Charaterization of the Cellular and Antitumor Effects of MPI-0479605, a Small-Molecule Inhibitor of the Mitotic Kinase Mps1

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Abstract

Mps1 is a dual specificity protein kinase that is essential for the bipolar attachment of chromosomes to the mitotic spindle and for maintaining the spindle assembly checkpoint until all chromosomes are properly attached. Mps1 is expressed at high levels during mitosis and is abundantly expressed in cancer cells. Disruption of Mps1 function induces aneuploidy and cell death. We report the identification of MPI-0479605, a potent and selective ATP competitive inhibitor of Mps1. Cells treated with MPI-0479605 undergo aberrant mitosis, resulting in aneuploidy and formation of micronuclei. In cells with wild-type p53, this promotes the induction of a postmitotic checkpoint characterized by the ATM- and RAD3-related-dependent activation of the p53–p21 pathway. In both wild-type and p53 mutant cells lines, there is a growth arrest and inhibition of DNA synthesis. Subsequently, cells undergo mitotic catastrophe and/or an apoptotic response. In xenograft models, MPI-0479605 inhibits tumor growth, suggesting that drugs targeting Mps1 may have utility as novel cancer therapeutics. Mol Cancer Ther; 10(12); 2267–75. ©2011 AACR.

Introduction

Genome integrity is critically dependent upon the accurate segregation of chromosomes during mitosis. This is monitored by the spindle assembly checkpoint (SAC), which functions to inhibit the metaphase-to-anaphase transition until each sister chromatid pair attaches to the mitotic spindle in a bipolar orientation (1). The SAC is facilitated by the assembly of a protein complex at unoccupied kinetochores that monitors the attachment and tension of the mitotic spindle. This protein complex includes Mad1, Mad2, Bub1, Bub3, BubR1, and Mps1 (also known as hMps1, TTK, or PYT). Together, these proteins generate a diffusible signal that inhibits Cdc20, a cofactor of the anaphase-promoting complex/cyclosome (APC/C). Once all kinetochores are properly attached, this diffusible signal is extinguished and the APC/C becomes active. The APC/C<sup>CTD20</sup> complex coordinates chromosome segregation with cell-cycle progression by targeting both securin and cyclin B for ubiquitin-mediated proteolysis. Degradation of these proteins promotes sister chromatid separation and mitotic exit, respectively.

Mps1 is a dual specificity protein kinase that phosphorylates tyrosine, serine, or threonine residues. Its expression and activity are regulated in a cell-cycle–dependent manner with maximal levels arising during mitosis. The localization of Mps1 to kinetochores during mitosis facilitates the trans-autophosphorylation and activation of Mps1 (2, 3). Mps1 has multiple functions during mitosis. Evidence suggests that it facilitates chromosome congression and alignment at the metaphase plate via the phosphorylation and recruitment of the kinesin protein, CENP-E (4, 5, 6). In addition, Mps1 promotes the proper bipolar attachment of microtubules to the kinetochores by eliminating misattachments. This likely occurs via the phosphorylation of BubR1 (7) as well as borealin (8, 9), a chromosomal passenger protein that regulates Aurora B. Finally, the kinase activity of Mps1 is required for full assembly of spindle checkpoint proteins at the kinetochore and for activation of this protein complex (4, 6, 10, 11).

Previous studies using short interfering RNA have shown that loss of Mps1 during an unperturbed mitosis...
results in gross chromosomal segregation defects including unaligned and/or unattached chromosomes during metaphase, lagging chromosomes during anaphase, and cytokinesis defects due to attempts of cells to divide with unsegregated DNA (12). Furthermore, reconstitution of Mps1-depleted cells with kinase-inactive Mps1 failed to restore proper chromosome segregation, highlighting the role of Mps1 kinase function. This was due in part to the inability of kinase-inactive Mps1 to recruit Mad2 to the kinetochore during activation of the SAC (10).

