Clinical Experience with Aurora Kinase Inhibitors: A Review

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Key Words. Aurora kinase • Mitosis • Targeted therapy • Drug development • Clinical trial

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ABSTRACT

The aurora kinase family of serine/threonine kinases comprises three members, designated auroras A, B, and C. Auroras A and B are essential components of the mitotic pathway, ensuring proper chromosome assembly, formation of the mitotic spindle, and cytokinesis. The role of aurora C is less clear. Overexpression of aurora A and B has been observed in several tumor types, and has been linked with a poor prognosis of cancer patients. Several small molecules targeting aurora kinases A and B or both have been evaluated preclinically and in early phase I trials. In this review we aim to summarize the most recent advances in the development of aurora kinase inhibitors, with a focus on the clinical data. The Oncologist 2009;14:780–793

Mitosis, the process by which a eukaryotic cell separates a complete copy of its duplicated genome into two identical sets in two daughter cells, is an extremely complex and tightly regulated process [1]. Central players in the mitotic process are the microtubules forming the mitotic spindle as well as the centrosome, an organelle that has an important role in regulating tubulin dynamics [2]. Defects in mitotic signaling pathways, including those involving tubulin dynamics, might lead to unrestrained growth, one of the hallmarks of cancer cells. The effectiveness of the taxanes and Vinca alkaloids in the treatment of many tumor types indicates the significance of the mitotic/tubulin machinery as a validated drug target [3, 4]. Recently, other mitosis-regulating molecules have been proposed as targets for anticancer drug development, including cyclin-dependent kinases [5], survivin [6], polo-like kinases [7], and the aurora kinases that will be discussed in this review. Members of the aurora kinase family have emerged as key regulators of the mitotic process. Furthermore, they are frequently overexpressed in various human malignancies, and overexpression in cancer patients correlates with a poor prognosis, exemplifying their significance for tumor formation and progression. These factors clearly demonstrate the feasibility of the aurora kinases as promising drug targets, which is underlined by the possibility to inhibit the auroras with small molecules. Several small molecules targeting one or more of the...
aurora family members have emerged and are currently being tested in clinical studies.

**THE AURORA FAMILY**

The aurora family of serine/threonine kinases contains three members in mammalian cells, designated auroras A, B, and C, which share the highest degree of sequence homology in their catalytic domains [8].

However, their expression pattern and cellular localization differ markedly. Auroras A and B are expressed in many different cell types, whereas the expression of aurora C seems to be restricted to testicular tissue [9]. Activation of the aurora kinases is dependent on cofactors, and many different cofactors are involved in the activation of auroras A, B, and C, respectively, although the precise mechanisms of aurora activation remain to be elucidated. The roles of aurora kinases A, B, and, to a much lesser extent, C, in mitosis have been extensively studied (Figure 1) as described in an excellent review by Vader and Lens [10]. Aurora A is required for centrosome maturation and division. Subsequently, the divided centrosomes migrate to opposite sites of the dividing cell to define the poles of the bipolar mitotic spindle [10]. This process, as well as the formation of the mitotic spindle, also requires aurora A function [11]. Entry into mitosis is also regulated by aurora A due to an effect on CDK1/cyclinB complexes, making aurora A also a cell cycle-regulating protein [12]. Aurora B is the catalytic component of the chromosomal passenger complex, which consists of three additional proteins: Survivin, borealin, and INCENP [13, 14]. This complex regulates chromosome condensation, probably via direct phosphorylation of histone H3 by aurora B [15]. Subsequently, aurora B directs the proper orientation of the chromosomes [16], the assembly of the mitotic spindle [17], and the correct attachment of the mitotic spindle to the chromosomes by destabilizing defective microtubule-chromosome attachments, leading to abrogation of the mitotic process [18]. These processes are controlled by the spindle checkpoint, a quality-control circuit that blocks the onset of mitosis until all chromosomes have achieved a bipolar attachment to the mitotic spindle [19]. The spindle checkpoint also requires aurora B function [14]. Finally, aurora B has a critical role in cytokinesis, the process...
whereby the cytoplasm of a single cell is divided to form two daughter cells. Abrogation of aurora B function results in polyploidy (a state when there are more than two homologous sets of chromosomes) as a result of cytokinesis failure [20]. The role of aurora C in mitosis is less well studied, although the protein has been implicated as a chromosomal passenger protein as well [21]. It is believed that aurora C function overlaps and complements the function of aurora B in mitosis [22]. The protein is known to play an important role during spermatogenesis [23, 24].

