Research Article

MK-5108, a Highly Selective Aurora-A Kinase Inhibitor, Shows Antitumor Activity Alone and in Combination with Docetaxel

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Abstract

Aurora-A kinase is one of the key regulators during mitosis progression. Aurora-A kinase is a potential target for anticancer therapies because overexpression of Aurora-A, which is frequently observed in some human cancers, results in aberrant mitosis leading to chromosomal instability and possibly tumorigenesis. MK-5108 is a novel small molecule with potent inhibitory activity against Aurora-A kinase. Although most of the Aurora-kinase inhibitors target both Aurora-A and Aurora-B, MK-5108 specifically inhibited Aurora-A kinase in a panel of protein kinase assays. Inhibition of Aurora-A by MK-5108 in cultured cells induced cell cycle arrest at the G2-M phase in flow cytometry analysis. The effect was confirmed by the accumulation of cells with expression of phosphorylated Histone H3 and inhibition of Aurora-A autophosphorylation by immunostaining assays. MK-5108 also induced phosphorylated Histone H3 in skin and xenograft tumor tissues in a nude rat xenograft model. MK-5108 inhibited growth of human tumor cell lines in culture and in different xenograft models. Furthermore, the combination of MK-5108 and docetaxel showed enhanced antitumor activities compared with control and docetaxel alone–treated animals without exacerbating the adverse effects of docetaxel. MK-5108 is currently tested in clinical trials and offers a new therapeutic approach to combat human cancers as a single agent or in combination with existing taxane therapies. Mol Cancer Ther; 9(1); 157–66. ©2010 AACR.

Introduction

The Aurora kinase family comprises three highly homologous serine/threonine kinases (Aurora-A, Aurora-B, and Aurora-C) that play critical roles in regulating many mitosis processes (1). Numerous studies using biological tools and small-molecule inhibitors have provided insight to the roles of Aurora kinases in mitosis. Aurora-A is involved in regulating centrosome maturation, mitotic entry, spindle assembly, and chromosome alignment in the cell cycle (2–5). Inhibition or depletion of Aurora-A results in the misalignment of chromosomes, inducing the activation of the spindle assembly checkpoint and the accumulation of cells in the G2-M phase (6). Aurora-B is a chromosomal passenger protein that regulates multiple functions including the spindle assembly checkpoint and cytokinesis (6–9). Inhibition or deletion of Aurora-B results in abrogation of the spindle assembly checkpoint, exit from mitosis without proper chromosome segregation and cytokinesis (7, 8), and polyploidy. Aurora-C is the least studied Aurora kinase and its role in mitosis is not well defined.

Both Aurora-A and Aurora-B are recognized as attractive targets for anticancer chemotherapy. Several Aurora kinase inhibitors, such as MK-0457 (Tozasertib), PHA-739358 (Danusertib), AZD-1152, and MLN8054, have been reported in the literature and are being investigated in clinical trials (10–13). MK-0457 and PHA-739358 are inhibitors of Aurora-A and Aurora-B, and AZD-1152 is a selective inhibitor of Aurora-B. These compounds show typical phenotypes of Aurora-B inhibition, including the induction of polyploidy and reduction of phosphorylated Histone H3 (pHH3). MLN8054 shows a typical phenotype of Aurora-A inhibition in cells at certain doses, including the accumulation of G2-M cells and increase in pH3. The phenotypic differences on the selective inhibition of Aurora-A, inhibition of both Aurora-A and Aurora-B, or the selective inhibition of Aurora-B suggest that selectively targeting Aurora-A or Aurora-B may provide different therapeutic outcomes and/or safety profiles (14).
Aurora-A is associated with tumorigenesis (15–20). Overexpression of Aurora-A is frequently observed in multiple tumor types and is correlated with poor prognosis (1). In breast cancer patients, high Aurora-A mRNA levels correlated with a low response rate to docetaxel (21). In HeLa cells, overexpression of Aurora-A abrogates the spindle assembly checkpoint and makes cells resistant to paclitaxel (22). Conversely, inhibition of Aurora-A by RNAi sensitizes tumor cells to paclitaxel or docetaxel (23–25). In addition, Aurora-A was identified by small interfering RNA (siRNA) screening as a gene that enhances the cytotoxicity of paclitaxel (26). These data indicate that selective inhibition of Aurora-A by a small-molecule inhibitor might sensitize tumor cells to taxanes.

