Aurora kinase inhibitors as anti-cancer therapy

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Aurora kinases are serine and threonine kinases that function as key regulators of the mitosis process. There are three distint human aurora kinases known as Aurora A, Aurora B, and Aurora C. Aurora A and Aurora B are overexpressed in a number of human cancers including non-small cell lung cancer, glioblastomas, and upper gastrointestinal adenocarcinomas. Given their association with tumorigenesis, both Aurora A and Aurora B have been targeted for cancer therapy. Currently, a number of selective and nonselective aurora kinase inhibitors are being tested in preclinical and clinical settings as anti-tumor agents. We review the biology of human aurora kinases, followed by an overview of inhibitors undergoing current clinical investigations. *Anti-Cancer Drugs* **21:339–350 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

In the late 1990s, a group of enzymes known as the Aurora kinases were described as important regulators of mitosis in mammalian cells [1,2]. Initially discovered in *Drosophila*, the first Aurora kinase was named after its mutation and was found to give rise to monopolar spindles, reminiscent of the North Pole [3]. The human Aurora family consists of highly conserved serine–threonine protein kinases. These kinases regulate various processes including mitotic entry, microtubule spindle assembly, centrosome duplication, chromosome segregation, and cytokinesis. Their dysfunction can lead to aneuploidy, resulting in the failure to maintain chromosomal integrity. Furthermore, they are overexpressed in a variety of cancer types. Increased levels of Aurora kinases can be associated with neoplastic transformation, serving as attractive targets for molecular therapies. In this review, we will focus on the roles of Aurora kinases both in normal cell division and human tumorigenesis. In addition, we will describe many novel inhibitors undergoing active clinical trials. Lastly, we will discuss the current development and future strategies for targeting these important regulators for cancer treatment.

Aurora kinase

The human genome encodes for three different subtypes of Aurora kinases, termed A, B, and C. All three entities are serine–threonine protein kinases that share a high degree of sequence homology in their catalytic domains [4,5]. Aurora A and B are expressed in many cell types, whereas Aurora C is mostly found in testicular tissue where it is involved with spermatogenesis [6]. Cell models have shown an association between abnormal levels of kinase activity and genetic instability [7]. Despite their shared sequences and common association with mitosis, all three subtypes have different activating mechanisms, subcellular distributions, and regulatory functions [6–9].

Activation

The activation of Aurora kinases is tightly regulated. The initial gene transcription occurs in a cell–cycle-dependent manner [10]. Various promoters of Aurora A and B contain specific sequences (CDE/CHR) that are required for transcription during the G_2 phase [11–13]. Additional transcription factors, such as E2F1, E2F4, DP2, and FoxM1, are also implicated in Aurora B expression [14]. Aurora C is similarly expressed in meiotically dividing cells, with its transcription at least in part controlled by a testis-specific transcription factor known as the testis zinc finger protein [15].

After synthesis, Aurora kinases require other cofactors for activation. For Aurora A, the most recognized cofactor is TPX2 [10]. TPX2 was initially described as a microtubule associated protein with involvement in bipolar spindle assembly [16]. It was later found to bind and direct Aurora A to the mitotic spindle [17]. Binding of TPX2 triggers the movement of the Aurora A-activating segment into the catalytic pocket, thereby preventing dephosphorylation of a phospho-threonine, which normally leads to deactivation of the kinase [18,19]. Other activating cofactors include the Ajuba, Bora, and HEF1 proteins. Ajuba is located on the centrosomes and promotes activation of Aurora A through autophosphorylation [20]. Bora is released from the nucleus into the cytoplasm during the late G_2 phase and enhances kinase activity [21]. HEF1 is a well-described protein that is involved with focal adhesion [22]. Although the intricate interactions between these proteins need to be further clarified, the cofactors are necessary for the appropriate activation of Aurora A.

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As with Aurora A, the activation of Aurora B requires additional cofactors including the inner centromere protein antigens (INCENP) as well as components of the chromosomal passenger complex [23]. INCENP itself undergoes autophosphorylation, which induces conformational change within the catalytic pocket of Aurora B [24]. Chromosomal passenger complex, together with other subunits such as Survivin and Borealin, is required for the proper localization of the kinase during initial mitosis [25]. Additional cofactors including Mps1, Chkl, and Tousled are themselves kinases that are collectively required for full activation [26–28]. Similar to Aurora B, Aurora C also binds INCENP during activation [29]. Altogether, these cofactors ensure the correct timing and location for kinase activity. When mitosis is complete, these kinases are degraded by the anaphase promoting complex/cyclosome in conjunction with cofactor Cdh1 [30,31]. This ensures appropriately low levels during the subsequent G_1 phase.

Distribution

All three kinase subtypes have different subcellular distributions. Aurora A initially localizes to the centrosomes during duplication in the S phase, and later migrates to the spindle poles during mitosis. Such distribution is consistent with its primary roles in centrosome regulation and mitotic spindle formation [17,19,32]. Aurora B undergoes dynamic distribution during mitosis, localizing first to the inner centromeres during prophase through metaphase, then to the spindle midzone during anaphase through cytokinesis. Aurora B migrates as part of the chromosomal passenger complex, whose function is to ensure accurate chromosome segregation and cell body division [33–35]. Similarly, Aurora C is a chromosomal passenger protein and migrates alongside Aurora B. However, Aurora C is predominantly expressed in the testes where it is involved with spermatogenesis [36].

Function

Aurora A and B are expressed in most cell types. These kinases regulate cell-cycle progression from G_2 through mitosis in a coordinated manner, with each having distinct roles. In contrast, Aurora C is restricted to the testicular tissue where it likely regulates meiosis of germ cells.

Aurora A

Aurora A regulates several key events during the cell cycle including centrosome maturation/separation, bipolar spindle assembly, and mitotic entry. During the G_2 phase, Aurora A localizes to the centrosomes where it initially promotes the development of the pericentrosomal material. In early mitosis, it extends to the bipolar spindles and increases the centrosomal microtubule nucleation activity. Aurora A depends on other enzymes such as Pak1, Plk1, and Cdk11 for centrosomal localization [37–39]. It also assists other centrosomal components such as centrosomin, NDEL1, LATS, and TACC protein for accurate targeting and proper activation [40–43]. Some of these proteins support centrosome maturation, whereas others stimulate microtubule nucleation.

Once the centrosomes have matured, both units migrate to opposite ends to become the centers for mitotic spindle formation. This process requires the function of Aurora A, which, if inhibited, can lead to monopolar spindles [3]. After centrosome separation, spindle assembly begins in early prophase. Spindle formation is mostly driven by microtubule nucleation at the centrosomes, with influence from the chromosomes [44,45]. Furthermore, as part of a large multi-protein complex (includes TPX2, Eg5, XMAP125/chTOG, and HURP), Aurora A resides on the spindle and provides bipolar orientation [46]. Finally, recent studies suggest that Aurora A may facilitate timely mitotic entry by activating CyclinB/Cdk1. It directly and indirectly (through Polo-like kinase 1) phosphorylates Cdc25B phosphatase, which is required for CyclinB/Cdk1 activation. This process occurs during the G_2/mitosis transition point, permitting passage into early mitosis [47,48].

Aurora B

Aurora B is a catalytic component of the chromosomal passenger complex, which incorporates other cofactors such as the INCENP, Survivin, and Borealin [23]. Throughout mitosis, it is involved with many processes including chromosomal condensation, microtubule–kinetochore attachment, chromatid segregation, cytokinesis, and mitotic spindle checkpoint regulation [33–35,49,50].

In the early prophase, the Aurora B function is associated with chromatin modification through the phosphorylation of histone H3 at Ser^{10} and Ser^{28} [51]. This process is thought to facilitate chromosomal condensation. During prometaphase and metaphase, Aurora B promotes proper localization of centromeric proteins, ensuring appropriate attachments of centromeres with bipolar spindles [50]. Accurate microtubule–kinetochore attachments and chromosome alignment require bi-orientation, with each sister kinetochore linked to spindles from the corresponding pole. If not corrected before the anaphase, defects in this process may lead to aneuploidy. When confronted with an improper microtubule–kinetochore attachment, Aurora B detects the absence of bipolar tension and breaks the attachment [33,49,52]. The unattached kinetochore then activates the mitotic spindle checkpoint, preventing the onset of chromatid segregation until the appropriate attachments are reassembled [49]. Aurora B also recruits other checkpoint proteins such as BubR1 and Mad2 to the unattached kinetochore, reinforcing the inhibition of the anaphase-promoting complex [53].

Once the chromatids have separated, Aurora B migrates from the centromeres to the spindle midzone where it resides until cytokinesis. During cytokinesis, an actinomyosin

ring encircles the cell equator, eventually dividing the cytoplasm into two equal parts [54]. Aurora B facilitates proper function of the contractile ring, ensuring that the cleavage furrow forms at the appropriate time and location [55]. Absence of kinase may lead to incomplete cytokinesis, resulting in unviable polyploid cells.

