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Inhibition of Wee1 sensitizes cancer cells to anti-metabolite chemotherapeutics in vitro and in vivo, independent of p53 functionality

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Abstract

Inhibition of Wee1 is emerging as a novel therapeutic strategy for cancer, and some data suggest that cells with dysfunctional p53 are more sensitive to Wee1 inhibition combined with conventional chemotherapy than those with functional p53. We and others found that Wee1 inhibition sensitizes leukemia cells to cytarabine. Thus, we sought to determine whether chemosensitization by Wee1 inhibition is dependent on p53 dysfunction and whether combining Wee1 inhibition is tolerable and effective in vivo. Synergistic inhibition of proliferation with a Wee1 inhibitor in clinical development, MK1775, and cytarabine was observed in all acute myeloid leukemia (AML) cell lines tested, regardless of p53 functionality. Mechanistic studies indicate that inhibition of Wee1 abrogates the S-phase checkpoint and augments apoptosis induced by cytarabine. In AML and lung cancer cell lines, genetic disruption of p53 did not alter the cells' enhanced sensitivity to antimetabolites with Wee1 inhibition. Lastly, mice with AML were treated with cytarabine and/or MK1775. The combination of MK1775 and cytarabine was well-tolerated in mice and enhanced the anti-leukemia effects of cytarabine, including survival. Thus, inhibition of Wee1 sensitizes hematologic and solid tumor cell lines to antimetabolite chemotherapeutics, whether p53 is functional or not, suggesting that the use of p53 mutation as a predictive biomarker for response to Wee1 inhibition may be restricted to certain cancers and/or chemotherapeutics. These data provide preclinical justification for testing MK1775 and cytarabine in patients with leukemia.

Keywords

Wee1; MK1775; antimetabolite; chemotherapy; chemosensitization; cell cycle; leukemia; in vivo; p53

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Introduction

Cell cycle checkpoint and DNA damage response proteins are critical mediators of successful DNA replication in the presence and absence of genotoxic stress. Cancer cells are particularly dependent on these processes, a phenomenon that could be exploited therapeutically (1, 2). For example, Chk1 has been studied extensively as an adjuvant therapeutic target in combination with anti-cancer therapy, including radiation and chemotherapy (3, 4). This strategy is expected to be particularly effective in tumors with disrupted p53 function, as they are highly dependent upon the G2/M checkpoint mediated by Chk1 (5).

Wee1 is a cell cycle checkpoint protein downstream of Chk1 that is activated during the normal cell cycle, as well as in the context of DNA damage. The primary function of WEE1 is inhibitory phosphorylation of CDKs at tyrosine 15 (Y15), thereby inhibiting cell cycle progression (6, 7). Most studies of Wee1 have focused on the phosphorylation of CDK1 in the context of DNA damage, which prevents progression through mitosis with levels of DNA damage that would result in mitotic catastrophe. Like Chk1, inhibition of Wee1 in combination with DNA damaging agents has been explored as a therapeutic strategy for tumors with dysregulated p53. Indeed, in published reports, inhibition of Wee1 with small-molecule inhibitors in combination with DNA damaging agents, including doxorubicin, has shown some specificity for *TP53* mutated tumor models (8–11). Using RNA interference screens, we and others have recently identified Wee1 as a critical mediator of AML cell survival after treatment with cytarabine, an antimetabolite that induces S-phase arrest, and a key component of successful AML therapy (12, 13). The addition of the Wee1 inhibitor, MK1775 (8), to cytarabine impairs the cell cycle checkpoint and induces more apoptosis than cytarabine alone (13). Notably, our data were generated in cell lines that are reported to have normal p53 function.