This study presents MK-5108, a novel, highly potent, and selective inhibitor of Aurora-A, which inhibits the growth of multiple human cancer cell lines in vitro and in human cancer xenograft models. Most importantly, MK-5108 significantly enhanced the efficacy of docetaxel without increasing its toxicity. MK-5108 is being developed in clinical trials in monotherapy and in combination with taxanes.

Materials and Methods

Compounds and Cell Lines
MK-5108 was synthesized at Banyu Tsukuba Research Institute. Docetaxel was purchased from Sigma-Aldrich and Sanoﬁ-Aventis. HeLa-luc cells were obtained from Xenogen Corp. All other cell lines were obtained from the American Type Culture Collection.

Biochemical Kinase Assays
Recombinant His-tagged human Aurora-A protein was expressed in Escherichia coli and was puriﬁed with HisTrap HP column (GE Healthcare). Puriﬁed recombinant human Aurora-B and Aurora-C protein were purchased from Carna Biosciences. Experiments were done in quintuplicate in 96-well plates. The Aurora-A assay reaction was conducted in the presence of 20 μmol/L ATP, 25 μmol/L Tetra-Kemptide [RRR(GLRRASLG)4R-NH2], 1.0 μCi per well [γ-33P]-ATP, 0.1 ng per well Aurora-A in 50 mmol/L Tris-HCl (pH 7.4), 15 mmol/L Mg(OAc)2, and 0.2 mmol/L EDTA at 30°C for 40 min. To investigate the inhibition mode of MK-5108 for Aurora-A, the IC50 values of MK-5108 were determined in the presence of different concentrations of ATP. Then, the IC50 value was plotted as a function of ATP concentration to analyze the effect of ATP concentration on the IC50 value of MK-5108. The Aurora-A assay reaction was conducted in the presence of 15 μmol/L ATP, 100 μmol/L Kemptide (GLRRASLG-NH2), 1.0 μCi per well [γ-33P]-ATP, 5.0 ng per well Aurora-B in 50 mmol/L Tris-HCl (pH 7.4), 15 mmol/L Mg(OAc)2, and 0.2 mmol/L EDTA at 30°C for 20 min. The Aurora-C assay reaction was conducted in the presence of 40 μmol/L ATP, 100 μmol/L Kemptide, 1.0 μCi per well [γ-33P]-ATP, 15 ng per well Aurora-C in 10 mmol/L MOPS-NaOH (pH 7.4), 5 mmol/L Mg(OAc)2, 1 mmol/L (±) DTT, and 1 mmol/L EDTA at 30°C for 20 min. After kinase reactions were terminated by adding 2.0% phosphoric acid, Tetra-Kemptide or Kemptide was trapped on the MultiScreen-PH plate. Wells were washed ﬁve times with 0.64% phosphoric acid and then monitored for radioactivity in a liquid scintillation counter (TOPCOUNT-HTS; Packard).

In vitro pH3 Assay
HeLa-S3 cells were synchronized at the G1-S phase boundary by double thymidine block with 2 mmol/L thymidine. Cells were washed and seeded to 96-well cell culture plates. After 4 h, an equal volume of medium

