

## Aurora Kinase A and Gastrointestinal Malignancies

Pokuri VK, Opyrchal M and Boland PM\*

Department of Medicine, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, USA

\*Corresponding author: Boland PM, Assistant Professor, Department of Medicine, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263, USA, E-mail: [Patrick.Boland@RoswellPark.org](mailto:Patrick.Boland@RoswellPark.org)

Received date: 23 Jul 2015; Accepted date: 23 Sep 2015; Published date: 29 Sep 2015.

Citation: Pokuri VK, Opyrchal M, Boland PM (2015) Aurora Kinase A and Gastrointestinal Malignancies. Int J Cancer Res Mol Mech 1(3): doi <http://dx.doi.org/10.16966/2381-3318.114>

Copyright: © 2015 Pokuri VK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

Aurora kinase A (AURKA) has emerged as a potential therapeutic target in gastrointestinal malignancies. It has been shown to not only regulate the process of mitosis, but also to have critical non-mitotic function. AURKA is involved in epithelial to mesenchymal transition and upregulation of stem cell-like cells. The AURKA gene has been localized to chromosome 20q13. Changes in expression levels of AURKA are frequently detected in human neoplasms including various GI malignancies. Aberrations of aurora kinase signaling can lead to dysregulation of cell growth, altering the activity of a growing number of partner proteins and pathways. In pre-clinical work, AURKA has been shown to play a role in carcinogenesis, propagation of tumors and resistance to chemotherapy in GI malignancies. Despite pre-clinical promise, initial clinical studies have yielded only limited successes. Novel agents and combinations, based upon an increasing understanding of AURKA's role, offer potential for new therapeutic anti-cancer strategies. This article represents a brief review of AURKA and its role in neoplasia, as well as a review of the clinical activity of various compounds in patients with GI malignancies.

**Keywords:** Aurora kinase; Gastrointestinal cancer; Targeted therapy

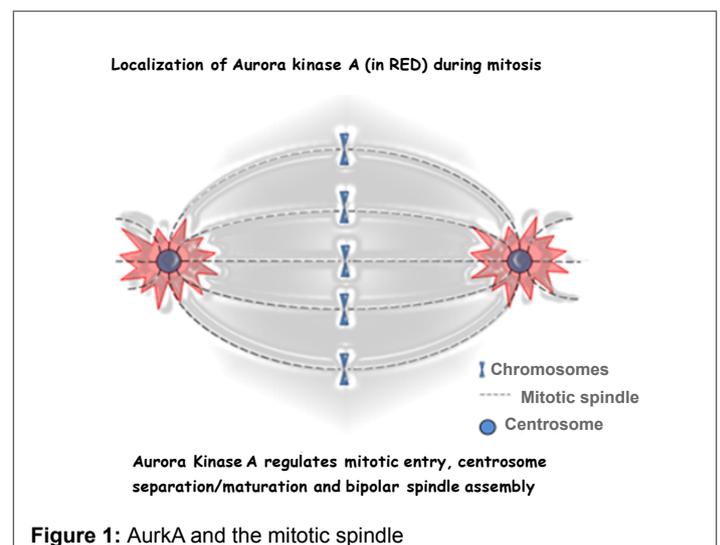
### Introduction

Aurora kinases (AURK/Ipl1p family) are a family of highly conserved serine/threonine protein kinases (A, B and C) that play a vital role in mitosis and/or meiosis [1]. The Aurora A and B (AURKA and B) were originally described as regulators of mitosis in cell cycle studies of model organisms like *Xenopus laevis* (African clawed frog) [2-4], *Drosophila melanogaster* (fruit fly) [5,6] and *Caenorhabditis elegans* (nematode) [7,8]. Subsequent studies in mammals identified the third Aurora kinase C (AURKC) apart from the other two [9]. These kinases have different subcellular localizations and functions. AURKA (STK15/BTAK) is localized to centrosomes and mitotic spindle poles/microtubules and thus regulates mitotic entry, centrosome maturation and bipolar spindle assembly (Figure 1) [10,11]. AURKB is localized to the kinetochores at the centromeres during early phases of mitosis and acts as a chromosomal passenger protein, serving in chromosome condensation, orientation and cytokinesis [11,12]. AURKC is similar to AURKB in localization and function; however, it is mainly expressed in gonads, regulating spermatogenesis and oocyte development (meiosis) [11,13,14]. Due to the importance of these molecules in the regulation of mitosis and chromosomal trafficking, aberrant expression of members of the aurora kinase family leads to genomic instability.

AURKA is the most extensively studied of the Aurora kinase family members. It is coded by a gene, Aurora A, located on chromosome 20q13, initially called Breast Tumor Activated Kinase (BTAK) as it was found to be overexpressed in transformed human breast cancer cell lines [15]. AURKA was found to be upregulated in colorectal malignancies as well as many other malignancies, including ovarian, prostate, neuroblastoma and cervical cancer cell lines [1,16]. It has been found that TPX2 and IQGAP1 proteins result in increased AURKA stability and their overexpression has been shown to promote oncogenesis [17-19]. Conversely, decreasing expression of CHFR, which results in degradation of AURKA, has also been implicated in oncogenesis [20]. Overexpression of AURKA causes

deregulation of centrosome duplication, distribution, chromosome segregation and cytokinesis resulting in aneuploidy [1,21]. Upregulated AURKA was also shown to phosphorylate p53 at serine315, causing its degradation via Mdm2 dependent ubiquitination [22]. Thus, there is a direct link between AURKA and dysregulation of p53. Collectively, AURKA induced alterations are capable of down-regulating cell cycle check-point pathways and promoting oncogenic transformation.