Therefore, we sought to determine whether the function of p53 influences the sensitivity to Wee1 inhibition with chemotherapy in a broad panel of AML cell lines with various molecular abnormalities. In contrast to data from solid tumor models sensitized to DNA damaging agents (8–11), we found that the functionality of p53 has no bearing on the chemosensitization of AML cells to cytarabine, as all of the cell lines tested were sensitized to cytarabine with Wee1 inhibition. Mechanistic studies indicate that inhibition of Wee1 abrogates the S-phase checkpoint and augments apoptosis induced by cytarabine. Furthermore, in isogenic models, in which wild-type p53 activity was impaired by RNA-interference or dominant negative p53 constructs, we did not find enhanced chemosensitization with impaired p53. Also, in contrast with data from solid tumor models, we did not observe chemosensitization to doxorubicin with Wee1 inhibition in AML cells, even in cells with non-functional p53. In addition, we found that the chemosensitization to antimetabolite chemotherapeutics is not limited to leukemia, as lung cancer cells were equally sensitized to cytarabine and pemetrexed, whether p53 function was impaired or not. Lastly, in mice with AML, we found that the combination of Wee1 inhibition with cytarabine slowed disease progression and prolonged survival better than cytarabine alone. These data support the development of clinical trials of antimetabolite chemotherapeutics and Wee1 inhibition for patients with cancers; however, distinct from DNA damaging agents that induce the G2/M checkpoint, our data do not support the use of *TP53* mutation as a biomarker to predict beneficial effects of Wee1 inhibition when combined with antimetabolites that induce the S-phase checkpoint.

Materials and Methods

Cell lines and tissue culture

Cell lines were generous gifts from the laboratories of Drs. Douglas Graham and James DeGregori. Cell lines were DNA fingerprinted by multiplex PCR using the Profiler Plus or Identifier Kits (ABI) and confirmed to match published or internal databases as previously described (14), prior to storage of stock vials in liquid nitrogen. All cells were cultured at 37°C in humidified air supplemented with 5% CO₂, in RPMI supplemented with 10% FBS and antibiotics, except OCI-AML3 and Kasumi-1 which were cultured in RPMI supplemented with 20% heat-inactivated FBS. All AML cell lines were seeded at 1–2×10⁵/ml prior to experimentation. A549 cells were plated at 1–2.5×10³ cells/well the day before experimentation. Cells were counted by propidium iodide (Sigma) exclusion and flow cytometry (Guava EasyCyte Plus, Millipore, Billerica, MA). Apoptosis and cell cycle were measured with the Guava EasyCyte Plus using the Guava Nexin and Guava Cell Cycle reagents per the manufacturer's protocol (Millipore).

Vectors

MSCV-ires-GFP (MiG), MSCV-DDp53-GFP (DDp53), and MSCV-DNp53-GFP (DNp53) plasmids (provided by Dr. DeGregori) were packaged into viral particles and transduced into OCI-AML3 cells as previously described (15). Transduced cells were sorted for GFP using a MoFlow fluorescence activated cell sorter (Dako Cytomation, Carpinteria, CA). Non-silencing shRNA and shRNA targeting p53 from the TRC collection (16) were purchased from the Functional Genomics Facility of the University of Colorado Cancer Center (Boulder, CO) and packaged as previously described (17). Transduced cells were selected in puromycin (Sigma-Aldrich, St. Louis, MO).

Antibodies, chemicals and reagents

Antibody directed against p21^{CIP1} was purchased from BD Biosciences (San Jose, CA); antibodies against phosphorylated CDK1 (Y15), CDK2 (Y15), and histone H3 (S10) were purchased from Cell Signaling Technology (Danvers, MA). Cytarabine and doxorubicin were purchased from Sigma-Aldrich and diluted in water. Nutlin-3 was purchased from Cayman Chemical (Ann Arbor, MI) and diluted in DMSO. Pemetrexed was purchased from Selleck (Houston, TX). MK1775 was provided by the National Cancer Institute and Merck Sharp & Dohme Corporation (Whitehouse Station, NJ).

Animal Studies

Female C57BL/6J mice, 6–8 weeks old, were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were housed in sterile micro-isolators in the Center for Comparative Medicine at the University of Colorado Denver. Mice were treated with cytarabine 50mg/kg/d by intraperitoneal injection and/or MK1775 40mg/kg/d by oral gavage. One million luciferase expressing AML cells (18) were injected into unirradiated recipients to induce leukemia. Luciferase activity was measured 5 minutes after injection of luciferin using a Xenogen IVIS2000 imaging system. Animal studies were approved by the Institutional Animal Care and Use Committee of the University of Colorado Denver.