![Figure 1. MK-5108 is a highly potent and selective inhibitor of Aurora-A kinase. A, chemical structure of MK-5108, trans-4-(3-chloro-2-fluorophenoxy)-1-[6-(1,3-thiazol-2-ylamino)pyridin to 2-yl]methyl) cyclohexanecarboxylic acid. B, inhibition curves of MK-5108 against Aurora-A (●), Aurora-B (▴), and Aurora-C (●). C, MK-5108 inhibited Aurora-A in an ATP-competitive manner. IC50 values of MK-5108 in the presence of different concentrations of ATP are shown.](image-url)
containing MK-5108 was added to each well. Nocodazole (300 nmol/L) was used as a 100% control. The cells were fixed overnight with cold methanol 12 h after seeding. Then, the cells were stained with rabbit anti-phosphohistone H3 Ser28 antibody (Stressgen) and then with anti-rabbit IgG-Cy5 (Chemicon). Total nuclei were stained with 10 mg/mL 4′,6-diamidino-2-phenylindole (Sigma-Aldrich). Immunostained images were acquired using the IN Cell Analyzer1000 (GE Healthcare) with ×10 objective lens. After acquisition of images, data were analyzed using the Developer Toolbox 1.0 software (GE Healthcare). The %pHH3-positive index was determined by measuring the %pHH3-positive cell counts per total nuclei counts for each sample, then by normalizing with respect to nocodazole-treated cells.

**Cell Cycle DNA Analysis**

HeLa-S3 cells were seeded in six-well plates and were incubated overnight, and then MK-5108, MLN8054, and docetaxel were added to each well. After incubation for the indicated time periods, cells were harvested by trypsinization, were fixed using 70% ice-cold ethanol, and were stored at −20°C until staining. After treatment with RNase, cells were stained with propidium iodide. DNA content of cells was measured by FACSCalibur (BD Biosciences). Cell cycle profile and sub-G1 population was calculated using the ModFitLT (BD Biosciences) and CellQuest (BD Biosciences) software, respectively.

**Cell Proliferation Assay**

Cells were seeded in 96-well plates then incubated overnight. A medium containing MK-5108, docetaxel, or DMSO (Sigma-Aldrich) control was added and was incubated for 72 h. The cell population densities were then measured by the WST-8 (Kishida Chemical Co., Ltd) colorimetric assay using a SpectraMax Plus384 plate reader (Molecular Devices). Concentration response curves were generated to give the decrease in cell population density in MK-5108– and docetaxel-treated samples relative to DMSO-treated control. Growth inhibition (IC50) values were determined from those curves.

![Figure 2. MK-5108 induces accumulation of mitotic cells in HeLa-S3 cells. HeLa-S3 cells were released from the G1-S block. MK-5108 (A) or MLN8054 (B) was added at 4 h and the percentage of pHH3-positive cells was determined at 18 h by immunofluorescent analysis as described in Materials and Methods. C, DNA profiles of asynchronously cultured HeLa-S3 cells treated by MK-5108 or MLN8054 for 24 h were evaluated by flow cytometry. The percentage of cells in G1, S, and G2-M phase is shown.](mct.aacrjournals.org/content/mct/9/1/159/F2.large.jpg)
In vivo Efficacy Studies

We handled the mice in strict accordance with good animal practice, as defined by the Institutional Animal Care and Use Committee at Banyu. All animal study was done with Institutional Animal Care and Use Committee approval and was conducted in a facility certified by the Association for Assessment and Accreditation of Laboratory Animal Care International. Immunodeficient nude rats (F344/N Jcl-mu strain, female) and severe combined immunodeficient (SCID) mice (C.B17/Icr-scid strain, female) were purchased from CLEA Japan. HCT116 cells were suspended in 50% Matrigel (BD Bioscience) and 50% PBS, and s.c. transplanted into the side flank of SCID mice (1 × 10^7 cells/100 μL). After 8 d, MK-5108 was suspended in 0.5% methyl cellulose/0.24% SDS and orally administered twice daily for 14 d. SW48 cells were suspended in 50% Matrigel/50% PBS and s.c. transplanted into the side flank of nude rats (1 × 10^7 cells/100 μL). After 7 d, MK-5108 was suspended in 0.5% methyl cellulose/0.24% SDS and orally administered twice daily for 2 d/wk for 3 wk. In a HeLa-luc and ES-2 dual flank xenograft model, HeLa-luc or ES-2 cells were suspended in 50% Matrigel and 50% PBS, and s.c. transplanted into the right or left side flank of nude rats (1 × 10^7 cells/100 μL), respectively. After 8 d, vehicle (5% ethanol-saline) or 7.5 mg/kg docetaxel was injected i.v. MK-5108 was orally administered twice daily for 2 d 24 h after the docetaxel injection. The volume (mm^3) of each tumor was determined from the tumor diameter, which was measured with digital calipers using the following formula: tumor volume (mm^3) = length × (width)^2 × 0.5. Relative tumor volume was then calculated as follows: relative tumor volume = V/V_0, where V_0 is the initial tumor volume.