ROLE OF THE AURORAS IN CANCER

The important roles of the aurora kinases A and B in mitotic processes raised the inevitable question whether these proteins might be involved in tumorigenesis. Both auroras A and B are overexpressed in various tumor types, as summarized recently by Gautschi et al. [25]. The human aurora A gene resides at chromosome 20q13.2, a region frequently amplified in breast cancer [26]. Overexpression of aurora A or amplification of the aurora A gene has been identified in several malignancies including breast [27, 28], lung [29], head and neck [30], and colon [31] cancers. In these studies, aurora A overexpression was associated with poor differentiated tumors [29], a poor prognosis [27, 30], and genomic instability [31]. Also, several polymorphisms in the gene encoding for aurora A have been identified, of which the polymorphic substitution of isoleucine for phenylalanine at residue 31 (F31I) has been correlated with an increased risk of developing colon and breast cancers [32, 33]. The human gene encoding for aurora B is located on chromosome 17p13.1, a region that is not commonly amplified in human tumors. Overexpression of the aurora B gene has been observed in non-small cell lung carcinoma (NSCLC) [34], glioblastoma [35], and oral squamous cell carcinoma [36], and overexpression was correlated with poor differentiation [34], lymph node involvement [34], metastatic potential [36], and a shortened survival [35]. One study found a polymorphism in the aurora kinase B gene that predisposes for breast cancer [37]. Finally, a mutation in the gene encoding for aurora kinase C was described in a patient with NSCLC [38].

Despite their overexpression in various tumors, no clear role for the aurora kinases in tumorigenesis has been established. Aurora A overexpression leads to centrosome amplification, chromosome instability, and oncogenic transformation in mammalian cells, probably via inactivation of the p53 pathway [39, 40]. On the other hand, aurora A activity also depends on p53, since p53 can inhibit aurora A function either by directly binding to its catalytic domain [41] or via induction of Gadd45, a protein that inhibits aurora A function [42]. The tight relation between aurora A and p53 is confirmed by the correlation between aurora A expression and p53 status in mouse models and human tumors [43]. Aurora A alone is not a potent inducer of cellular transformation in primary cells and it is thought that additional oncogenic events, such as Ras activation, are needed for this to occur [44]. Moreover, it is not known whether the observed effects following aurora A overexpression can be attributed to aurora A kinase activity, since overexpression of kinase-dead aurora A caused a similar phenotype [45]. Altogether, aurora A is thought to be an important oncoprotein, despite the lack of direct evidence linking this protein with the process of tumor formation. Aurora B is believed to be of less importance for tumorigenesis, although one study concluded that aurora B expression increased invasiveness in xenograft experiments [46], and another study reported enhanced cellular transformation in cells expressing oncogenic Ras when aurora B was overexpressed [47]. Finally, no role in tumorigenesis has been suggested for aurora C.

TARGETING THE AURORAS

The clearly established role for the aurora kinases A and B in mitosis, accompanied by the evidence suggesting that deregulated aurora A and B expression is linked to tumorigenesis, raised the hypothesis that inhibiting these kinases might be a powerful antitumor strategy. Preclinical experiments revealed that knocking out either aurora A or aurora

Figure 2. Expected phenotypes from inhibition of aurora A and aurora B. The given phenotypes were observed in RNA interference experiments, and in experiments with selective small molecules directed against the aurora kinases.
B leads to distinct phenotypes, in accordance with the different functions of both kinases (Figure 2). RNA interference experiments showed that loss of aurora A leads to incorrect centrosome duplication and misalignment of chromosomes during metaphase [48]. Also, aurora A inhibition delayed the entry in mitosis, and finally caused cells to arrest at the G2/M checkpoint [49]. In contrast with aurora A-deficient cells, cells without aurora B function can progress through mitosis without delay. However, several defects arise without proper aurora B function, including abnormal chromosome-spindle attachments and cytokinesis failure [50]. The progression through subsequent rounds of mitosis without cell division depends on p53 function. p53 wild-type cells will arrest following endoreduplication, whereas p53-mutated cells are able to pass through another cell cycle, leading to polyploidy [51]. Eventually, these cells will die by apoptosis. These results led to the development of several small-molecule inhibitors of these kinases. Hesperadin (Boehringer Ingelheim, Ingelheim, Germany) and ZM447439 (AstraZeneca, Wilmington, DE) were the first proven small-molecule inhibitors of aurora kinases. Hesperadin is a specific inhibitor of aurora kinase B (Hespera was the opponent of Aurora in Greek mythology). The compound induced polyploidy in HeLa cells [52], and stopped cell growth of prostate and breast cancer cells [53]. In vitro, ZM447439 inhibits both aurora kinases A and B, and leads to failure of chromosome alignment, segregation, and cytokinesis, followed by cell death [54, 55]. Both compounds never entered clinical trials, probably due to the emergence of more potent and specific inhibitors of the aurora kinases. We will discuss the most relevant aurora kinase inhibitors that are in clinical development, starting with the pan-aurora kinase inhibitors, inhibitors that target multiple aurora family members. Subsequently, we will discuss inhibitors targeting aurora kinase A and, finally, we will discuss AZD1152, the only selective aurora kinase B inhibitor that has entered clinical trials. An overview of all compounds is given in Table 1; the chemical structures are presented in Figure 3.

