Targeting the Mitotic Checkpoint for Cancer Therapy with NMS-P715, an Inhibitor of MPS1 Kinase

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Abstract

MPS1 kinase is a key regulator of the spindle assembly checkpoint (SAC), a mitotic mechanism specifically required for proper chromosomal alignment and segregation. It has been found aberrantly overexpressed in a wide range of human tumors and is necessary for tumoral cell proliferation. Here we report the identification and characterization of NMS-P715, a selective and orally bioavailable MPS1 small-molecule inhibitor, which selectively reduces cancer cell proliferation, leaving normal cells almost unaffected. NMS-P715 accelerates mitosis and affects kinetochore components localization causing massive aneuploidy and cell death in a variety of tumoral cell lines and inhibits tumor growth in preclinical cancer models. Inhibiting the SAC could represent a promising new approach to selectively target cancer cells.

Introduction

In eukaryotic cells, the spindle assembly checkpoint (SAC) is an evolutionarily conserved mitotic control mechanism specifically required for proper chromosomal segregation. The SAC ensures that cells do not divide until all sister chromatids align at the metaphase plate and are correctly captured by the mitotic spindle, resulting in bipolar amphitelic attachment of chromosomes (1, 2). Although not all the molecular aspects of this checkpoint are understood, it ultimately produces a diffusible "wait anaphase" signal that results in the suppression of the ubiquitin E3 ligase activity of the anaphase promoting complex/cyclosome (APC/C) by inhibiting its activator cdc20 (3, 4). This results in a mitotic arrest by preventing proteasome-mediated degradation of 2 key mitotic components: cyclin B1 and Securin (4).

Human monopolar spindle 1 (MPS1) kinase, also known as TTK, is a dual serine/threonine kinase that plays a critical role in SAC signaling by controlling chromosome alignment and influencing the stability of the kinetochore–microtubule interaction (5–7). MPS1 is a highly dynamic SAC member expressed only in proliferating cells and is activated upon phosphorylation during mitosis, where it is required for proper kinetochore recruitment of essential SAC proteins such as MAD1 and MAD2 (7–11).

In zebrafish, MPS1 mutations are embryonic lethal, and homozygous knockout mice as well as RNA interference (RNAi) studies on other SAC components have revealed that a functional spindle checkpoint is essential for viability (2, 15–17).

Aneuploidy is a common feature of cancer cells seen in 70%–95% of human tumors, and over a century ago it was hypothesized that this may be a possible cause of cancer (18, 19). Although SAC inhibition promotes aneuploidization, genes encoding mitotic checkpoint components are rarely mutated in tumors (20). Specifically, MPS1 has been found upregulated in a number of tumors of different origins including bladder, anaplastic thyroid, breast, lung, esophagus, and prostate (20–25). In addition, MPS1 has been identified in the signature of the top 25 genes overexpressed in CIN and
aneuploid tumors (26). As the other SAC components have also been found to be upregulated in different tumors, and MAD2 overexpression was shown to promote tumorigenesis in mice, it is probable that tumors become "addicted" to SAC functions and the mitotic checkpoint could be required to sustain their proliferation in the presence of aneuploidy (27–31).

Here we report the discovery of NMS-P715, the first orally bioavailable, potent, and selective small-molecule inhibitor of MPS1 kinase activity. The crystal structure of NMS-P715 bound to MPS1 indicates an ATP-competitive binding mode and treatment of tumor cells with NMS-P715 promotes SAC override, affects kinetochore component localization and interactions, and causes massive aneuploidization leading to cell death in a wide range of cancer cell types.

Proliferation data performed on a large panel of cellular lines indicate selectivity toward tumoral cells, and oral administration of NMS-P715 to tumor-bearing mice resulted in tumor growth inhibition at well-tolerated doses.

Our data provide, for the first time, compelling evidence that selective inhibition of MPS1 kinase, SAC abrogation, and consequent aneuploidy induction may represent a promising new approach to cancer therapy.