In the present study, we characterize MPI-0479605, a potent inhibitor of Mps1, and show that Mps1 inhibition impairs the SAC, resulting in chromosome segregation defects and aneuploidy. This triggers the activation of a postmitotic checkpoint, characterized by inhibition of DNA synthesis and growth arrest. Furthermore, the p53–p21 pathway is induced in cells with wild-type p53 in an ATM- and RAD3-related (ATR)-dependent manner. Ultimately, cells undergo mitotic catastrophe and cell death. MPI-0479605 was cytotoxic across a broad range of tumor cell lines and exhibited antitumor activity in nude mice bearing human tumor xenografts.

Materials and Methods

Cell culture and reagents

Cell lines were obtained from American Type Culture Collection or DMSZ (German Collection of Microorganisms and Cell Cultures) and cultured at 37°C in 5% CO2 in the recommended media supplemented with 10% fetal calf serum. The TIP5/hTERT cells were kindly provided by Steve Lessnick (University of Utah, Salt Lake City, UT). The ML-2, a rat fibroblast cell line, and the TIP5/hTERT cells were not authenticated by the authors. Cells were synchronized in G1 using a standard double thymidine block or in mitosis using 250 ng/mL nocodazole. Cells were treated with a C-terminal tGFP tag was purchased from Origene (Sigma). CGK733 (Sigma) and KU-55933 (Tocris) were used to inhibit JAK/STAT and Src kinases, respectively. Mouse monoclonal antibodies to centromeres with anti-human centromere antibody (1:100, Antibodies Inc.); human centromeres with anti-human centromere antibody (1:1,000, Abcam); centrosomes with pericentrin rabbit polyclonal antibody (1:100, Sigma); alpha-tubulin mouse monoclonal antibody (1:200; Sigma); and 1-micron CDK1 (496nm) were stained with 7-aminoactinomycin (7-AAD; Invitrogen) and released from arrest into media containing dimethyl sulfoxide (DMSO) or MPI-0479605. Cells were fixed and analyzed by flow cytometry. To measure bromodeoxyuridine (BrdUrd) incorporation, cells were labeled with BrdUrd and stained by the BrdU Flow Kit according to the manufacturer’s protocol (BD Biosciences). Flow cytometric analysis was conducted with a FACScan flow cytometer (BD Biosciences) or a Guava EasyCyte (Millipore).

Quantitative real-time PCR

mRNA was isolated from cells by the RNeasy Kit (Qiagen). RNA from each sample was used as template for quantitative real-time PCR (qRT-PCR) with primers for CDKN1A (p21) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Applied Biosystems). The CDKN1A mRNA levels were normalized to GAPDH mRNA levels, and data were plotted as a percentage of the vehicle-treated samples.

Immunofluorescence microscopy

For mitotic imaging studies, cells were fixed with 4% paraformaldehyde for 15 minutes at 37°C, permeabilized in 0.2% Triton X-100 for 5 minutes, and incubated with primary antibodies. Microtubules were visualized with β-tubulin mouse monoclonal antibody (1:200; Sigma); centrosomes with pericentrin rabbit polyclonal antibody (1:1,000, Abcam); centromeres with anti-human centromere antibody (1:100, Antibodies Inc.); and DNA with Hoechst 33342 dye. Images were taken with a 40x objective lens on a confocal microscope using 1-μm slices.

Cell viability and caspase assays

Cell viability was measured with CellTiter-Glo (Promega), and caspase-3/7 activity was measured with Caspase-Glo 3/7 (Promega). The pan-caspase inhibitor Z-VAD-fmk (Sigma) was used at 100 μmol/L on cells.
In vivo antitumor studies

HCT-116 or Colo-205 cells were transplanted subcutaneously into the flanks of nude (nu+/nu+) mice, and compound treatment was initiated when tumor masses reached an average size of 100 mm³. MPI-0479605 was formulated in 5% dimethylacetamide (DMA)/12% ethanol/40% PEG-300, ispinesib was formulated in 2% cremophor/2% DMA, and 5-fluorouracil was formulated in 2% sodium bicarbonate. Tumor volume was measured with vernier calipers, and tumor growth inhibition (TGI) was calculated as follows: %TGI = 100 – (change in median tumor volume of treated)/(change in median tumor volume control). These studies conformed to the recommendations set forth in the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Results

