido demonstrada por outros estudos, contudo, ainda não existem pesquisas que avaliem o efeito de inibidores de AURKB na formação de esferas.

Como citado anteriormente, verificamos que diversos genes associados ao ciclo celular estavam diferencialmente expressos após os tratamentos. Por esse motivo, realizamos ensaios com marcação com IP a fim de verificar se o ciclo celular foi alterado após

exposição às drogas. As três linhagens estudadas apresentaram comportamento semelhante quando tratadas com ZM447439, INH1 ou 5-FU + ZM447439 (Tabela 1). De maneira geral, o tratamento com INH1 não induziu grandes alterações no ciclo celular de nenhuma das linhagens, resultado similar ao relatado por WU *et al* (2008). Contudo, o inibidor ZM447439 induziu alterações em diversas fases do ciclo, sendo o acúmulo de células em G2/M e de células com conteúdo de DNA>4N, as mais frequentemente relatadas pela literatura. Diversos estudos descreveram essas alterações como consequência da endoreduplicação ocasionada pela inibição da proteína AURKB, impedindo a divisão celular já que possui papel essencial na citocinese (CRISPI *et al.*, 2010; KAESTNER *et al.*, 2009). Em contrapartida, a combinação de 5-FU com ZM447439 teve como principal efeito o acúmulo de células na fase S, sendo observado aumento de células com >4N somente em ACP03.

É importante considerar também que os tumores apresentam uma heterogeneidade intra tumoral quanto a fatores genéticos, epigenéticos e fenotípicos (GAY *et al.,* 2016). Portanto, após os tratamentos, é possível que os resultados observados sejam referentes a uma população selecionada de células, uma vez que as células sobreviventes poderiam ser resistentes ou tolerantes às drogas. Recentemente, os conceitos de tolerância e resistência, já bem definidos em áreas como a microbiologia (BRAUNER *et al.*, 2010), vêm sendo aplicados no tratamento contra o câncer (DILLMAN & SCHNEIDER, 2015). Células tolerantes a um determinado tratamento possuem mecanismos transitórios que permitem sua sobrevivência, contudo posteriormente podem voltar a ser sensíveis, enquanto células resistentes possuem essa capacidade de sobrevivência à droga permanentemente (BRAUNER *et al.*, 2010).

Em resumo, os dados obtidos nesta pesquisa mostram que os tratamentos com ZM447439, um inibidor de AURKB, e sua combinação com o agente quimioterápico 5-FU,

apresentaram elevada atividade antitumoral em células de adenocarcinoma gástrico humano. Estes foram capazes de induzir apoptose, senescência, catástrofe mitótica, alterações do ciclo celular e de inibir a formação de esferas (testado somente no tratamento isolado) (Tabela 1). Além disso, a combinação das drogas foi capaz de induzir apoptose após 24 horas de tratamento, se mostrando mais eficaz do que os tratamentos isolados, e possibilitou a utilização de uma concentração menor dos dois compostos, o que é clinicamente desejável, a fim de reduzir possíveis efeitos colaterais. A observação do potencial antitumoral desta combinação *in vitro* é de suma importância, uma vez que testes clínicos realizados com outros antimitóticos, incluindo inibidores de aurora cinases, mostraram baixa eficácia quando utilizados como monoterapia. Contudo, houve considerável aumento nas taxas de resposta quando os mesmos foram combinados com tratamentos já estabelecidos na clínica.

Tabela 1. Tabela com a síntese dos principais resultados observados nos diferentes ensaios após tratamento com 5-FU, ZM447439, INH1 ou 5-FU + ZM447439 nas linhagens MN01, ACP02 e ACP03.

	Apoptose			Senescência			Catástrofe mitótica			Migração celular			Formação de esferas			Fase do ciclo em que houve aumento no número de células		
	MN01	ACP02	ACP03	MN01	ACP02	ACP03	MN01	ACP02	ACP03	MN01	ACP02	ACP03	MN01	ACP02	ACP03	MN01	ACP02	ACP03
5-FU	↑	↑	ND	↑	NS	NS	NS	NS	NS	NS	NS	NS	NS	↓	↓	S e G2/M	S	S, G2/M e >4n
ZM447439	↑	↑	ND	↑	↑	↑	↑	↑	NS	NS	NS	NS	NS	↓	↓	S, G2/M e >4n	S, G2/M e >4n	S, G2/M e >4n
INH1	NS	↑	ND	NS	NS	NS	NS	NS	NS	NS	NS	↓	ND	ND	ND	NS	NS	NS
5-FU+ZM447439	NS	↑	↑	↑	↑	↑	NS	↑	↑	NS	NS	NS	ND	ND	ND	S	S	S e >4n

ND= Não determinado; NS= Não significante (p>0.05 em comparação com controle não tratado)

CONCLUSÕES

Os resultados apresentados por diversos estudos com agentes antimitóticos, mostram que altas taxas de resposta clínica foram observadas somente quando as drogas foram combinadas com tratamentos utilizados convencionalmente. O presente estudo observou elevada atividade antitumoral de ZM447439 e, principalmente, de sua associação com o agente quimioterápico 5-FU em células de adenocarcinoma gástrico humano. Desse modo, frente aos dados obtidos por esse trabalho, podemos concluir que:

- A expressão diferencial de diversos genes envolvidos em vias relacionadas com apoptose, senescência, EMT e ciclo celular foi verificada após monoterapia com as drogas 5-FU, ZM447439 e INH1, em MN01, ACP02 e ACP03;
- Ambas as linhagens de adenocarcinoma gástrico expressam LGR5 e CD24, além de serem capazes de formar esferas, evidenciando a presença de CTTs entre as células estudadas;
- A formação de esferas pelas linhagens ACP02 e ACP03 foi inibida tanto por 5-FU como por ZM447439;
- O tratamento com o composto ZM447439 bem como sua combinação com 5-FU induziram apoptose, senescência e catástrofe mitótica nas linhagens estudadas;
- As terapias com 5-FU, ZM447439 ou a combinação destes dois agentes, induziram alterações significativas em diversas fases do ciclo celular, diferentemente de INH1;
- Entre os tratamentos avaliados neste estudo, INH1 apresentou o maior valor de IC_{50} e menor atividade antitumoral;

- A combinação do quimioterápico 5-FU com ZM447439 permitiu a utilização de doses menores dos compostos, além de ser capaz de induzir apoptose em menor tempo quando comparada ao tratamento com as drogas isoladas.