Materials and Methods

Cell culture and treatments

HeLa cervical carcinoma cells (ECACC) were cultured in E-MEM medium, U2OS osteosarcoma cells (American Type Culture Collection) in DMEM, HCT116 colon carcinoma cells (ATCC) in McCoy’s, and BJ Human normal fibroblasts (ATCC) in McCoy’s. All cell lines were supplemented with 10% fetal calf serum (FCS) and 2 mmol/L-glutamine (Gibco). MG132 (Sigma) was utilized at 15 μmol/L and aphi-dicolin was added 12 hours before additional treatment at 0.5 μmol/L. Cells were then lysed in hypotonic solution (75 mmol/L KCl) for 20 minutes at 37°C and fixed in methanol/glacial acetic acid (3:1) solution for 10 minutes at 4°C. Two or 3 drops of suspended cells were applied to glass slides and chromosomes were stained with DAPI. Images were acquired using an Axiovert 100 microscope (Zeiss) and chromosomes were counted using Image pro-plus 5.0 (Media Cybernetics).

Immunoblotting

For Western blot analysis, cell extracts were prepared in SDS-Buffer (125 mmol/L Tris-HCl, pH 6.8, 2% SDS) and after sonication and boiling, total extracts of the indicated samples were loaded on SDS-PAGE precast gels (Invitrogen) or on 8% acrylamide SDS-PAGE gels containing 12.5 μmol/L phos-tag (32) and immunoblotted.

The following antibodies have been used: phospho-histone H3 Ser10, phospho-H2AX Ser139 and MPS1 (Millipore), cyclin B1, BUB3, MAD2, and BUBR1 (BD Biosciences), histone H3 and Securin (Abcam), ubiquitin (Invitrogen), Parp, anti-cdc20, B1, BUB3, MAD2, and BUBR1 (BD Biosciences), histone H3 and Securin (Abcam), ubiquitin (Invitrogen), Parp, anti-cdc20, or cdc27 (Santa Cruz Biotechnology). Immunoreactive signals were detected with SuperSignal West Pico Chemiluminescent Substrate (Pierce).

Coimmunoprecipitation

HeLa cells were lysed in E1A buffer (50 mmol/L HEPES, pH 7.0, 250 mmol/L NaCl, 0.1% NP-40, and protease inhibitors). Immunoprecipitations (IP) were done by incubating 2 mg of total extract with 2 μg of cdc20 (p55CDC—E7) or cdc27 (C-20) specific antibodies (Santa Cruz Biotechnology). Normal rabbit IgG (Santa Cruz Biotechnology) was used as a negative control.

Flow cytometry

Cells were harvested, resuspended in cold phosphate buffer saline (PBS), fixed with cold (−20°C) 70% ethanol in PBS, and stored at 4°C for at least 1 hour. Cells were then stained with propidium iodide (25 μg/mL) in sodium citrate containing 0.001% nonidet and 25 μg/mL RNase A. The DNA content was analyzed using a FACS Calibur flow cytometer (BD Biosciences).

Colony assay

BJ cells were plated in 12-well plates at 1,000 cells/well and after 72 hours of treatment, cells were incubated in
compound-free medium at 37°C for 18 days. U2OS cells were plated at 500 cells/well in 12-well plates and colony staining was done after 9 days. HeLa cells (4,000 cells) were plated after 48 hours of RNAi-oligos transfection in 10-cm plates and allowed to grow in complete medium for 15 days. Colonies were fixed in 5% formaldehyde for 5 minutes and stained with 1% crystal violet solution. Colonies were counted with Image pro-plus 5.0 software (Media Cybernetics).

Accession codes

MPS1 crystal structure in complex with NMS-P715: Protein Data Bank code 2X9E.

Results

Discovery of NMS-P715 as a selective inhibitor of MPS1 kinase

NMS-P715 is a pyrazolo-quinazoline (Fig. 1A; Supplementary Fig. S1), which was identified after a high-throughput library screening using a biochemical MPS1 kinase assay followed by a medicinal chemistry expansion.