Data Analyses

Excel and GraphPad Prism 5 (GraphPad Software, San Diego, CA) were used for data sorting, analysis and graphical depiction of data. Student's t-test was used to compare 2 samples; analysis of variance (ANOVA) and Bonferroni's post-test were used to compare more than 2 samples. The Mantel-Cox (log-rank) test was used to test for significant differences in survival. Except as indicated in figure legends, figures demonstrate means of

data collected from at least 3 independent experiments completed in duplicate or triplicate for each condition. Error bars in each figure depict the standard error of the mean, and may be obscured when narrow. For determination of synergistic effects, the extent of inhibition relative to control treated cells were input into CalcuSyn (Biosoft, Cambridge, UK) to calculate CI values. CI values less than 1 are considered to represent synergistic inhibition of cellular proliferation (19). Chemiluminescence signals from Western blots were quantified using ImageJ software (20).

Results

We previously reported synergistic inhibition of proliferation in AML cells with cytarabine and MK1775, a small molecule inhibitor of Wee1, in Molm13, MV4-11 and U937 cell lines (21). These data suggested that the combinatorial effect of cytarabine and Wee1 inhibition in AML cells is independent of p53 functionality, FLT3 mutation, and MLL rearrangement (Supplemental Table 1). We sought to confirm this in a broader panel of AML cell lines with wild-type and mutated *TP53*, as well as other AML specific oncogenes (Supplemental Table 1). We first treated cells with the MK1775 as a single agent with a range of doses to determine the EC₅₀ for each cell line. Consistent with a recent publication (22), we observed inhibition of proliferation of all cell lines with MK1775 as a single agent at clinically achievable concentrations, with a median EC₅₀ value of 396.8 nM (Supplemental Figure 1 and Supplemental Table 1). Similar to findings in sarcoma cell lines (23), the mutational status of *TP53* was not associated with sensitivity to MK1775 as a single agent ($p=0.47$; Supplemental Table 1 and Supplemental Figure 1).

Despite single agent activity, MK1775 would most likely be incorporated into clinical trials as a chemosensitizing agent in combination with conventional chemotherapeutics. We chose OCI-AML3 and HL60 for detailed study of the chemosensitizing effects of Wee1 inhibition, as they reportedly have wild-type and mutated *TP53*, respectively (24, 25). To confirm the function of p53, we treated cells with nutlin-3a, a small molecule that interferes with the interaction of Mdm2 and p53, resulting in activation of p53 in cells in which it is not mutated (26). We found that OCI-AML3 cells were sensitive to treatment with nutlin-3a, while HL60 were not, consistent with the reported function of p53 in these cell lines (Figure 1A). As a second test of p53 function, we performed Western blotting for p21 induction with DNA damage induced by doxorubicin (27). As expected, p21 was induced in OCI-AML3 cells, but not in HL60 (Figure 1B). Despite discrepant p53 functionality, we found that in both cell lines, the concomitant treatment with MK1775 and cytarabine was synergistic (Figure 1C and 1D and Supplemental Figure 2). We observed synergistic inhibition in Nomo1 and Kasumi1 cells as well (Supplemental Figure 3). Thus, 3 cell lines with mutated *TP53* (HL60, Nomo1 and Kasumi1) and 3 cell lines with wild-type *TP53* (OCI-AML3, Molm13, and MV4-11 (13)) are sensitized to cytarabine with MK1775, indicating that the beneficial effects of this combination are not dependent upon p53 dysfunction.