**Table 1. IC_{50}s of MK-5108 for cell proliferation**

<table>
<thead>
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<th>Cell line</th>
<th>Origin</th>
<th>IC_{50} (μmol/L)</th>
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<tr>
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</tr>
<tr>
<td>T47D</td>
<td>Breast</td>
<td>&gt;30</td>
</tr>
<tr>
<td>HCC1419</td>
<td>Breast</td>
<td>&gt;30</td>
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<tr>
<td>MIAPaCa-2</td>
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</table>

NOTE: Numbers represent average IC_{50} from two independent experiments.
*Single experiment was completed for HCT116.

Shimomura et al.

Mol Cancer Ther; 9(1) January 2010

Molecular Cancer Therapeutics

Figure 3. MK-5108 induces the accumulation of mitotic cells in HeLa-luc tumors and skin hair bulbs. HeLa-luc tumor–bearing nude rats were treated with MK-5108 by four repeat oral doses at 12-h intervals. pH3-positive cells in tumor and skin tissues were detected by immunohistochemistry. **A**, the dose dependency of induction of pH3-positive cells in HeLa-luc tumor was evaluated 12 h after the last dose of MK-5108 at the indicated doses (n = 4 rats per dose; columns, mean; bars, SD). **B**, the induction of pH3 was evaluated at multiple time points after the last dose of MK-5108 at 16 mg/kg in HeLa-luc tumor and skin hair bulb regions (n = 4 rats per time point; columns, mean; bars, SD). **C** and **D**, representative pH3 and hematoxylin staining in HeLa-luc tumors (**C**) and skin hair bulbs (**D**) at 4 h after the last dose of MK-5108 at 16 mg/kg. Scale bars, 50 μm.
tumor volume at day 0 and \( V \) is the tumor volume at each observation day. Antitumor activity was evaluated by calculating the change in mean tumor volume for the treatment group as a percentage of the mean change in the control group (%T/C) for relative tumor volume. %T/C is calculated as follows: 

\[
%T/C = \frac{\Delta T}{\Delta C} \times 100 \quad \text{if} \quad \Delta T > 0; \quad %T/C = \frac{\Delta T}{T_0} \times 100 \quad \text{if} \quad \Delta T < 0. 
\]

\( T_0 \) is the mean relative tumor volume at day 0, and \( \delta \) volumes are calculated by subtracting the mean relative tumor volume on day 0 from the mean relative tumor volume on the particular observation day for each treated group (\( \Delta T \)) and control group (\( \Delta C \)). Statistical analysis was done using repeated measure ANOVA followed by Dunnett's test for relative tumor volume. MK-5108 concentrations in plasma were determined by a liquid chromatography tandem mass spectrometry method.

**In vivo pH3 Assay**

HeLa-luc cells were suspended in 50% Matrigel/50% PBS and s.c. transplanted into the side flank of nude rats (1 × 10^7 cells/100 μL). MK-5108 was suspended with 0.5% methyl cellulose and orally administered twice daily (12-h interval) for 2 d. HeLa-luc tumor and skin tissues were collected for staining for pH3.
Ser28. Briefly, excised tumors and skins were fixed overnight in 10% buffered formalin (Mildform, Wako), and then samples were paraffin embedded and sectioned (1.5 μm). Deparaffinized sections were immunostained with rabbit polyclonal anti–phospho-Histone H3 Ser28 antibody (Stressgen) followed by incubation with biotinylated goat anti-rabbit IgG (H+L) antibody (Chemicon) and then Avidin/Biotinylated peroxidase Complex (Vectastain Elite ABC kit, Vector Laboratories). Tumor and skin sections immunostained with phospho-Histone H3 Ser28 antibody were quantified by automated image acquisition and analysis. Images were acquired using an Olympus AX80 microscope fitted with a motorized stage and a digital camera. A positively immunostained area was quantified from the acquired images using Image Pro Plus software (Media Cybernetics). Necrotic regions in the tumor were excluded from the analysis. In skin sections, a region consisted of a skin layer between the top and bottom of hair bulbs containing >10 hair bulbs used for the analysis. Positive signals were calculated as the percentage of positively immunostained area in the total area measured in each sample. The results were expressed as a fold increase compared with the vehicle control group.