MPI-0479605 is a potent, selective inhibitor of Mps1 kinase

MPI-0479605 is a potent, ATP-competitive inhibitor of Mps1 identified by compound screening and subsequent medicinal chemistry optimization. It inhibits Mps1 with an IC₅₀ value of 1.8 nmol/L (Fig. 1A) and is highly selective for Mps1 when tested against 120 other kinases (Supplementary Table S1). MPI-0479605 is structurally similar to reversine and MPS1-IN-1, 2 recently published inhibitors of Mps1 (13, 14), but differs from reversine in that it lacks activity against Aurora kinases.

MPI-0479605 inhibited Mps1 function in cells as shown by the ability to override the SAC induced by the microtubule-destabilizing drug nocodazole. In nocodazole-arrested cells, MPI-0479605 triggered the time-dependent degradation of both cyclin B and securin, which normally allow for mitotic exit, as well as a decrease in BubR1 phosphorylation (Fig. 1B). As a result, cells exited mitosis and failed to undergo cytokinesis, as evidenced by an increase in the percentage of cells with more than 4N DNA content (Fig. 1C). In addition, the percentage of cells that stained positive for phosphohistone-H3 (pHH3), a marker of mitotic cells, decreased in a dose-dependent manner in response to treatment with MPI-0479605 (Supplementary Fig. S2). Consistent with inhibition of Mps1 in cells, MPI-0479605 blocked apparent autophosphorylation of Mps1 at threonine 676 in HEK293T cells overexpressing Mps1 (Fig. 1D).

![Figure 1. In vitro activity of MPI-0479605 and cellular inhibition of Mps1. A, structure of MPI-0479605 and dose-dependent inhibitory activity of MPI-0479605 on Mps1 enzymatic activity in vitro at different ATP concentrations using myelin basic protein (MBP) as substrate. The effect of MPI-0479605 on Mps1 enzymatic activity is reported relative to samples treated with vehicle only (% DMSO). The K₅₀ for ATP was determined to be 20 μmol/L. B, nocodazole-arrested HeLa cells were treated with DMSO or 1 μmol/L MPI-0479605 (9605) for various times. Cell lysates were analyzed by Western blotting with anti-cyclin B1 and GAPDH antibodies (top) or with antibodies for securin, BubR1, or GAPDH (bottom). C, nocodazole-arrested HCT-116 cells were treated with DMSO or MPI-0479605 (9605) for 0 or 6 hours. Cells were stained with propidium iodide and analyzed by fluorescence-activated cell sorting. D, HEK-293T cells transfected with GFP-tagged Mps1 wild-type were treated with MPI-0479605 for 4 hours. After 48 hours, cell lysates were analyzed by Western blotting with antibodies for Mps1-pT676 and Mps1. U, untreated.](https://aacrtjournals.org/mct/2011/10/0479605)
Effects of Mps1 inhibition on mitosis and cell-cycle progression

The effect of MPI-0479605 on chromosome segregation was examined by imaging A549 cells during mitosis. Cells treated with MPI-0479605 exhibited severe defects in the ability to align chromosomes at the metaphase plate. A significant fraction of cells treated with MPI-0479605 entered anaphase with at least one unaligned chromosome, as was evident by the presence of lagging chromosomes during anaphase (Fig. 2A). Following completion of mitosis and reentry into interphase, cells treated with MPI-0479605, or the related compound MPI-0485812 (structure in Supplementary Fig. S3), contained micronuclei that stained positive for centromeres, but not γH2AX (Fig. 2B and data not shown), consistent with the presence of whole chromosomes, and not DNA fragments, within these micronuclei. In some cells, we observed the presence of multiple nuclei, but this was not the predominant phenotype.