PERSPECTIVAS

- Verificar a expressão da proteína AURKB fosforilada antes após tratamento com ZM447439;
- Verificar a expressão de proteínas associadas ao ciclo celular como, por exemplo, ciclina B1, após o tratamento com ZM447439, a fim de confirmar se o elevado número de células observadas em G2/M deve-se ao escape prematuro da mitose sem a ocorrência de citocinese;
- Analisar a presença de células senescentes por β-galactosidase após o tratamento;
- Realizar ensaio cometa para verificar se o tratamento induz dano ao DNA.

REFERÊNCIAS

AJANI, J. A. et al. Gastric cancer, version 2.2013: featured updates to the NCCN Guidelines. **Journal of the National Comprehensive Cancer Network : JNCCN**, v. 11, n. 5, p. 531–46, 1 maio 2013.

ANDREWS, P. D. et al. Mitotic mechanics: the auroras come into view. **Current opinion in cell biology**, v. 15, n. 6, p. 672–83, dez. 2003.

BASS, A. J. et al. Comprehensive molecular characterization of gastric adenocarcinoma. **Nature**, v. 513, n. 7517, p. 202–209, 23 jul. 2014.

BERGER, S. I.; IYENGAR, R. Network analyses in systems pharmacology. **Bioinformatics**, v. 25, n. 19, p. 2466–2472, 1 out. 2009.

BOHL, S. R.; PIRCHER, A.; HILBE, W. Cancer Stem Cells: Characteristics and Their Potential Role for New Therapeutic Strategies. **Onkologie**, v. 34, n. 5, p. 269–274, 2011.

BONNET, D.; DICK, J. E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. **Nature medicine**, v. 3, n. 7, p. 730–7, jul. 1997.

BORGES, K. S. et al. Inhibition of Aurora kinases enhances chemosensitivity to temozolomide and causes radiosensitization in glioblastoma cells. **Journal of cancer research and clinical oncology**, v. 138, n. 3, p. 405–14, mar. 2012.

BORNSCHEIN, J. et al. H. pylori Infection Is a Key Risk Factor for Proximal Gastric Cancer. **Digestive Diseases and Sciences**, v. 55, n. 11, p. 3124–3131, 29 nov. 2010.

BRAUNER, A. et al. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. **Nature reviews. Microbiology**, v. 14, n. 5, p. 320–30, 15 abr. 2016.

BRUNGS, D. et al. Gastric cancer stem cells: evidence, potential markers, and clinical implications. **Journal of gastroenterology**, v. 51, n. 4, p. 313–26, 1 abr. 2016.

CALCAGNO, D. Q. et al. MYC, FBXW7 and TP53 copy number variation and expression in Gastric Cancer. **BMC Gastroenterology**, v. 13, n. 1, p. 141, 23 dez. 2013.

CALLUS, B. A.; VAUX, D. L. Caspase inhibitors: viral, cellular and chemical. **Cell Death and Differentiation**, v. 14, n. 1, p. 73–78, 8 jan. 2007.

CARMENA, M. et al. The chromosomal passenger complex (CPC): from easy rider to the godfather of mitosis. **Nature reviews. Molecular cell biology**, v. 13, n. 12, p. 789–803, 23

dez. 2012.

CARMENA, M.; EARNSHAW, W. C. The cellular geography of aurora kinases. **Nature reviews. Molecular cell biology**, v. 4, n. 11, p. 842–54, nov. 2003.

CATALANO, V. et al. Gastric cancer. **Critical reviews in oncology/hematology**, v. 71, n. 2, p. 127–64, ago. 2009.

CHAN, K.-S.; KOH, C.-G.; LI, H.-Y. Mitosis-targeted anti-cancer therapies: where they stand. **Cell death & disease**, v. 3, p. e411, jan. 2012.

CHANG, H. et al. Synergistic action of master transcription factors controls epithelial-to-mesenchymal transition. **Nucleic acids research**, v. 44, n. 6, p. 2514–27, 7 abr. 2016.

CHANG, J. C. Cancer stem cells. **Medicine**, v. 95, n. 1 Suppl 1, p. S20–S25, set. 2016.

CHOI, Y. Y.; NOH, S. H.; CHEONG, J.-H. Evolution of Gastric Cancer Treatment: From the Golden Age of Surgery to an Era of Precision Medicine. **Yonsei Medical Journal**, v. 56, n. 5, p. 1177, set. 2015.

CORREA, P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. **Cancer research**, v. 52, n. 24, p. 6735–40, 15 dez. 1992.

COWAN, M. K. **Microbiology fundamentals : a clinical approach + connect plus 1 semester access card + obenauf ...** 2nd. ed. [s.l.] Mcgraw-Hill, 2012.

CRISPI, S. et al. Antiproliferative effect of Aurora kinase targeting in mesothelioma. **Lung cancer (Amsterdam, Netherlands)**, v. 70, n. 3, p. 271–9, dez. 2010.

DELUCA, J. G. et al. Kinetochore microtubule dynamics and attachment stability are regulated by Hec1. **Cell**, v. 127, n. 5, p. 969–82, 1 dez. 2006.

