Preclinical Evaluation of AMG 900, a Novel Potent and Highly Selective Pan-Aurora Kinase Inhibitor with Activity in Taxane-Resistant Tumor Cell Lines

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Abstract
In mammalian cells, the aurora kinases (aurora-A, -B, and -C) play essential roles in regulating cell division. The expression of aurora-A and -B is elevated in a variety of human cancers and is associated with high proliferation rates and poor prognosis, making them attractive targets for anticancer therapy. AMG 900 is an orally bioavailable, potent, and highly selective pan-aurora kinase inhibitor that is active in taxane-resistant tumor cell lines. In tumor cells, AMG 900 inhibited autophosphorylation of aurora-A and -B as well as phosphorylation of histone H3 on Ser10, a proximal substrate of aurora-B. The predominant cellular response of tumor cells to AMG 900 treatment was aborted cell division without a prolonged mitotic arrest, which ultimately resulted in cell death. AMG 900 inhibited the proliferation of 26 tumor cell lines, including cell lines resistant to the antimitotic drug paclitaxel and to other aurora kinase inhibitors (AZD1152, MK-0457, and PHA-739358), at low nanomolar concentrations. Furthermore, AMG 900 was active in an AZD1152-resistant HCT116 variant cell line that harbors an aurora-B mutation (W221L). Oral administration of AMG 900 blocked the phosphorylation of histone H3 in a dose-dependent manner and significantly inhibited the growth of HCT116 tumor xenografts. Importantly, AMG 900 was broadly active in multiple xenograft models, including 3 multidrug-resistant xenograft models, representing 5 tumor types. AMG 900 has entered clinical evaluation in adult patients with advanced cancers and has the potential to treat tumors refractory to anticancer drugs such as the taxanes. Cancer Res; 70(23); 1–9. ©2010 AACR.

Introduction
Somatic cell division is a complex and highly coordinated process that ensures faithful segregation of duplicated chromosomes into 2 daughter cells. Deregulation of the cell cycle is a hallmark of cancer, characterized by uncontrolled proliferation and defects in chromosome segregation. Antimitotic drugs that block tumor cell division are a proven intervention strategy in the treatment of cancer. However, the clinical benefits of classical antimitotic drugs may be hampered by development of multidrug resistance (MDR) and collateral damage to nondividing cells, including peripheral neuropathy (1). Aurora kinases are essential mitotic regulators and their potential role in tumorigenesis makes them attractive targets for anticancer therapy (2–4).

In mammalian cells, the aurora family of serine/threonine protein kinases is composed of 3 paralogous genes (aurora-A, -B, and -C). Aurora-A and -B are essential regulators of mitotic entry and progression, whereas aurora-C function is primarily restricted to male meiosis during spermatogenesis (5–10). Aurora-A can function as an oncogene and is amplified in a subset of human tumors. The expression of aurora-A and -B is frequently elevated in human cancers and is associated with advanced clinical staging (11).

The mitotic checkpoint, also referred to as the spindle assembly checkpoint (SAC), is a surveillance mechanism responsible for controlling proper alignment, microtubule–kinetochore attachments, and segregation of duplicated chromosomes (12). In tumor cells, genetic depletion or pharmacologic inhibition of aurora-A results in abnormal spindle formation and SAC activation. In contrast, depletion or inhibition of aurora-B inactivates the SAC, resulting in aborted cell division without a mitotic arrest. Importantly, dual suppression of aurora-A and -B phenocopies the effects of inhibiting aurora-B alone (13, 14). The silencing of the SAC leads to an accumulation of tumor cells that contain 4N DNA content in the G1 phase of the cell cycle. Continued suppression of
aurora-B activity leads to further rounds of genome replication without division, a process referred to as endoreduplication, which ultimately results in tumor cell death (15, 16). This mechanism of action is distinct from that of microtubule-binding antimitotic drugs (e.g., taxanes, vinca alkaloids, and epothilones) in that death of tumor cells is primarily driven by continued cell-cycle progression rather than by SAC activation and prolonged cell arrest in mitosis (16, 17).