### Clinical Experience with Inhibitors Targeting Multiple Aurora Family Members (Pan-Aurora Inhibitors)

#### Tozasertib
Tozasertib, originally developed as VX-680 by Vertex (Cambridge, MA) and later renamed MK-0457 by Merck

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<tr>
<th>Pan-aurora inhibitors</th>
<th>Aurora inhibition</th>
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<th>DLT</th>
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<td>Aurora A (IC&lt;sub&gt;50&lt;/sub&gt; 79 nM)</td>
<td>Aurora C (IC&lt;sub&gt;50&lt;/sub&gt; 61 nM)</td>
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<td>Diarrhea Mucositis Neutropenic infection</td>
<td>PR</td>
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<td>Aurora B (IC&lt;sub&gt;50&lt;/sub&gt; 83 nM)</td>
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<td>Aurora B (IC&lt;sub&gt;50&lt;/sub&gt; 31 nM)</td>
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<td>Aurora B (IC&lt;sub&gt;50&lt;/sub&gt; 172 nM)</td>
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<td>MLN8237</td>
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**Table 1. Aurora kinase inhibitors in clinical development**

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**Table 1. Aurora kinase inhibitors in clinical development**

**Abbreviations:** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; DLT, dose-limiting toxicity; IC<sub>50</sub>, half maximal inhibitory concentration; K<sub>i</sub>, inhibitory constant; PR, partial response; SD, stable disease.
(Whitehouse Station, NY), was the first aurora kinase inhibitor to be tested in clinical trials. The drug, a pyrimidine derivative, has affinity for all aurora family members at nanomolar concentrations with inhibitory constant values (K_i(app)) of 0.6, 18, and 4.6 nM for aurora A, aurora B, and aurora C, respectively [56]. Preclinical studies confirmed that tozasertib inhibited both aurora A and aurora B kinase activity [57], and activity has been reported against prostate [58], thyroid [59], ovarian [60], and oral squamous [61] cancer cell lines. Upon treatment with tozasertib, cells accumulate with a 4N DNA content due to a failure of cytokinesis. This ultimately leads to apoptosis, preferentially in cells with a compromised p53 function [62]. The first phase I study in patients with advanced solid tumors reported a good tolerability of tozasertib up to doses of 8 mg/m^2/hour, when administered by continuous 5-day intravenous infusion every 28 days. The dose-limiting toxicity (DLT) was asymptomatic neutropenia at 12 mg/m^2/hour, and escalation up to 10 mg/m^2/hour was under way at the time of the report at American Society of Clinical Oncology (ASCO) 2006. Three of the 16 treated patients achieved stable disease, and 2 of them completed 6 cycles [63]. Of interest is the observed activity of tozasertib in patients with T315I Abl-mutated chronic myeloid leukemia (CML) or Philadelphia chromosome-positive acute lymphocytic leukemia (ALL) [64]. This phenomenon is caused by the binding of the drug to the active conformation of the Abl kinase domain, thereby preventing phosphorylation of the protein kinase domain. This, together with the avoidance of the innermost cavity of the Abl kinase domain by tozasertib, explains the significant effectiveness of this compound against imatinib- and dasatinib-resistant forms of Abl [65]. Further structural studies explained the high affinity of tozasertib for both the aurora kinases, and imatinib-resistant forms of Abl by showing that the compound exploits a hydrophobic pocket in the active site that is present only in an inactive kinase conformation [66]. Tozasertib appeared to be particularly effective in bone marrow mononuclear cells obtained from acute myeloid leukemia (AML) patients with high aurora A expression. Therefore, aurora A expression may serve as a prognostic marker for leukemia patients treated with tozasertib [67]. A phase I study in patients with

![Figure 3](http://theoncologist.alphamedpress.org/)

Figure 3. Chemical structures of aurora kinase inhibitors that are currently in clinical trials. References indicate the source of the chemical structures.
leukemia was initiated in 2005. The study has been completed, but no reports have entered the public domain. Other clinical studies that were initiated include a phase I study in patients with CML and Philadelphia-positive ALL, a study in patients with colorectal cancer, a phase II study in patients with lung cancer, and a phase II study in patients with T315I mutant CML and Philadelphia chromosome-positive ALL (data obtained from http://clinicaltrials.gov). In November 2007, Merck suspended enrollment in clinical trials with tozasertib, pending a full analysis of all efficacy and safety data for this drug. The decision was based on preliminary safety data, in which a QTc prolongation was observed in one patient. However, recently at ASCO 2009, data of a phase I study of concomitant and consecutive treatment with dasatinib and tozasertib in CML and ALL patients were presented. Three patients (two patients with Ph+ ALL, and one with CML in blast crisis), all previously unsuccessfully treated with imatinib, were enrolled. The two ALL patients, both in hematological response after 3 months of treatment with dasatinib, subsequently received a 6-hour biweekly infusion of tozasertib at 64 mg/m²/hour. Both patients maintained the hematological response. The CML patient progressed on dasatinib, and was subsequently treated with a 5-day continuous infusion of tozasertib at 10 mg/m²/hour, every 4 weeks. This patient obtained a complete hematological response after one cycle of treatment, and the authors concluded that the sequential and concomitant administration of dasatinib and tozasertib is a promising strategy for refractory Ph+ CML and ALL [68].