Aurora C

Aurora C is the least studied of the three subtypes. Unlike Aurora A and B, Aurora C is expressed in germ cells but not in other tissues or cancer cell lines. Recent evidence shows that it has a mitotic distribution pattern similar to that of Aurora B [56]. Studies also suggest that it functions as a catalytic subunit of the chromosomal passenger protein [29,57]. In experimental settings, Aurora C has been shown to rescue Aurora B-depleted cells, suggesting similar functions [57]. Given the expression in testis, Aurora C is likely involved with chromosome segregation during spermatogenesis [36,58]. However, as it does not appear to regulate mitosis in somatic or tumor cells, further discussion on tumor development will focus on Aurora A and B.

Aurora kinase and tumorigenesis

Aurora A

Among the kinase subtypes, Aurora A has been most consistently implicated in tumorigenesis. Human Aurora A is located on chromosome 20q13.2, which is often amplified in many malignant diseases including breast, colon, bladder, ovarian, and pancreatic cancers [59–63]. Similarly, the levels of Aurora A transcriptions and protein expressions are increased in those tumors [64–67]. Overexpression has also been associated with malignant transformation in several cell lines including the NIH 3T3 and Rat1 fibroblasts [59,60]. In human tumors, it remains unclear whether the levels of expression correlate with histological grade. Some studies associate overexpression with higher grades and worse prognosis, whereas other studies find elevated levels in early stage diseases (i.e. ovarian cancer) [66,68,69]. Despite our limited understanding, current evidence suggests that Aurora A may be an important tumor-promoting factor.

There are several proposed mechanisms by which Aurora A overexpression gives rise to cancer. In normal cells, Aurora A is expressed during the G_2/mitosis transition point, with distribution to the centrosomes and mitotic spindles. In contrast, many tumor cells constitutively express Aurora A throughout the cytoplasm, regardless of the cell-cycle phase [64–66]. This process may allow over-phosphorylation of normal substrates during the G_1/S phases or improper targeting of cytoplasmic proteins, leading to neoplastic transformation.

Elevated levels of Aurora A also interfere with cell-cycle checkpoints. Normal cells have a late G_2 checkpoint, which prevents mitotic initiation in the presence of DNA damage. Aurora A overexpression disrupts this checkpoint, allowing genetically aberrant cells to enter mitosis and undergo cell division [70]. Interestingly, overexpression also interferes with a second checkpoint known as the spindle assembly checkpoint (SAC) during mitosis. Dysfunction of SAC permits progression from metaphase to anaphase despite improper spindle formation [71]. This may lead to resistance against chemotherapeutic agents such as taxanes, which depend on the activation of the mitotic checkpoint for cell arrest and eventual apoptosis.

Aurora A overexpression is also associated with polyploidy as a consequence of cytokinesis failure and unrestrained multinucleation [72]. Despite no evidence showing direct involvement with cytokinesis, given the similar phosphorylation motifs among the kinase subtypes, increased levels of Aurora A may alter the regulatory function of Aurora B. Furthermore, tumor cells that overexpress the kinase often form abnormal mitotic spindles that disrupt the SAC, leading to exit from mitosis without cytokinesis [73–75]. This produces tetraploid cells with duplicated genetic content and centrosomes.

Aneuploid cells are normally arrested by the post-mitotic G_1 checkpoint, eventually undergoing apoptosis [76,77]. However, the G_1 checkpoint relies on the p53 signaling pathway, which may be suppressed by Aurora A overexpression [77–82]. Consequently, aneuploid cells proceed through the cell cycle while undergoing further DNA replication. This allows the propagation of genetically aberrant cells. In theory, it remains unclear whether the levels of kinase activity correlate with cellular effects. Overexpression of inactive Aurora A appears to cause similar tetraploidy [72]. Several studies have suggested that even if kinase activity is not required for DNA duplication, it is important for malignant transformation and tumor growth [59,60].

Aside from checkpoint regulation, Aurora A also interacts with several tumor suppressor proteins including p53, BRCA1, and Chfr [82–86]. The association with p53 plays a critical role in tumorigenesis. Aurora A directly phosphorylates p53 at Ser^{315}, leading to Mdm2-mediated degradation of the tumor suppressor protein [82]. It also phosphorylates p53 at Ser^{215}, inhibiting p53 transcription and suppressing downstream targets such as p21 and PTEN [84]. Conversely, p53 induces the expression of Gadd45a, which inhibits Aurora A [87]. p53 may also directly disrupt the kinase by binding to its catalytic domain [88]. Other than p53, Aurora A also binds and phosphorylates BRCA1 at Ser^{308} [85]. In mouse embryo fibroblasts, the inhibition of serine phosphorylation leads to decreased mitotic activity. Aurora A also interacts with Chfr, which serves as a mitotic checkpoint regulator [86]. Chfr in turn ubiquinates the kinase, modifying its activity. Altogether, the tumor suppressor proteins and Aurora A are closely associated. Given that Aurora A overexpression

on its own may not cause cancer, additional factors such as inactivation of tumor suppressor proteins can play significant roles. This may explain why certain tumors (i.e. hepatocellular carcinomas) with both Aurora A overexpression and p53 mutation have worse prognosis than those with p53 mutation alone [68].

Besides the inactivation of tumor suppressor proteins, Aurora A also participates in other cellular pathways. In pancreatic cancer, Aurora A is a downstream target of MAPK1/ERK3 [89]. The expression of DUSP6/MKP3, an inhibitor of MAPK1/ERK3, is often suppressed in primary pancreatic cancers. As such, the constitutive activation of MAPK1 may lead to Aurora A overexpression. Furthermore, in human ovarian and breast cancers, Aurora A induces mRNA transcription of the human telomerase reverse transcriptase [90]. Aurora A binds to c-Myc sites on the promoter, leading to increased human telomerase reverse transcriptase expression and telomerase activity. Given that increased telomerase activity is associated with prolonged survival time, this may confer a survival advantage for the cancer.

Aurora B

Aurora B plays a less clear role in tumorigenesis. Aurora B is located on chromosome 17p13.1, which has not been associated with significant amplification. Despite reports of overexpression in certain cancers, the increased expression may simply reflect hyperproliferation rather than carcinogenesis [91]. This is especially relevant for mitotic products, which are often upregulated during rapid cell division. However, many studies now support an association between Aurora B and malignant transformation, with the involvement of additional factors. In one study, Aurora B overexpression alone did not transform rodent fibroblast cells [92]. However, increased kinase activity did facilitate Ras-induced transformation, which led to the production of aneuploid cells. In another study, overexpression of Aurora A and B gave rise to multinucleation with centrosome duplication [72]. This was enhanced by the absence of a p53-induced G_1 checkpoint, suggesting that the failure to detect polyploidy likely contributed to chromosomal aberrations.

In humans, Aurora B overexpression is found in several tumor types. In advanced colorectal cancer, increased kinase levels have been associated with poor prognosis [93]. Aurora B normally phosphorylates histone H3 at Ser^{10}, which may be important for chromatin stability following condensation. However, excessive phosphorylation has been linked to chromosome instability as well as increased tumor invasiveness. In astrocytoma, a strong correlation was found between Aurora B expression and histological grading [94]. Survival time was inversely related with mRNA/protein levels. A similar finding in endometrial carcinoma showed a close correlation between Aurora B expression and Ki-67 [95]. Patients with increased kinase levels had poor prognosis compared with those with normal levels. Finally, in primary lung carcinoma, aberrant transcriptional regulation was associated with Aurora B overexpression [96]. In addition, several lung stem-cell-associated genes, such as SERPINB5, TERT, and PRAME, were also upregulated. This was thought to contribute to allelic imbalance and progressive genetic instability.

Aurora kinase inhibitors

Aurora kinases are attractive targets for anti-tumor therapies, given their clear involvement with mitosis and apparent association with tumorigenesis. Early experiments validating these kinases as potential therapeutic targets were performed using gene-silencing techniques such as small interfering RNA and antisense oligonucleotides. More recent evidence linking kinase overexpression and/or dysfunction with neoplastic transformation has led to the development of multiple potent inhibitors.

In terms of Aurora A, many studies have shown that inhibition will lead to cell cycle delay at the G_2/M transition point followed by aberrant microtubule assembly. Although this finding initially made Aurora A the more attractive therapeutic target, recent studies also point to Aurora B as an important mitotic regulator. Inhibition of Aurora B gives rise to polyploid cells, which are less viable with greater tendency to undergo apoptosis. When both kinase subtypes are deactivated, a phenotype consistent with Aurora B inhibition alone is found. This finding suggests that Aurora B deficiency may bypass the Aurora A function during mitosis [97].