Drug resistance is a major problem limiting the efficacy of many current anticancer therapies. The underlying mechanisms of clinical resistance to microtubule-binding agents are multifactorial and not fully understood (18). In cultured tumor cells, 2 prominent mechanisms of resistance to the taxanes are overexpression of drug efflux pumps and tubulin modifications (19–21). One strategy to overcome susceptibility to the effects of MDR would be to design a novel antimitotic drug candidate whose activity is not influenced by drug efflux, mediated by ATP-binding cassette (ABC) transporters such as P-glycoprotein (P-gp) (ABCB1) and BCRP (ABCG2) (22). Moreover, a small molecule inhibitor that is equipotent against 2 essential mitotic kinases may reduce the potential for resistance driven by target-modifying mutations (23, 24).

Currently, a variety of ATP-competitive inhibitors that target 1 or more of the aurora kinases and that have varying degrees of kinase specificity are in early clinical development (25). This report describes the preclinical activities of AMG 900, an orally bioavailable, potent, and selective pan-aurora kinase inhibitor with activity in MDR tumor cell lines. In contrast to paclitaxel and 3 well-characterized aurora kinase inhibitors (AZD1152, MK-0457, and PHA-739358), AMG 900 showed uniform potency across tumor cell lines, including P-gp and BCRP-expressing cell lines. Furthermore, AMG 900 was active in an HCT116 cell line adapted to grow in the presence of AZD1152. This HCT116 variant cell line carries a missense mutation in 1 allele of the aurora-B gene, resulting in an amino acid substitution (H222L) in its activation loop. In vivo, AMG 900 blocks the phosphorylation of histone H3, a proximal substrate of aurora-B (26), and inhibits the growth of multiple tumor xenografts, including 3 MDR xenograft models resistant to paclitaxel or docetaxel. Our data provide compelling evidence that AMG 900 may be active in tumors resistant to taxanes and 3 other well-characterized inhibitors that target aurora-B. AMG 900 is presently under clinical evaluation in adult patients with advanced cancers.

Materials and Methods

Chemistry

AMG 900 N-(4-((3-(2-amino-4-pyrimidinyl)-2-pyridinyl)oxy)phenyl)-4-(4-methyl-2-thienyl)-1-phthalazinamine) was synthesized at Amgen (WO 2007087276). The molecular structures have been disclosed for the following compounds: paclitaxel and docetaxel (18), MLN8054 (25), MK-0457 (4), AZD1152 (25), and PHA-739358 (25).

Cell lines

Tumor cell lines were obtained from the American Type Culture Collection (ATCC) unless otherwise specified. Cell lines were grown under recommended conditions. The HCT116 p21− and p21+ cell lines were obtained from Johns Hopkins University under a licensing agreement. The CAL51 cell line was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Brunswick, Germany). The MCF-7 p33− and p53− cell lines were generated as previously described (27). The MDA-MB-231-PTX and NCI-H460-PTX cell lines were established by growing the cells in the presence of increasing concentrations of paclitaxel over a period of 6 months. The HCT116 AZD1152-resistant cell line was established by growing the cells in the presence of AZD1152 at 80 nmol/L. The histone H2B-GFP (BD Biosciences) HeLa cell line was established at Amgen.

Animals

All experimental procedures were conducted in accordance with Institutional Animal Care and Use Committee and U.S. Department of Agriculture regulations. Four- to six-week-old female athymic nude mice (Harlan Sprague Dawley) were housed in sterilized cages and maintained under aseptic conditions. Eight-week-old female BDF1 mice (Charles River Laboratories) were housed 5 per filter-capped cage under pathogen-free conditions. The laboratory housing the animals provided alternating light and dark cycles (12 hours each) and met the standards of the Association for Assessment and Accreditation of Laboratory Animal Care specifications. Food, water, and nutritional supplements were offered ad libitum. All drugs were administered on the basis of individual body weight of each mouse.