DILLMAN, A. R.; SCHNEIDER, D. S. Defining Resistance and Tolerance to Cancer. **Cell Reports**, v. 13, n. 5, p. 884–887, 3 nov. 2015.

DITCHFIELD, C. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. **The Journal of Cell Biology**, v. 161, n. 2, p. 267–280, 28 abr. 2003.

FIGUEIREDO, C. et al. Pathogenesis of Gastric Cancer. **Helicobacter**, v. 20, p. 30–35, set. 2015.

GALLUZZI, L. et al. Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. **Cell Death and Differentiation**, v. 19, n.

1, p. 107–120, 15 jan. 2012.

GAY, L.; BAKER, A.-M.; GRAHAM, T. A. Tumour Cell Heterogeneity. **F1000Research**, v. 5, p. 238, 29 fev. 2016.

GHANIZADEH-VESALI, S. et al. Significance of AZD1152 as a potential treatment against Aurora B overexpression in acute promyelocytic leukemia. **Annals of Hematology**, v. 95, n. 7, p. 1031–1042, 19 jun. 2016.

GHOSHAL, K.; JACOB, S. T. An alternative molecular mechanism of action of 5-fluorouracil, a potent anticancer drug. **Biochemical pharmacology**, v. 53, n. 11, p. 1569–75, 1 jun. 1997.

GIRDLER, F. et al. Validating Aurora B as an anti-cancer drug target. **Journal of Cell Science**, v. 119, n. 17, p. 3664–3675, 1 set. 2006.

GLINKA, A. et al. LGR4 and LGR5 are R-spondin receptors mediating Wnt/β-catenin and Wnt/PCP signalling. **EMBO reports**, v. 12, n. 10, p. 1055–1061, 9 set. 2011.

HANAHAN, D.; WEINBERG, R. A. The hallmarks of cancer. **Cell**, v. 100, n. 1, p. 57–70, 7 jan. 2000.

HANAHAN, D.; WEINBERG, R. A. Hallmarks of Cancer: The Next Generation. **Cell**, v. 144, n. 5, p. 646–674, 4 mar. 2011.

HONMA, K. et al. Contribution of Aurora-A and -B expression to DNA aneuploidy in gastric cancers. **Surgery Today**, v. 44, n. 3, p. 454–461, 10 mar. 2014.

HWANG, H.; DWYER, J.; RUSSELL, R. M. Diet, Helicobacter pylori infection, food preservation and gastric cancer risk: are there new roles for preventative factors? **Nutrition reviews**, v. 52, n. 3, p. 75–83, mar. 1994.

INOHARA, N. et al. CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. **Proceedings of the National Academy of Sciences of the United States of America**, v. 94, n. 20, p. 10717–22, 30 set. 1997.

JIA, M. et al. Proteome profiling of immortalization-to-senescence transition of human breast epithelial cells identified MAP2K3 as a senescence-promoting protein which is downregulated in human breast cancer. **PROTEOMICS - Clinical Applications**, v. 4, n. 10–11, p. 816–828, nov. 2010.

JIANG, N. et al. Advances in mitotic inhibitors for cancer treatment. **Mini reviews in medicinal chemistry**, v. 6, n. 8, p. 885–95, ago. 2006.

JORDAN, M. A.; WILSON, L. Microtubules as a target for anticancer drugs. **Nature reviews. Cancer**, v. 4, n. 4, p. 253–65, abr. 2004.

KAESTNER, P.; STOLZ, A.; BASTIANS, H. Determinants for the efficiency of anticancer drugs targeting either Aurora-A or Aurora-B kinases in human colon carcinoma cells. **Molecular cancer therapeutics**, v. 8, n. 7, p. 2046–56, jul. 2009.

KATSUNO, Y. et al. Coordinated expression of REG4 and aldehyde dehydrogenase 1 regulating tumourigenic capacity of diffuse-type gastric carcinoma-initiating cells is inhibited by TGF-β. **The Journal of pathology**, v. 228, n. 3, p. 391–404, nov. 2012.

KIM, K. S. et al. Regulation of replicative senescence by insulin-like growth factor-binding protein 3 in human umbilical vein endothelial cells. **Aging cell**, v. 6, n. 4, p. 535–45, ago. 2007.

KIM, S. Y. et al. Deregulation of Immune Response Genes in Patients With Epstein-Barr Virus-Associated Gastric Cancer and Outcomes. **Gastroenterology**, v. 148, n. 1, p. 137–147.e9, jan. 2015.

KITANO, H. A robustness-based approach to systems-oriented drug design. **Nature reviews. Drug discovery**, v. 6, n. 3, p. 202–10, 23 mar. 2007.

KUMAR, S. Caspase function in programmed cell death. **Cell Death and Differentiation**, v. 14, n. 1, p. 32–43, 3 jan. 2007.

LAMKANFI, M.; KANNEGANTI, T.-D. Caspase-7: A protease involved in apoptosis and inflammation. **The International Journal of Biochemistry & Cell Biology**, v. 42, n. 1, p. 21–24, jan. 2010.

LARSSON, D. E. et al. Combination analyses of anti-cancer drugs on human neuroendocrine tumor cell lines. **Cancer Chemotherapy and Pharmacology**, v. 65, n. 1, p. 5–12, 19 dez. 2009.

LEE, J. H. et al. Current Management and Future Strategies of Gastric Cancer. **Yonsei Medical Journal**, v. 53, n. 2, p. 248, mar. 2012.

LI, J.; WANG, S.-X. Synergistic enhancement of the antitumor activity of 5-fluorouracil by bornyl acetate in SGC-7901 human gastric cancer cells and the determination of the underlying mechanism of action. **Journal of B.U.ON. : official journal of the Balkan Union of Oncology**, v. 21, n. 1, p. 108–17, 2016.