Early development of inhibitors

The first inhibitors to be described are Hesperadin, ZM447439, and VX680 (MK0457). Hesperadin specifically targets Aurora B ($IC_{50} = 50$ nm), inducing aberrant microtubule–kinetochore attachments and cytokinesis failure [33]. This agent promotes polyploidy formation, along with decreased histone H3 phosphorylation. Although Hesperadin has been a useful experimental tool for studying Aurora B function, it has not been further developed in clinical trials.

ZM447439 is a potent inhibitor of Aurora A and B ($IC_{50} = 110$ and 130 nm, respectively). Despite being a dual inhibitor, this agent produces a phenotype consistent with Aurora B inhibition. Neither the delayed mitotic entry nor the centrosome separation defect typical of Aurora A inhibition is seen [33,49]. Treated cells undergo apoptosis, after the aneuploid genome triggers the p53-dependent G_1 checkpoint. Like Hesperadin, ZM447439 is commercially available as a research tool but not as a therapeutic agent.

VX680 is an inhibitor of all Aurora kinase subtypes (IC = 0.6, 18, and 4.6 nm for A, B, and C respectively). It also inhibits the FMS-related tyrosine kinase 3 (FLT3),

which is frequently associated with refractory acute myelogenous leukemia. In addition, it has activity against BCR-ABL, including the T315I mutant that is resistant against imatinib, dasatinib, and nilotinib [98,99]. In preclinical studies, VX680 induced significant apoptosis in leukemia, lymphoma, and colorectal cell lines [100]. As with ZM447439, VX680-treated cells had increased polyploidy formation with decreased histone H3 phosphorylation. On the basis of encouraging preclinical data, Merck (Merck & Co., Inc., New Jersy, USA) sponsored several clinical trials under the new label MK0457. Unfortunately, the studies were discontinued after reports of treatment-associated QTc prolongation.

Inhibitors in recent development

Since the initial development of inhibitors, many other agents have been discovered and are undergoing clinical evaluation. Some of these drugs have selective activity against one Aurora kinase subtype whereas others exhibit pan-inhibitory effects. To facilitate drug delivery, several agents have been developed as orally bioavailable small molecule inhibitors. One example is MLN8054, which was the first orally administered and Aurora A-selective inhibitor ($IC_{50} = 4$ nm) to enter clinical trials. *In vivo*, MLN8054 in low concentrations led to mitotic accumulation with aberrant spindle assembly consistent with Aurora A selectivity [101]. Early experiments showed tumor shrinkage in several xenografts representing human lung, colon, and prostate cancers. Despite encouraging preclinical data, MLN8054 has now been replaced by a second generation agent known as MLN8237. This new inhibitor along with others in active clinical trials will be discussed.

AMG900

AMG900 (Amgen; One Amgen Center Drive, Thousand Oaks, California, USA) is an orally administered small molecule inhibitor of Aurora A, B, and C. A phase I study with dose escalation and expansion is at an early stage. Of interest, one taxane-resistant tumor type will be evaluated in each cohort of the expansion arm.

AT9283

AT9283 (Astex Therapeutics, Cambridge, UK) is a multitargeted kinase inhibitor of tyrosine and serine/threonine kinases with an IC_{50} of less than 10 nmol/l including Aurora A and B (both $IC_{50} = 3$ nm), JAK2, and Abl. Exposure of solid tumor and leukemic cell lines to AT9283 induces an 'aurora inhibitory' phenotype creating large aneuploid cells with limited replication potential. Cell survival decreases with increased duration of exposure.

A dose-escalation study carried in patients with refractory solid tumors used a 72 h intravenous (i.v.) infusion every 3 weeks in 22 patients [102]. Biological evidence of activity was seen across all dose levels with a dose-dependent reduction in histone H3 phosphorylation. Decrease of H3 phosphorylation was associated with p53 stabilization, which was more evident in patients with complete inhibition. The maximum tolerated dose was 27 mg/m^2/72 h (9 mg/m^2/day) and dose-limiting toxicity was febrile neutropenia. The best response seen was stable disease with three patients receiving at least six cycles of therapy (squamous cell carcinoma of the lung, colorectal adenocarcinoma). In another phase I study, AT9283 was administered by 72 h i.v. infusion to patients with refractory acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), high-risk myelodysplastic syndromes, and imatinib/dasatinib refractory chronic myeloid leukemia (CML) [103]. Once again, biological activity was correlated with reduction in histone H3 phosphorylation and caspase activation. The maximum tolerated dose was 108 mg/m^2/day, with dosing above this level associated with grade IV elevation in serum transaminases and myocardial infarction. Approximately one-third of patients with refractory AML experienced a significant reduction in bone marrow blasts. Furthermore, two patients with refractory CML exhibited a hematological response with one exhibiting ongoing partial cytogenetic response after four cycles of treatment.

AZD1152

AZD1152 (Astra Zeneca; AstraZeneca Pharmaceuticals LP, Washington, District of Columbia, USA) is a highly selective inhibitor of Aurora B ($IC_{50} = 0.37$ nm), with much less activity against Aurora A ($IC_{50} = 1369$ nm) or other serine–threonine/tyrosine kinases including FLT3, JAK2, and ABL. Consistent with the inhibition of Aurora B, AZD1152 induces chromosome misalignment, disrupts chromatid separation, and prevents proper cytokinesis. In an early study, AZD1152 exhibited significant inhibition of growth in human colon, lung, and hematologic tumor xenografts [104]. Furthermore, pharmacodynamic analysis revealed a temporal sequence of events including transient suppression of histone H3 phosphorylation followed by accumulation of tetraploid cells, associated with aberrant cell division and eventual apoptosis. In another study, AZD1152 inhibited the proliferation of cell lines representing AML, ALL, biphenotypic leukemia, acute eosinophilic leukemia, and the blast crisis of CML [105]. Interestingly, AZD1152 synergistically enhanced the anti-tumor activity of vincristine (tubulin depolymerizing agent) and daunorubicin (topoisomerase II inhibitor) against the leukemic cells *in vitro* and *in vivo*. More recently, a new study also showed that treatment of myeloma cell lines with AZD1152 induced apoptotic cell death, in a manner consistent with the expected cell cycle phenotype of Aurora B inhibition [106]. In some cases, the combination of AZD1152 with dexamethasone increased cell killing, which may warrant further clinical investigation. Taken together, these studies suggest that AZD1152 is a selective inhibitor of

tumor proliferation, with further activity enhancement in combination with other agents.

In a phase I dose escalation study, AZD1152 was administered to 13 patients as a 2 h i.v. infusion given weekly [107]. The patients had various tumor types including colon cancer ($n = 3$), melanoma ($n = 2$), prostate ($n = 2$), nasopharynx ($n = 1$), adenoid cystic carcinoma ($n = 1$), mesothelioma ($n = 1$), renal ($n = 1$), esophagus ($n = 1$), and pancreas ($n = 1$). Doses were escalated from 100 to 200, 300, and 450 mg. Dose-limiting toxicity was grade IV neutropenia in three patients at 450 mg, indicating a nontolerated dose on this schedule. Disease stabilization was observed in five patients who have remained on therapy for more than 12 weeks. Additional patients are being enrolled to confirm tolerance up to 300 mg.

CYC116

CYC116 (Cyclacel, Cyclacel Pharmaceuticals, New Jersey, USA) is an orally administered small molecule multikinase inhibitor with antineoplastic activity. In preclinical studies, CYC116 exhibits inhibitory effects on Aurora A and B as well as vascular endothelial growth factor receptor 2 resulting in the disruption of the cell cycle, rapid cell death, and the inhibition of angiogenesis. Phase I studies are in early stages.

ENMD2076

ENMD2076 (EntreMed, Inc., Rockville, Maryland, USA) is an orally administered Aurora A inhibitor with activity against other tyrosine kinases including vascular endothelial growth factor receptor 2. In preclinical studies, ENMD2076 caused dose-dependent inhibition in multiple xenograft models derived from breast cancer, colon cancer, and leukemia. In addition, the combination of ENMD2076 and lenalidomide showed synergistic cytotoxic activity toward several multiple myeloma cell lines. A similar effect was also seen in triple negative breast cancer models when ENMD2076 was given with cisplatin. Phase I studies are now underway.

MK5108

MK5108 (Merck & Co., Inc.,) is an orally administered, highly selective small molecule inhibitor of Aurora A ($IC_{50} = 0.084$ nm). Like other small molecule inhibitors, it competes for the ATP binding site on the Aurora kinase. The development of MK5108 follows earlier studies with MK0457, which was a pan-inhibitor of Aurora A and B. Clinical trials with MK0457 have since been discontinued given reports of treatment-associated QTc prolongation. As a selective inhibitor of Aurora A, MK5108 has been shown to delay entry into mitosis followed by the induction of the spindle checkpoint and eventual cell cycle arrest. Furthermore, preclinical studies indicate that MK5108 enhances the antiproliferative effects of other standard chemotherapies including the taxanes. In particular, the combination with docetaxel exhibited significant antitumor activity in various xenograft models representing human cervical, ovarian, and colorectal cancers. Interestingly, the combination also showed no effect in noncycling cells and minimal to no additive toxicity in animal models. A phase I dose escalation and pharmacodynamic study with MK5108 alone and in combination with docetaxel has recently been initiated.