When evaluated against full-length MPS1 in a biochemical assay, NMS-P715 was found to be a potent inhibitor with a half-maximal inhibitory concentration (IC50) value of 182 nmol/L in the presence of 8 μmol/L ATP, which is approximately twice its calculated K_m value. Furthermore, inclusion of a preincubation step significantly increased the compound potency up to an IC50 of 8 nmol/L, suggesting a time-dependent mechanism of inhibition with a K_i of 0.99 ± 0.16 nmol/L (Supplementary Fig. S2A–F). Substrate and ATP titration studies showed a pure ATP-competitive mechanism with a reversible dissociation (Supplementary Fig. S2G and H).

To evaluate selectivity, NMS-P715 was profiled in vitro against a panel of 60 kinases belonging to the different branches of the human kinome (Supplementary Table S1). NMS-P715 was found to be highly specific for MPS1, with no other kinases inhibited below an IC50 value of 5 μmol/L and with only 3 kinases inhibited below 10 μmol/L (CK2, MELK, NEK6).
Ile663, the residue immediately before the DFG motif that partially responsible for the compound high level of selectivity in the loop where it packs against Ile663, Asp664, and the side chain of the diethylphenyl moiety sits underneath the glycine-rich ring. In MPS1, this group binds in a pocket formed by Gln541 and Gly605. The pyrazolo-quinazoline core of NMS-P715 is sandwiched between Val539 and Leu654 and the diethylphenyl moiety sits underneath the glycine-rich loop where it packs against Ile663, Asp664, and the side chain of Lys553. Presumably, the diethylphenyl moiety is partially responsible for the compound high level of selectivity because the parent compound with the simple amide makes extensive hydrophobic contacts with the inhibitor. Isoleucine residues in this position are relatively rare so that the majority of other kinases would not have the proper “fit” for NMS-P715. Another factor contributing to the selectivity is the presence of the trifluoromethoxy group on the phenyl ring. In MPS1, this group binds in a pocket formed by Gln541 and the hinge segment Cys604-Gly605-Asn606. However, in kinases with a residue bulkier than cysteine in the corresponding position, the size of the pocket would be significantly reduced, thus leading to a steric clash with the trifluoromethoxy group.

To better understand the source of affinity and selectivity for MPS1, we solved the crystal structure of the MPS1 catalytic domain bound to NMS-P715. The 3.1 A structure (PDB accession code 2X9E; Supplementary Table S2) reveals that the compound binds in the ATP-pocket (Fig. 1C; Supplementary Fig. S3), where it makes a series of donor–acceptor–donor hydrogen bonds with the MPS1 hinge residues (Glu603 and Gly605). The pyrazolo-quinazoline core of NMS-P715 is sandwiched between Val539 and Leu654 and the diethylphenyl moiety sits underneath the glycine-rich loop where it packs against Ile663, Asp664, and the side chain of Lys553. Presumably, the diethylphenyl moiety is partially responsible for the compound high level of selectivity because the parent compound with the simple amide is significantly less selective. This may be in part due to the high level of shape complementarity between NMS-P715 and Ile663, the residue immediately before the DFG motif that makes extensive hydrophobic contacts with the inhibitor. Isoleucine residues in this position are relatively rare so that the majority of other kinases would not have the proper “fit” for NMS-P715. Another factor contributing to the selectivity is the presence of the trifluoromethoxy group on the phenyl ring. In MPS1, this group binds in a pocket formed by Gln541 and the hinge segment Cys604-Gly605-Asn606. However, in kinases with a residue bulkier than cysteine in the corresponding position, the size of the pocket would be significantly reduced, thus leading to a steric clash with the trifluoromethoxy group.

Taken together, these data show that NMS-P715 is the most potent and selective ATP-competitive MPS1 kinase inhibitor described to date.

**Cellular effects upon NMS-P715 treatment**

MPS1 inhibition is expected to promote checkpoint override in the presence of spindle poisons and to increase the velocity of the mitotic phase in unperturbed conditions (9, 12). To verify the mechanism of action of NMS-P715, we treated nocodazole-arrested U2OS cells at different compound concentrations and mitotic cells were then identified by following histone H3 phosphorylation (Supplementary Fig. S4A). Similarly to MPS1 silencing by RNAi (Supplementary Fig. S4B–D; ref. 9), we confirmed that NMS-P715 promotes massive SAC override with a half-maximal effective concentration (EC<sub>50</sub>) of 65 mM/L (Fig. 1D). In the same assay, NMS-P715 results were far more potent than those for SP600125, the PKC inhibitor G60967, or the Aurora inhibitors MLN8054, ZM447439, and Danusertib (PHA-739358), compounds previously described to have the same effect (Supplementary Fig. S5A; refs. 13, 33, 34).