LI, M. et al. Aurora kinase inhibitor ZM447439 induces apoptosis via mitochondrial

pathways. **Biochemical pharmacology**, v. 79, n. 2, p. 122–9, 15 jan. 2010.

LI, X. et al. An individualized prognostic signature for gastric cancer patients treated with 5-Fluorouracil-based chemotherapy and distinct multi-omics characteristics of prognostic groups. **Oncotarget**, v. 7, n. 8, p. 8743–55, 22 fev. 2016.

LONGLEY, D. B.; HARKIN, D. P.; JOHNSTON, P. G. 5-Fluorouracil: mechanisms of action and clinical strategies. **Nature Reviews Cancer**, v. 3, n. 5, p. 330–338, maio 2003.

MAIATO, H. et al. The dynamic kinetochore-microtubule interface. **Journal of cell science**, v. 117, n. Pt 23, p. 5461–77, 1 nov. 2004.

MALUMBRES, M.; PÉREZ DE CASTRO, I. Aurora kinase A inhibitors: promising agents in antitumoral therapy. **Expert opinion on therapeutic targets**, v. 18, n. 12, p. 1377–93, dez. 2014.

MARJANOVIC, N. D.; WEINBERG, R. A.; CHAFFER, C. L. Cell Plasticity and Heterogeneity in Cancer. **Clinical Chemistry**, v. 59, n. 1, p. 168–179, 1 jan. 2013.

MARQUÉS-LESPIER, J. M.; GONZÁLEZ-PONS, M.; CRUZ-CORREA, M. Current Perspectives on Gastric Cancer. **Gastroenterology clinics of North America**, v. 45, n. 3, p. 413–28, set. 2016.

MATSUOKA, J. et al. Role of the stemness factors sox2, oct3/4, and nanog in gastric carcinoma. **The Journal of surgical research**, v. 174, n. 1, p. 130–5, 1 maio 2012.

MEYER, H.-J.; WILKE, H. Treatment strategies in gastric cancer. **Deutsches Arzteblatt international**, v. 108, n. 41, p. 698–705; quiz 706, out. 2011.

MISLEH, J. G. et al. Multidisciplinary Management of Gastric Cancer. **Surgical Oncology Clinics of North America**, v. 22, n. 2, p. 247–264, abr. 2013.

MOJARDÍN, L. et al. New insights into the RNA-based mechanism of action of the anticancer drug 5'-fluorouracil in eukaryotic cells. **PloS one**, v. 8, n. 11, p. e78172, 1 nov. 2013.

NAGATA, S. Fas Ligand-Induced Apoptosis. **Annual Review of Genetics**, v. 33, n. 1, p. 29–55, dez. 1999.

PÉREZ DE CASTRO, I.; DE CÁRCER, G.; MALUMBRES, M. A census of mitotic cancer genes: new insights into tumor cell biology and cancer therapy. **Carcinogenesis**, v. 28, n. 5, p. 899–912, maio 2007.

PERRY, M. C. (MICHAEL C.; DOLL, D. C.; FRETER, C. E. **Perry's the chemotherapy**

source book. 5th. ed. [s.l.] Wolters Kluwer Health/Lippincott Williams & Wilkins, 2012.

PLUMMER, M.; FRANCESCHI, S.; MUÑOZ, N. Epidemiology of gastric cancer. **IARC scientific publications**, n. 157, p. 311–26, 2004.

Portal - Instituto Nacional de Câncer - INCA. Disponível em: <http://www2.inca.gov.br/wps/wcm/connect/inca/portal/home>. Acesso em: 15 fev. 2017.

PORTELLA, G.; PASSARO, C.; CHIEFFI, P. Aurora B: a new prognostic marker and therapeutic target in cancer. **Current medicinal chemistry**, v. 18, n. 4, p. 482–96, 2011.

QIAO, X. T. et al. Prospective identification of a multilineage progenitor in murine stomach epithelium. **Gastroenterology**, v. 133, n. 6, p. 1989–98, dez. 2007.

QU, Y. et al. Hec1/Ndc80 is overexpressed in human gastric cancer and regulates cell growth. **Journal of gastroenterology**, v. 49, n. 3, p. 408–18, 17 mar. 2014.

RASSOULI, F. B.; MATIN, M. M.; SAEINASAB, M. Cancer stem cells in human digestive tract malignancies. **Tumor Biology**, v. 37, n. 1, p. 7–21, 7 jan. 2016.

ROSADO, J. O.; HENRIQUES, J. P.; BONATTO, D. A systems pharmacology analysis of major chemotherapy combination regimens used in gastric cancer treatment: predicting potential new protein targets and drugs. **Current cancer drug targets**, v. 11, n. 7, p. 849–69, set. 2011.

SADAIE, M. et al. Cell-based screen for altered nuclear phenotypes reveals senescence progression in polyploid cells after Aurora kinase B inhibition. **Molecular Biology of the Cell**, v. 26, n. 17, p. 2971–2985, 1 set. 2015.

SCAFFIDI, C. et al. The role of c-FLIP in modulation of CD95-induced apoptosis. **The Journal of biological chemistry**, v. 274, n. 3, p. 1541–8, 15 jan. 1999.

SCHMIDT, M.; BASTIANS, H. Mitotic drug targets and the development of novel anti-mitotic anticancer drugs. **Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy**, v. 10, n. 4–5, p. 162–81, ago. 2007.

SHACKLETON, M. et al. Heterogeneity in Cancer: Cancer Stem Cells versus Clonal Evolution. **Cell**, v. 138, n. 5, p. 822–829, 4 set. 2009.

SHAY, J. W.; RONINSON, I. B. Hallmarks of senescence in carcinogenesis and cancer therapy. **Oncogene**, v. 23, n. 16, p. 2919–2933, 12 abr. 2004.