**PHA-739358**

PHA-739358 (Nerviano Medical Sciences, Milan, Italy) is a small-molecule 3-aminopyrazole derivative with strong activity against aurora kinases A, B, and C (half maximal inhibitory concentration \[IC_{50}\] values of 13, 79, and 61 nM, respectively) [69]. Interestingly, this compound also inhibits several cancer-related tyrosine kinases at the nanomolar range, including fibroblast growth factor receptor-1, Ret, Trk-a, and Abl [69]. The crystal structure of PHA-739358 in complex with the T315I Abl mutant has been unraveled, providing an explanation for the activity of PHA-739358 on the T315I mutation [70]. In accordance with these findings, PHA-739358 is very effective against BCR-ABL-positive leukemia cell lines, including the imatinib-resistant cell lines harboring the T315I mutation [71]. Other sensitive tumor cell lines included several carcinomas, including those of the colon, breast, ovaries, and prostate [69]. Data of the first clinical phase I studies were presented at ASCO 2008. In the first study, PHA-739358, administered as 24-hour infusions once every 2 weeks, was well tolerated up to doses of 500 mg/m², which was considered the maximal tolerated dose (MTD). The DLT was febrile neutropenia. The most frequently observed hematological toxicities included grade 3 and 4 neutropenia and lymphocytopenia; nonhematological toxicities were mostly mild (grade I/II). The pharmacokinetic parameters were independent of dose and time, and characterized by a low interpatient variability. Clinically relevant disease stabilizations were observed in several patients. The second part of the study, where patients received colony-stimulating factor as bone marrow support, was still recruiting patients at the time of the report [72]. The second study investigated the tolerability, pharmacokinetics, and safety of PHA-739358 when administered as a 6-hour or 3-hour infusion on days 1, 8, and 15 every 4 weeks. During the first part of the study, patients received the drug as a 6-hour infusion. DLTs in this part of the study were observed in six patients, and neutropenia was the DLT for this schedule. The recommended phase II dose was identified at 330 mg/m². The most frequently observed adverse events included grade I/II anorexia, nausea, fatigue, and diarrhea. Pharmacokinetics were dose/time-independent, and clinically relevant disease stabilizations were observed in several patients. Pharmacodynamic analyses of skin biopsy samples revealed inhibition of histone H3 phosphorylation, a biomarker of aurora B inhibition, at doses of 190 mg/m² and higher. The second part of the study was ongoing at the time of the report, but the 330-mg/m² dose as a 3-hour infusion appeared to be less well tolerated, with two DLTs (grade 4 neutropenia and grade 3 fatigue) in seven treated patients [73]. Phase II studies with PHA-739358 are ongoing in patients with CML and refractory prostate cancer, and results of the study in patients with CML were reported recently. Twelve patients were included, and received doses from 250 to 400 mg/m²/day by a once-weekly 6-hour infusion, for 3 consecutive weeks every 4 weeks. Two patients, both with T315I-mutated BCR-ABL, achieved a complete hematologic response at 330 mg/m². The drug was, in accordance with the phase I results, well tolerated, with neutropenia as the principal toxicity. Pharmacodynamic data were also in agreement with phase I data, and pharmacodynamic analyses revealed treatment-associated decreases of CRKL (V-ckr sarcoma virus CT10 oncogene homolog [avian]-like) phosphorylation, a biomarker of BCR-ABL activity, in 10 of 11 evaluable patients [74].

**AS703569**

AS703569 (R763), developed by Merck Serono (Darmstadt, Germany) and Rigel Pharmaceuticals (South San Francisco, CA), is an orally available ATP-competitive inhibitor of auroras A, B, and C. The compound also inhibits other cancer-related kinases, including FLT3, making this
compound a good candidate for evaluation in patients with hematological malignancies. The drug demonstrated potent inhibition of several tumor cell lines in vitro, and in xenograft studies [75]. Three phase I studies are ongoing, two of which were recently presented at international meetings. The first trial, presented at ASCO 2007, is a two-arm phase I study that is conducted in patients with advanced solid tumors. Patients included in this study received AS703569 orally on days 1 and 8 (arm 1), or on days 1, 2, and 3 (arm 2) in 21-day cycles. At the time of the report, a total of 15 patients was included at two dose levels (6 and 12 mg/m²). The drug was well tolerated, and no study-related DLTs or serious adverse events were observed. No pharmacokinetic and pharmacodynamic data were available at the time of the report [76]. Recently, data of a second phase I trial with AT9283 was presented. In this phase I study in patients with advanced hematological malignancies, two dosing regimens were tested: days 1–3 and 8–10 of a 21-day cycle (regimen 1) and days 1–6 of a 21-day cycle (regimen 2). In regimen 1, 24 patients were treated up to dose levels of 47 mg/m². At this dose level, 2 DLTs were reported (both grade 3 diarrhea). In regimen 2, 21 patients were treated up to dose levels of 47 mg/m², and three DLTs were seen at this dose level (neutropenic infection and two cases of grade 4 mucositis). Consequently, in both regimens the doses were de-escalated to 37 mg/m², and enrollment was ongoing at the time of the report. The most frequently observed grade ≥3 toxicities included infections, neutropenia, thrombocytopenia, anemia, and gastrointestinal disorders. One patient with CML (T315I) had a hematological and cytogenetic response, three patients with AML achieved reduction in bone marrow (BM) and/or peripheral blasts, and one patient with CML achieved a partial response. Several other patients had disease stabilizations [77]. The third phase I study is evaluating R763/AS703569 in combination with a standard-of-care therapy in patients with advanced malignancies. This study was recently initiated, and is currently recruiting patients.