Results

MK-5108 Is a Novel, Highly Potent, and Selective Inhibitor of Aurora-A Kinase

MK-5108 is a novel small molecule discovered in the course of our internal medicinal chemistry research (Fig. 1A). MK-5108 inhibited Aurora-A activity with an IC50 value of 0.064 nmol/L in an ATP-competitive manner (Fig. 1B and C). It showed robust selectivity against
the other family kinases Aurora-B (220-fold) and Aurora-C (190-fold) in the biochemical assay (Fig. 1B). MK-5108 also exhibited high selectivity for Aurora-A over other protein kinases. Of the 233 kinases tested, MK-5108 inhibited only one kinase (TrkA) with <100-fold selectivity as shown in Supplementary Table S1. MLN8054 has been reported to be an Aurora-A inhibitor with an IC₅₀ value of 4 nmol/L and 43-fold selectivity over Aurora-B kinase (13), but one of its liabilities is its potent inhibitory activity of ɣ-amino butyric acid α₁, based on its benzodiazepine core structure (1). In contrast to MLN8054, MK-5108 does not have a benzodiazepine core and showed little binding activity to ɣ-amino butyric acid receptors in a series of binding assays (data not shown).

**MK-5108 Selectively Inhibits Aurora-A**

It has been reported that Aurora-A inhibition by siRNA and the small-molecule inhibitor MLN8054 induce accumulation of cells in the G₂M phase in the cell cycle with increased levels of Histone H3 phosphorylation. Conversely, inhibition of Aurora-B shows the opposite effect: inhibition of Histone H3 phosphorylation has been used as a marker for Aurora-B inhibition by small-molecule inhibitors such as MK-0457, AZD-1152, and PHA-73958 (10). Because pH3 Ser28 is only phosphorylated in prophase to anaphase in mitosis, the positive pH3 Ser28 signal indicates cells in mitosis (27). In synchronized HeLa-S3 cells, accumulation of pH3-positive cells was observed by treatment with MK-5108 at concentrations between 300 nmol/L and 10 μmol/L, suggesting the selective inhibition of Aurora-A (Fig. 2A). Maximum pH3 induction was achieved at MK-5108 concentrations between 1 and 10 μmol/L. The induction of pH3 was correlated with the target inhibition; Aurora-A autophosphorylation at Thr288 partially and almost fully inhibited at 300 nmol/L and 1 μmol/L, respectively (Supplementary Fig. S1). Therefore, the induction of pH3 would be a surrogate marker for Aurora-A selective inhibition. MLN8054 also induced pH3 phosphorylation, but only at 1 and 3 μmol/L, and the level of induction was lower (Fig. 2B). These data suggest that MK-5108 may be more Aurora-A selective than MLN8054. The effect of MK-5108 and MLN8054 on cell cycle progression was further evaluated by monitoring cellular DNA content by flow cytometry in asynchronously cultured HeLa-S3 cells. Consistent with the induction of pH3-positive cells, both MK-5108 and MLN8054 induced accumulation of cells in the G₂M phase (Fig. 2C). The effect of MK-5108 on Histone H3 phosphorylation in vivo was evaluated using a HeLa-luc xenograft rat model. MK-5108 was administered orally twice daily for 2 days. HeLa-luc tumors were removed 12 hours after the dose and were evaluated by immunohistochemistry for pH3. MK-5108 induced pH3-positive cells at doses of 16 and 32 mg/kg, but induction was not apparent at lower doses (Fig. 3A). Plasma concentration of MK-5108 at 8 and 16 mg/kg were 1.7 and 4.4 μmol/L, respectively. pH3 induction in HeLa-luc tumor and skin tissues was evaluated after oral administration of MK-5108 at 16 mg/kg at several time points. MK-5108 treatment resulted in the induction of pH3 in tumor and skin tissues, which started at 2 hours and reached a maximum at 4 hours (Fig. 3B, C, and D). No reduction of pH3 was observed in these experiments, suggesting that MK-5108 selectively inhibits Aurora-A over a wide dose range.