SHIMOYAMA, S. Pharmacogenetics of fluoropyrimidine and cisplatin. A future

application to gastric cancer treatment. **Journal of gastroenterology and hepatology**, v. 24, n. 6, p. 970–81, jun. 2009.

SHINOZAKI-USHIKU, A.; KUNITA, A.; FUKAYAMA, M. Update on Epstein-Barr virus and gastric cancer (Review). **International Journal of Oncology**, v. 46, n. 4, p. 1421–34, 28 jan. 2015.

STANTON, R. A. et al. Drugs that target dynamic microtubules: a new molecular perspective. **Medicinal research reviews**, v. 31, n. 3, p. 443–81, maio 2011.

TAMATANI, T. et al. Antitumor efficacy of sequential treatment with docetaxel and 5-fluorouracil against human oral cancer cells. **International journal of oncology**, v. 41, n. 3, p. 1148–56, 4 set. 2012.

TANAKA, K.; HIROTA, T. Chromosome segregation machinery and cancer. **Cancer science**, v. 100, n. 7, p. 1158–65, jul. 2009.

TAO, Y. et al. The Aurora B kinase inhibitor AZD1152 sensitizes cancer cells to fractionated irradiation and induces mitotic catastrophe. **Cell Cycle**, v. 8, n. 19, p. 3172–3181, 28 out. 2009.

TAVANTI, E. et al. Preclinical validation of Aurora kinases-targeting drugs in osteosarcoma. **British Journal of Cancer**, v. 109, n. 10, p. 2607–2618, 12 nov. 2013.

Tocris Bioscience. Disponível em: <https://www.tocris.com/>. Acesso em: 15 fev. 2017.

VADER, G.; LENS, S. M. A. The Aurora kinase family in cell division and cancer. **Biochimica et Biophysica Acta (BBA) - Reviews on Cancer**, v. 1786, n. 1, p. 60–72, set. 2008.

VAN CUTSEM, E. et al. Gastric cancer. **The Lancet**, v. 388, n. 10060, p. 2654–2664, 26 nov. 2016.

VAN DER HORST, A.; LENS, S. M. A. Cell division: control of the chromosomal passenger complex in time and space. **Chromosoma**, v. 123, n. 1–2, p. 25–42, mar. 2014.

VARMA, D.; SALMON, E. D. The KMN protein network--chief conductors of the kinetochore orchestra. **Journal of cell science**, v. 125, n. Pt 24, p. 5927–36, 15 dez. 2012.

VIDARSDOTTIR, L. et al. Sensitivity of BRCA2 mutated human cell lines to Aurora kinase inhibition. **Investigational new drugs**, v. 30, n. 2, p. 425–34, abr. 2012.

WADDELL, T. et al. Gastric cancer: ESMO–ESSO–ESTRO clinical practice guidelines for diagnosis, treatment and follow-up. **European Journal of Surgical Oncology (EJSO)**, v. 40, n.

5, p. 584–591, maio 2014.

WALCZAK, C. E.; CAI, S.; KHODJAKOV, A. Mechanisms of chromosome behaviour during mitosis. **Nature reviews. Molecular cell biology**, v. 11, n. 2, p. 91–102, fev. 2010.

WALSBY, E. et al. Effects of the aurora kinase inhibitors AZD1152-HQPA and ZM447439 on growth arrest and polyploidy in acute myeloid leukemia cell lines and primary blasts. **Haematologica**, v. 93, n. 5, p. 662–9, maio 2008.

WAN, X. et al. Protein architecture of the human kinetochore microtubule attachment site. **Cell**, v. 137, n. 4, p. 672–84, 15 maio 2009.

WANG, B. et al. LGR5 Is a Gastric Cancer Stem Cell Marker Associated with Stemness and the EMT Signature Genes NANOG, NANOGP8, PRRX1, TWIST1, and BMI1. **PLOS ONE**, v. 11, n. 12, p. e0168904, 29 dez. 2016.

WANG, W. et al. Sequential activation of the MEK-extracellular signal-regulated kinase and MKK3/6-p38 mitogen-activated protein kinase pathways mediates oncogenic ras-induced premature senescence. **Molecular and cellular biology**, v. 22, n. 10, p. 3389–403, maio 2002.

WHITEBAY, E. A. et al. In Silico Prediction of Mechanism of Action for Cancer Therapeutics. **Molecular informatics**, v. 32, n. 8, p. 735–41, ago. 2013.

WHO | World Health Organization. Disponível em: <http://www.who.int/en/>. Acesso em: 15 fev. 2017.

WIST, A. D.; BERGER, S. I.; IYENGAR, R. Systems pharmacology and genome medicine: a future perspective. **Genome medicine**, v. 1, n. 1, p. 11, 22 jan. 2009.

WU, G. et al. Small molecule targeting the Hec1/Nek2 mitotic pathway suppresses tumor cell growth in culture and in animal. **Cancer research**, v. 68, n. 20, p. 8393–9, 15 out. 2008.

YANG, H. H. et al. Involvement of IGF binding protein 5 in prostaglandin E2-induced cellular senescence in human fibroblasts. **Biogerontology**, v. 12, n. 3, p. 239–252, 30 jun. 2011.

YANG, I.; NELL, S.; SUERBAUM, S. Survival in hostile territory: the microbiota of the stomach. **FEMS microbiology reviews**, v. 37, n. 5, p. 736–61, set. 2013.

YANG, P. et al. Overweight, obesity and gastric cancer risk: Results from a meta-analysis of cohort studies. **European Journal of Cancer**, v. 45, n. 16, p. 2867–2873, nov.

2009.

YOON, M. J. et al. Aurora B confers cancer cell resistance to TRAIL-induced apoptosis via phosphorylation of survivin. **Carcinogenesis**, v. 33, n. 3, p. 492–500, mar. 2012.