MLN8237

MLN8237 (Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts, USA) is a second-generation, orally bioavailable, highly selective small molecule inhibitor of Aurora A. The potential benefits of MLN8237 over its parent compound, MLN8054, are an increased potency of inhibition accompanied by less benzodiazepine-like central nervous system effects. Potential antineoplastic activity may involve disruption of mitotic spindle assembly, chromosome segregation, and cell proliferation. In a pediatric preclinical testing program, MLN8237 exhibited significant in-vitro activity against the ALL cell line panel as well as high level of in-vivo activity against the ALL and neuroblastoma panels [108]. A second pediatric preclinical study showed that MLN8237 at 50% of its maximum tolerated dose induced complete remission of all three ALL xenografts and two out of three neuroblastoma xenografts [109]. Additional experiments also showed that MLN8237 can induce cytotoxicity and cell cycle arrest in multiple myeloma and diffuse large B-cell lymphoma models [110,111]. MLN8237 has recently entered several phase II trials to investigate its clinical activity against a wide range of solid and hematologic malignancies.

An early phase I study included 40 adult patients with various tumor types including colorectal (23%), non-small cell lung cancer (15%), head and neck (13%), and ovarian (10%) [112]. Anti-tumor activity was observed at the dose of MLN8237 (50 mg twice daily) for 7 days every 21 days. One patient with vaginal adenocarcinoma and metastatic lung disease had prolonged stable disease of almost 1 year. Another patient with platinum-refractory, radiation-resistant, metastatic ovarian cancer had tumor shrinkage after only two cycles. In the preliminary report, the main treatment-related adverse events were somnolence (30%), nausea (30%), neutropenia (20%), and fatigue (20%).

PF03814735

PF03814735 (Pfizer, New York, USA) is an orally administered reversible small molecule inhibitor of Aurora A and B with a broad spectrum of preclinical activity.

A phase I accelerated dose-escalation, pharmacokinetic, and pharmacodynamic study administered PF03814735 daily for 5 or 10 consecutive days in 3-week cycles [113]. In the preliminary report, 20 patients received a median of two cycles across seven doses ranging from 5 to 100 mg/day for 5 days. The patients had various tumor types including colorectal (five), breast (three), non-small

cell lung cancer (four), SCLC (two), bladder, melanoma, ovarian, renal, head and neck, and cancer of unknown primary (one each). Dose-limiting febrile neutropenia was observed in two of seven patients treated with 100 mg/day. Other common treatment-related adverse events include mild-to-moderate diarrhea (50%), vomiting (25%), anorexia, fatigue, and nausea (19% each). The maximum tolerated dose was defined as 80 mg/day for 5 days. Dose escalation in the 10-day schedule is still being conducted. No objective response has yet been reported.

PHA739358

PHA739358 (Nerviano Medical Sciences, Italy) is a potent inhibitor of all Aurora kinase members ($IC_{50} = 13$, 79, and 61 nm for A, B, and C respectively). Interestingly, PHA739358 also inhibits several receptor tyrosine kinases such as Abl (including the T315I mutant), Ret, and TrkA. These tyrosine kinases are involved in various malignancies including chronic myelogenous leukemia, acute lymphoblastic leukemia, thyroid cancer, and prostate cancer [114]. Despite activity against all Aurora kinase members, PHA739358 exhibits a dominant Aurora B inhibition phenotype with decrease of histone H3 phosphorylation and failure of cytokinesis, leading to polyploidy with eventual apoptosis. This effect has been observed in earlier RNA interference experiments in which the simultaneous inhibition of Aurora A and B resulted in a phenotype consistent with the inhibition of Aurora B alone [99]. In a preclinical study, PHA739358 showed significant antitumor activity against various xenografts representing acute myelogenous leukemia, colon cancer, breast cancer, ovarian cancer, and prostate cancer [114]. In the case of chronic myelogenous leukemia, PHA739358 binds with high affinity to the Abl kinase domain, including the T315I mutant, which might predict the therapeutic value in patients who are resistant to current first-line and second-line Abl kinase inhibitors [115]. By exhibiting activity against multiple kinase members, PHA739358 appears to be an attractive candidate for anti-tumor therapy.

In a phase I study, 40 patients with advanced solid tumors were given PHA739358 as a 24 h infusion without/with granulocyte colony-stimulating factor (GCSF) every 14 days per cycle [116]. Without GCSF, two patients developed neutropenic fever at 650 mg/m^2, leading to the recommended dose of 500 mg/m^2 for phase II trials. With GCSF, one patient developed grade IV neutropenia and three patients had grade II creatinine elevations at 1000 mg/m^2. As such, 750 mg/m^2 appears to be the maximum tolerated dose when given with growth factor support. In the preliminary report, 46% of the evaluable patients had stable disease as best response.

SNS314

SNS314 (Sunesis Pharmaceuticals, Inc., South San Francisco, California, USA) is an aminothiazole-derived urea with inhibitory effects on Aurora A ($IC_{50} = 9$ nm), B ($IC_{50} = 31$ nm), and C ($IC_{50} = 3$ nm).

In the preliminary report from a phase I study, nine patients with advanced solid tumors were given 3 h i. v. infusion once weekly × 3 (28 day cycle) [117]. The initial dose was 30 mg/m^2 with subsequent dose escalation of up to 120 mg/m^2 with no observed dose-limiting toxicities. Enrollment and dose escalation are ongoing.

Current development and future directions

In light of their association with tumorigenesis, Aurora kinases are attractive targets for cancer therapy. As we attempt to develop more effective inhibitors, several important issues are being addressed.

During the development of new drugs, the selection of appropriate biomarkers is essential for establishing target validation and confirming cellular activity. This is especially important for therapies that may be more successful at stabilizing disease than decreasing measurable tumor volume. An example of useful biomarkers is the phosphorylated histone H3 at Ser^{10}. This protein is abundant in mitotic cells and is easy to detect by western blot analysis. Inhibition of Aurora A and B may be differentiated based on the levels of phosphorylated histone H3. Given that Aurora A inhibition results in mitotic accumulation, the biomarker level is expected to increase. In contrast, Aurora B inhibition will decrease the biomarker level because histone H3 is a direct substrate of the kinase [34,51]. Initially developed for in-vitro experiments, phosphorylated histone H3 is now a useful biomarker in clinical studies.

In addition to phosphorylated histone H3, several other biomarkers have undergone recent investigation. In a preclinical study on PHA739358, bromodeoxyuridine incorporation and cyclin A levels were measured to follow cell proliferation *in vitro* and *in vivo* [118]. Meanwhile, caspase 3 was used to follow apoptosis. After treatment, bromodeoxyuridine incorporation and cyclin A-positive cells were decreased, whereas caspase 3 expression was slightly increased. In another study on MLN8054, Aurora A autophosphorylation on Thr^{288} was found to reflect the level of kinase activity [101]. Following drug exposure, tumor cells displayed decreased Thr^{288} phosphorylation along with increase mitotic index, consistent with mitotic accumulation. Altogether, these biomarkers are critical for confirming target selection and predicting clinical response. With the appropriate use of biomarkers in clinical trials, we can accelerate the development of new therapeutic agents.

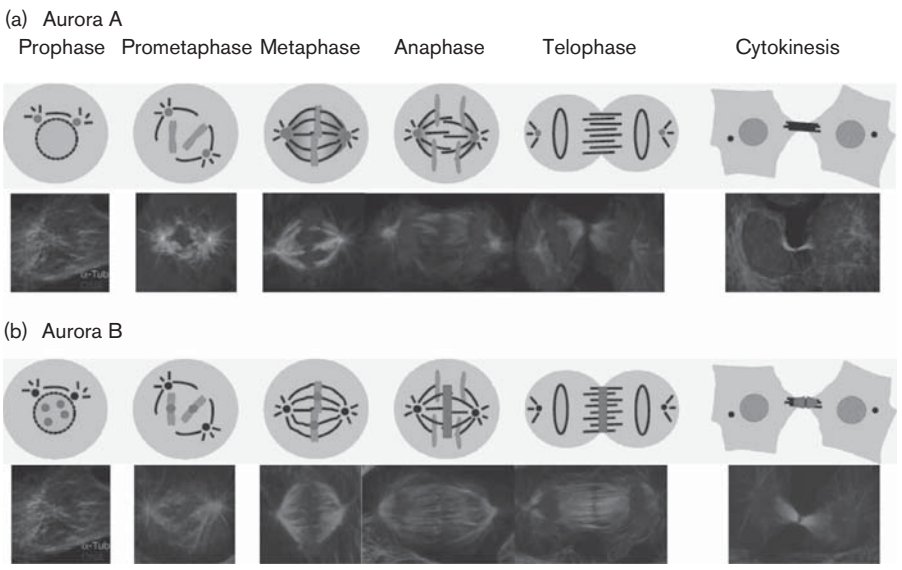
As more drugs are discovered, the identification of additional molecular targets can improve the success of clinical development. Many Aurora kinase inhibitors are active against other tyrosine kinases, which are involved with specific malignancies. For instance, VX680 (MK0457) was

shown to abolish colony formation of AML cells possessing mutated FLT3 kinases [100]. Given that these mutations are associated with constitutive activation and poor prognosis, VX680 may be a useful therapeutic agent. In another study, the same drug induced clinical response in patients with T315I phenotype refractory CML and Ph-positive ALL [119]. Inhibition of the T315I variant BCR-ABL kinase, which is typically associated with drug resistance, may yield significant clinical benefits. Other than VX680, many newer drugs including AT9283, AZD1152, and PHA739358 have shown activity against multiple tyrosine kinases. With the discovery of additional therapeutic targets, our understanding and clinical application of these novel agents become more sophisticated.