**MK-5108 Inhibits Proliferation of Cultured Human Tumor Cell Lines and Growth of Xenograft Tumors**

To investigate the effect of Aurora-A inhibition on cell proliferation, the effects of MK-5108 were tested in 17 cultured human cancer cell lines. MK-5108 inhibited the growth of 14 cell lines with IC₅₀ values between 0.16 and 6.4 μmol/L (Table 1). Although IC₅₀ values could not be obtained in the other three cell lines, MK-5108 showed 20% to 50% growth inhibition in the same dose range (data not shown), indicating that MK-5108 also penetrated into these cell lines.

To evaluate the antitumor activity of Aurora-A inhibition in vivo, we examined the ability of MK-5108 to inhibit the growth of xenograft tumors in immunodeficient animals. SCID mice bearing HCT116 tumors were treated with oral MK-5108 twice daily for 12 days. In the HCT116 tumor model, MLN8054 exhibited significant tumor growth inhibition (13). The doses for MK-5108 were determined by the in vivo pH3 assay, in which nearly maximum induction of pH3 was achieved at 30 mg/kg (Supplementary Fig. S2). MK-5108 treatments at 15 and 30 mg/kg resulted in significant tumor growth inhibition with the change in mean tumor volume for the treatment group as a percentage of the mean change in the control group (%T/C) of 10% and 6% at day 11, and 17% and 5% at day 18, respectively (P < 0.001; Fig. 4A). MK-5108 was well tolerated at both doses, with minimal reduction in body weight. MK-5108 also exhibited significant antitumor activity through intermittent dosing in nude rats bearing SW48 tumors, which is the most sensitive cell lines tested in the in vitro cell proliferation assay (Table 1; Fig. 4B). MK-5108 was orally administered twice daily for 2 days per week for 3 weeks. On this schedule, MK-5108 at 15 and 45 mg/kg caused dose-dependent tumor growth inhibition with a %T/C of 35% and 7% at day 10, and 58% and 32% at day 27, respectively (Fig. 4B). MK-5108 was well tolerated in nude rats, with no body weight reduction and moderate effect on blood cells; 41% and 50% of WBC (49% and 50% of neutrophil, 28% and 40% of lymphocyte) reduction on day 20 at doses of 15 and 45 mg/kg, respectively, were observed.

**MK-5108 Sensitizes Tumor Cells to Docetaxel and Enhances Cell Death Induction**

Aurora-A inhibition by siRNA has been reported to sensitize tumor cells to taxanes (23–26). We evaluated the effect on cell proliferation of MK-5108 in combination
with docetaxel treatment using HeLa-S3 and ES-2 cell lines. In HeLa-S3 cells, docetaxel alone showed 34% and 87% growth inhibition at 0.3 and 1 nmol/L, respectively (Fig. 5A). Little growth inhibition was induced when the cells were treated with MK-5108 alone at 0.3 μmol/L. However, MK-5108 enhanced the effect of docetaxel when the two agents were given together. Although docetaxel was less effective in ES-2 cells than in HeLa-S3 cells, its enhancement in combination with MK-5108 was observed in both cell types (Fig. 5B).

To investigate the effect of the combination of MK-5108 and docetaxel on cell death, the percentage of sub-G1 fraction in HeLa-S3 cells was measured by flow cytometry. Both docetaxel and MK-5108 alone induced cell death in a dose-dependent manner (Fig. 5C), and the percentage of dead cells was greater with the combination of MK-5108 and docetaxel than with either agent alone.