Despite defects in chromosome segregation, the majority of MPI-0479605–treated cells were able to complete cytokinesis, as indicated by the absence of a large population of tetraploid cells (cells with 8N DNA content). Instead, there seemed to be gain or loss of chromosomes, as shown by the divergence of DNA content from the 2N and 4N peaks, in the majority of cells (Fig. 3A). Interestingly, p53-proficient HCT-116 cells showed only a small increase in the apoptotic sub-G1 population of cells within 72 hours of compound exposure, whereas p53-deficient Colo-205 cells exhibited a strong increase in this population. This suggests that cells with wild-type p53 may be partially protected from apoptosis. To better understand whether cells are actively dividing, cells were synchronized in late G1 and treated with CSFE, which labels intracellular proteins. Upon cell division, the fluorescence intensity decreases, allowing cell division to be monitored. Labeled cells were released from the G1 block into media containing DMSO or MPI-0479605, and the number of cell divisions occurring during a 72-hour period was determined. Cells treated with MPI-0479605 were able to complete one cell division in a manner similar to the DMSO-treated cells, but thereafter, MPI-0479605–treated cells showed a significant delay and/or arrest in cell-cycle progression (Fig. 3B). DNA synthesis was monitored by the incorporation of BrdUrd. Following exposure to MPI-0479605 for 48 hours, BrdUrd incorporation was greatly suppressed, indicating that most cells were not actively cycling through S-phase (Fig. 3C). This occurred in both p53 wild-type and p53-deficient cells, suggesting that this effect is not dependent on the presence of functional p53. We conclude that inhibition of Mps1 abrogated the SAC and allowed anaphase progression in the presence of misattached chromosomes, resulting in chromosome segregation defects and aneuploidy. Subsequent to this,

Figure 2. Effect of MPI-0479605 on chromosome segregation. Immunofluorescence images of A549 cells treated with vehicle (DMSO), 1 μmol/L MPI-0479605, or 1 μmol/L MPI-0485812 (a more potent analogue of MPI-0479605) for 24 hours. Pericentrin (a marker for centrosomes), centromeres, and DNA are shown in red, white, and blue, respectively. Black and white images of the DNA stain highlight the defects in chromosome segregation during mitosis (A) and the formation of micronuclei (arrows) in interphase (B).
cell-cycle progression was retarded, presumably due to the activation of a postmitotic checkpoint or initiation of mitotic catastrophe.

**Molecular characterization of the postmitotic checkpoint**

Recent work has shown that inactivation of the spindle checkpoint induces aneuploidy followed by activation of a p53 response and growth arrest and/or apoptosis (15, 16). To determine whether aneuploidy induced by MPI-0479605 promotes a similar response, we examined the p53 signaling pathway. In p53-proficient cells, MPI-0479605 induced the expression of p53 in a time-dependent manner with maximal levels occurring by 48 hours, a time frame that is consistent with the observed inhibition of DNA synthesis (Fig. 4A). Induction of p21 protein and mRNA also occurred in a time- and dose-dependent manner, suggesting that p53 was transcriptionally active (Fig. 4A and B). Previous studies indicated that Mps1 phosphorylates p53 on threonine 18 during a postmitotic checkpoint in response to microtubule destabilization (17). Surprisingly, inhibition of endogenous Mps1 in the absence of microtubule-targeting drugs induced the phosphorylation of p53 on serine 15 rather than on threonine 18 (Fig. 4C and data not shown). Because ATR is normally activated in response to DNA damage, phosphorylation of histone H2AX, a marker for DNA damage and a direct substrate of ATR, was examined. At 48 hours posttreatment with MPI-0479605, approximately 15% of cells showed diffuse nuclear staining for γH2AX but did not display the typical focal staining observed in response to DNA damage (data not shown). By Western blotting, an increase in γH2AX was detected with the maximal signal occurring by 36 hours (Fig. 4A). Phosphorylation of H2AX in response to MPI-0479605 was inhibited with CGK733 but not KU-55933 (Fig. 4C and data not shown). Thus, both p53 and H2AX, known substrates of ATR, are phosphorylated in response to MPI-0479605 treatment.