ZALI, H.; REZAEI-TAVIRANI, M.; AZODI, M. Gastric cancer: prevention, risk factors and treatment. **Gastroenterology and hepatology from bed to bench**, v. 4, n. 4, p. 175–85, 2011.

ZANIBONI, A.; MERIGGI, F. The emerging role of oxaliplatin in the treatment of gastric cancer. **Journal of chemotherapy (Florence, Italy)**, v. 17, n. 6, p. 656–62, 18 dez. 2005.

ZEKRI, A. et al. Inhibitor of Aurora Kinase B Induces Differentially Cell Death and Polyploidy via DNA Damage Response Pathways in Neurological Malignancy: Shedding New Light on the Challenge of Resistance to AZD1152-HQPA. **Molecular Neurobiology**, v. 53, n. 3, p. 1808–1823, 11 abr. 2016.

ZHANG, L.; ZHANG, S. ZM447439, the Aurora kinase B inhibitor, suppresses the growth of cervical cancer SiHa cells and enhances the chemosensitivity to cisplatin. **The journal of obstetrics and gynaecology research**, v. 37, n. 6, p. 591–600, jun. 2011.

ZHAO, Y.; FENG, F.; ZHOU, Y.-N. Stem cells in gastric cancer. **World Journal of Gastroenterology**, v. 21, n. 1, p. 112, 7 jan. 2015.

APÊNDICE

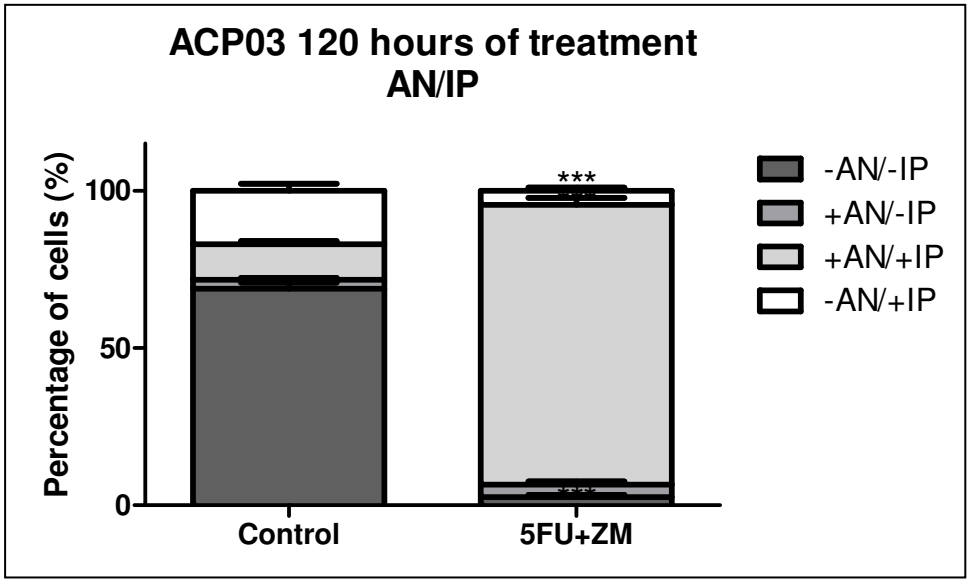

Figura 1. Resultados da marcação com AN/IP após 120 horas do tratamento combinado (5-FU+ZM447439) de ACP03. ***p<0,001

ANEXO

Larissa Siqueira Penna

Curriculum Vitae

Abril/2017

Larissa Siqueira Penna
Curriculum Vitae

Identificação

Nome Larissa Siqueira Penna
Nome em citações bibliográficas Penna, L.S.

Endereço

Endereço profissional Universidade Federal do Rio Grande do Sul, Centro de Biotecnologia
Avenida Bento Gonçalves, 9500 - Laboratório de Biologia Computacional e Molecular, 219, Prédio 43421
Agronomia
90501970 – Porto Alegre, RS – Brasil
Telefone: (51) 33086080
E-mail: larispenna@gmail.com

Formação acadêmica/titulação

2012 Doutorado em Biologia Celular e Molecular.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil
Título: Análise de novos agentes quimioterápicos em linhagens celulares de câncer gástrico humano
Orientador: Diego Bonatto e João Antônio Pegas Henriques
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico

2008 - 2009 Mestrado em Medicina: Ciências Médicas.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil
Título: Frequencia alélica de 14 locos do cromossomo X de indivíduos da região Sul do Brasil, Ano de obtenção: 2010
Orientador: José Antônio Magalhães
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

2007 - 2008 Aperfeiçoamento em Identificação Humana pelo DNA.
Hospital de Clínicas de Porto Alegre, HCPA, Porto Alegre, Brasil

2006 - 2007 Aperfeiçoamento em Histocompatibilidade.
Hospital de Clínicas de Porto Alegre, HCPA, Porto Alegre, Brasil

2002 - 2005 Graduação em Biomedicina.
Universidade Feevale, FEEVALE, Novo Hamburgo, Brasil

Formação complementar

2013 - 2013 Extensão universitária em III ESTUDO DA SINALIZAÇÃO CELULAR NO CÂNCER - METODOLOGIAS. (Carga horária: 15h).
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2011 - 2011 Curso de curta duração em Curso de Extensão SPSS Aplicado à Gestão. (Carga horária: 8h).
Pontifícia Universidade Católica do Rio Grande do Sul, PUCRS, Porto Alegre, Brasil

2008 - 2008 Extensão universitária em Identificação Humana pelo DNA: Genética Forense. (Carga horária: 40h).
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2008 - 2008 Curso de curta duração em Curso de Capacitação em Biossegurança e Qualidade. (Carga horária: 1h).
Hospital de Clínicas de Porto Alegre, HCPA, Porto Alegre, Brasil

2008 - 2008 Treinamento Restrito de PCR em Tempo Real. . (Carga horária: 32h).
Life Tecnologies , LIFE TEC, Sao Paulo, Brasil