To better understand the mechanisms of chemosensitization to cytarabine with MK1775 we first examined inhibitory phosphorylation of tyrosine 15 of CDK2 by western blot, as a measure of S-phase checkpoint activation. Treatment with cytarabine alone led to enhanced phosphorylation of CDK2 (Y15), while the addition of MK1775 abrogated this modification, whether p53 was functional or not (Figure 2A). Consistent with this, measurement of the cell cycle demonstrated S-phase arrest in cells treated with cytarabine that was diminished with the addition of MK1775 in both cell lines (Figure 2B). Moreover, the addition of MK1775 enhanced the induction of apoptosis by cytarabine in both cell lines with discrepant p53 functionality (Figure 2C). We also observed diminished phosphorylation of tyrosine 15 of CDK1 in cells treated with MK1775 (Supplemental Figure 4A). However, in contrast to other reports in which MK1775 leads to unscheduled mitosis in

chemotherapy treated cells (8, 10, 22), we did not observe a dramatic increase in phosphorylated histone H3, (Supplemental Figure 4B) in either cell line. These data indicate that the function of Wee1 in arresting cells in the S-phase of the cell cycle is critical to the survival of leukemia cells exposed to cytarabine, independent of p53 functionality.

While cytarabine is one of the most active agents in AML, successful therapy also includes anthracyclines, such as doxorubicin, and MK1775 has been reported to sensitize a *TP53* mutated colorectal cancer cell line (WiDr) to doxorubicin (28). Surprisingly, we found that the combination of MK1775 and doxorubicin did not have much synergistic effect in HL60 or OCI-AML3 (Figure 3 and Supplemental Figure 5), suggesting that dysfunctional p53 by itself is insufficient to sensitize cancer cells to this combination. In fact, the CI values at many of the dose combinations suggested an antagonistic effect of these two compounds, foreboding caution in the development of clinical trials incorporating MK1775 into induction regimens for AML.

While these data suggest that the functionality of p53 has no bearing on the chemosensitization of AML cells to cytarabine, interpretation of data from genotypically diverse cell lines should be cautious. To more definitively test the relevance of p53 function in this context, we created isogenic cell lines with disrupted p53 activity by retroviral transduction of OCI-AML3 cells with a dominant negative form of p53 (DDp53) or the empty control vector (MiG). These cells were then treated with cytarabine or doxorubicin with or without MK1775. Cells expressing DDp53 were less sensitive to nutlin-3a and had impaired p21 induction with doxorubicin treatment, as compared to cells expressing the empty vector, indicating impaired p53 activity (Figure 4A). Cells expressing DDp53 were equally as sensitive to the combination of cytarabine and MK1775 as those cells expressing the empty vector (Figure 4B). Experiments with isogenic cells in which p53 function has been disrupted also indicate that even in cells with dysfunctional p53, inhibition of Wee1 does not sensitize AML cells to doxorubicin (Figure 4B). Similar observations were made with a second dominant negative form of p53 (DNp53), although the extent of p53 impairment was not as great as with DDp53 (Supplemental Figure 6), and with shRNAs directed against p53 (Figure 4C and Supplemental Figure 6). These data confirm that the synergistic anti-leukemic activity of cytarabine and MK1775 is independent of p53 functionality.

To determine if our observations are specific to AML, we tested the combination of cytarabine and MK1775 in the lung cancer derived A549 cell line. These cells have been described to have functional p53 (29), which we confirmed using nutlin-3a (Figure 5A). Consistent with our data in AML cell lines, A549 cells were synergistically sensitized to cytarabine when treated with MK1775 (Figure 5B, Supplemental Figure 7). We next tested a more clinically relevant antimetabolite used for lung cancer treatment, pemetrexed, in combination with MK1775 and again found synergistic inhibition of proliferation (Figure 5C, Supplemental Figure 7). A549 cells were then transduced with MiG and DDp53 and the functionality of p53 probed with nutlin-3a (Figure 5D). These cells were treated with pemetrexed and/or MK1775, and we again observed similar sensitivity to combination therapy (Figure 5E), indicating that the beneficial effect of combining Wee1 inhibition with antimetabolite chemotherapeutics is not limited to AML and may be beneficial whether *TP53* is mutated or not.