Moreover, NMS-P715 promoted mitotic bypass of both nocodazole or Taxol-arrested cells in an MG132-dependent manner, showing NMS-P715 independence on the type of checkpoint triggering agent and confirming, as expected, that proteasomal activity is required for the compound-mediated mitotic exit (Supplementary Fig. S5B).

To analyze NMS-P715 activity in growing unperturbed mitotic cells, we conducted time-lapse video microscopy experiments in U2OS cells overexpressing YFP-α-tubulin. In agreement with RNAi data (Supplementary Fig. S6; refs. 9, 12), MPS1 inhibition by NMS-P715 leads to mitotic acceleration, with anaphase starting before metaphase plate formation (Fig. 2A). Mitotic-phase length was reduced by approximately 3 times (Fig. 2B) and, as a consequence, we observed a strong reduction in mitotic cells (Fig. 2C). Notably, no defects were detected in bipolar spindle formation or in cytokinesis (Fig. 2A) and, differently from what has been reported in budding yeast (35), centrosomal splitting was normal, thus excluding a major role of the enzymatic activity of MPS1 in centrosomal functions in human cells.

MPS1 was previously reported to be heavily phosphorylated during mitosis and a number of autophosphorylation sites were identified (7, 11, 36). To determine a direct in vivo regulation of MPS1 autophosphorylation by NMS-P715, cellular lysates of nocodazole-arrested HeLa cells were analyzed following treatment with the compound. NMS-P715 caused a specific shift in the migration of MPS1. This shift was also observed in the presence of MG132, administered to keep cells in mitosis and thus exclude the possibility that this change was due to mitotic exit (Fig. 2D). Notably, however, NMS-P715 did not cause a complete MPS1 dephosphorylation, which was achieved only in the absence of MG132 upon mitotic exit, indicating that only a subset of MPS1 phosphorylation sites are directly affected by compound treatment. In addition, Securin and cyclin B1 polyubiquitylation was induced, indicating an NMS-P715-dependent activation of the APC/C ubiquitin E3 ligase activity.

As MPS1 kinase has also been implicated in the recruitment of essential SAC components to the kinetochore (8, 9), we investigated the kinetochore localization of different mitotic checkpoint components upon NMS-P715 treatment in the presence of MG132 in nocodazole-arrested HeLa cells. NMS-P715 leads to an almost complete delocalization of MAD1, MAD2, BUB1, and BUB3 (Fig. 2E; Supplementary Fig. S7). Interestingly, MPS1 itself was also reduced, although not to the same extent as the other SAC components. We also observed a delocalization of Borealin, which was not accompanied by a change in Aurora B localization. This confirms a specific role for MPS1 on Borealin, which was recently described as an MPS1 substrate (12). No modulation was observed on CENPB, HEC1, and BUBR1. These data are in full agreement with published data (Supplementary Table S3), the only exception being the marked BUB1 reduction not previously reported. We can exclude the activity of NMS-P715 on BUB1 kinase in vitro (Supplementary Table S1) and an explanation of this observation might be that complete inhibition of MPS1, achieved only by...
small-molecule inhibitors, is required to cause BUB1 kinetochoore disappearance.