2007 - 2007 Curso de curta duração em Curso de Epidemiologia, Bioestatística e Bioética. (Carga horária: 30h).
MW Consultoria Científica, MWC, Brasil

2006 - 2007 Extensão universitária em Imunologia Básica & Clínica. (Carga horária: 90h).
Hospital de Clínicas de Porto Alegre, HCPA, Porto Alegre, Brasil

2004 - 2004 Curso de curta duração em Curso Internacional de MIcologia. (Carga horária: 22h).
Universidade Feevale, FEEVALE, Novo Hamburgo, Brasil

2004 - 2004 Curso de curta duração em Genética Molecular Humana. (Carga horária: 8h).
Universidade Luterana do Brasil, ULBRA, Canoas, Brasil

2002 - 2002 Extensão universitária em Hemoglobinopatias. (Carga horária: 4h).
Universidade Feevale, FEEVALE, Novo Hamburgo, Brasil

Atuação profissional

1. Micropathology Ltd - MP

Vínculo institucional

2010 - 2010 Vínculo: Treinamento , Enquadramento funcional: Treinamento , Carga horária: 35, Regime: Dedicação exclusiva

2. Future- Sistema de Ensino

Vínculo institucional

2007 - 2007 Vínculo: Professor substituto , Enquadramento funcional: Professora substituta , Carga horária: 2, Regime: Parcial

3. Hospital de Clínicas de Porto Alegre - HCPA

Vínculo institucional

2005 - 2005 Vínculo: Estágio Voluntário , Enquadramento funcional: Voluntário , Carga horária: 16, Regime: Parcial

Atividades

07/2005 - 10/2005 Estágio, Serviço de Imunologia - Setor de Biologia Molecular

4. Essência da Terra Farmácia de Manipulação - ETFM

Vínculo institucional

2005 - 2005 Vínculo: Estágio Voluntário , Enquadramento funcional: Voluntária , Carga horária: 40, Regime: Integral

5. Universidade Federal do Rio Grande do Sul - UFRGS

Vínculo institucional

2015 - 2015 Vínculo: Docente , Enquadramento funcional: Estágio docente , Carga horária: 2, Regime: Parcial
Outras informações:
Docente nas aulas teórico-práticas da disciplina de Biologia Molecular Básica do curso de Ciências Biológicas da UFRGS, no semestre 2015/2.

2013 - 2013 Vínculo: Apoio pedagógico , Enquadramento funcional: Professor em EAD, Regime: Parcial
Outras informações:
Curso ministrado: IDENTIFICAÇÃO HUMANA PELO DNA: GENÉTICA FORENSE E SISTEMA HLA

2012 - 2012 Vínculo: Apoio pedagógico , Enquadramento funcional: Professor em EAD, Regime: Parcial
Outras informações:
Curso ministrado: IDENTIFICAÇÃO HUMANA PELO DNA: GENÉTICA FORENSE E SISTEMA HLA

2010 - 2010 Vínculo: Professor , Enquadramento funcional: Professor em EAD, Regime: Parcial
Outras informações:
Professor de curso EAD de Imunologia Básica e Clínica

2010 - 2010 Vínculo: Professor , Enquadramento funcional: Professor em EAD, Regime: Parcial
Outras informações:
Professor de Curso de Extensão em Identificação Humana pelo DNA.

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. PENNA, LARISSA SIQUEIRA; SILVA, FERNANDA GAMIO; SALIM, PATRICIA HARTSTEIN; EWALD, GISELE; JOBIM, Mariana; MAGALHÃES, JOSÉ ANTÔNIO DE AZEVEDO; JOBIM, LUIZ FERNANDO
Development of two multiplex PCR systems for the analysis of 14 X-chromosomal STR loci in a southern Brazilian population sample. International Journal of Legal Medicine (Print). , v.126, p.327 - 330, 2012.

2. JOBIM, M.; SALIM, P. H.; Portela, P.; Wilson, T. J.; Fraportti, J.; Baronio, D.; Gil, B.; PENNA, L. S.; Roesler, R.; JOBIM, L. F.; Schwartsmann, G.
Killer cell immunoglobulin-like receptor gene diversity in a Caucasian population of Southern Brazil. International Journal of Immunogenetics (Print). , p.1 - 7, 2010.

3. CHESKY, Marisa; COUTINHO, V. L. S.; JOBIM, Mariana; SALIM, P. H.; GIL, Beatriz Chamun; PENNA, L. S.; OZAKI, L.S.; JOBIM, Luiz Fernando Job
Molecular analysis of pathogens in cerebrospinal fluid by the polymerase chain reaction in HIV-infected patients. Revista AMRIGS. , v.53, p.46 - 51, 2009.

Artigos aceitos para publicação

1. Penna, L. S.; Henriques, J.A.P.; Bonatto, D.
Anti-mitotic agents: are they emerging molecules for cancer treatment?. PHARMACOLOGY & THERAPEUTICS. , 2017.

Livros publicados

1. GAMIO, F.; EWALD, G. M.; PENNA, L. S.; JOBIM, M. R.; JOBIM, M.
Identificação Humana. Campinas : Millenium Editora, 2012, v.1. p.181-202.

Trabalhos publicados em anais de eventos (resumo)

1. Penna, L. S.; Henriques, J.A.P.; Bonatto, D.
New small molecule inhibitors from AURKB and NDC80 as a treatment of gastric cancer cells In: European Cancer Congress, 2015, Viena.

2. Portela, P.; MERZONI, J.; SALIM, P. H.; JOBIM, M.; SCHLOLTTFELDT, J.; KRUGER, M.; GIL, B. C.; STOLZ, J.; PENNA, L. S.; MONTEIRO, L.; JOBIM, L. F.
Tipagem HLA pelos métodos de PCR-SSO e PCR-SSP: identificação e resolução de ambiguidades In: XXXVI Congresso Brasileiro de Alergia e Imunopatologia, 2009, Porto de Galinhas.