Western blot analysis

Cell lysates were processed for Western blot analysis as previously described (27). Immunoblots were probed with the following antibodies: anti-aurora-A, anti-aurora-B (BD Biosciences), anti-p-aurora-A Thr288, anti-p-aurora-B Thr232, anticaspase-7 Asp198, and anti-total p53 (Cell Signaling), anti-β-actin (Sigma), and anti-p21 (Santa Cruz). Next, the immunoblots were probed with either anti-rabbit or antimouse IgG ( Vectastain kit; Vector Laboratories) and the protein bands were detected using the Lightning-Enhanced Chemiluminescence kit from PerkinElmer.

Details regarding enzyme assays, nocodazole block, cell imaging assays, colony formation assays, flow cytometry assays, aurora kinase gene analysis, pharmacodynamic assays, tumor xenograft models, neutrophil assessment study, and statistical analyses are described in the Supplementary Materials and Methods.

Results

AMG 900 is a potent and highly selective pan-aurora kinase inhibitor

The discovery of a novel class of ATP-competitive phthalazinamine small molecule inhibitors of aurora kinases led to the identification of AMG 900 (S. Geuns-Meyer, manuscript in preparation). AMG 900 inhibits the enzyme activity of all 3 aurora kinase family members with IC50 values of 5 nmol/L or less (Fig. 1A). To determine the specificity of AMG 900 across
the kinome, a panel of 26 kinases was screened and only p38α and TYK2 enzymes were inhibited (>50%) at concentrations of less than 500 nmol/L (Fig. 1A). Further screening was performed against a panel of 353 distinct kinases, using an ATP site–dependent competition binding assay (28). AMG 900 exhibited low nanomolar binding affinity for aurora kinases as well as interactions (K_d < 50 nmol/L) with DDR1, DDR2, and LTK receptor tyrosine kinases (Fig. 1A).

To investigate the inhibition of aurora kinase activity in cells by AMG 900, the levels of phosphorylated aurora-A Thr288, aurora-B Thr232, and histone H3 Ser10 (p-histone H3) were determined using Western blotting and quantitative cell imaging techniques. In HeLa cells, AMG 900 inhibited autophosphorylation of aurora-A and -B in a concentration-dependent manner (Fig. 1B). MLN8054 (aurora-A inhibitor) and AZD1152 (aurora-B inhibitor) selectively blocked the autophosphorylation of aurora-A Thr288 and aurora-B Thr232, respectively. In the cell imaging assay, HeLa cells treated with AMG 900 for 6 hours showed a concentration-dependent decrease in p-aurora-A Thr288 and p-histone H3 immuno-fluorescence staining with IC_{50} values of 6.5 and 8.2 nmol/L, respectively (Fig. 1C). Next, the effects of AMG 900 on chromosome dynamics and cell-cycle progression were examined by live-cell imaging of HeLa cells that express a histone H2B-GFP fusion protein. The top image panel of Figure 1D shows a representative DMSO-treated cell entering and exiting mitosis to yield 2 daughter cells. In contrast, a representative AMG 900–treated cell entering mitosis failed to properly congress chromosomes to the metaphase plate, which subsequently resulted in aborted cell division (bottom panel of Fig. 1D). These data demonstrate that AMG 900 does not induce a prolonged cell arrest in mitosis, consistent with inhibition of aurora-B and SAC silencing.