NMS-P715 treatment causes SAC inactivation and delocalization of kinetochore components. MAD2, BUBR1, BUB3, and cdc20 have been shown to be components of the mitotic checkpoint complex (MCC) that is required to sustain the mitotic checkpoint activity inhibiting cdc20-dependent activation of APC/C (2). For this reason, MCC complex integrity was evaluated upon NMS-P715 treatment by coimmunoprecipitation (Co-Ip) experiments. Interestingly, in treated cells, cdc20 interaction with MAD2 and BUB3 was lost whereas BUBR1 binding was slightly reduced (Fig. 3A). This was observed not only upon cdc20 Co-Ip but also upon Co-Ip of the APC/C component Cdc27. We also noticed that NMS-P715

Figure 2. NMS-P715 has the expected mechanism of action in cells. A, time-lapse of YFP-α-tubulin overexpressing U2OS cells. Brightfield and YFP-dependent fluorescent signals of a representative mitotic cell are shown in controls (DMSO) or 1 μmol/L NMS-P715–treated cells. Images were taken every 6 minutes. Scale bar, 10 micrometers/L. B, mitotic length was calculated as the average of 20 mitotic cells treated as described above. C, mitotic index (percentage of mitotic cells) of U2OS cells treated for 20 hours with NMS-P715 (1 μmol/L) or DMSO (control). A total of 800 cells were counted in 3 independent experiments. D, Western blots were obtained as indicated in extracts of nocodazole (Noc.)–arrested mitotic HeLa cells, harvested by shake-off, and then treated for 1 hour as shown. MPS1 was evaluated by Phos-tag gel technology. The ubiquitylated forms of Securin and cyclin B1 are indicated. E, bar graph quantifying pixel intensities of the indicated mitotic proteins at the kinetochore (KT) upon NMS-P715 treatment (1 μmol/L), compared with control treatment (DMSO). Values are normalized to the total kinetochore signal obtained by Crest-specific antibody staining and represent the mean derived from the analysis of at least 5 cells.
causes cdc20 deubiquitylation (Fig. 3B); this indicates that MPS1 inhibition leads to mitotic override affecting both of the two putative SAC quality controls on cdc20: it affects MCC integrity and stability and abrogates cdc20 ubiquitylation allowing it to trigger APC/C activation (37).

In summary, the observed cellular effects indicate that MPS1 is the main target of NMS-P715 in cells.

**NMS-P715 inhibits tumoral cell proliferation**

MPS1 depletion causes chromosomal segregation errors and massive aneuploidy not compatible with cellular survival (Supplementary Fig. S8; refs. 9, 12). Indeed, NMS-P715 treatment also causes SAC inhibition and mitotic acceleration, which ultimately promotes chromosomal misalignment in metaphase and anaphase (Supplementary Fig. S9A and B). As a consequence, chromosome counts after mitotic spreads revealed a dramatic increase in aneuploidy upon treatment with NMS-P715 (Fig. 4A; Supplementary Fig. S9C).

To assess the ability of NMS-P715 to promote aneuploidy-mediated cell death, A2780 ovarian cancer cells were treated at different doses for 2, 24, and 72 hours (Fig. 4B and C). Already after 24 hours, NMS-P715 treatment caused a reduction in G1 phase and a flattening in G2/M phase of the cell cycle as measured by flow cytometry. A similar profile was observed upon MPS1 RNAi (Supplementary Fig. S8) and was also confirmed in the colon-derived cell line HCT116 (Supplementary Fig. S10). Aneuploidization, together with the appearance of a sub-G1 population, further increased at 72 hours (Fig. 4B). This was accompanied by histone H3 dephosphorylation, Parp cleavage, and histone H2AX phosphorylation, indicating mitotic exit and confirming subsequent induction of apoptosis (Fig. 4C).

To follow the behavior of individual cells over a long period of treatment, we analyzed U2OS cells upon NMS-P715 administration by time-lapse microscopy and established a fate profile (Fig. 4D; ref. 38). After drug addition, we confirmed...
that only the mitotic phase was affected. For an average of 39 hours virtually all the cells were able to grow and divide. Faster mitotic division occurred in mitotic cells a few minutes after drug treatment and caused aneuploidy-mediated cell death that starts to be seen after 52 hours of treatment and occurred more frequently after 2 aberrant mitoses and upon a prolonged aneuploid interphase. After 86 hours, almost all the cells died (Supplementary Fig. S11A). In addition, when cells were arrested in early S phase by aphidicolin treatment, we found an almost complete inhibition of NMS-P715–mediated apoptotic induction (Fig. 4E); thus the passage through an aberrant mitosis is critical and essential for induction of cell death.