AURKA has also been proposed to interact with multiple other pathways of importance in GI malignancies. AURKA has been linked to control of STAT3 activity, with overexpression of AURKA enhancing STAT3 phosphorylation and nuclear translocation [23]. Conversely, inhibition of AURKA via siRNA as well as specific inhibitors results in decreased phosphorylation and transcription of STAT3, mediated



through JAK2. Potentially as a consequence of STAT inhibition, the anti-apoptotic targets, BCL-2 and MCL1, are also downregulated via AURKA inhibition in multiple tumor types, including gastrointestinal cancers [23,24]. Several other mechanisms through which increased expression of AURKA could promote cancer progression were described, increased NFκB transcription factor activity [25], increased activity of Akt pathway in a p53-dependent manner [26], increased oncogenic Ras signaling [27], increased expression of c-Myc [28] and N-Myc [29] (Table 1).

AURKA has been implicated in inducing resistance of cancer cells to standard treatments [26,30,31]. In various models, Aurora kinase inhibitors have been shown to have increased activity in combination with chemotherapy [32-34], underscoring the potential of aurora kinase inhibition to overcome chemo-resistance in cancer cells. One of the proposed mechanisms has been that AURKA leads to cancer progression and metastasis through induction of epithelial to mesenchymal transition (EMT) and increased cancer cell population with stem cell-like characteristics [35,36]. EMT and the acquisition of stem cell-like properties have been linked to resistance to standard treatment regimens [37]. In a colorectal cancer model, knockdown of AURKA resulted in growth inhibition of colorectal cancer stem cells, with decreased tumorigenic potential as demonstrated by decreased ability to engraft and form viable tumors in immunocompromised mice. AURKA knockdown has also resulted in decreased metastatic potential of the cancer cells [38]. These studies highlight the oncogenic role of AURKA in malignant transformation, tumor progression and resistance to current therapies.

### Prognostic Significance

Prognostic significance of AURKA expression has been studied in gastrointestinal cancers. In colorectal carcinoma (CRC), studies evaluating the prognostic significance of AURKA expression have shown conflicting results, though most demonstrate that increased expression of AURKA correlates with worse clinical outcomes. Several studies showed a strong association between accumulation of gains in 20q13, the site of AURKA among other putative oncogenes, and adenoma-to-carcinoma progression in colorectal cancer [39-42]. These data highlight the potential mechanistic role for AURKA as one of the driving forces of carcinogenesis. Gains of 20q13.2 (by fluorescence in situ hybridization), where in one series were identified in 53% of sporadic CRC patients, correlate with faster tumor progression and worse patient survival, independent of tumor size and lymph node involvement [43]. In this data set, 20q13 gain was inversely correlated with tumor grade and associated with left-sided colon cancers.

Multiple retrospective studies have looked at protein expression of AURKA or gene copy number. In one study, including 200 patients with CRC of various stages, AURKA protein expression by immunohistochemistry (IHC) was noted in 48.5% of samples, more frequently in those with well

or moderately differentiated colorectal carcinomas, as well as left sided tumors [44]. There was a trend toward worse outcomes with AURKA expression, not reaching statistical significance. A second group examined 517 CRC cases, stages I-IV, and identified AURKA overexpression by IHC in 19% of patients. A significant association between AURKA overexpression and chromosomal instability was observed, which was not related to site of origin. However, in this analysis, AURKA overexpression was not significantly associated with survival [45]. On the other hand, in an analysis of 386 patients with stages II-III colon cancer, a slightly more uniform population, higher AURKA expression by IHC was associated with higher risk of disease recurrence [46]. In the metastatic setting, 61 patients were examined for AURKA gene copy number, with 68% having increased gene copy number by real time PCR [47]. Contrary to the prior data sets, the increase in AURKA was associated with improved overall and progression free survival in this population. Only a limited sub-set of patients were able to undergo IHC based analysis. Finally, in yet another analysis of metastatic patients, 343 colorectal liver metastasis resection specimens were analyzed for AURKA expression by IHC [48]. Expression within the hepatic lesions correlated with that seen in the primary tumors. Importantly, when comparing the 30% of patients with strong expression to those with lesser expression of AURKA, significantly worse survival was observed. This difference was maintained upon multivariate Cox regression analysis, which included established clinicopathologic predictors of outcome. In summary, while there have been differences in methodology, most studies correlate AURKA expression to worse prognosis, particularly when looking at larger data sets and those with a more homogenous patient population.

In non-colorectal gastrointestinal cancers, a number of studies have highlighted the poor prognostic impact of AURKA protein overexpression or gene amplification. Greater than 50% of upper GI cancers demonstrate overexpression of AURKA by IHC [49]. Elevated AURKA expression in primary duodenal adenocarcinoma is predictive of poorer overall survival in multivariate regression analysis [50]. In gastric cancers, AURKA expression by IHC appears to correlate with nodal involvement, lymphovascular invasion, and advanced stage [51]. In multivariate analysis, AURKA overexpression remains an unfavorable predictor of survival. Another study demonstrated upregulation of Aurora kinase A mRNA by RT-PCR in 30% and protein expression in >50% of esophageal squamous cell carcinomas [52]. Upregulation by either methodology correlated with the presence of distant lymph node metastasis and poorer prognosis. Again, on multivariate analysis protein expression remained an independent negative prognostic variable for survival. In a study of resectable gastroesophageal cancer patients who underwent neoadjuvant chemo-radiation and surgical resection, analysis of single nucleotide polymorphisms (SNPs) in the coding region of AURKA (STK15) was performed [53]. The Phe31/Ile variant of T91A was associated with worsened odds of recurrence, as well as inferior survival. An additional SNP was evaluated, with the presence of at least one variant allele at each locus markedly increasing risk of recurrence (adjusted OR=6.21). Limited further validation of these data sets has been published. In completely resected primary gastrointestinal stromal tumors (GISTs) and metastatic GISTs, AURKA overexpression was identified as an unfavorable prognostic marker with worse recurrence-free survival and overall survival [54,55]. In summary, AURKA expression, by various methods, appears to be adversely linked to patient outcomes in GI cancers.