**Effects of AMG 900 on proliferating cells are consistent with inhibition of aurora-B activity**

Suppression of aurora-B activity in mitosis is known to induce polyploidy in tumor cells as a consequence of cytokinesis failure. To confirm this, we first determined the concentration of AMG 900 that completely suppressed p-histone H3 immunofluorescence staining (IC_{50} = 28 nmol/L) and p-aurora-A Thr288 (IC_{50} = 47 nmol/L) as a percentage of the DMSO control (POC). IC_{50} values represent 2 independent experiments run in duplicate (error bars, ±SD). D, representative live-cell imaging analysis of histone H2B-GFP HeLa cells treated with DMSO or AMG 900 at 50 nmol/L. Cells were imaged over a period of 75 minutes (lower right-hand corner of each image denotes the time in minutes).

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**Table 1.** Inhibition of enzymatic activity (n = 26) and enzyme binding affinity (n = 353)

<table>
<thead>
<tr>
<th>Kinase</th>
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<th>K_d (nmol/L)</th>
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<td>&gt;10,000</td>
<td>340 kinases</td>
<td>&gt;20% control at 1,000 nmol/L</td>
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**Figure 1.** AMG 900 is a potent and selective inhibitor of aurora-A, -B, and -C. A, chemical structure and enzyme selectivity profile of AMG 900. B, Western blotting of cell lysates from HeLa cells synchronized in mitosis and treated with DMSO, AMG 900 at the indicated concentrations, MLN8054 (aurora-A inhibitor) at 500 nmol/L, or AZD1152 (aurora-B inhibitor) at 1,000 nmol/L for 3 hours. C, Immunofluorescence imaging assay (ArrayScan VTi; Cellomics) was used to determine the inhibition of aurora kinase activity in HeLa cells. The concentration–response curves were calculated on the basis of decrease in the fluorescence intensity of p-histone H3 Ser10(*) and p-aurora-A Thr288(♦) as a percentage of the DMSO control (POC). IC_{50} values represent 2 independent experiments run in duplicate (error bars, ±SD). D, representative live-cell imaging analysis of histone H2B-GFP HeLa cells treated with DMSO or AMG 900 at 50 nmol/L. Cells were imaged over a period of 75 minutes (lower right-hand corner of each image denotes the time in minutes).
H3 in HCT116 cells (Fig. 2A). Treatment of HCT116 cells with 50 nmol/L of AMG 900 for 48 hours resulted in polyploidy and suppressed the formation of colonies after cell replating (Fig. 2B and C). Several reports have established that inhibition of aurora-B activates the postmitotic G1 checkpoint mediated by the p53 tumor suppressor protein (29, 30). Consistent with these findings, AMG 900 induced a significant increase in p53 and p21<sup>Cip1</sup> proteins that correlated with a corresponding decrease in 5-bromo-2′-deoxyuridine (BrdUrd) incorporation, a direct measure of DNA synthesis (Supplementary Fig. S1). To assess the kinetics of cell death induction by AMG 900, HCT116 cells were treated with the compound for 48 hours, washed twice, and then cultured in complete media lacking the compound for 0, 24, 48, and 72 hours. Treatment with AMG 900 induced a time-dependent increase in apoptosis measured by the induction of cleaved caspase-7 (Fig. 2D). Other mechanisms of cell death (e.g., mitotic catastrophe, multipolar cell division, and giant-cell necrosis) may also contribute to the loss of tumor cell viability after treatment with AMG 900 (unpublished observations; 31). Consistent with the highly selective profile of AMG 900, Jurkat cells treated with the compound for 48 hours over a 5,000-fold concentration range (0.0012–5 μmol/L) maintained a stable polyploidy phenotype, indicating that the dominant phenotype of AMG 900 inhibition is through inhibition of aurora-B (data not shown). AMG 900 had no obvious toxic effects on noncycling human foreskin fibroblast cells up to a concentration of 25 μmol/L; however, it did inhibit cell-cycle progression (without endoreduplication) and induced cell death in proliferating human bone marrow mononuclear cells at low nanomolar concentrations as expected with an on-mechanism effect of this agent (data not shown).