Similar to other targeted agents, many Aurora kinase inhibitors can enhance the activity of standard chemotherapeutic drugs. Examination for synergism is a critical aspect of drug development, with significant clinical implications. Many different combinations have shown encouraging results in in-vitro and in-vivo systems. In one preclinical study, AZD1152 increased the antiproliferative effects of vincristine and daunorubicin in human leukemic cell lines [105]. Another study showed that the combination of AZD1152 and CPT-11 is superior to either single agent alone in suppressing the growth of human colon carcinoma cell line [120]. Moreover, AZD1152 can radiosensitize tumor cell lines, resulting in greater cytocidal effects [121]. Other combinations with potential synergism include MK0457 and etoposide in ovarian and lung cancer cells [122]. MK0457 has also been shown to increase the activity of doxorubicin in prostate cancer cells [123]. In our endeavor to develop more successful treatments, the combination of Aurora kinase inhibitors with other agents allows further optimization of therapy (Fig. 1, Table 1).

Many issues that affect the current development of Aurora kinase inhibitors also influence our future approach toward creating new therapies. The application of selective biomarkers, the identification of secondary molecular targets, and the optimization of combination treatments are all important facets of future drug development. With more agents now undergoing investigation, additional challenges need to be addressed. For instance, several new drugs are formulated for oral administration, which can be associated with variable bioavailability. Hence, the determination of appropriate dosage and scheduling is critical to ensure adequate therapeutic levels. Furthermore, the toxicities of new drugs need to be fully examined. Up to now, clinical tolerability has generally been good with neutropenia being the most common dose-limiting toxicity in phase I studies. To evaluate additional acute effects or chronic sequela, longer follow-ups are required. Lastly, the standard response criteria by which drug efficacy is determined may not be suitable for new agents. Similar to other targeted drugs, Aurora kinase inhibitors may be more effective at stabilizing disease than reducing tumor bulk. Therefore, future clinical trials should incorporate additional endpoints based on time to tumor progression and quality-of-life improvements.

Fig. 1



Aurora kinase A and B (red) have different subcellular distributions during mitosis, as shown with DNA (blue) and microtubule spindles (green). (a) Aurora A initially localizes to the duplicated centrosomes from the S phase. During metaphase and anaphase, the kinase migrates to the spindle poles and remains at the centrosomes until the end of mitosis. (b) Aurora B initially appears in the nucleus during prophase. It then migrates to the kinetochores during prometaphase and metaphase, and later relocates to the mid-spindles during anaphase and telophase. Finally, it localizes to the contractile ring during cytokinesis. Adapted from Vader and Lens [10].

Table 1 Agents in active trials (enlisted with NIH)

Drug	Target	Route	Status	*ClinicalTrials.gov* identifier	Sponsor
AMG900	Aurora A, B, C	PO	Phase I (solid tumors)	NCT00858377	Amgen
AT9283	Aurora A, B (both $IC_{50}=3$ nm); JAK2 ($IC_{50}=1.2$ nm); Abl (T315I) ($IC_{50}=4$ nm); Flt3 ($IC_{50}=10$ nm)	IV	Phase I/II (leukemias) Phase I (solid tumors, NHL)	NCT00522990 NCT00443976	Astex Therapeutics
AZD1152	Aurora A ($IC_{50}=1369$), B ($IC_{50}=0.37$ nm), C ($IC_{50}=17$ nm); Flt3 (Kd=8 nm); Kit (Kd=17 nm)	IV	Phase I (AML) Phase I/II (AML) Phase I (solid tumors) Phase I (solid tumors)	NCT00530699 NCT00497991 NCT00338182 NCT00497731	Astra Zeneca
CYC116	Aurora A ($IC_{50}=44$), B ($IC_{50}=19$), C ($IC_{50}=65$); VEGFR2 ($IC_{50}=69$); Flt3 ($IC_{50}=88$)	PO	Phase I (solid tumors) Phase I (solid tumors)	NCT00530465 NCT00560716	Cyclacel
ENMD2076	Aurora A ($IC_{50}=14$ nm), B ($IC_{50}=290$ nm); Flt3 ($IC_{50}=3$ nm); VEGFR2 ($IC_{50}=36$); KIT ($IC_{50}=120$); Abl ($IC_{50}=295$); Abl (T315I) ($IC_{50}=81$ nm)	PO	Phase I (multiple myeloma) Phase I (advanced malignancies)	NCT00806065 NCT00658671	EntreMed
MK5108	Aurora A ($IC_{50}=0.084$ nm), B ($IC_{50}=27$ nm), C ($IC_{50}=19$ nm)	PO	Phase I (solid tumors)	NCT00543387	Merck
MLN8237	Aurora A ($IC_{50}=1$ nm), B ($IC_{50}=1100$ nm)	PO	Phase I (solid tumors) Phase I (advanced malignancies) Phase I (hematological malignancies) Phase I/II (solid tumors or ALL) Phase II (AML or MDS) Phase II (solid tumors) Phase II (NHL)	NCT00500903 NCT00651664 NCT00697346 NCT00739427 NCT00830518 NCT00853307 NCT00807495	Millennium Pharmaceuticals
PF03814735	Aurora A ($IC_{50}=5$ nm), B ($IC_{50}=0.8$ nm)	PO	Phase I (solid tumors)	NCT00424632	Pfizer
PHA739358	Aurora A ($IC_{50}=13$ nm), B ($IC_{50}=79$ nm), C ($IC_{50}=61$ nm); Abl ($IC_{50}=25$ nm); VEGFR3 ($IC_{50}=161$ nm)	IV	Phase II (CML) Phase II (prostate cancer) Phase II (multiple myeloma)	NCT00335868 NCT00766324 NCT00872300	Nerviano
SNS314	Aurora A ($IC_{50}=9$ nm), B ($IC_{50}=31$ nm), C ($IC_{50}=3$ nm)	IV	Phase I (solid tumors)	NCT00519662	Sunesis

ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; IV, intravenous; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; PO, oral; VEGFR2, vascular endothelial growth factor receptor 2.

References

1 Adams R, Carmena M, Earnshaw W. Chromosomal passengers and the (aurora) ABCs of mitosis. *Trends Cell Biol* 2001; **11**:49–54.

2 Nigg E. Mitotic kinases as regulators of cell division and its checkpoints. *Nat Rev Mol Cell Biol* 2001; **2**:21–32.

3 Glover D, Leibowitz M, McLean D, Parry H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. *Cell* 1995; **81**:95–105.

4 Fu J, Bian M, Jiang Q, Zhang C. Roles of aurora kinases in mitosis and tumorigenesis. *Mol Cancer Res* 2007; **5**:1–10.

5 Ducat D, Zheng Y. Aurora kinases in spindle assembly and chromosome segregation. *Exp Cell Res* 2004; **301**:60–67.

6 Kimura M, Matsuda Y, Yoshioka T, Okano Y. Cell cycle dependent expression and centrosome localization of a third human aurora/ lpl1 related protein kinase, AIK3. *J Biol Chem* 1999; **274**:7334–7340.

7 Giet R, Petretti C, Prigent C. Aurora kinases, aneuploidy and cancer, a coincidence or a real link? *Trends Cell Biol* 2005; **15**:241–250.

8 Andrews P. Aurora kinases: shining lights on the therapeutic horizon? *Oncogene* 2005; **24**:5005–5015.

9 Marumoto T, Zhang D, Saya H. Aurora A–a guardian of poles. *Nat Rev Cancer* 2005; **5**:42–50.

10 Vader G, Lens S. The Aurora kinase family in cell division and cancer. *Biochim Biophys Acta* 2008; **1786**:60–72.

11 Kimura M, Kotani S, Hattori T, Sumi N, Yoshioka T, Todokoro K, *et al*. Cell cycle dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of Drosophila and yeast Ipl1. *J Biol Chem* 1997; **272**:13766–13771.