3. PENNA, L. S.; GAMIO, F.; EWALD, G.; SALIM, P. H.; GIL, Beatriz Chamun; JOBIM, L. F.
Aplicação de Dois novos Sistemas Multiplex de STRs dos Cromossmos X e Y em

Casos de Identificação Humana In: I Congresso Internacional de Bioanálises e IV Congresso Sul-Brasileiro de Biomedicina, 2008, Novo Hamburgo.

4. PENNA, L. S.; GAMIO, F.; EWALD, G.; P.H., SALIM; GIL, Beatriz Chamun; JOBIM, R.; MAGALHAES, J. A.; JOBIM, Luiz Fernando Job
Desenvolvimento de Sistemas Multiplex para Análise de 14 STRs dos Cromossomos X e Y em Testes de Paternidade In: 28ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2008, Porto Alegre.

5. PENNA, L. S.; GAMIO, F.; EWALD, G.; GIL, B. C.; P.H., SALIM; JOBIM, M.; JOBIM, R.; MAGALHAES, J. A.; JOBIM, L. F.
Desenvolvimento de 2 sistemas multiplex de locos STR dos cromossomos X eY In: XXV Congresso Brasileiro de Genética Médica, 2008, Gramado.

6. PENNA, L. S.; MAGALHAES, J. A.
Hérnia Diafragmática: diagnóstico pré-natal e seguimento In: 28ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2008, Porto Alegre.

7. PENNA, L. S.; MAGALHAES, J. A.
Hérnia diafragmática: diagnóstico pré-natal e seguimento. In: 2º Congresso de atualização das rotinas de ginecologia e obstetrícia do Hospital de Clínicas de Porto Alegre, 2008, Gramado.

8. JOBIM, L. F.; JOBIM, M.; GIL, Beatriz Chamun; PENNA, L. S.; SALIM, P. H.; MONTEIRO, L.; PIETCHER, O.; TROTTA, E.
Proposta para novo tratamento da Síndrome Hemofagocítica In: 28ª Semana Científica do Hospital de Clínicas de Porto Alegre, 2008, Porto Alegre.

9. JOBIM, M.; GIL, B. C.; PENNA, L. S.; SALIM, P. H.; MONTEIRO, L.; JOBIM, L. F.
Síndrome Hemofagocítica: Proposta de novo tratamento In: XXXV Congresso Brasileiro de Alergia e Imunopatologia e XIII Congresso Luso-Brasileiro de Alergia e Imunologia Clínica, 2008

Apresentação de trabalho e palestra

1. PENNA, L. S.
DNA DO CROMOSSOMO X: BASES GENÉTICAS E APLICAÇÃO NA INVESTIGAÇÃO DE PATERNIDADE, 2010. (Conferência ou palestra,Apresentação de Trabalho)

Eventos

Eventos

Participação em eventos

1. **18th ECCO – 40th ESMO European Cancer Congress**, 2015. (Congresso)

2. **10th International Congress on Cell Biology and the XVI Meeting of the Brazilian Society for Cell Biology**, 2012. (Congresso)

3. **Encontro Internacional em Terapia Celular e Engenharia de Tecidos**, 2011. (Encontro)

4. **IV Congresso Internacional de Bioanálises, VII Congresso Sul-Brasileiro de Biomedicina e XI Semana Gaúcha de Biomedicina**, 2011. (Congresso)

5. **Simpósio Sobre Medicina Molecular e Terapias Inovadoras**, 2011. (Simpósio)

6. **III Congresso Internacional de Bioanálises, VI Congresso Sul - Brasileiro de Biomedicina, X Semana Gaúcha de Biomedicina**, 2010. (Congresso)

7. **Bioética em debate: os animais**, 2009. (Encontro)

8. **II Congresso Internacional de Bioanálises, V Congresso Sul - Brasileiro de Biomedicina e IX Semana Gaúcha de Biomedicina**, 2009. (Congresso)

9. **I Bioanalysis Conference MN in Brazil**, 2008. (Seminário)

10. **I Congresso Internacional de Bioanálises, IV Congresso Sul Brasileiro de Biomedicina e VIII Semana Gaúcha de Biomedicina**, 2008. (Congresso)

11. **III International Meeting of Urology, X Congresso da ABEIS, XIX Jornada de Urologia da Santa Casa de Porto Alegre**, 2008. (Congresso)

12. **XX Congresso Brasileiro de Genética Médica**, 2008. (Congresso)

13. **3º Congresso Sul Brasileiro de Biomedicina e VII Semana Gaúcha de Biomedicina**, 2007. (Congresso)

14. **I Simpósio Gaúcho sobre Terapia Gênica e Celular**, 2007. (Simpósio)

15. **XI Congresso da Sociedade Brasileira de Transplante de Medula Óssea**, 2007. (Congresso)

16. **II Congresso Sul Brasileiro de Biomedicina e VI Semana Gaúcha de Biomedicina**, 2006. (Congresso)

17. **1º Simpósio Nacional sobre Células-Tronco**, 2005. (Simpósio)

18. **5ª Semana Gaúcha de Biomedicina**, 2005. (Simpósio)

19. **14º Encontro de Geneticistas**, 2004. (Encontro)

20. **4ª Semana Gaúcha de Biomedicina**, 2004. (Simpósio)

21. **1º Ciclo Biomédico AGAB**, 2003. (Simpósio)

22. **1º Encontro Sulbrasileiro de Biomedicina**, 2003. (Encontro)

23. **3ª Semana Gaúcha de Biomedicina**, 2003. (Simpósio)

24. **II Semana Gaúcha de Biomedicina**, 2002. (Simpósio)