**AMG 900 blocks the proliferation of multiple human tumor cell lines including cell lines resistant to paclitaxel, AZD1152, MK-0457, and PHA-739358**

To investigate the antiproliferative effects of AMG 900, a panel of 26 cell lines from diverse tumor types were treated with the compound for 24 hours, washed twice in complete media lacking AMG 900, and then cultured for an additional 48 hours. Concentration–response curves and corresponding cell count EC<sub>50</sub> values were established for each cell line (Fig. 3A and B). The results show that AMG 900 inhibited cell proliferation, with EC<sub>50</sub> values ranging from 0.7 to 5.3 nmol/L. Importantly, 4 of these AMG 900-sensitive cell lines (HCT-15, MES-SA-Dx5, 769P, and SNU449) are resistant to paclitaxel and other anticancer agents (32–34). To further investigate the activity of AMG 900 in MDR cells, 4 tumor cell lines expressing P-gp were compared with 3 non-P-gp–expressing parental cell lines. As shown in Figure 3C, in a colony formation assay, there was a profound decrease in the number of detectable colonies in all of the cell lines treated with AMG 900 at 5 or 50 nmol/L. In contrast, paclitaxel failed to inhibit colony formation in all 4 of the P-gp–expressing tumor cell lines at concentrations effective in the parental cell lines. Intrigued by this potentially important finding, we sought to understand whether this was a common property of aurora kinase inhibitors. We, therefore, evaluated 3 well-characterized aurora kinase inhibitors (AZD1152, MK-0457, and PHA-739358) in a subset of MDR tumor cell lines expressing either P-gp or BCRP drug efflux.
transporters. Interestingly, AMG 900 inhibited p-histone H3 or induced polyploidy across all the cell lines tested irrespective of P-gp or BCRP status with uniform potency (IC\textsubscript{50} or EC\textsubscript{50} values ranging from 2 to 3 nmol/L). In contrast, the other aurora kinase inhibitors were less potent in 1 or more of the MDR cell lines compared with the matched sensitive tumor cell lines (Table 1). Inhibition of P\textsubscript{-}gp and BCRP drug pumps with GF120918 (21) sensitized HCT-15 and RPMI-8226 cells to AZD1152, suggesting that the loss of potency in the MDR cell lines was due to drug efflux (data not shown).

To investigate alternative mechanisms of resistance to aurora kinase inhibitors (35), HCT116 cells were adapted to grow in the presence of AZD1152. The activity of AMG 900 was then evaluated in the HCT116 parental and AZD1152-resistant cell lines. The cellular 4N or greater DNA content EC\textsubscript{50} values for AMG 900 were 2 and 5 nmol/L compared with 34 and 672 nmol/L for AZD1152, respectively (Supplementary Fig. S2A). AMG 900 inhibited the colony formation in both HCT116 cell lines at concentration levels 5 nmol/L or greater, whereas the variant subline was insensitive to AZD1152 at 50 nmol/L (Supplementary Fig. S2B). Both of the HCT116 cell lines were equally sensitive to paclitaxel and were negative for the expression of P-gp and BCRP (Supplementary Fig. S2C). Interestingly, the HCT116 variant subline harbors a missense mutation in 1 allele of the aurora-B gene (TGG → TTG; W221L), whereas no mutations were detected in the aurora-A and -C genes. These results suggest that AMG 900 maintains activity in tumor cells carrying a heterozygous mutation in aurora-B that may be responsible for resistance to AZD1152. Somatic point mutations at the analogous residue adjacent