### Pre-Clinical Studies of Aurora Kinase Inhibitors in GI Malignancies

The aurora kinase inhibiting compounds can be divided into AURKA selective or pan-Aurora kinase inhibitors. Inhibition of aurora kinases has shown promising pre-clinical activity in gastrointestinal tumors of various origins. R1498, a multi-kinase inhibitor whose target includes

Select signaling molecules / pathways regulated by AURKA
• p53
• MDM2
• STAT3
• JAK2
• SRC
• BCL2
• MCL1
• GSK3β
• NFκB
• Akt/mTOR
• c-Myc
• N-Myc
• Ras-MAP kinase

**Table 1:** Summary of key molecules interacting with AURKA

Aurora kinases, showed significant antitumor activity in xenograft models of gastric and hepatocellular carcinomas [56]. Another group demonstrated the antitumor activity of the pan-aurora kinase inhibitor Danusertib (PHA-739358) in an orthotopic xenograft model of metastatic gastroenteropancreatic neuroendocrine tumors [57].

*In vitro* and *in vivo* studies utilizing the selective oral first generation AURKA inhibitor, MLN8054, resulted in proliferation arrest in multiple human tumor cell lines including human colorectal cancer xenografts; mechanistically this appeared to occur via mitotic accumulation and apoptosis, resulting in phenotypes consistent with Aurora A inhibition [58]. In combination with docetaxel, Alisertib (MLN8237) resulted in significant antitumor activity in upper gastrointestinal adenocarcinoma cell lines and xenograft models. Anti-tumor activity was observed irrespective of p53 status, inducing aberrant mitosis, polyploidy and apoptosis [59]. Alisertib has also exhibited promising antitumor activity in combination with cisplatin in esophageal adenocarcinoma cell lines, as well as docetaxel in gastric and esophageal models [59,60]. Finally, a novel aurora-A inhibitor, BPR1K0609S1, was shown to sensitize human colon carcinoma cell lines and xenograft models to 5-fluorouracil [61].

The exact mechanism for chemosensitization is unclear. AURKA's role as a mitotic checkpoint represents one possible explanation. An alternative hypothesis focuses on the non-mitotic function of AURKA and its ability to induce EMT and increase the percentage of cells with stem cell-like characteristics; both of which have been associated with resistance to chemotherapy agents in GI malignancies. Studies have demonstrated inhibition and reversal of EMT with AURKA signaling blockade. In both pancreatic and gastric cancer cell lines, compounds which inhibit AURKA induce cell cycle arrest and inhibit and reverse the process of EMT [62,63]. Through a series of experiments which examined populations of CD133+ colorectal cancer stem-cell like cells, AURKA inhibited Twist and Snail expression (two genes closely linked to EMT), markedly diminishing the migratory capacity of cancer cells. Inhibition of AURKA further increased the chemosensitivity of this relatively resistant cell population to a multitude of agents, including 5-FU and oxaliplatin [38].

Combined targeting of AURKA and the EGFR-RAS-MAPK pathway has seen some preclinical benefit. A synthetic lethality screen identified AURKA as a target which might synergize with anti-EGFR inhibition. Initial experiments supported this avenue of investigation [64]. In melanoma cell lines, AURKA inhibitors demonstrated marked activity when combined with MEK and/or BRAF inhibitors [65]. More recently, in colorectal cell lines and xenografts, the combined inhibition of MEK with TAK-733 and AURKA with alisertib was studied; double KRAS/PIK3CA mutant cells lines demonstrated synergistic inhibition of proliferation with the combination treatment as compared to either treatment alone [66]. Beyond the EGFR-MAPK pathway, alisertib has been combined with inhibitors of KIT and ABL (e.g. imatinib) where synergy was exhibited in GIST cell lines [55].

## Clinical Studies of Aurora Kinase Inhibitors in GI Malignancies

Multiple aurora kinase inhibitors have been developed (Table 2), with many undergoing the initial process of clinical development: single agent testing in phase I studies. MLN8054 was studied in several phase I trials, producing initial signals of durable antitumor activity in multiple tumor types. In a phase I study of MLN8054 in 61 patients, 22 of whom had refractory colorectal cancer, 9 patients (15%) achieved stable disease for at least 4 cycles of treatment [67]. No complete or partial responses were seen. One colorectal cancer patient in this study received the minimum dose of 5 mg orally daily for 7 days (and off for 14 days, constituting a 21 day cycle) and experienced stable disease lasting for 8 cycles of treatment. The dose limiting toxicity (DLT) was somnolence, predicted based upon

structural similarity to benzodiazepines. Unfortunately, despite alternate dosing schedules or use of psychostimulants (e.g. methylphenidate or modafinil) this toxicity proved insurmountable [67]. An additional study produced similar results: disease stabilization in a minority of patients, with the DLT of transaminitis [68]. Very few patients with other gastrointestinal cancers were included, precluding any assessment of clinical benefit. Pharmacokinetic/pharmacodynamic studies of MLN8054 revealed that at the maximum tolerated dose, Aurora A inhibition was incomplete. Given marked toxicity, which precluded further escalation and adequate target inhibition, further development was halted.

At present, Alisertib is the AURKA inhibitor which is furthest in clinical development, correspondingly with the greatest amount of clinical data. Two phase I studies of Alisertib in advanced solid cancers showed similar responses of stable disease over 3 to 6 months in 10-23% patients [69,70]. Among the gastrointestinal cancers in these studies, most were colorectal cancers (42 patients) with others being pancreatic (5), anal (5), gastroesophageal (3) and gall bladder (2). In these initial phase I studies, 1 patient with metastatic colorectal cancer experienced stable disease for greater than 12 months. The most common dose limiting toxicities of Alisertib were neutropenia and stomatitis, while fatigue, nausea and neutropenia were the most common adverse events in these studies [69,70]. The recommended phase 2 dose of Alisertib was 50 mg orally twice daily for 7 days in a 21 day cycle [70,71]. This dose is currently being studied in several phase 2 clinical trials.