**MK-5108 Enhances the Antitumor activity of Docetaxel without Exacerbating Toxicity In vivo**

The antitumor activity of MK-5108 in combination with docetaxel was evaluated using a dual flank HeLa-luc and ES-2 tumor xenograft nude rat model. Because these xenograft tumors are resistant to docetaxel in the nude rats at the maximum tolerated dose (7.5 mg/kg), the model seems to be suitable to evaluate the effect of MK-5108 in combination with docetaxel. Docetaxel was administered by i.v. injection at 7.5 mg/kg, and 24 hours postinjection, MK-5108 was administered orally twice daily for 2 days at 16 mg/kg, the dose at which maximum levels of pHH3 were induced in the HeLa-luc tumor (Fig. 3A). In the HeLa-luc tumor, docetaxel showed significant antitumor activity with a %T/C of 28% and 30% at day 6 and day 10, respectively (P < 0.01 versus vehicle control; Fig. 6A). Although MK-5108 alone showed little antitumor activity, the combination of MK-5108 with docetaxel exhibited greater antitumor activity: %T/C of ~42% and ~28% at day 6 and day 10, respectively (P < 0.01 versus docetaxel alone and P < 0.01 versus vehicle control). In the ES-2 tumor, the combination of MK-5108 and docetaxel was also more effective than either agent alone (P < 0.05 versus docetaxel alone and P < 0.01 versus vehicle control; Fig. 6B). Surprisingly, MK-5108 did not exacerbate the toxicity of docetaxel, which is manifested as the reduction of body weight (Fig. 6C) and the reduction of WBC count (Fig. 6D).

**Discussion**

MK-5108 is a highly potent and a selective inhibitor of Aurora-A kinase. However, it exhibited large discrepancy between the biochemical and the cell-based assays. Because MK-5108 is an ATP-competitive inhibitor of Aurora-A kinase, this discrepancy could be explained by the different ATP concentration in cells and in the biochemical assay. In addition, the plasma protein binding of MK-5108 would also affect the difference between the biochemical potency and cell-based activity. The IC50 values of MK-5108 for Aurora-A and Aurora-B indicate that MK-5108 has a higher affinity to Aurora-A than Aurora-B. It is conceivable that the lower binding affinity to Aurora-B induces the larger shift in the Aurora-B inhibitory activity in the cell-based assay, resulting in wider window between Aurora-A inhibition and Aurora-B inhibition in the cell-based assay than in the biochemical assay.

In our study, BT474, T47D, and HCC1419 were resistant to MK-5108; however, partial growth inhibition was observed in the same dose range, suggesting that MK-5108 was penetrated into the cells. Considering the role of Aurora-A in tumorigenesis, the expression levels of Aurora-A are expected to predict the sensitivity to MK-5108. It has been reported that the protein expression levels of Aurora-A are varied among breast cancer cell lines (28). In the report, the Aurora-A expression level is high in BT474 and low in T47D. Thus, the data may not support the correlation between Aurora-A expression and the sensitivity to MK-5108. Because the data are limited, it is not sufficient to neglect the possibility to predict the sensitivity to MK-5108 by the levels of Aurora-A expression. We need more data not only for MK-5108-sensitive but also MK-5108-resistant cells. Further study is currently ongoing to clarify the correlation.