Figure 3. AMG 900 inhibits cell proliferation and colony formation in human tumor cell lines at low nanomolar concentrations, including MDR cell lines resistant to paclitaxel. A and B, fluorescence-based cell count imaging assay (ArrayScan VTi) was performed on a panel of 26 tumor cell lines treated with AMG 900 (concentration range = 0.3–156 nmol/L). Cells were treated for 24 hours, washed twice, and incubated in complete media lacking inhibitor for 48 hours. A, a representative concentration–response curve of PC3 cells treated with AMG 900. B, cell lines rank ordered by sensitivity to AMG 900 and color coded by tumor origin. C, colony formation assay was performed in 3 paclitaxel-sensitive cell lines (parental) and in 4 MDR cell lines (P-gp expressing). Cells were treated with paclitaxel or AMG 900 at the indicated concentrations for 48 hours and replated in complete media lacking inhibitor. After the DMSO-treated cells reached confluence, the cells were stained with crystal violet and imaged (duplicate wells).
to the invariant DFG motif have been found in epidermal growth factor receptor and BRAF genes (L858R and L596R, respectively) in primary human tumors (36, 37). Studies are underway to more fully characterize the biological effects of this aurora-B mutation.

**AMG 900 inhibits the phosphorylation of histone H3 and suppresses the growth of human tumor xenografts in vivo**

To confirm that AMG 900 inhibits aurora-B activity in vivo, mice bearing established HCT116 tumors were administered a single oral dose of vehicle alone or AMG 900 at 3.75, 7.5, or 15 mg/kg. As shown in Figure 4A and B, administration of AMG 900 resulted in significant dose-dependent inhibition of p-histone H3 in tumors compared with the vehicle-treated control group (*P* ≤ 0.008). Furthermore, the degree of p-histone H3 suppression in tumors correlated with plasma drug concentrations. AMG 900 also inhibited p-histone H3 in mouse bone marrow cells at comparable doses and plasma drug concentrations (data not shown). In a separate multiple dose study using the HCT116 tumor xenograft model, AMG 900 induced a marked increase in the percentage of tumor cells with 4N or greater DNA content (Supplementary Fig. S3).

Next, we compared the inhibition of aurora-B activity, as measured by the degree and duration of p-histone H3 inhibition, with suppression of tumor growth in vivo. Mice bearing established HCT116 tumors were orally administered vehicle alone or AMG 900 at 3.75, 7.5, or 15 mg/kg twice daily (b.i.d.) for 2 consecutive days per week for 3 weeks. As shown in Figure 4C, intermittent administration of AMG 900 resulted in a dose-dependent inhibition of tumor growth compared with the vehicle-treated control group [3.75 mg/kg (40%), 7.5 mg/kg (64%), 15 mg/kg (75%); *P* ≤ 0.0001]. The main adverse effects observed with AMG 900 at the highest dose were moderate body weight loss (average of 7%; Supplementary Fig. S4A) with transient and reversible myelosuppression (data not shown). An alternative schedule based on daily dosing was also tested. Mice with established HCT116 tumors were orally administered vehicle alone or AMG 900 at 1.5, 2.25, or 3 mg/kg b.i.d. for 3 weeks (Fig. 4D). This continuous schedule with lower doses of AMG 900 resulted in significant tumor growth inhibition (TGI) when compared with the vehicle-treated control group [2.25 mg/kg (51% inhibition), *P* = 0.0019; and 3 mg/kg (74% inhibition), *P* < 0.0001]. Daily dosing with AMG 900 was well tolerated with no adverse effect on body weight (Supplementary Fig. S4B), although a decrease in neutrophil counts was observed at the end of the study (data not shown).

The effect of AMG 900 on tumor growth was further evaluated in a panel of human xenografts, including 3 MDR xenograft models, from 5 different tumor types (breast, colon, lung, pancreatic, and uterine). Mice bearing established tumors were orally administered AMG 900 at 15 mg/kg b.i.d. for 2 consecutive days per week or at 3 mg/kg b.i.d. every day for the duration of the study. As shown in Figure 5A, AMG 900 exhibited significant antitumor activity in all 9 xenograft models tested (50%–97% TGI compared with the vehicle-treated control group, *P* < 0.0005). Importantly, AMG 900 was active in the MES-SA-Dx5 (84% TGI, *P* < 0.0001) and NCI-H460-PTX (66% TGI, *P* < 0.0001) xenograft models that were resistant to either docetaxel or paclitaxel administered at their respective maximum tolerated doses (Fig. 5B and C). Together, these in vivo data provide evidence that AMG 900 inhibits the activity of aurora-B in HCT116 tumors and suppresses the growth of multiple xenografts that represent diverse tumor types. Notably, our data indicate that AMG 900 has the potential to treat tumors resistant to standard-of-care antimitotic drugs.