**Inhibition of Mps1 induces a time-dependent cell death**

Treatment of HCT-116 cells with MPI-0479605 resulted in a significant decrease in cell viability at 48 and 72 hours (Fig. 5A). At the same time, caspase-3/7 activity was induced, with induction levels highest at 48 hours (Fig. 5B). Caspase induction was observed in both p53 wild-type and mutant cells but pretreatment of cells with the pan-caspase inhibitor z-VAD-fmk did not affect MPI-0479605–mediated cell death (Supplementary Fig. S4). To explore commitment to cell death, cells were exposed to Mps1 inhibitors for 24 hours, followed by incubation in compound-free media for up to 9 additional days. At lower doses of inhibitor, cell killing was incomplete and cell growth recovered, whereas at higher doses, cell killing...
was essentially complete (Fig. 5C and D). Treatment of a panel of tumor cell lines for 72 hours revealed many lines with minimal sensitivity. In contrast, treatment for 7 days led to cytotoxicity in all of the lines tested, with half-maximal growth inhibition (GI50) values ranging from 30 to 100 nmol/L in most cell lines (Supplementary Table S2). The extended time course of cell death may reflect the time required to accumulate a lethal level of chromosome segregation errors, or it may reflect the delayed cell-cycle kinetics exhibited upon Mps1 inhibition.

**MPI-0479605 displays antitumor activity in colon cancer xenograft models**

To ascertain the effect of Mps1 inhibition on tumor growth, we treated mice bearing subcutaneous HCT-116 or Colo-205 human tumor cell xenografts with...
Ispinesib 7 days resulted in 63% TGI (did not show antitumor activity, whereas dosing every 4 mice (Fig. 6A). For the Colo-205 xenografts, daily dosing resulted in 74% TGI (xenografts, daily dosing at 30 mg/kg resulted in 49% TGI 4 days with 150 mg/kg of MPI-0479605. For the HCT-116 ated dose, mice were treated daily with 30 mg/kg or every (body weight loss and death) using these dosing regi-

Figure 6. Effect of MPI-0479605 on xenograft tumor growth. A, nude mice bearing HCT-116 tumors were treated (n = 10 per group) intraperitoneally with MPI-0479605 at 30 mg/kg daily (QD) or at 150 mg/kg every fourth day (Q4D) for a period of 21 days. 5-Fluorouracil (5-FU) dosed weekly at 100 mg/kg was used as a reference treatment. Median tumor volume (left) and body weight (right) were measured. There was one death at day 11 in the 30-mg/kg group and one death at day 15 in the 150-mg/kg group. B, in vivo effect of MPI-0479605 on neutrophils. Swiss Webster mice were injected intraperitoneally with 150 mg/kg MPI-0479605, and neutrophil counts were determined at the indicated time points (n = 4 animals per time point). C, effect of MPI-0479605 on the viability of TIPS fibroblasts immortalized with hTERT or transformed with hTERT plus vRas.

MPI-0479605. On the basis of pharmacokinetic analysis (Supplementary Fig. S5) and studies of maximum tolerated dose, mice were treated daily with 30 mg/kg or every 4 days with 150 mg/kg of MPI-0479605. For the HCT-116 xenografts, daily dosing at 30 mg/kg resulted in 49% TGI (P = 0.1), whereas dosing every 4 days at 150 mg/kg resulted in 74% TGI (P = 0.005) relative to vehicle-treated mice (Fig. 6A). For the Colo-205 xenografts, daily dosing did not show antitumor activity, whereas dosing every 4 days resulted in 63% TGI (P = 0.07; Supplementary Fig. S6). These animal studies provide validation for Mps1 as a cancer target, but indicate that there is associated toxicity (body weight loss and death) using these dosing regi-
mens. The specific cause of the toxicity is unclear; how-
ever, mice treated with a single dose of MPI-0479605 exhibited significant neutropenia by day 5 (Fig. 6B). This suggests that the effects of MPI-0479605 are not limited to tumor cells.