**Figure 4.** NMS-P715 induces aneuploidy and inhibits tumoral cell proliferation. A, mitotic spread results showing chromosomal number distribution of HCT116 cells treated for 24 hours with NMS-P715 (1 μmol/L) or DMSO (control). Spreads of 68 cells were analyzed. B, DNA content assessed by flow cytometry of A2780 cells treated with DMSO (control) or NMS-P715. C, Western blots were obtained as indicated from extracts of A2780 cells treated with DMSO (Ctr.) or NMS-P715. D, fate profile of U2OS cells exposed to DMSO (control) or NMS-P715 (1 μmol/L). Each lane represents 1 cell followed for 86 hours (5,160 minutes) by time-lapse. E, bar graph quantifying apoptotic induction measured by flow cytometry in HCT116 cells. NMS-P715 (1 μmol/L) was administered with or without aphidicolin. Values are normalized versus control (DMSO). F, colony assay of U2OS cells treated as indicated for 22 hours and grown in compound-free medium for 8 days (washout) or treated continuously for 9 days (prolonged treatment) before staining. Percentages of colony numbers are normalized to control (DMSO). Values are the average of 2 experiments.
death. Importantly, colony assay experiments done in U2OS cells treated with NMS-P715 for one entire cell cycle (22 hours) followed by drug washout, revealed that one single aberrant mitotic event is sufficient to commit cells to apoptosis (Fig. 4F; Supplementary Fig S11B).

To evaluate NMS-P715 antiproliferation effects in a large cell line panel, 127 cell lines were treated for 72 hours at different compound concentrations. The IC\textsubscript{50} for inhibition of cell growth was determined for each cell line (Supplementary Table S4), and the activity ratio was calculated (Supplementary Fig S12; ref. 39). Growth inhibition was observed in a wide range of cell lines. Highest sensitivity was observed in colon, breast, renal, and melanoma cell lines with IC\textsubscript{50} values close to 1 \(\mu\text{mol/L}\), whereas no correlation was observed between antiproliferative effects and cellular doubling time or chromosomal number, which is known for the 52 cell lines belonging to the NCI60 cell line panel (40). Notably, normal cells were much less sensitive than tumoral cell lines to the antiproliferative activity of NMS-P715 (Supplementary Fig S12). To confirm this finding, normal human fibroblast BJ cells were tested in colony-forming assay experiments and no effect on colony growth was observed (Supplementary Fig S13). Thus, NMS-P715 has potent antiproliferative activity and shows selectivity toward cancer cell lines.

**NMS-P715 inhibits tumor xenograft growth**

To evaluate the antitumor activity of NMS-P715 and to examine the role of MPS1 kinase in tumor proliferation *in vivo*, we administered the compound to nude mice bearing subcutaneous implanted human tumor cell xenografts and monitored tumor growth rate. At a dose of 10 mg/kg NMS-P715 showed an oral bioavailability of 37% and good pharmacokinetic properties, with an area under the concentration–time curve (AUC) of 8 \(\mu\text{mol/L-h}\). This was accompanied by low clearance, long half-life, and good volume of distribution in tissues (Supplementary Fig S14).

NMS-P715 was administered by oral route at 90 mg/kg once a day for 7 consecutive days in an A2780 ovary carcinoma xenograft model, resulting in 53% tumor growth inhibition at the end of the treatment (Fig. 5A). At this dose, the compound was well tolerated and there were no signs of body weight loss or other overt toxicities. NMS-P715 antitumor activity was also confirmed in the A375 melanoma xenograft model, with a tumor growth inhibition of approximately 43% measured after 10 days of treatment at 100 mg/kg (Fig. 5B).

After administration of 100 mg/kg, pharmacokinetic parameters indicate a \(C_{\text{max}}\) of 1.8 \(\mu\text{mol/L}\) that is reached 6 hours after initiation of treatment, a prolonged half-life, and an AUC of 50 \(\mu\text{mol/L-h}\) (Supplementary Fig S14). At this dose NMS-P715 leads to a reduction of histone H3 phosphorylation in tumor samples (Fig. 5C), indicating that sufficient exposure levels were reached to inhibit MPS1 kinase activity, causing rapid mitotic exit.