Because inhibition of aurora-B activity is known to cause myelosuppression (11, 25), studies were conducted to examine the effects of PEGylated-granulocyte colony-stimulating factor

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**Table 1. AMG 900 exhibits uniform potency in human tumor cell lines expressing P-gp and BCRP resistant to 1 or more aurora kinase inhibitors**

<table>
<thead>
<tr>
<th>Cell line designation</th>
<th>P-gp status</th>
<th>P-histone H3 assay, IC(_{50}) value (nmol/L)</th>
<th>≥ 4N DNA content assay, EC(_{50}) value (nmol/L)</th>
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(SD/02) administration on AMG 900–induced neutropenia. Mice were treated with AMG 900 at 15 mg/kg b.i.d. for 2 consecutive days alone or with SD/02 at 1 mg/kg, 1 day subsequent to the first day of AMG 900 treatment. Administration of AMG 900 alone showed a marked reduction in neutrophils by day 4 after treatment followed by a full recovery of neutrophils by day 8. Mice administered SD/02 the day after the initiation of AMG 900 treatment showed a significant decrease in the duration of neutropenia compared with AMG 900 treatment alone [days 6, 7, and 8 (P < 0.0018); Supplementary Fig. S5].

**Discussion**

Targeting the structural components of the mitotic machinery is a proven intervention strategy in the treatment of cancer. Microtubule-binding agents such as the taxanes and vinca alkaloids are highly active against a variety of human cancer types, although MDR and peripheral neuropathy remain persistent problems. Consequently, there is a need for novel antimitotic drugs that target nonmicrotubule proteins such as the mitotic kinases (38, 39). In the past decade, aurora-A and -B have gained prominence as essential regulators of somatic cell division and represent promising mitotic targets for anticancer therapy.

In this report, we have described the preclinical activities of AMG 900, a novel potent and highly selective pan-aurora kinase inhibitor. The predominant response of tumor cells to AMG 900 treatment was abortcd cell division without a protracted mitotic arrest, resulting in polyploidy-specific lethality. AMG 900 induces p53 and p21Cip1 proteins, which may act to regulate the rate of endoreduplication. Remarkably, AMG 900 is active in all of the tested tumor cell lines at low nanomolar concentrations, suggesting that it can inhibit the proliferation of tumor cells irrespective of genomic alterations or tumor origin. It is possible that the underlying profile of genetic and epigenetic modifications in tumor cells may play an important role in determining the fate of remnant cells that survive AMG 900 treatment. Because cells must pass through G2/M phase of the cell cycle in order for AMG 900 to inhibit aurora kinase activity, other factors such as mitotic and proliferation indices may impact tumor cell response. Further efforts will be required to define potential susceptibility markers to help identify the most AMG 900 vulnerable tumors as well as determining whether combinatorial approaches lead to durable tumor regression.

Despite the ongoing debate regarding the relevance of drug efflux–mediated clinical resistance, the consistent potency of AMG 900 against tumor cells irrespective of P-gp or BCRP status may circumvent a mechanism by which tumor cells...
could otherwise escape the activity of this experimental agent. Furthermore, we found that AMG 900 is active in an AZD1152-resistant cell line harboring a missense mutation in aurora-B, resulting in an amino acid substitution (W221L) in its kinase domain activation loop. A recent report has shown that other aurora-B mutant alleles (Y156H, G160V) located in the ATP-binding pocket confer resistance to multiple aurora inhibitors (35). It will be of interest to test whether AMG 900 inhibits aurora-B activity in these catalytic domain mutants.