Discussion

Tubulin-targeting drugs are successful chemothera-
papeutic agents, presumably due to their antimotic effects on tumor cells. In the last decade, a number of mitosis-specific targets have been identified, such as the kinesin motor proteins Eg5 and CENP-E, and the mitotic kinases Aurora A, Aurora B, and Polo-like kinase (20–24), whose expression or activity is limited to dividing cells. The recently identified kinase Mps1 is another novel mitotic target, whose inhibition would be expected to selectively block growth of rapidly dividing cells. We have charac-
terized MPI-0479605, a potent small-molecule inhibitor of Mps1, and described the mechanisms leading to tumor cell death. Consistent with recently published inhibitors of Mps1 (6, 13, 14), MPI-0479605 impairs the SAC and the bipolar attachment of chromosomes to the mitotic spindle, resulting in chromosome segregation defects and aneu-
plody. This leads to growth arrest and ultimately pro-
motes cell death by apoptosis or mitotic catastrophe. Furthermore, MPI-0479605 was shown to inhibit tumor growth in vivo.

Aneuploidy is a common event in cancer and is thought to provide a selective survival advantage to tumor cells (25). Thus, it seems counterintuitive that MPI-0479605, which promotes aneuploidy, would be a useful therapeutic in the treatment of cancer. We sought to determine how MPI-0479605–induced aneuploidy promoted cell death. Recent work from Thompson and Compton (16) and Li and colleagues (15) shows that aneuploidy caused by impairment of the spindle checkpoint results in a p53-
dependent senescence-like growth arrest and/or apopto-
sis. Similarly, we found that following chromosome missegregation, MPI-0479605–treated cells induce the p53–p21 pathway and this correlates with a reduction in BrdUrd incorporation, indicative of growth arrest. In these cells, ATR is the dominant kinase for the MPI-0479605–induced phosphorylation of both p53 and H2AX, as shown by the ability of CGK733, but not an ATM-
specific compound, to inhibit these events (Fig. 4C and data not shown). Typically, ATR and ATM are activated in response to DNA damage and phosphorylate H2AX at sites of DNA damage, resulting in the formation of distinct γH2AX-positive foci (26–29). However, activation of an ATR–p53 pathway that results in a senescence-like growth arrest was recently reported by Toledo and colleagues (30) and was shown to be independent of
DNA damage. In our studies, we saw an increase in H2AX phosphorylation by Western blotting but did not see the formation of γH2AX-positive foci by image analysis. Moreover, the micronuclei formed as a consequence of chromosome missegregation failed to stain positive for γH2AX, indicating that undamaged chromosomes are being missegregated. Interestingly, the phosphorylation of H2AX, but not p53, was inhibited by pretreatment with the caspase inhibitor Z-VAD-fmk (Supplementary Fig. S7), suggesting that H2AX phosphorylation occurs during apoptosis as a consequence of caspase-induced DNA fragmentation, as has been previously reported (31).