Recently, the results of a multi-arm phase II study of single agent Alisertib in advanced, heavily pretreated solid tumors were reported. Patients were selected for tumors which have been shown to overexpress AURKA and included breast cancer, small cell lung cancer, non-small cell lung cancer, head and neck squamous cell carcinoma and gastroesophageal adenocarcinoma [72]. The toxicity profile was similar to that seen in the phase 1 trials; neutropenia was the most common grade 3-4 adverse event [72]. Alisertib demonstrated differential activity in this trial, with a response rate (RR) of 18% and 21% in breast and small cell lung cancers, respectively, while only 9% (4/47 patients) of patients with gastroesophageal adenocarcinomas experienced responses. Due to the small fraction of patients experiencing benefit, the median progression free survival (PFS) was uninspiring in gastroesophageal adenocarcinoma (1.5 months).

Danusertib is a pan-aurora kinase inhibitor which was studied in a phase 1 dose escalation study of patients advanced refractory solid tumors. The established the maximum tolerated dose was 500 mg/m<sup>2</sup>, given as 24 hour intravenous infusion every 2 weeks [73]. Nineteen colorectal cancer patients were involved in this study and prolonged disease stabilization (23.9-52.3 weeks) was observed only in two of them (11%). An additional phase II study utilizing this dosing schema was conducted in heavily treated patients with advanced solid tumors [74]. The safety profile of Danusertib was similar to prior studies and neutropenia was the most frequent adverse event. This study showed only marginal activity in GI cancers with a progression free survival of 4 months in 10% (3/30 patients) in pancreatic cancers and 0% (0/28 patients) in colorectal cancers.

AT9283 is an AURKA and B inhibitor which has been investigated in the phase I setting. DLT was found to be neutropenia. At MTD there was evidence of target inhibition and modest clinical activity was observed. Though there were no RECIST responses, best clinical response observed was stable disease seen in 1/6 (17%) esophageal cancer patients and in 1/12 (8%) colorectal cancer patients.

MSC199237A is a pan-aurora kinase inhibitor, which has seen investigation as a single agent, as well as in combination with gemcitabine. In the monotherapy phase I study, cytopenia and fatigue represented the most common grade 3/4 toxicities [75]. Again, no partial or complete

AURK inhibitor	AURK selective IC <sub>50</sub> in nM	Major Additional Targets	Dosing Formulation, frequency	# Currently open active clinical studies*
MLN-8237 (Alisertib; Millennium)	AurkA=1 AurkB>200 AurkC=Not Available [79]	N/A	Oral, bid	22
VX-689/MK-5108 (Vertex/Merck)	AurkA=0.064 AurkB=14 AurkC=12 [80]	N/A	Oral, bid	0
ENMD-2076 (CASI)	AurkA=14; AurkB=700 AurkC=Not Available [81]	VEGFR, FGFR, FLT3, c-KIT, CSFR1	Oral, daily	2
TAS-119 (Taiho)	Not available	N/A	Oral, bid	1
MLN8054 (Millennium)	AurkA=0.064 AurkB=14 AurkC=12 [58]	LCK, PKA, Chk2, PLK1	Oral, bid	0
AT9283 (ASTEX)	AurkA=3 AurkB=3	JAK2, JAK3	Intravenous, 24 hour continuous infusion	0
AMG900 (Amgen)	AurkA=5 AurkB=4 AurkC=1	p38α	Oral, daily	0

**Table 2:** Profiles of Select Aurora Kinase Inhibitors

responses were achieved. Stable disease was achieved in a substantial proportion of evaluable patients (41%). This included one patient with a neuroendocrine tumor who experienced minor response (17% reduction in tumor burden) of 18 months duration. An additional study of MSC199237A in combination with gemcitabine was conducted [76]. This drug proved tolerable with standard doses of gemcitabine (1000 mg/m<sup>2</sup>), with expected neutropenia as DLT. There were only 2 patients with confirmed partial responses (3%). However, several patients with GI cancers saw some clinical benefit. One out of 6 patients with hepatocellular carcinoma (HCC) experienced a partial response, remaining on study for 11 cycles. One patient of two (50%) with esophagogastric cancer attained stable disease lasting over 9 months, and one additional patient of 8 (13%) with pancreatic cancer also achieved disease stability lasting greater than 7 months.

There are multiple ongoing, completed and planned studies of aurora kinase inhibitors in combination with chemotherapy. The AURKA inhibitor, Alisertib, has seen the greatest use in this setting. Tolerability has been demonstrated with weekly paclitaxel as well as docetaxel with some signs of clinical benefit [77]. Recently, data was presented on alisertib combined with gemcitabine utilizing an alternate schedule: days 1-3, 8-10, and 15-17 of a 28 day cycle. In a study of multiple tumor types (most commonly NSCLC), there were 18 evaluable patients, with 1 (6%) patient experiencing a PR and 10 (56%) experiencing stable disease as best response. Though there was a significant incidence of grade III/IV hematologic toxicity, the vast majority of patients received full doses of both drugs [78]. An expansion cohort of patients with pancreatic cancer is ongoing and will be of great interest. Multiple combinatorial investigations are ongoing across tumor types with studies of particular interest targeting GI tumors highlighted in Table 3.