Consistent with our in vitro observations, AMG 900 inhibits the phosphorylation of histone H3 and growth of multiple human tumor xenograft models, using either intermittent or continuous dosing schedules. Most relevant to our goal of developing an antimitotic agent that is effective against drug resistant tumors, AMG 900 shows promising antitumor activity in tumor xenografts resistant to taxanes. As anticipated, treatment of mice at efficacious doses of AMG 900 leads to a transient loss of body weight and reversible myelosuppression.

In summary, AMG 900 is an orally bioavailable, potent, and highly selective pan-aurora kinase inhibitor with activity in taxane-resistant tumor cell lines. Together, these features distinguish AMG 900 from other antimitotic drugs as well as other aurora kinase inhibitors (AZD1152, MK-0457, and PHA-739358). These key attributes contribute to the profile of this attractive clinical candidate; AMG 900 has entered phase 1 evaluation in adult patients with advanced cancers.

### Table 1: Tumor xenograft models

<table>
<thead>
<tr>
<th>Tumor xenograft model</th>
<th>Origin</th>
<th>Maximum TGI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231 Breast</td>
<td>82**</td>
<td></td>
</tr>
<tr>
<td>COLO 205 Colon</td>
<td>73*</td>
<td></td>
</tr>
<tr>
<td>HCT-15 (MDR) Colon</td>
<td>50*</td>
<td></td>
</tr>
<tr>
<td>HCT116 Colon</td>
<td>85**</td>
<td></td>
</tr>
<tr>
<td>NCI-H460 Lung</td>
<td>85**</td>
<td></td>
</tr>
<tr>
<td>NCI-H460-PTX (MDR) Lung</td>
<td>65**</td>
<td></td>
</tr>
<tr>
<td>MiaPaCa2 Pancreas</td>
<td>60**</td>
<td></td>
</tr>
<tr>
<td>MES-SA Uterine</td>
<td>87**</td>
<td></td>
</tr>
<tr>
<td>MES-SA-Dx5 (MDR) Uterine</td>
<td>84**</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5.** AMG 900 inhibits the growth of multiple human tumor xenografts *in vivo*, including multidrug resistant models. A, mice bearing established tumor xenografts were orally administered AMG 900 at 15 mg/kg b.i.d. for 2 consecutive days per week for 3 weeks or at 3 mg/kg b.i.d. everyday for 3 weeks. The maximum percentage of tumor growth inhibition (TGI) is reported for each xenograft model. Statistically significant TGI compared with the vehicle-treated control is denoted by asterisks (*, P < 0.005, **, P < 0.0005). B and C, mice bearing established multidrug resistant MES-SA-Dx5 or NCI-H460-PTX tumor xenografts were orally administered vehicle alone (○), AMG 900 at 15 mg/kg b.i.d. (●) for 2 consecutive days per week or at 3 mg/kg b.i.d. (△) everyday. As a control, mice were intraperitoneally administered docetaxel at 30 mg/kg (□) once per week or paclitaxel at 12.5 mg/kg (■) 5 days per week. Tumor volumes (mm³) are represented as mean ± SE (n = 10). Statistically significant TGI compared with the vehicle-treated control is denoted by an asterisk (*, P < 0.0019).
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank their Aurora team colleagues in the Departments of Lead Discovery, Oncology and Hematology Research, Pathology, Toxicology, Medicinal Chemistry, Pharmacokinetics and Drug Metabolism, and Pharmacaceutics for their contributions and to Julie Bailis, Greg Friberg, Robert Lobeg, and Glenn Begley for their critical reading of the manuscript. The authors also gratefully acknowledge the excellent technical assistance of Brian Belmontes, Jacob Corcoran, Tibor Gyuris, Scott Heller, Raheemuddin Khaja, and Ji-Rong Sun.

References

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Cancer Res  Published OnlineFirst October 8, 2010.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-10-3001

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