AT9283
AT9283 (Astellas Therapeutics, Cambridge, U.K.) is a multitargeted kinase inhibitor, with potent activity against aurora kinases A and B (IC₅₀ of approximately 3 nM) [78]. A variety of other cancer-related protein kinases is also inhibited by AT9283, including Abl kinase, JAK2, JAK3, Ret, and GSK3 beta. The inhibitory potential of AT9283 against the T315I Abl mutant makes this compound an attractive option for the treatment of imatinib-resistant patients with CML [79]. Clinical studies with this compound are ongoing, and preliminary results of a phase I study in patients with refractory leukemia were presented at ASCO 2008. Twenty-nine patients were included at the time of the report. AT9283 was administered by a 72-hour continuous intravenous (i.v.) infusion once every 3 weeks. The MTD was identified at a dose level of 108 mg/m²/day, as DLTs were observed at the next higher dose level of 162 mg/m²/day. The DLTs included grade IV elevation of serum aminotransferases in two patients. Another patient died from myocardial infarction shortly after completion of the AT9283 infusion. AT9283 administration was associated with predictable myelosuppression, particularly neutropenia. Several patients experienced clinical benefit following AT9283 treatment, including reductions in BM blasts in approximately 1/3 of the treated patients with AML. Also, two patients with refractory CML exhibited a hematological response. Pharmacodynamic and clinical observations in this trial suggest that expanding the duration of the infusion will increase the biological effect of AT9283, a hypothesis that will be explored in the near future [80]. A second phase I study, conducted in patients with advanced solid malignancies, was presented at ASCO 2009. Thirty-three patients had been treated up to doses of 12 mg/m²/day. This dose level was found to be intolerable, because three of six treated patients experienced a DLT (neutropenia in all cases). The MTD was set at 9 mg/m²/day. Pharmacokinetic analyses suggested an exposure that increased linearly with dose. Seven patients included in the study received an oral dose of AT9283 1 week prior to starting i.v. treatment. Pharmacokinetic analysis revealed a median oral bioavailability of 27%. Biological evidence of aurora B inhibition, manifest as a reduction in histone H3 phosphorylation in skin biopsies during the infusion, was observed at all dose levels. Best response in this study was a partial response in a patient with NSCLC, who was ongoing at the time of the report. Prolonged disease stabilizations were observed in 4 patients with various tumor types [81]. Currently, another phase I study is ongoing in patients with solid tumors or refractory non-Hodgkin lymphoma, investigating 24-hour infusions on days 1 and 8 every 3 weeks.

SNS-314
SNS-314 (Sunesis Pharmaceuticals, South San Francisco, CA) is a novel aminothiazole-derived urea that selectively inhibits auroras A, B, and C in the low nanomolar range, exhibits potent activity against various tumor cell lines, and also displays activity in xenograft models [82]. Recently, preliminary data of the first phase I study in patients with advanced solid tumors were presented at ASCO 2009. Thirty-two patients were included at the time of the report, at doses ranging from 30 to 1800 mg/m². The drug was administered as a 3-hour i.v. infusion on days 1, 8, and 15 in 28-day cycles. A DLT of grade 3 neutropenia preventing...
administration of all three doses was observed at 1,440 mg/m², but the MTD was not established at the time of the report. SNS-314 was generally well tolerated at the studied dose levels, with grade 1–2 gastrointestinal complaints and fatigue as the most frequently observed toxicities. The plasma pharmacokinetics were dose proportional, and there were no signs of drug accumulation following weekly administration of SNS-314. Pharmacodynamic analyses of pre- and post-dose skin biopsies suggested aurora B inhibition at doses of 240 mg/m² and higher. Six patients had stable disease as their best response, and the study was ongoing at the time of the report [83].

PF-03814735
PF-03814735 (Pfizer, New York) is an orally available aurora kinase inhibitor that is currently in phase I clinical trials. The compound inhibits aurora kinases A and B, and has a broad spectrum of clinical activity. Preclinical data have not been published; data of the first phase I study were presented at ASCO 2008. Twenty patients were recruited, and received doses up to 100 mg/day for 5 consecutive days in 3-week cycles. At this dose level, 2 of 7 patients experienced a DLT (neutropenia), and the next lower dose level was considered the MTD (80 mg/day). Pharmacokinetics were linear up to the highest dose level, and two patients had prolonged disease stabilization. The second part of the study will investigate a treatment schedule of 10 consecutive days in 3-week cycles. The study was recruiting patients for this schedule at the time of the report [84].