### Future Directions

Aurora kinase inhibitors, with alisertib being most advanced, are currently being evaluated in multiple phase I and II trials, alone and in various combinations. As single agents, their efficacy appears to be limited

in gastrointestinal tumors with best responses predominantly being stable disease. Given that alisertib demonstrated promise in preclinical investigations of upper GI malignancies when added to chemotherapeutics and demonstrates evidence of single agent clinical efficacy, further investigation is merited. Alternate schedules might be worthy of investigation, due to class-related toxicity of myelosuppression. With gemcitabine, which has the potential to induce significant myelotoxicity, a slight adjustment in dosing strategy allowed for the addition of alisertib with reasonable tolerability at full doses and some signs of benefit.

Though in the small phase II study of alisertib in gastroesophageal cancers the median PFS was limited, robust responses were observed, suggesting that there may be clinical benefit for select patients. Notably, preclinical activity has been demonstrated when AURKA inhibition is combined with fluoropyrimidines, platinum compounds and taxanes, across multiple tumor types, particularly in gastric and esophageal cancer cell lines. These three classes of agents are instrumental in the treatment of advanced gastroesophageal cancers. In the future, such combinations may be tested. There is intensive interest in combining AURKA inhibitors with hypothesis driven targeted agents with non-overlapping toxicities. JAK2-STAT3 pathway activation by AURKA was recently found to be an important driver of carcinogenesis in upper GI malignancies; more extensive work is needed to determine if these combinations have future in clinical development. Pre-clinical data suggest potential in exploring combination therapies with Aurora inhibition together with inhibitors of EGFR-RAS-MAPK pathway. Additional work is needed to identify patient population which may benefit from this approach.

In order to fully establish AURKA inhibition as viable treatment option for patients, development of reliable clinical predictors of efficacy is needed. Ongoing and future studies will hopefully identify prospective biomarkers of clinical benefit or lack of thereof, which will have to be tested in a prospective manner. Additional pre-clinical work fully delineating non-mitotic function of AURKA will help to develop innovative correlative studies.

Drug combination	Indication	Identifier
Alisertib+FOLFOX	Gastrointestinal malignancies	NCT02319018
Alisertib+Gemcitabine	Solid tumors/pancreatic cancer	NCT01924260
Alisertib+Irinotecan	Solid tumors/colorectal cancer	NCT01923337
Alisertib+Nab-paclitaxel	Solid tumors	NCT01677559
TAS-119+docetaxel	Solid tumors	NCT02448589
Alisertib+MLN2480 (pan-RAF inhibitor)	Solid tumors	NCT02327169

**Table 3:** Ongoing Clinical Studies of Interest Utilizing Aurora Kinase Inhibitors

## Conclusion

Although the preclinical data of AURKA inhibition in gastrointestinal cancers is promising, the initial clinical activity of this class of compounds in patients with GI malignancies has been modest at best. Pharmacokinetic and pharmacodynamic testing has suggested that AURKA inhibition in tumor tissue is attainable at MTD. More work is needed to identify the optimal partners for combination therapies and biomarkers for predicting therapeutic benefit. We are eagerly awaiting the results of the ongoing clinical trials to determine if inhibitors of AURKA will have a role in treatment of patients with GI malignancies.

## References

- Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, et al. (1998) Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat Genet* 20: 189-193.
- Roghi C, Giet R, Uzbekov R, Morin N, Chartrain I, et al. (1998) The *Xenopus* protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. *J Cell Sci* 111 : 557-572.
- Andresson T, Ruderman JV (1998) The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. *EMBO J* 17: 5627-5637.
- Adams RR, Wheatley SP, Gouldsworthy AM, Kandels-Lewis SE, Carmena M, et al. (2000) INCENP binds the Aurora-related kinase AIRK2 and is required to target it to chromosomes, the central spindle and cleavage furrow. *Curr Biol* 10: 1075-1078.
- Glover DM, Leibowitz MH, McLean DA, Parry H (1995) Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell* 81: 95-105.
- Reich A, Yanai A, Mesilaty-Gross S, Chen-Moses A, Wides R, et al. (1999) Cloning, mapping, and expression of ial, a novel *Drosophila* member of the lpl1/aurora mitotic control kinase family. *DNA Cell Biol* 18: 593-603.
- Schumacher JM, Golden A, Donovan PJ (1998) AIR-2: An Aurora/lpl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. *J Cell Biol* 143: 1635-1646.
- Hannak E, Kirkham M, Hyman AA, Oegema K (2001) Aurora-A kinase is required for centrosome maturation in *Caenorhabditis elegans*. *J Cell Biol* 155: 1109-1116.
- Kimura M, Matsuda Y, Yoshioka T, Okano Y (1999) Cell cycle-dependent expression and centrosome localization of a third human aurora/lpl1-related protein kinase, AIK3. *J Biol Chem* 274: 7334-7340.
- Marumoto T, Honda S, Hara T, Nitta M, Hirota T, et al. (2003) Aurora-A kinase maintains the fidelity of early and late mitotic events in HeLa cells. *J Biol Chem* 278: 51786-51795.
- Carmena M, Earnshaw WC (2003) The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol* 4: 842-854.
- Adams RR, Maiato H, Earnshaw WC, Carmena M (2001) Essential roles of *Drosophila* inner centromere protein (INCENP) and aurora B in histone H3 phosphorylation, metaphase chromosome alignment, kinetochore disjunction, and chromosome segregation. *J Cell Biol* 153: 865-880.
- Li X, Sakashita G, Matsuzaki H, Sugimoto K, Kimura K, et al. (2004) Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora-C. *J Biol Chem* 279: 47201-47211.
- Kimmins S, Crosio C, Kotaja N, Hirayama J, Monaco L, et al. (2007) Differential functions of the Aurora-B and Aurora-C kinases in mammalian spermatogenesis. *Mol Endocrinol* 21: 726-739.
- Sen S, Zhou H, White RA (1997) A putative serine/threonine kinase encoding gene BTAK on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. *Oncogene* 14: 2195-2200.
- Bischoff JR, Anderson L, Zhu Y, Mossie K, Ng L, (1998) A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J* 17: 3052-3065.
- Giubettini M, Asteriti IA, Scrofani J, De Luca M, Lindon C, et al. (2011) Control of Aurora-A stability through interaction with TPX2. *J Cell Sci* 124: 113-122.
- Yin N, Shi J, Wang D, Tong T, Wang M, et al. (2012) IQGAP1 interacts with Aurora-A and enhances its stability and its role in cancer. *Biochem Biophys Res Commun* 421: 64-69.
- Aguirre-Portolés C, Bird AW, Hyman A, Cañamero M, Pérez de Castro I, et al. (2012) Tpx2 controls spindle integrity, genome stability, and tumor development. *Cancer Res* 72: 1518-1528.
- Sanbhnani S, Yeong FM (2012) CHFR: a key checkpoint component implicated in a wide range of cancers. *Cell Mol Life Sci* 69: 1669-1687.
- Meraldi P, Honda R, Nigg EA (2004) Aurora kinases link chromosome segregation and cell division to cancer susceptibility. *Curr Opin Genet Dev* 14: 29-36.
- Katayama H, Sasai K, Kawai H, Yuan ZM, Bondaruk J, et al. (2004) Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53. *Nat Genet* 36: 55-62.
- Katsha A, Arras J, Soutto M, Belkhir A, El-Rifai W (2014) AURKA regulates JAK2-STAT3 activity in human gastric and esophageal cancers. *Mol Oncol* 8: 1419-1428.
- Li JP, Yang YX, Liu QL, Pan ST, He ZX, et al. (2015) The investigational Aurora kinase A inhibitor alisertib (MLN8237) induces cell cycle G2/M arrest, apoptosis, and autophagy via p38 MAPK and Akt/mTOR signaling pathways in human breast cancer cells. *Drug Des Devel Ther* 9: 1627-1652.
- Chefetz I, Holmberg JC, Alvero AB, Visintin I, Mor G (2011) Inhibition of Aurora-A kinase induces cell cycle arrest in epithelial ovarian cancer stem cells by affecting NFκB pathway. *Cell Cycle* 10: 2206-2214.
- Yang H, He L, Kruk P, Nicosia SV, Cheng JQ (2006) Aurora-A induces cell survival and chemoresistance by activation of Akt through a p53-dependent manner in ovarian cancer cells. *Int J Cancer* 119: 2304-2312.