Lastly, to determine if the combination of antimetabolite chemotherapy plus Wee1 inhibition is tolerable and effective in vivo, we modeled therapy in mice. First, we treated mice without leukemia with cytarabine (50mg/kg/d) with or without MK1775 (40mg/kg/d) for 5, 7 or 10 days, and found that the longer courses of single or combination therapy were toxic, resulting in pancytopenia (not shown). In a second toxicity study, mice without

leukemia were treated with cytarabine and/or MK1775 for 5 of 7 days for 3 consecutive weeks. The addition of MK1775 did not enhance the hematologic effects of cytarabine with this regimen (Figure 6A). We then used an aggressive model of murine AML expressing MLL-ENL, FLT3-ITD and luciferase (18), to determine if Wee1 inhibition would enhance the anti-leukemia effects of cytarabine. Consistent with the previous report (18), cytarabine alone slowed the progression of the leukemia, as measured by luciferase expression over time (Figure 6B and Supplemental Figure 8). The addition of MK1775 to cytarabine markedly enhanced the effects of cytarabine in slowing disease progression. Moreover, the addition of MK1775 to cytarabine significantly enhanced survival as compared to cytarabine alone (Figure 6C).

Discussion

Targeting Wee1 to sensitize cancer cells to chemotherapy has emerged as a novel therapeutic strategy, and is currently being tested in early phase clinical trials with the Wee1 inhibitor, MK1775. While others have demonstrated that cell lines with *TP53* mutation are particularly sensitive to the combination of MK1775 and DNA damaging chemotherapy, we have shown here that the functionality of p53 does not influence sensitization to antimetabolite chemotherapeutics by MK1775 in AML cells and lung cancer cells. This conclusion is based on data not only from a panel of AML cell lines with discrepant molecular abnormalities, including *TP53* mutation, but also isogenic cell line models of AML and lung cancer. We also demonstrate that inhibition of Wee1 does not sensitize AML cells to doxorubicin, even when p53 function is disrupted. Thus, the use of *TP53* mutation as a biomarker to predict likely responders to chemosensitization with MK1775 must be cautious and considered in the context of the chemotherapy with which it will be paired, as well as the preclinical data supporting the combination. Moreover, we demonstrate for the first time that Wee1 inhibition can be effectively combined with cytarabine to slow leukemia progression in vivo.

As noted, tumors with dysfunctional p53 may be particularly susceptible to the combination of MK1775 and DNA damaging agents (8–11), hypothetically due to the impaired G1 checkpoint and high dependence upon the G2/M checkpoint (5). The specificity of chemosensitization by MK1775 for *TP53* mutated tumors was first reported in an ovarian cancer cell line (TOV21G) in which p53 had been knocked down by shRNA (8). In these experiments, cells with knockdown of p53 had greater sensitization to gemcitabine, carboplatin and cisplatin as measured by sub-G1 DNA content, when treated with a single dose of MK1775 (8). Conversely, lung cancer cells with impaired p53 function (H1299) transduced with inducible p53, were sensitized to ionizing irradiation with MK1775 if p53 was not expressed, but not sensitized when p53 expression was induced (9). Other reports of the specificity of chemosensitization in *TP53* mutated cell lines rely on data from non-isogenic cell lines (10, 11). Notably, in a large panel of breast cell lines, while there was an association with mutated *TP53* and synergy with gemcitabine and MK1775, many of the cell lines with mutated *TP53* did not have CI values reflecting synergy, indicating that dysfunctional p53 does not always confer sensitivity to that particular combination (10). Our data do not directly contradict these reports, as we have looked specifically in the context of antimetabolite chemotherapeutics with distinct mechanisms of action. Rather, our data highlight the function of Wee1 in the S-phase checkpoint activated by anti-metabolites (13), independent of p53, and broaden the potential clinical applicability of Wee1 inhibition. The fact that lung cancer cells were also sensitized to cytarabine and pemetrexed indicates that the beneficial effect of combining Wee1 inhibition with antimetabolite chemotherapeutics is not limited to AML and may be beneficial whether *TP53* is mutated or not.

Importantly, we did not observe synergy with the anthracycline, doxorubicin, in combination with MK1775 in any of the AML cell lines tested. To the contrary, CI values suggested antagonism at some of the dose combinations tested. This information will be critical to consider in the design of clinical trials incorporating MK1775 into induction regimens for AML that typically include both cytarabine and an anthracycline.