CLINICAL EXPERIENCE WITH SELECTIVE INHIBITORS OF AURORA KINASE A
MLN8054
MLN8054 (Millennium Pharmaceuticals, Cambridge, MA) is an orally available selective inhibitor of aurora kinase A (IC₅₀ = 4 nM), with a high selectivity over aurora kinase B (IC₅₀ = 172 nM), and a panel of other selected kinases [85]. Inhibition of aurora A by MLN8054 leads to the formation of abnormal mitotic spindles, and alignment defects during metaphase, ultimately resulting in aneuploidy and cell death [86]. The compound inhibited the growth of colorectal, prostate, NSCLC, breast, and ovarian cancer cell lines, and prostate and colorectal xenografted tumors in preclinical experiments [85]. Data of the first phase I study were presented at ASCO 2007. Initially, the drug was given once daily for 7 consecutive days, repeated every 21 days. At the time of the report, 22 patients had been treated, at doses up to 40 mg/day. Reversible grade 3 somnolence was observed in two of four patients treated at 40 mg/day, after which it was decided to change drug administration to divided doses on a q.i.d. schedule. Sixteen additional patients were treated at this schedule up to doses of 55 mg/day. Two of four patients treated at 55 mg/day experienced reversible grade 3 somnolence, resulting in a MTD of 45 mg/day at a q.i.d. schedule. In contrast with the other aurora kinase inhibitors, no myelosuppression was observed at any dose with either schedule. The occurrence of somnolence can be explained by the structural similarity of MLN8054 to benzodiazepines. MLN8054 binds to the gamma-aminobutyric acid alpha 1 benzodiazepine receptor. Therefore, it was decided to continue dose escalation with the coadministration of methylphenidate. Unfortunately, no accumulation of cells in mitosis was observed in skin biopsies of patients treated with MLN8054, suggesting insufficient target inhibition [87]. Recently, the final results of another phase I study with MLN8054 were presented. In this study, patients with advanced solid tumors received the drug once daily on days 1–5 and 8–12 or on days 1–14 in four divided doses, in 28-day cycles. Forty-three patients received doses up to 80 mg/day; dose escalation was stopped at this dose-level because of DLTs (grade 3 somnolence, liver function elevations, neutropenia, and mucositis, respectively). No responses were reported, although pharmacodynamic markers suggested aurora A inhibition at the higher dose levels [88, 89]. Following these results, MLN8054 was replaced by MLN8237, a second-generation aurora kinase A inhibitor.

MLN8237
MLN8237 (Millennium Pharmaceuticals) is the follow-up compound for MLN8054, with an increased potency of inhibition and a decreased benzodiazepine-like effect on the central nervous system [90]. MLN8237 demonstrated promising antitumor activity in preclinical models, particularly against neuroblastoma and ALL xenograft panels [91]. Results of the first phase I study were presented at an European Organisation of Research and Treatment of Cancer symposium last year. The compound was administered orally once daily to patients with advanced solid tumors for 7 days in 21-day cycles. Twenty-three patients had been treated at doses up to 150 mg/day, DLTs were observed in three of six patients treated at the highest dose level (neutropenia, grade 3 mucositis, and grade 3 somnolence). Upon administration, MLN8237 was rapidly absorbed, exposure increased with dose, and efficacious exposure was observed at the highest dose levels. One patient with metastatic ovarian cancer had preliminary evidence of antitumor activity; four patients had prolonged disease stabilization following treatment with MLN8237. Interestingly, no clinically significant benzodiazepine-like side effects were observed. The investigators planned to evaluate alternate dose groups including 110 mg once daily, 70–100 mg twice
AZD1152 (AstraZeneca) is a dihydrogen phosphate prodrug of a pyrazoloquinazoline aurora kinase inhibitor (AZD1152-hydroxyquinazoline pyrazol analide, AZD1152-HQPA), and is converted rapidly to the active drug in plasma [94]. AZD1152-HQPA is a potent and selective inhibitor of aurora kinase B (Ki(app) = 0.36 nM), compared with aurora kinase A (Ki(app) = 1369 nM), and has a high specificity over a panel of 50 other kinases [95]. AZD1152 potently inhibited the growth of human colon, NSCLC, and promyelocytic leukemia tumor xenografts in preclinical studies [96]. Moreover, the compound induced growth arrest and apoptosis in various leukemia cell lines, suggesting that AZD1152 is a promising new agent for treatment of individuals with leukemia [97, 98]. Another study showed encouraging antitumor activity of AZD1152 against a panel of myeloma cells, expressing high levels of aurora B, and suggested AZD1152 alone or in combination with dexamethasone as a potential treatment for patients with myeloma [99]. The first clinical study is completed, although no definitive report has been published. In this phase I trial, presented at ASCO 2006, AZD1152 was administered as a 2-hour infusion once every week to patients with advanced solid malignancies, and was tolerated well up to doses of 300 mg. DLT was grade 4 neutropenia in 3 patients treated at 450 mg, and bone marrow recovery was generally noted by 2 weeks after dose. Pharmacokinetic analyses revealed a rapid conversion to the active drug in plasma, and linear pharmacokinetics of both the prodrug and the active moiety. Significant disease stabilizations were observed in 5 of 13 treated patients [100]. Several other studies with AZD1152 are ongoing, including a phase I and a phase I/II study in patients with AML.