27. Lim KH, Brady DC, Kashatus DF, Ancrile BB, Der CJ, et al. (2010) Aurora-A phosphorylates, activates, and relocalizes the small GTPase RalA. *Mol Cell Biol* 30: 508-523.
28. Dimova I, Raitcheva S, Dimitrov R, Doganov N, Toncheva D (2006) Correlations between c-myc gene copy-number and clinicopathological parameters of ovarian tumours. *Eur J Cancer* 42: 674-679.
29. Otto T, Horn S, Brockmann M, Eilers U, Schüttrumpf L, et al. (2009) Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. *Cancer Cell* 15: 67-78.
30. Anand S, Penrhyn-Lowe S, Venkitaraman AR (2003) AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. *Cancer Cell* 3: 51-62.
31. Opyrchal M, Salisbury JL, Zhang S, McCubrey J, Hawse J, et al. (2014) Aurora-A Mitotic Kinase Induces Endocrine Resistance through Down-Regulation of ERalpha Expression in Initially ERalpha+ Breast Cancer Cells. *PloS one* 9: e96995.
32. Scharer CD, Laycock N, Osunkoya AO, Logani S, McDonald JF, et al. (2008) Aurora kinase inhibitors synergize with paclitaxel to induce apoptosis in ovarian cancer cells. *J Transl Med* 6: 79.
33. Sun C, Chan F, Briassouli P, Linardopoulos S (2007) Aurora kinase inhibition downregulates NF-kappaB and sensitises tumour cells to chemotherapeutic agents. *Biochem Biophys Res Commun* 352: 220-225.
34. VanderPorten EC, Taverna P, Hogan JN, Ballinger MD, Flanagan WM, et al. (2009) The Aurora kinase inhibitor SNS-314 shows broad therapeutic potential with chemotherapeutics and synergy with microtubule-targeted agents in a colon carcinoma model. *Mol Cancer Ther* 8: 930-939.
35. D'Assoro AB, Liu T, Quatraro C, Amato A, Opyrchal M, et al. (2014) The mitotic kinase Aurora-a promotes distant metastases by inducing epithelial-to-mesenchymal transition in ERα(+) breast cancer cells. *Oncogene* 33: 599-610.
36. Regan JL, Sourisseau T, Soady K, Kendrick H, McCarthy A, et al. (2013) Aurora A kinase regulates mammary epithelial cell fate by determining mitotic spindle orientation in a Notch-dependent manner. *Cell Rep* 4: 110-123.
37. Kemper K, de Goeje PL, Peeper DS, van Amerongen R (2014) Phenotype switching: tumor cell plasticity as a resistance mechanism and target for therapy. *Cancer Res* 74: 5937-5941.
38. Cammareri P, Scopelliti A, Todaro M, Eterno V, Francescangeli F, et al. (2010) Aurora-a is essential for the tumorigenic capacity and chemoresistance of colorectal cancer stem cells. *Cancer Res* 70: 4655-4665.
39. Hermsen M, Postma C, Baak J, Weiss M, Rapallo A, et al. (2002) Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. *Gastroenterology* 123: 1109-1119.
40. Fijneman RJ, Carvalho B, Postma C, Mongera S, van Hinsbergh VW, et al. (2007) Loss of 1p36, gain of 8q24, and loss of 9q34 are associated with stroma percentage of colorectal cancer. *Cancer Lett* 258: 223-229.
41. Sillars-Hardebol AH, Carvalho B, Tijssen M, Beliën JA, de Wit M, et al. (2012) TPX2 and AURKA promote 20q amplicon-driven colorectal adenoma to carcinoma progression. *Gut* 61: 1568-1575.
42. Carvalho B, Postma C, Mongera S, Hopmans E, Diskin S, et al. (2009) Multiple putative oncogenes at the chromosome 20q amplicon contribute to colorectal adenoma to carcinoma progression. *Gut* 58: 79-89.
43. Aust DE, Muders M, Kohler A, Schmidt M, Diebold J, et al. (2004) Prognostic relevance of 20q13 gains in sporadic colorectal cancers: a FISH analysis. *Scand J Gastroenterol* 39: 766-772.
44. Lam AK, Ong K, Ho YH (2008) Aurora kinase expression in colorectal adenocarcinoma: correlations with clinicopathological features, p16 expression, and telomerase activity. *Hum Pathol* 39: 599-604.