Although antitumor activities induced by MLN8054 may be mainly mediated by Aurora-A, inhibition of pHH3 was observed in xenograft tumors immediately after high plasma concentrations of MLN8054 were achieved, suggesting that Aurora-B inhibition could have some involvement in the antitumor activity of MLN8054 in vivo (29). MK-5108 exhibited greater Aurora-A kinase selectivity than MLN8054 in the biochemical assay and exhibited induction of pHH3-positive cells in culture. In addition, MLN8054 binds to the γ-aminobutyric acid α1 benzodiazepine receptor, which causes reversible somnolence (1). This toxicity might limit dose escalation and prevent the achievement of sufficient Aurora-A inhibition in patients. MLN8237 has been reported as a follow-up compound of MLN8054; however, it was also exhibited transient Aurora-B inhibition at the effective doses (1, 29). Thus, MK-5108 could be a better inhibitor to clarify the therapeutic value of Aurora-A inhibitors in cancer patients. In clinical studies, MK-0457, PHA-739358, and AZD1152 caused disease stabilization: grade 3 to 4 neutropenia was the most common dose-limiting toxicity (1, 29). From these and from other biological studies, inhibition of Aurora-B kinase is expected to cause severe neutropenia. Considering the effect on cell cycle progression, it is expected that the toxicity caused by the mitotic arrest with Aurora-A selective inhibition is moderate than that by the aberrant and irreversible mitotic progression with Aurora-B inhibition. In xenograft models, MK-5108 induced ~50% reduction of neutrophils at the efficacious dose, suggesting that MK-5108 may have wider therapeutic index than Aurora-B inhibitors. The safe profiles of MK-5108 were further
showed by the rat 2-day oral toxicity study with a 21-day recovery period, in which no adverse effect level of MK-5108 was 500 mg/kg/day (data not shown).

Paclitaxel and docetaxel are microtubule-stabilizing taxanes that have been used in the clinic to treat various types of cancer. Aurora-A inhibition by antisense oligonucleotide or RNAi sensitizes cancer cells to taxanes. Consistently, MK-5108 sensitized cancer cells to docetaxel in cell culture and in xenograft models. Docetaxel and paclitaxel also showed benefits in combination with other Aurora kinase inhibitors. Concurrent treatment with VE-465 and paclitaxel exhibited synergistic induction of apoptosis at low doses specific to Aurora-A in cultured ovarian cancer cells (30), suggesting the importance of Aurora-A selectivity for the combination treatment with taxanes. MK-0457 alone significantly reduced tumor burden compared with control in the ovarian cancer models (31). Combination treatment with docetaxel showed improved reduction in tumor growth beyond that afforded by docetaxel alone. The timing of MK-0457 did not affect on the efficacy in the combination treatment. Interestingly, a potent pan-Aurora inhibitor SNS-314 exhibited profound antiproliferative effects with sequential administration of SNS-314 followed by docetaxel in the HCT116 xenograft model (32). The inverse sequence with docetaxel first followed by SNS-314 did not exhibit the enhanced antiproliferative effect than docetaxel alone. In our study, MK-5108 enhanced the antitumor effects of docetaxel by a concurrent treatment and a sequential administration of docetaxel followed by MK-5108 in the in vitro and in vivo study, respectively. The difference in treatment sequences for efficacious combination treatments suggests that SNS-314 and MK-5108 used distinct mechanisms to sensitize tumor cells to docetaxel. Docetaxel treatment activates spindle assembly checkpoint. Inhibition of Aurora-B by siRNA or Hesperadin overrides mitotic arrest by Aurora-A siRNA or paclitaxel (6, 7); conversely, inhibition of Aurora-A by siRNA or an Aurora-A selective inhibitor is expected to enhance mitotic arrest by taxanes through the activation of the spindle assembly checkpoint (25). The different effect of Aurora-A or Aurora-B inhibition on the spindle assembly checkpoint would explain the distinct mechanisms in combination with taxanes. In addition to docetaxel, Aurora-A silencing and vincristine treatment exhibit a synergistic effect on human tumor cell lines (33). MK-5108 might exhibit robust antitumor activity in combination with antimiotic agents such as taxanes and Vinca alkaloids.

In conclusion, MK-5108 is a novel, highly potent, and selective inhibitor of Aurora-A that shows a typical Aurora-A inhibition phenotype in cell culture and in vivo. Furthermore, it exhibited marked effects on the growth of tumor cells in vitro and in vivo. Importantly, MK-5108 enhances the antitumor activity of docetaxel without exacerbating the toxicity of docetaxel. Thus, the selective inhibition of Aurora-A by MK-5108 provides a new approach to combat human cancers, with potential both as a single agent and in combination with existing therapies. Clinical studies are in progress to assess the potential of MK-5108 as an anticancer drug.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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