12 Tanaka M, Ueda A, Kanamori H, Ideguchi H, Yang J, Kitajima S, *et al*. Cell cycle dependent regulation of human Aurora A transcription is mediated by periodic repression of E4TF1. *J Biol Chem* 2002; **277**:10719–10726.

13 Kimura M, Uchida C, Takano Y, Kitagawa M, Okano Y. Cell cycle dependent regulation of the human Aurora B promoter. *Biochem Biophys Res Commun* 2004; **316**:930–936.

14 Wang I, Chen Y, Hughes D, Petrovic V, Major ML, Park HJ, *et al*. Forkhead box M1 regulates the transcriptional network of genes essential for mitotic progression and genes encoding the SCF (Skp2-Cks1) ubiquitin ligase. *Mol Cell Biol* 2005; **25**:10875–10894.

15 Tang C, Chuang C, Hu H, Tang T. The zinc finger domain of Tzfp binds to the tbx motif located at the upstream flanking region of the Aie1 (Aurora C) kinase gene. *J Biol Chem* 2001; **276**:19631–19639.

16 Wittmann T, Wilm M, Karsenti E, Vernos I. TPX2, A novel xenopus MAP involved in spindle pole organization. *J Cell Biol* 2000; **149**:1405–1418.

17 Kufer T, Silljé H, Körner R, Gruss O, Meraldi P, Nigg E. Human TPX2 is required for targeting Aurora-A kinase to the spindle. *J Cell Biol* 2002; **158**:617–623.

18 Bayliss R, Sardon T, Vernos I, Conti E. Structural basis of Aurora-A activation by TPX2 at the mitotic spindle. *Mol Cell* 2003; **12**:851–862.

19 Eyers P, Erikson E, Chen L, Maller J. A novel mechanism for activation of the protein kinase Aurora A. *Curr Biol* 2003; **13**:691–697.

20 Hirota T, Kunitoku N, Sasayama T, Marumoto T, Zhang D, Nitta M, *et al*. Aurora-A and an interacting activator, the LIM protein Ajuba, are required for mitotic commitment in human cells. *Cell* 2003; **114**:531–532.

21 Hutterer A, Berdnik D, Wirtz-Peitz F, Zigman M, Schleiffer A, Knoblich J. Mitotic activation of the kinase Aurora-A requires its binding partner Bora. *Dev Cell* 2006; **11**:133–134.

22 Pugacheva E, Golemis E. The focal adhesion scaffolding protein HEF1 regulates activation of the Aurora-A and Nek2 kinases at the centrosome. *Nat Cell Biol* 2005; **7**:937–946.

23 Klein U, Nigg E, Gruneberg U. Centromere targeting of the chromosomal passenger complex requires a ternary subcomplex of Borealin, Survivin, and the N-terminal domain of INCENP. *Mol Biol Cell* 2006; **17**:2547–2558.

24 Sessa F, Mapelli M, Ciferri C, Tarricone C, Areces LB, Schneider TR, *et al*. Mechanism of Aurora B activation by INCENP and inhibition by hesperadin. *Mol Cell* 2005; **18**:379–391.

25 Vader G, Medema R, Lens S. The chromosomal passenger complex: guiding Aurora-B through mitosis. *J Cell Biol* 2006; **173**:833–837.

26 Jelluma N, Brenkman A, Van den Broek N, Cruijsen CW, Van Osch MH, Lens SM, *et al*. Mps1 phosphorylates Borealin to control Aurora B activity and chromosome alignment. *Cell* 2008; **132**:181–182.

27 Zachos G, Black E, Walker M, Scott MT, Vagnarelli P, Earnshaw WC, *et al*. Chk1 is required for spindle checkpoint function. *Dev Cell* 2007; **12**:167–168.

28 Han Z, Riefler G, Saam J, Mango S, Schumacher J. The C. elegans tousled-like kinase contributes to chromosome segregation as a substrate and regulator of the Aurora B kinase. *Curr Biol* 2005; **15**:894–904.

29 Li X, Sakashita G, Matsuzaki H, Sugimoto K, Kimura K, Hanaoka F, *et al*. Direct association with inner centromere protein (INCENP) activates the novel chromosomal passenger protein, Aurora-C. *J Biol Chem* 2004; **279**:47201–47211.

30 Littlepage L, Ruderman J. Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit. *Genes Dev* 2002; **16**:2274–2285.

31 Stewart S, Fang G. Destruction box-dependent degradation of aurora B is mediated by the anaphase-promoting complex/cyclosome and Cdh1. *Cancer Res* 2005; **65**:8730–8735.

32 Berdnik D, Knoblich J. Drosophila Aurora-A is required for centrosome maturation and actin-dependent asymmetric protein localization during mitosis. *Curr Biol* 2002; **12**:640–647.

33 Hauf S, Cole R, LaTerra S, Zimmer C, Schnapp G, Walter R, *et al*. The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J Cell Biol* 2003; **161**:281–294.

34 Giet R, Glover D. Drosophila aurora B kinase is required for histone H3 phosphorylation and condensin recruitment during chromosome condensation and to organize the central spindle during cytokinesis. *J Cell Biol* 2001; **152**:669–682.

35 Goto H, Yasui Y, Kawajiri A, Nigg EA, Terada Y, Tatsuka M, *et al*. Aurora-B regulates the cleavage furrow-specific vimentin phosphorylation in the cytokinetic process. *J Biol Chem* 2003; **278**:8526–8530.

36 Kimmins S, Crosio C, Kotaja N, Hirayama J, Monaco L, Höög C, *et al*. Differential functions of the Aurora-B and Aurora-C kinases in mammalian spermatogenesis. *Mol Endocrinol* 2007; **21**:726–739.

37 Zhao Z, Lim J, Ng Y, Lim L, Manser E. The GIT-associated kinase PAK targets to the centrosome and regulates Aurora-A. *Mol Cell* 2005; **20**:237–249.

38 De Luca M, Lavia P, Guarguaglini G. A functional interplay between Aurora-A, Plk1 and TPX2 at spindle poles: Plk1 controls centrosomal localization of Aurora-A and TPX2 spindle association. *Cell Cycle* 2006; **5**:296–303.

39 Petretti C, Savoian M, Montembault E, Glover D, Prigent C, Giet R. The PITSLRE/CDK11p58 protein kinase promotes centrosome maturation and bipolar spindle formation. *EMBO Rep* 2006; **7**:418–424.

40 Terada Y, Uetake Y, Kuriyama R. Interaction of Aurora-A and centrosomin at the microtubule-nucleating site in Drosophila and mammalian cells. *J Cell Biol* 2003; **162**:757–763.

41 Mori D, Yano Y, Toyo-oka K, Yoshida N, Yamada M, Muramatsu M, *et al*. NDEL1 phosphorylation by Aurora-A kinase is essential for centrosomal maturation, separation, and TACC3 recruitment. *Mol Cell Biol* 2007; **27**:352–367.

42 Toji S, Yabuta N, Hosomi T, Nishihara S, Kobayashi T, Suzuki S, *et al*. The centrosomal protein Lats2 is a phosphorylation target of Aurora-A kinase. *Genes Cells* 2004; **9**:383–397.

43 Barros T, Kinoshita K, Hyman A, Raff J. Aurora A activates D-TACC-Msps complexes exclusively at centrosomes to stabilize centrosomal microtubules. *J Cell Biol* 2005; **170**:1039–1046.

44 Maiato H, Rieder C, Khodjakov A. Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis. *J Cell Biol* 2004; **167**:831–840.

45 Khodjakov A, Cole R, Oakley B, Rieder C. Centrosome-independent mitotic spindle formation in vertebrates. *Curr Biol* 2000; **10**:59–67.

46 Koffa M, Casanova C, Santarella R, Köcher T, Wilm M, Mattaj I. HURP is part of a Ran-dependent complex involved in spindle formation. *Curr Biol* 2006; **16**:743–754.

47 Seki A, Coppinger J, Jang C, Yates J, Fang G. Bora and the kinase Aurora a cooperatively activate the kinase Plk1 and control mitotic entry. *Science* 2008; **320**:1655–1658.

48 Macùrek L, Lindqvist A, Lim D, Lampson MA, Klompmaker R, Freire R, *et al*. Polo-like kinase-1 is activated by aurora A to promote checkpoint recovery. *Nature* 2008; **455**:119–123.

49 Ditchfield C, Johnson V, Tighe A, Ellston R, Haworth C, Johnson T, *et al*. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J Cell Biol* 2003; **161**:267–280.

50 Murata-Hori M, Wang Y. The kinase activity of aurora B is required for kinetochore-microtubule interactions during mitosis. *Curr Biol* 2002; **12**:894–899.

51 Monier K, Mouradian S, Sullivan K. DNA methylation promotes Aurora-B-driven phosphorylation of histone H3 in chromosomal subdomains. *J Cell Sci* 2007; **120**:101–114.