**Clinical Experience with a Selective Inhibitor of Aurora Kinase B**

**AZD1152**

AZD1152 is a selective inhibitor of aurora kinase B (Ki(app) = 0.36 nM), compared with aurora kinase A (Ki(app) = 1369 nM), and has a high specificity over a panel of 50 other kinases [95]. AZD1152 potently inhibited the growth of human colon, NSCLC, and promyelocytic leukemia tumor xenografts in preclinical studies [96]. Moreover, the compound induced growth arrest and apoptosis in various leukemia cell lines, suggesting that AZD1152 is a promising new agent for treatment of individuals with leukemia [97, 98]. Another study showed encouraging antitumor activity of AZD1152 against a panel of myeloma cells, expressing high levels of aurora B, and suggested AZD1152 alone or in combination with dexamethasone as a potential treatment for patients with myeloma [99]. The first clinical study is completed, although no definitive report has been published. In this phase I trial, presented at ASCO 2006, AZD1152 was administered as a 2-hour infusion once every week to patients with advanced solid malignancies, and was tolerated well up to doses of 300 mg. DLT was grade 4 neutropenia in 3 patients treated at 450 mg, and bone marrow recovery was generally noted by 2 weeks after dose. Pharmacokinetic analyses revealed a rapid conversion to the active drug in plasma, and linear pharmacokinetics of both the prodrug and the active moiety. Significant disease stabilizations were observed in 5 of 13 treated patients [100]. Several other studies with AZD1152 are ongoing, including a phase I and a phase I/II study in patients with AML.

**Other Aurora Kinase Inhibitors**

Other aurora kinase inhibitors that are currently being evaluated in phase I clinical trials include CYC116 (Cyclacel Pharmaceuticals, Berkeley Heights, NJ) and MK5108 (VX-689), developed by Merck and Vertex. However, data from these studies have not entered the public domain as far as we know. Recently, the development of another aurora kinase inhibitor MK6592 (VX-667) was terminated by Merck and Vertex, after the compound did not meet pharmacokinetic objectives in a phase I study. Aurora kinase inhibitors that demonstrated antitumor activity in preclinical studies, but have not entered clinical trials (yet), include the aforementioned hesperadin [52, 53] and ZM447439 [54, 55], PHA-680632 [101], PHA-680626 [102], AKI-001 [103], jadomycin B [104], and reversion [105].

**Discussion**

The mitotic/tubulin machinery is a validated drug target, exemplified by the success of the taxanes and Vinca alkaloids. Aurora kinase inhibitors potentially have a benefit over these agents, because they target only those cells that enter mitosis, possibly improving specificity for dividing cells. The aurora kinases have shown to be promising drug targets in preclinical models, and several agents have entered the clinic.

In most phase I studies described in this review, the aurora kinase inhibitors were well tolerated, showing reversible neutropenia as the DLT in the great majority of the tested compounds. This might constitute a problem in future combination studies with chemotherapeutic agents, since neutropenia is a dose-limiting side effect of several anticancer drugs. A strategy to reduce the severity of myelosuppression might be to coadminister granulocyte colony-stimulating factor, which has already been done in one of the phase I studies described [72].Interestingly, no thrombocytopenia was observed following treatment with the aurora kinase inhibitors described in this overview. This might be due to downregulation of aurora A and aurora B during maturation of megakaryocytes, the thrombocyte-producing bone marrow cells [106]. This downregulation leads to polyploidization, a step that is thought to be essen-
tial for megakaryocyte maturation and subsequent thrombocyte production [107].

Overall, the responses seen in the phase I studies reported in this overview in patients with solid tumors are rather disappointing. The majority of reports had disease stabilization as best response, with the exception of two partial responses in patients treated with AT9283 (NSCLC) and MLN8237 (ovarian). The promising activity seen in patients with hematological malignancies is probably due to cross-reactivity of the aurora kinase inhibitors with BCR-ABL, the fusion protein that is aberrantly expressed in these patients. The lack of objective responses in patients with solid tumors could be due to many different factors, including incomplete target inhibition or the occurrence of mutations in the targeted proteins. The latter issue was recently addressed [108]. Combining the existing aurora inhibitors with novel agents targeting mutated aurora kinases might overcome these problems in the future.