45. Baba Y, Noshio K, Shima K, Irahara N, Kure S, et al. (2009) Aurora-A expression is independently associated with chromosomal instability in colorectal cancer. *Neoplasia* 11: 418-425.
46. Belt EJ, Brosens RP, Delis-van Diemen PM, Bril H, Tijssen M, et al. (2012) Cell cycle proteins predict recurrence in stage II and III colon cancer. *Ann Surg Oncol* 19: S682-S692.
47. Dotan E, Meropol NJ, Zhu F, Zambito F, Bove B, et al. (2012) Relationship of increased aurora kinase A gene copy number, prognosis and response to chemotherapy in patients with metastatic colorectal cancer. *Br J Cancer* 106: 748-755.
48. Goos JA, Coupe VM, Diosdado B, Delis-Van Diemen PM, Karga C, et al. (2013) Aurora kinase A (AURKA) expression in colorectal cancer liver metastasis is associated with poor prognosis. *Br J Cancer* 109: 2445-2452.
49. Dar AA, Zaika A, Piazzuelo MB, Correa P, Koyama T, et al. (2008) Frequent overexpression of Aurora Kinase A in upper gastrointestinal adenocarcinomas correlates with potent antiapoptotic functions. *Cancer* 112: 1688-1698.
50. Chen J, Lin Q, Wen JY, Li X, Ma XK, et al. (2014) Prognosis value of mitotic kinase Aurora-A for primary duodenal adenocarcinoma. *Tumour Biol* 35: 9361-9370.
51. Wang J, Yang S, Zhang H, Song Y, Zhang X, et al. (2011) Aurora-A as an independent molecular prognostic marker in gastric cancer. *Oncol Rep* 26: 23-32.
52. Tanaka E, Hashimoto Y, Ito T, Okumura T, Kan T, et al. (2005) The clinical significance of Aurora-A/STK15/BTAK expression in human esophageal squamous cell carcinoma. *Clin Cancer Res* 11: 1827-1834.
53. Pan JY, Ajani JA, Gu J, Gong Y, Qin A, et al. (2012) Association of Aurora-A (STK15) kinase polymorphisms with clinical outcome of esophageal cancer treated with preoperative chemoradiation. *Cancer* 118: 4346-4353.
54. Yen CC, Yeh CN, Cheng CT, Jung SM, Huang SC, et al. (2012) Integrating bioinformatics and clinicopathological research of gastrointestinal stromal tumors: identification of aurora kinase A as a poor risk marker. *Ann Surg Oncol* 19: 3491-3499.
55. Yeh CN, Yen CC, Chen YY, Cheng CT, Huang SC, et al. (2014) Identification of aurora kinase A as an unfavorable prognostic factor and potential treatment target for metastatic gastrointestinal stromal tumors. *Oncotarget* 5: 4071-4086.
56. Zhang C, Wu X, Zhang M, Zhu L, Zhao R, et al. (2013) Small molecule R1498 as a well-tolerated and orally active kinase inhibitor for hepatocellular carcinoma and gastric cancer treatment via targeting angiogenesis and mitosis pathways. *PLoS One* 8: e65264.
57. Fraedrich K, Schrader J, Ittrich H, Keller G, Gontarewicz A, et al. (2012) Targeting aurora kinases with danusertib (PHA-739358) inhibits growth of liver metastases from gastroenteropancreatic neuroendocrine tumors in an orthotopic xenograft model. *Clin Cancer Res* 18: 4621-4632.
58. Manfredi MG, Ecsedy JA, Meetze KA, Balani SK, Burenkova O, et al. (2007) Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase. *Proc Natl Acad Sci U S A* 104: 4106-4111.
59. Sehdev V, Katsha A, Ecsedy J, Zaika A, Belkhiri A, et al. (2013) The combination of alisertib, an investigational Aurora kinase A inhibitor, and docetaxel promotes cell death and reduces tumor growth in preclinical cell models of upper gastrointestinal adenocarcinomas. *Cancer* 119: 904-914.
60. Sehdev V, Peng D, Soutto M, Washington MK, Revetta F, et al. (2012) The aurora kinase A inhibitor MLN8237 enhances cisplatin-induced