Not surprisingly, we observed hematopoietic toxicity in mice treated with cytarabine with or without MK1775 when given daily for more than 5 days. Nonetheless, mice with leukemia survived longer due to enhanced disease control when treated with combination therapy as compared to cytarabine alone. Notably, we observed a decrease in disease burden with each course of combination therapy. Whether reducing the cytarabine dose and giving combined therapy continuously would be tolerable and more efficacious remains to be determined.

Taken together, these data suggest that the combination of Wee1 inhibition and cytarabine is a broadly applicable therapeutic strategy for AML, independent of several known molecular abnormalities, including mutation in *TP53*. Further, the inhibition of Wee1 in combination with other clinically relevant antimetabolites should be tested, as this strategy may be applicable across a number of different cancer types including lung cancer. While the use of *TP53* mutation as a biomarker predictive of response for certain chemosensitization strategies may be appropriate, its use for combinations with antimetabolites appears to be limited.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009; 136:823–37. [PubMed: 19269363]
2. Zhou BB, Bartek J. Targeting the checkpoint kinases: chemosensitization versus chemoprotection. *Nat Rev Cancer*. 2004; 4:216–25. [PubMed: 14993903]
3. Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA, O'Connor PM. UCN-01: a potent abrogator of G2 checkpoint function in cancer cells with disrupted p53. *J Natl Cancer Inst*. 1996; 88:956–65. [PubMed: 8667426]
4. Sampath D, Cortes J, Estrov Z, Du M, Shi Z, Andreeff M, et al. Pharmacodynamics of cytarabine alone and in combination with 7-hydroxystaurosporine (UCN-01) in AML blasts in vitro and during a clinical trial. *Blood*. 2006; 107:2517–24. [PubMed: 16293603]
5. Ma CX, Cai S, Li S, Ryan CE, Guo Z, Schaiff WT, et al. Targeting Chk1 in p53-deficient triple-negative breast cancer is therapeutically beneficial in human-in-mouse tumor models. *J Clin Invest*. 2012; 122:1541–52. [PubMed: 22446188]
6. Parker LL, Piwnicka-Worms H. Inactivation of the p34cdc2-cyclin B complex by the human WEE1 tyrosine kinase. *Science*. 1992; 257:1955–7. [PubMed: 1384126]
7. McGowan CH, Russell P. Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. *Embo J*. 1993; 12:75–85. [PubMed: 8428596]
8. Hirai H, Iwasawa Y, Okada M, Arai T, Nishibata T, Kobayashi M, et al. Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. *Mol Cancer Ther*. 2009; 8:2992–3000. [PubMed: 19887545]