A matter of debate in the field of the aurora kinases is which of the auroras is the best drug target. This issue was addressed by several groups, and contradictory results have been reported. Results of experiments in pancreatic cell lines, using antisense oligonucleotides, showed that targeting aurora A has advantages over targeting aurora B, whereas no advantage was observed when both proteins were targeted simultaneously [109]. On the other hand, experiments in colon cells showed an advantage of aurora B inhibition over aurora A inhibition [110], suggesting that the role of the different aurora kinases might be cell line-dependent. Intriguing are the preclinical results with the dual aurora kinase inhibitors tozasertib (VX-680) and ZM447439. Following administration of these small molecules, phenotypes identical to inactivation of aurora B alone were observed, indicating that dual inhibitors act primarily via inhibition of aurora B [56, 110]. Also contributing to the ongoing debate are several recent publications regarding the role of aurora kinase A. One study found no role for aurora kinase A in RAS-MAPK-mediated cellular transformation [111], whereas another study concluded that aurora A is essential for maintaining genomic stability, and that aurora A is a tumor suppressor protein [112]. Altogether, these results question whether aurora A is the favorable drug target [113]. It is also uncertain whether in tumor cells aurora overexpression is the cause or consequence of tumorigenesis. It is plausible that the upregulation of cell cycle-regulating proteins is due to the increased turnover rate of cancer cells, and that inhibition of these proteins will not destroy the source of the malignant process. This hypothesis does not apply to leukemias expressing BCR-ABL. This aberrant protein is known to be the cause of the malignant transformation, thereby explaining the impressive activity of aurora kinase inhibitors with cross-reactivity toward this protein.

To increase the antitumor activity of the aurora kinase inhibitors in the clinic, combination therapy with cytotoxic anticancer agents, radiotherapy, or other targeted agents might be used in the future. Several preclinical studies have addressed this issue. Transcriptional silencing of aurora kinase A potentiates the effect of tubulin-targeting agents, including vincristine [114] and taxanes [115]. This synergy is also observed after treatment with tozasertib in combination with docetaxel [60]. Treatment with AZD1152, an aurora B inhibitor, was found to be synergistic with a variety of chemotherapeutic agents, including irinotecan, docetaxel, vinorelbine, gemcitabine, oxaliplatin, and 5-fluorouracil [116], and with vincristine and topoisomerase inhibitors in leukemia cell lines [98]. Moreover, this compound also potentiates the radiation response in p53-deficient cancer cells, suggesting synergy with radiotherapy [117]. Recently, synergy between tozasertib and vorinostat, a histone deacetylase inhibitor, was demonstrated in several leukemia cell lines [118, 119]. Finally, at ASCO 2009, it was reported that MLN8237 and rituximab, an anti-CD20 monoclonal antibody, reduced tumor burden in a synergistic mechanism in multiple diffuse large B-cell lymphoma tumor models [120].

Future studies should use validated biomarkers to assess the degree of target inhibition in patients who are treated with aurora kinase inhibitors. In some of the phase I trials described in this review, phosphorylation of histone H3 was used as a biomarker [73, 84, 121, 122]. This event is associated with aurora B activity, since aurora B directly phosphorylates histone H3 on serine 10, making it a useful tool for evaluating the degree of aurora B inhibition [123, 124]. Histone H3 phosphorylation is critical for the transformation of cancer cells, and might therefore be an anticancer target on its own [125]. The mitotic index constitutes a biomarker for aurora A activity, because inhibition of aurora A in cancer cells resulted in accumulation in mitosis [87, 92]. Another biomarker for aurora A activity is the degree of autophosphorylation on threonine 10, making it a useful tool for evaluating the degree of aurora B inhibition [123, 124]. Histone H3 phosphorylation is critical for the transformation of cancer cells, and might therefore be an anticancer target on its own [125]. The mitotic index constitutes a biomarker for aurora A activity, because inhibition of aurora A in cancer cells resulted in accumulation in mitosis [87, 92]. Another biomarker for aurora A activity is the degree of autophosphorylation on threonine 10, making it a useful tool for evaluating the degree of aurora B inhibition [123, 124]. Histone H3 phosphorylation is critical for the transformation of cancer cells, and might therefore be an anticancer target on its own [125].

Patient selection based on aurora A and/or aurora B overexpression may be a useful strategy to test whether these subpopulations will benefit from therapy with aurora kinase inhibitors. Some histologically aggressive lymphomas, for instance, are known to overexpress the aurora kinase A gene [50]. Finally, more work needs to be done to
determine the optimal treatment regimen for this class of molecules. One of the phase I studies described herein suggested that prolonged inhibition of the aurora kinases is beneficial [80]. If this holds true, there is a need for orally available aurora kinase inhibitors. Fortunately, several of these agents have entered clinical trials.

In conclusion, the aurora kinases were considered promising drug targets when their inhibitors first entered the clinic. However, the clinical activity of the aurora kinase inhibitors in patients with solid tumors has been rather disappointing. Future studies with aurora kinase inhibitors should focus on the possibility of combining these agents with radiotherapy, chemotherapy, or other targeted anticancer drugs.

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