Taken together, the *in vivo* data show the proof of concept that MPS1 inhibitors reduce tumor growth *in vivo* and, for the first time, propose a strong rationale for the development of MPS1 or SAC inhibitors as a valid approach for cancer therapy. In particular, we believe that slow-growing tumors that better resemble human tumor growth might show improved antitumor activity after a prolonged and continuous treatment by accumulation of chromosomal segregation errors.

**Discussion**

In the last decade, a number of mitotic kinases have been identified as playing essential roles in the orchestration of the cell cycle and some of these are the subject of targeted therapy approaches in oncology (41).

Here we describe NMS-P715 as the first selective, ATP-competitive, and orally bioavailable inhibitor of MPS1, a...
mitotic kinase expressed only in proliferating normal tissues but aberrantly overexpressed in a wide range of human tumors and required for proper SAC functions (1, 10). Similar to MPS1 silencing experiments, NMS-P715 treatment promotes mitotic checkpoint override, reduces mitotic length, and causes chromosomal misalignment, kinetochore and MCC complex destabilization, and massive aneuploidization leading to cell death in a wide range of cancer cell types.

Importantly, our studies show for the first time that selective inhibition of MPS1 and targeting of the SAC causes tumor growth inhibition in xenograft models, and proliferation analysis done on a large panel of cell lines indicates higher activity toward cancer cell proliferation while leaving normal cell growth almost unaffected.

MPS1 inhibition and mitotic checkpoint abrogation introduce a completely new strategy to cancer therapy. Differently from the classical antiimitotic therapy, the objective is not to arrest cell proliferation but to inactivate the SAC with the consequence of an accelerated mitosis, which increases aneuploidy and results in cell death.

Whether aneuploidy is a cause or consequence of malignant transformation is still under debate, with recent evidence indicating that aneuploidy may not be a strong driver of tumorigenesis (42). Complete inactivation of the mitotic checkpoint is lethal in mice and in cancer cell lines, whereas mice expressing reduced levels of mitotic checkpoint components, despite a high aneuploidy rate, rarely develop tumors and in this case only after a long latency (16, 43). Finally, in yeast cells and in mouse embryonic fibroblasts even the addition of a single chromosome causes cellular stress and fitness reduction and in mouse models of chemically or genetically induced tumorigenesis, an increased rate of aneuploidy acts as a tumor suppressor (44, 45).

As most cancers are aneuploid, they must evolve a strategy to overcome this initial fitness reduction. Although some cancer cell lines show a weakened mitotic checkpoint signaling, no clear correlation has been reported with chromosomal instability status and a number of reports indicate that most aneuploid tumoral cell lines have an intact mitotic checkpoint, often with high levels of checkpoint components (20, 30, 42, 46, 47). Thus, MPS1 functions and checkpoint activity could be selectively required to sustain and favor aneuploid tumor proliferation and this could be the reason why we observe higher activity of NMS-P715 against tumoral cell lines. This view is supported by a recent report that shows that MPS1 depletion is selectively toxic in tumor cell lines if added in combination with Taxol (5). Current studies are in progress to determine if different levels of checkpoint components, or a specific genetic background, sensitizes cancer cells to MPS1 inhibition. There is growing evidence that the SAC function is particularly critical in the presence of extra chromosomes in human cells or in a tetraploid background in yeast (29, 48). In Drosophila cells, SAC functions have been shown to be specifically required to maintain proliferation of polycentromosomal cells (49), and in a recent report a new MPS1 inhibitor (MPS1-IN-1) was described to increase the frequency of multipolar mitosis in a cell line containing extra centrosomes (14).

Our data provide strong evidence that inhibition of the SAC by MPS1 inhibition may represent a novel approach to cancer therapy with the potential to selectively block tumor cell proliferation and makes NMS-P715 a suitable candidate for further development toward clinical studies.

Disclosure of Potential Conflicts of Interest

The authors do not have a potential conflict of interest to disclose. All authors declare to be full-time employees of Nerviano Medical Sciences S.r.l.

Authors Contributions


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References


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