52 Lan W, Zhang X, Kline-Smith S, Rosasco SE, Barrett-Wilt GA, Shabanowitz J, *et al*. Aurora B phosphorylates centromeric MCAK and regulates its localization and microtubule depolymerization activity. *Curr Biol* 2004; **14**:273–286.

53 Fang G. Checkpoint protein BubR1 acts synergistically with Mad2 to inhibit anaphase-promoting complex. *Mol Biol Cell* 2002; **13**:755–766.

54 Glotzer M. The molecular requirements for cytokinesis. *Science* 2005; **307**:1735–1739.

55 Yokoyama T, Goto H, Izawa I, Mizutani H, Inagaki M. Aurora-B and Rho-kinase/ROCK, the two cleavage furrow kinases, independently regulate the progression of cytokinesis: possible existence of a novel cleavage furrow kinase phosphorylates ezrin/radixin/moesin (ERM). *Genes Cells* 2005; **10**:127–137.

56 Tang C, Lin C, Tang T. Dynamic localization and functional implications of Aurora-C kinase during male mouse meiosis. *Dev Biol* 2006; **290**:398–410.

57 Sasai K, Katayama H, Stenoien D, Fujii S, Honda R, Kimura M, *et al*. Aurora-C kinase is a novel chromosomal passenger protein that can complement Aurora-B kinase function in mitotic cells. *Cell Motil Cytoskeleton* 2004; **59**:249–263.

58 Dieterich K, Soto Rifo R, Faure A, Hennebicq S, Ben Amar B, Zahi M, *et al*. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. *Nat Genet* 2007; **39**:661–665.

59 Bischoff J, Anderson L, Zhu Y, Mossie K, Ng L, Souza B, *et al*. A homologue of Drosophila aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J* 1998; **17**:3052–3065.

60 Zhou H, Kuang J, Zhong L, Kuo WL, Gray JW, Sahin A, *et al*. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. *Nat Genet* 1998; **20**:189–193.

61 Tanner M, Grenman S, Koul A, Johannsson O, Meltzer P, Pejovic T, *et al*. Frequent amplification of chromosomal region 20q12–q13 in ovarian cancer. *Clin Cancer Res* 2000; **6**:1833–1839.

62 Watanabe T, Imoto I, Katahira T, Hirasawa A, Ishiwata I, Emi M, *et al*. Differentially regulated genes as putative targets of amplifications at 20q in ovarian cancers. *Jpn J Cancer Res* 2002; **93**:1114–1122.

63 Rojanala S, Han H, Muñoz R, Browne W, Nagle R, Von Hoff DD, *et al*. The mitotic serine threonine kinase, Aurora-2, is a potential target for drug development in human pancreatic cancer. *Mol Cancer Ther* 2004; **3**:451–457.

64 Tanaka T, Kimura M, Matsunaga K, Fukada D, Mori H, Okano Y. Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. *Cancer Res* 1999; **59**:2041–2044.

65 Takahashi T, Futamura M, Yoshimi N, Sano J, Katada M, Takagi Y, *et al*. Centrosomal kinases, HsAIRK1 and HsAIRK3, are overexpressed in primary colorectal cancers. *Jpn J Cancer Res* 2000; **91**:1007–1014.

66 Gritsko T, Coppola D, Paciga J, Yang L, Sun M, Shelley SA, *et al*. Activation and overexpression of centrosome kinase BTAK/Aurora-A in human ovarian cancer. *Clin Cancer Res* 2003; **9**:1420–1426.

67 Li D, Zhu J, Firozi PF, Abbruzzese JL, Evans DB, Cleary K, *et al*. Overexpression of oncogenic STK15/BTAK/Aurora A kinase in human pancreatic cancer. *Clin Cancer Res* 2003; **9**:991–997.

68 Jeng Y, Peng S, Lin C, Hsu H. Overexpression and amplification of Aurora-A in hepatocellular carcinoma. *Clin Cancer Res* 2004; **10**:2065–2071.

69 Miyoshi Y, Iwao K, Egawa C, Noguchi S. Association of centrosomal kinase STK15/BTAK mRNA expression with chromosomal instability in human breast cancers. *Int J Cancer* 2001; **92**:370–373.

70 Marumoto T, Hirota T, Morisaki T, Kunitoku N, Zhang D, Ichikawa Y, *et al*. Roles of aurora-A kinase in mitotic entry and G2 checkpoint in mammalian cells. *Genes Cells* 2002; **7**:1173–1182.

71 Anand S, Penrhyn-Lowe S, Venkitaraman A. AURORA-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol. *Cancer Cell* 2003; **3**:51–62.

72 Meraldi P, Honda R, Nigg E. Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53–/– cells. *EMBO J* 2002; **21**:483–492.

73 Minn A, Boise L, Thompson C. Expression of Bcl-xL and loss of p53 can cooperate to overcome a cell cycle checkpoint induced by mitotic spindle damage. *Genes Dev* 1996; **10**:2621–2631.

74 Lanni J, Jacks T. Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. *Mol Cell Biol* 1998; **18**:1055–1064.

75 Rieder C, Maiato H. Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. *Dev Cell* 2004; **7**:637–651.

76 Andreassen P, Lohez O, Lacroix F, Margolis R. Tetraploid state induces p53-dependent arrest of nontransformed mammalian cells in G1. *Mol Biol Cell* 2001; **12**:1315–1328.

77 Margolis R, Lohez O, Andreassen P. G1 tetraploidy checkpoint and the suppression of tumorigenesis. *J Cell Biochem* 2003; **88**:673–683.

78 Di Leonardo A, Khan S, Linke S, Greco V, Seidita G, Wahl G. DNA rereplication in the presence of mitotic spindle inhibitors in human and mouse fibroblasts lacking either p53 or pRb function. *Cancer Res* 1997; **57**:1013–1019.

79 Khan S, Wahl G. p53 and pRb prevent rereplication in response to microtubule inhibitors by mediating a reversible G1 arrest. *Cancer Res* 1998; **58**:396–401.

80 Casenghi M, Mangiacasale R, Tuynder M, Caillet-Fauquet P, Elhajouji A, Lavia P, *et al*. p53-independent apoptosis and p53-dependent block of DNA rereplication following mitotic spindle inhibition in human cells. *Exp Cell Res* 1999; **250**:339–350.

81 Vogel C, Kienitz A, Hofmann I, Müller R, Bastians H. Crosstalk of the mitotic spindle assembly checkpoint with p53 to prevent polyploidy. *Oncogene* 2004; **23**:6845–6853.

82 Katayama H, Sasai K, Kawai H, Yuan ZM, Bondaruk J, Suzuki F, *et al*. Phosphorylation by aurora kinase A induces Mdm2-mediated destabilization and inhibition of p53. *Nat Genet* 2004; **36**:55–62.

83 Fujiwara T, Bandi M, Nitta M, Ivanova E, Bronson R, Pellman D. Cytokinesis failure generating tetraploids promotes tumorigenesis in p53-null cells. *Nature* 2005; **437**:1043–1047.

84 Liu Q, Kaneko S, Yang L, Feldman RI, Nicosia SV, Chen J, *et al*. Aurora-A abrogation of p53 DNA binding and transactivation activity by phosphorylation of serine 215. *J Biol Chem* 2004; **279**:52175–52182.

85 Ouchi M, Fujiuchi N, Sasai K, Katayama H, Minamishima YA, Ongusaha PP, *et al*. BRCA1 phosphorylation by Aurora-A in the regulation of G2 to M transition. *J Biol Chem* 2004; **279**:19643–19648.

86 Yu X, Minter-Dykhouse K, Malureanu L, Zhao WM, Zhang D, Merkle CJ, *et al*. Chfr is required for tumor suppression and Aurora A regulation. *Nat Genet* 2005; **37**:401–406.

87 Shao S, Wang Y, Jin S, Song Y, Wang X, Fan W, *et al*. Gadd45a interacts with aurora-A and inhibits its kinase activity. *J Biol Chem* 2006; **281**:28943–28950.

88 Eyers P, Maller J. Regulation of Xenopus Aurora A activation by TPX2. *J Biol Chem* 2004; **279**:9008–9015.

89 Furukawa T, Kanai N, Shiwaku H, Soga N, Uehara A, Horii A. AURKA is one of the downstream targets of MAPK1/ERK2 in pancreatic cancer. *Oncogene* 2006; **25**:4831–4839.

90 Yang H, Ou C, Feldman R, Nicosia S, Kruk P, Cheng J. Aurora-A kinase regulates telomerase activity through c-Myc in human ovarian and breast epithelial cells. *Cancer Res* 2004; **64**:463–467.

91 Katayama H, Brinkley W, Sen S. The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metas Rev* 2003; **22**:451–464.