- cell death in esophageal adenocarcinoma cells. *Mol Cancer Ther* 11: 763-774.
61. Shionome Y, Lin WH, Shiao HY, Hsieh HP, Hsu JT, et al. (2013) A novel aurora-A inhibitor, BPR1K0609S1, sensitizes colorectal tumor cells to 5-fluorouracil (5-FU) treatment. *Int J Biol Sci* 9: 403-411.
  62. Yuan CX, Zhou ZW, Yang YX, He ZX, Zhang X, et al. (2015) Danusertib, a potent pan-Aurora kinase and ABL kinase inhibitor, induces cell cycle arrest and programmed cell death and inhibits epithelial to mesenchymal transition involving the PI3K/Akt/mTOR-mediated signaling pathway in human gastric cancer AGS and NCI-N78 cells. *Drug Des Devel Ther* 9: 1293-1318.
  63. Wang F, Li H, Yan XG, Zhou ZW, Yi ZG, et al. (2015) Alisertib induces cell cycle arrest and autophagy and suppresses epithelial-to-mesenchymal transition involving PI3K/Akt/mTOR and sirtuin 1-mediated signaling pathways in human pancreatic cancer cells. *Drug Des Devel Ther* 9: 575-601.
  64. Astsaturov I, Ratushny V, Sukhanova A, Einarson MB, Bagnyukova T, et al. (2010) Synthetic lethal screen of an EGFR-centered network to improve targeted therapies. *Sci Signal* 3: ra67.
  65. Caputo E, Miceli R, Motti ML, Taté R, Fratangelo F, et al. (2014) Aurka inhibitors enhance the effects of B-RAF and MEK inhibitors in melanoma treatment. *J Transl Med* 12: 216.
  66. Davis SL, Robertson KM, Pitts TM, Tentler JJ, Bradshaw-Pierce EL, et al. (2015) Combined inhibition of MEK and Aurora A kinase in KRAS/PIK3CA double-mutant colorectal cancer models. *Front Pharmacol* 6: 120.
  67. Dees EC, Infante JR, Cohen RB, O'Neil BH, Jones S, et al. (2011) Phase 1 study of MLN8054, a selective inhibitor of Aurora A kinase in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 67: 945-954.
  68. Macarulla T, Cervantes A, Elez E, Rodríguez-Braun E, Baselga J, et al. (2010) Phase I study of the selective Aurora A kinase inhibitor MLN8054 in patients with advanced solid tumors: safety, pharmacokinetics, and pharmacodynamics. *Mol Cancer Ther* 9: 2844-2852.
  69. Dees EC, Cohen RB, von Mehren M, Stinchcombe TE, Liu H, 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. *Clin Cancer Res* 18: 4775-4784.
  70. Cervantes A, Elez E, Roda D, Ecsedy J, Macarulla T, et al. (2012) Phase I pharmacokinetic/pharmacodynamic study of MLN8237, an investigational, oral, selective aurora A kinase inhibitor, in patients with advanced solid tumors. *Clin Cancer Res* 18: 4764-4774.
  71. Falchook G, Kurzrock R, Gouw L, Hong D, McGregor KA, et al. (2014) Investigational Aurora A kinase inhibitor alisertib (MLN8237) as an enteric-coated tablet formulation in non-hematologic malignancies: phase 1 dose-escalation study. *Invest New Drugs* 32: 1181-1187.
  72. Melichar B, Adenis A, Lockhart AC, Bennouna J, Dees EC, 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 phase 2 study. *Lancet Oncol* 16: 395-405.
  73. Cohen RB, Jones SF, Aggarwal C, von Mehren M, Cheng J, 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. *Clin Cancer Res* 15: 6694-6701.
  74. Schoffski P, Besse B, Gauler T, de Jonge MJ, Scambia G, et al. (2015) Efficacy and safety of biweekly i.v. administrations of the Aurora kinase inhibitor danusertib hydrochloride in independent cohorts of patients with advanced or metastatic breast, ovarian, colorectal, pancreatic, small-cell and non-small-cell lung cancer: a multi-tumour, multi-institutional phase II study. *Ann Oncol* 26: 598-607.
  75. Mita M, Gordon M, Rejeb N, Gianella-Borradori A, Jeco V, et al. (2014) A phase I study of three different dosing schedules of the oral aurora kinase inhibitor MSC1992371A in patients with solid tumors. *Target Oncol* 9: 215-224.
  76. Raymond E, Alexandre J, Faivre S, Goldwasser F, Besse-Hammer T, et al. (2014) A phase I schedule dependency study of the aurora kinase inhibitor MSC1992371A in combination with gemcitabine in patients with solid tumors. *Invest New Drugs* 32: 94-103.
  77. Falchook GS, Goff BA, Kurzrock R, Gray HJ, Martin LP, et al. (2012) Phase I/II study of weekly paclitaxel with or without MLN8237 (alisertib), an investigational aurora A kinase inhibitor, in patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer (OC), or breast cancer (BrC): Phase I results. 2012 ASCO Annual Meeting *J Clin Oncol* 30: 5021.
  78. Kim EJ, Semrad TJ, Gandara DR, Riess J, Li T, et al. (2015) Phase I study of the combination of alisertib (MLN8237) and gemcitabine in advanced solid tumors. 2015 ASCO Annual Meeting, *J Clin Oncol* 33: 2526.
  79. Gorgun G, Calabrese E, Hideshima T, Ecsedy J, Perrone G, et al. (2010) A novel Aurora-A kinase inhibitor MLN8237 induces cytotoxicity and cell-cycle arrest in multiple myeloma. *Blood* 115: 5202-5213.
  80. Shimomura T, Hasako S, Nakatsuru Y, Mita T, Ichikawa K, et al. (2010) MK-5108, a highly selective Aurora-A kinase inhibitor, shows antitumor activity alone and in combination with docetaxel. *Mol Cancer Ther* 9: 157-166.
  81. Tentler JJ, Bradshaw-Pierce EL, Serkova NJ, Hasebroock KM, Pitts TM, et al. (2010) Assessment of the in vivo antitumor effects of ENMD-2076, a novel multitargeted kinase inhibitor, against primary and cell line-derived human colorectal cancer xenograft models. *Clin Cancer Res* 16: 2989-2998.