While aneuploidy induced a p53 response followed by growth arrest in p53-proficient cells, we also observed a growth arrest phenotype in cells with transcriptionally inactive p53 (Fig. 3), suggesting other unknown mechanisms may contribute to the growth arrest phenotype. Indeed, MPI-0479605 killed multiple cancer cell lines regardless of p53 status. Furthermore, inhibiting caspase activity failed to delay or inhibit cell death. These results indicate that neither p53 nor caspase activity are essential for MPI-0479605–mediated cell death (Fig. 4A and Supplementary Fig. S4). Recent data suggest that altering the sensitivity of cells to apoptosis, particularly cells of epithelial origin, has little overall effect on cell survival and that there are compensatory increases in nonapoptotic mechanisms of cell death such as mitotic catastrophe (32, 33). A hallmark feature of mitotic catastrophe is the presence of micronuclei following mitosis, which was readily apparent in our experiments (Fig. 2B). On the basis of our studies, we would argue that growth arrest followed by apoptosis and/or mitotic catastrophe contribute to MPI-0479605–mediated TGI and may be influenced by the genetic background of the cell as well as the specific chromosomes affected during chromosome missegregation. Interestingly, whereas MPI-0479605 induced chromosome segregation defects and aneuploidy after only 24 hours (Fig. 2), drug washout experiments showed that complete cell death, even at high doses of compound, took many days (Fig. 5). Short-term exposure of cells to sublethal doses of Mps1 inhibitors resulted in an initial decrease in viability followed by full recovery. Depending on which chromosomes are affected during missegregation, commitment to cell death may require more than one round of mitosis in order for a lethal level of aneuploidy to occur. This would suggest that extended inhibition of Mps1 will be necessary to achieve sustained antitumor effects. It is conceivable that the frequency of chromosome missegregation would be increased by combining Mps1 inhibitors with tubulin-targeting drugs (34). In preliminary experiments, cotreatment of cancer cells with MPI-0479605 and tubulin-targeting drugs had an additive effect on cell viability depending on the dose and order of administration of compounds (data not shown).

Mps1 has been proposed to play a role in Aurora B signaling, but this remains controversial. Early studies showed that Mps1 phosphorylates borealin, a chromosomal passenger protein that regulates Aurora B (8), suggesting that Mps1 functions upstream of Aurora B during chromosome biorientation. In addition, the Mps1 inhibitor, MPS1-IN-1 was found to inhibit both Aurora B autophosphorylation on threonine 232 and phosphorylation of its substrate histone H3 on serine 10 (13). In contrast, 2 other newly characterized inhibitors of Mps1 failed to affect Aurora B kinase activity (6, 14). Inhibition of Aurora B overrides the SAC and impedes cytokinesis, resulting in multinucleated cells. Furthermore, cells treated with Aurora B inhibitors fail to effectively engage the postmitotic checkpoint, resulting in endoreduplication and subsequent polyploidy (35, 36). These phenotypes were not highly observed in response to treatment with MPI-0479605. Cell-cycle analysis failed to show a significant increase in 8N cells after 72 hours of treatment (Fig. 3A). Also, whereas there were some multinucleated cells, the vast majority were not (Fig. 2), indicating that MPI-0479605 does not impair cytokinesis. Furthermore, in p53-proficient cells, the postmitotic checkpoint seems to be intact following treatment with MPI-0479605, as indicated by an increase in p53 and p21 and the inhibition of DNA synthesis. As a result, the majority of cells do not become polyploid. These results show that inhibition of Mps1 does not affect the ability of Aurora B to promote cytokinesis or engage the postmitotic checkpoint, suggesting Aurora B functions independently of Mps1 in this context.

Administration of MPI-0479605 to mice bearing human tumor xenografts led to partial TGI that was associated with significant toxicity. Related compounds with greater potency or superior pharmacokinetic properties (to be described elsewhere) did not show improved antitumor activity. In viability assays, MPI-0479605 inhibited the growth of normal colon cell lines (data not shown) as well as human fibroblasts immortalized with hTERT (Fig. 6C) and MPI-0479605 induced significant neutropenia in mice (Fig. 6B), indicating a lack of tumor selectivity. Recently, a novel Mps1 inhibitor was shown to have antitumor activity with no associated toxicity in 10- and 13-day xenograft studies using A2780 and A375 tumor cell lines (37). It will be of great interest to learn whether these responses are durable. If longer term exposure to Mps1 inhibitors as a single agent is intolerable, these compounds may still find utility when used in combination regimens, such as with tubulin-targeting agents (34).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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Molecular Cancer Therapeutics

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Mol Cancer Ther 2011;10:2267-2275. Published OnlineFirst October 6, 2011.

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