92 Kanda A, Kawai H, Suto S, Kitajima S, Sato S, Takata T, *et al*. Aurora-B/AIM-1 kinase activity is involved in Ras-mediated cell transformation. *Oncogene* 2005; **24**:7266–7272.

93 Ota T, Suto S, Katayama H, Han ZB, Suzuki F, Maeda M, *et al*. Increased mitotic phosphorylation of histone H3 attributable to AIM-1/Aurora-B overexpression contributes to chromosome number instability. *Cancer Res* 2002; **62**:5168–5177.

94 Araki K, Nozaki K, Ueba T, Tatsuka M, Hashimoto N. High expression of Aurora-B/Aurora and Ipll-like midbody-associated protein (AIM-1) in astrocytomas. *J Neurooncol* 2004; **67**:53–64.

95 Kurai M, Shiozawa T, Shih H, Miyamoto T, Feng YZ, Kashima H, *et al*. Expression of Aurora kinases A and B in normal, hyperplastic, and malignant human endometrium: Aurora B as a predictor for poor prognosis in endometrial carcinoma. *Hum Pathol* 2005; **36**:1281–1288.

96 Smith S, Bowers N, Betticher D, Gautschi O, Ratschiller D, Hoban PR, *et al*. 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. *Br J Cancer* 2005; **93**:719–729.

97 Yang H, Burke T, Dempsey J, Diaz B, Collins E, Toth J, *et al*. Mitotic requirement for aurora A kinase is bypassed in the absence of aurora B kinase. *FEBS Lett* 2005; **579**:3385–3391.

98 Gizatullin F, Yao Y, Kung V, Harding M, Loda M, Shapiro G. The Aurora kinase inhibitor VX-680 induces endoreduplication and apoptosis preferentially in cells with compromised p53-dependent postmitotic checkpoint function. *Cancer Res* 2006; **66**:7668–7677.

99 Young M, Shah N, Chao L, Seeliger M, Milanov ZV, Biggs WH III, *et al*. Structure of the kinase domain of an imatinib-resistant Abl mutant in complex with the Aurora kinase inhibitor VX-680. *Cancer Res* 2006; **66**:1007–1014.

100 Harrington E, Bebbington D, Moore J, Rasmussen RK, Ajose-Adeogun AO, Nakayama T, *et al*. VX-680, a potent and selective small-molecule inhibitor of the Aurora kinases, suppresses tumor growth in vivo. *Nat Med* 2004; **10**:262–267.

101 Manfredi M, Ecsedy J, Meetze K, Balani SK, Burenkova O, Chen W, *et al*. Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase. *Proc Natl Acad Sci U S A* 2007; **104**:4106–4111.

102 Plummer E, Calvert H, Arkenau H, Lock V, Squires M, Smith DM, *et al*. A dose-escalation and pharmacodynamic study of AT9283 in patients with refractory solid tumours [abstract 2519]. *J Clin Oncol* 2008; **20** (**Suppl**):3158–3168.

103 Foran J, Ravandi F, O'Brien S, Borthakur G, Rios M, Boone P, *et al*. Phase I and pharmacodynamic trial of AT9283, an aurora kinase inhibitor, in patients with refractory leukemia [abstract 2518]. *J Clin Oncol* 2008; **20** (**Suppl**):7987–7990.

104 Wilkinson R, Odedra R, Heaton S, Wedge SR, Keen NJ, Crafter C, *et al*. AZD1152, a selective inhibitor of aurora B kinase, inhibits human tumor xenograft growth by inducing apoptosis. *Clin Cancer Res* 2007; **13**:3682–3688.

105 Yang J, Ikezoe T, Nishioka C, Tasaka T, Taniguchi A, Kuwayama Y, *et al*. AZD1152, a novel and selective aurora B kinase inhibitor, induces growth arrest, apoptosis, and sensitization for tubulin depolymerizing agent or topoisomerase II inhibitor in human acute leukemia cells in vitro and in vivo. *Blood* 2007; **110**:2034–2040.

106 Evans RP, Naber C, Steffler T, Checkland T, Maxwell CA, Keats JJ, *et al*. The selective Aurora B kinase inhibitor AZD1152 is a potential new treatment for multiple myeloma. *Br J Haematol* 2008; **140**:295–302.

107 Schellens J, Boss D, Witteveen P, Zandvliet A, Beijnen JH, Voogel-Fuchs M, *et al*. Phase I and pharmacological study of the novel aurora kinase inhibitor AZD1152 [abstract 3008]. *J Clin Oncol* 2006; **20** (**Suppl**):295–302.

108 Houghton P, Morton C, Maris J, Courtright J, Carol H, Lock RB, *et al*. Pediatric preclinical testing program (PPTP) evaluation of the Aurora A Kinase Inhibitor MLN8237. Pediatric Preclinical Testing Program Meeting 2008; abstract 2997.

109 Smith M, Houghton P, Morton C, Maris JM, Courtright J, Carol H, *et al*. Pediatric preclinical testing program (PPTP) stage 2 testing of the Aurora A kinase inhibitor MLN8237. Pediatric Preclinical Testing Program Meeting 2008; abstract 286.

110 Gorgun G, Calabrese E, Mani M, Hideshima T, Ikeda H, Perrone G, *et al*. A novel Aurora-a kinase inhibitor MLN8237 induces cytotoxicity and cell cycle arrest in experimental multiple myeloma models. ASH Meeting 2008; abstract 1719.

111 Huck J, Zhang M, Hyer M, Manfredi M. Anti-tumor activity of the Aurora a inhibitor MLN8237 in diffuse large B-cell lymphoma preclinical models. ASH Meeting 2008; abstract 1592.

112 Infante J, Dees E, Cohen R, Burris H, O'Neil B, Murphy P, *et al.* Phase I study of the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8237, a selective Aurora A kinase inhibitor, in the United States. EORTC-NCI-AACR 20th International Symposium 2008; abstract 280.

113 Jones S, Burris HA, Dumez H, Infante JR, Fowst C, Gerletti P, *et al*. Phase I accelerated dose-escalation, pharmacokinetic (PK) and pharmacodynamic study of PF-03814735, an oral aurora kinase inhibitor, in patients with advanced solid tumors: preliminary results [abstract 2517]. *J Clin Oncol* 2008; **20** (**Suppl**).

114 Carpinelli P, Ceruti R, Giorgini ML, Cappella P, Gianellini L, Croci V, *et al*. PHA-739358, a potent inhibitor of Aurora kinases with a selective target inhibition profile relevant to cancer. *Mol Cancer Ther* 2007; **6** (**12 Pt 1**):3158–3168.

115 Modugno M, Casale E, Soncini C, Rosettani P, Colombo R, Lupi R, *et al*. Crystal structure of the T315I Abl mutant in complex with the aurora kinases inhibitor PHA-739358. *Cancer Res* 2007; **67**:7987–7990.

116 Cohen R, Jones S, Von Mehren M, Cheng J, Spiegel DM, Laffranchi B, *et al*. Phase I study of the pan aurora kinases (AKs) inhibitor PHA-739358 administered as a 24 h infusion without/with G-CSF in a 14-day cycle in patients with advanced solid tumors [abstract 2520]. *J Clin Oncol* 2008; **20** (**Suppl**).

117 Robert F, Hurwitz H, Verschraegen C, Advani R, Berman C, Taverna P, *et al*. Phase 1 trial of SNS-314, a novel selective inhibitor of aurora kinases A, B, and C, in advanced solid tumor patients [abstract 14642]. *J Clin Oncol* 2008; **20** (**Suppl**).

118 Carpinelli P, Moll J. Aurora kinases and their inhibitors: more than one target and one drug. *Adv Exp Med Biol* 2008; **610**:54–73.

119 Giles F, Cortes J, Jones D, Bergstrom D, Kantarjian H, Freedman S. MK-0457, a novel kinase inhibitor, is active in patients with chronic myeloid leukemia or acute lymphocytic leukemia with the T315I BCR-ABL mutation. *Blood* 2007; **109**:500–502.

120 Nair J, De Stanchina E, Schwartz G. The topoisomerase I poison CPT-11 enhances the effect of the aurora B kinase inhibitor AZD1152 both in vitro and in vivo. *Clin Cancer Res* 2009; **15**:2022–2030.

121 Tao Y, Zhang P, Girdler F, Frascogna V, Castedo M, Bourhis J, *et al*. Enhancement of radiation response in p53-deficient cancer cells by the Aurora-B kinase inhibitor AZD1152. *Oncogene* 2008; **27**:3244–3255.

122 Sun C, Chan F, Briassouli P, Linardopoulos S. Aurora kinase inhibition downregulates NF-kappaB and sensitises tumour cells to chemotherapeutic agents. *Biochem Biophys Res Commun* 2007; **352**:220–225.

123 Lee E, Frolov A, Li R, Ayala G, Greenberg N. Targeting Aurora kinases for the treatment of prostate cancer. *Cancer Res* 2006; **66**:4996–5002.