9. Bridges KA, Hirai H, Buser CA, Brooks C, Liu H, Buchholz TA, et al. MK-1775, a novel Wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. *Clin Cancer Res.* 2011; 17:5638–48. [PubMed: 21799033]
10. Aarts M, Sharpe R, Garcia-Murillas I, Gevensleben H, Hurd MS, Shumway SD, et al. Forced Mitotic Entry of S-Phase Cells as a Therapeutic Strategy Induced by Inhibition of WEE1. *Cancer Discov.* 2012; 2:524–39. [PubMed: 22628408]
11. Rajeshkumar NV, De Oliveira E, Ottenhof N, Watters J, Brooks D, Demuth T, et al. MK-1775, a potent Wee1 inhibitor, synergizes with gemcitabine to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts. *Clin Cancer Res.* 2011; 17:2799–806. [PubMed: 21389100]
12. Tibes R, Bogenberger JM, Chaudhuri L, Hagelstrom RT, Chow D, Buechel ME, et al. RNAi screening of the kinome with cytarabine in leukemias. *Blood.* 2012; 119:2863–72. [PubMed: 22267604]
13. Porter CC, Kim J, Fosmire S, Gearheart CM, van Linden A, Baturin D, et al. Integrated genomic analyses identify WEE1 as a critical mediator of cell fate and a novel therapeutic target in acute myeloid leukemia. *Leukemia.* 2012; 26:1266–76. [PubMed: 22289989]
14. Schweppe RE, Klopfer JP, Korch C, Pugazhenti U, Benezra M, Knauf JA, et al. Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification. *J Clin Endocrinol Metab.* 2008; 93:4331–41. [PubMed: 18713817]
15. Marusyk A, DeGregori J. Replicational stress selects for p53 mutation. *Cell Cycle.* 2007; 6:2148–51. [PubMed: 17786047]
16. Moffat J, Grueneberg DA, Yang X, Kim SY, Kloepper AM, Hinkle G, et al. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. *Cell.* 2006; 124:1283–98. [PubMed: 16564017]
17. Porter CC, DeGregori J. Interfering RNA-mediated purine analog resistance for in vitro and in vivo cell selection. *Blood.* 2008; 112:4466–74. [PubMed: 18587011]
18. Pardee TS, Zuber J, Lowe SW. Flt3-ITD alters chemotherapy response in vitro and in vivo in a p53-dependent manner. *Exp Hematol.* 2011; 39:473–85. e4. [PubMed: 21288478]
19. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul.* 1984; 22:27–55. [PubMed: 6382953]
20. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012; 9:671–5. [PubMed: 22930834]
21. Porter, CC.; Kim, J.; Fosmire, S.; Gearheart, CM.; van Linden, A.; Baturin, D., et al. *Leukemia* : official journal of the Leukemia Society of America. Leukemia Research Fund; UK: 2012. Integrated genomic analyses identify WEE1 as a critical mediator of cell fate and a novel therapeutic target in acute myeloid leukemia.
22. Guertin AD, Li J, Liu Y, Hurd MS, Schuller AG, Long B, et al. Preclinical Evaluation of the WEE1 Inhibitor MK-1775 as Single Agent Anticancer Therapy. *Mol Cancer Ther.* 2013
23. Krehling JM, Gemmer JY, Reed D, Letson D, Bui M, Altiock S. MK1775, a selective Wee1 inhibitor, shows single-agent antitumor activity against sarcoma cells. *Mol Cancer Ther.* 2012; 11:174–82. [PubMed: 22084170]
24. Wolf D, Rotter V. Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells. *Proc Natl Acad Sci U S A.* 1985; 82:790–4. [PubMed: 2858093]
25. Kojima K, Konopleva M, Samudio IJ, Shikami M, Cabreira-Hansen M, McQueen T, et al. MDM2 antagonists induce p53-dependent apoptosis in AML: implications for leukemia therapy. *Blood.* 2005; 106:3150–9. [PubMed: 16014563]
26. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science.* 2004; 303:844–8. [PubMed: 14704432]
27. el-Deiry WS, Harper JW, O'Connor PM, Velculescu VE, Canman CE, Jackman J, et al. WAF1/CIP1 is induced in p53-mediated G1 arrest and apoptosis. *Cancer Res.* 1994; 54:1169–74. [PubMed: 8118801]

28. Hirai H, Arai T, Okada M, Nishibata T, Kobayashi M, Sakai N, et al. MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. *Cancer Biol Ther.* 2010; 9:514–22. [PubMed: 20107315]
29. O'Connor PM, Jackman J, Bae I, Myers TG, Fan S, Mutoh M, et al. Characterization of the p53 tumor suppressor pathway in cell lines of the National Cancer Institute anticancer drug screen and correlations with the growth-inhibitory potency of 123 anticancer agents. *Cancer Res.* 1997; 57:4285–300. [PubMed: 9331090]

Figure 1. MK1775 sensitizes AML cells to cytarabine independent of p53 function
HL60 and OCI-AML3 cells were tested for p53 function and response to cytarabine and/or MK1775. **A.** Cells were treated with nutlin-3a at the indicated doses for 72 hours, and then counted by flow cytometry and PI exclusion. The number of live cells, relative to DMSO treated, is depicted. **B.** Cells were treated with doxorubicin (Dox) at the indicated doses for 24 hours, then protein lysates were subject to Western blot for p21 and actin. **C.** Cell lines were treated with cytarabine and/or MK1775 for 72 hours and then counted. **D.** Normalized isobolograms of CI values are depicted. Individual data points indicate different combinations of cytarabine and MK1775. Points below/left of the diagonal indicate synergistic cellular inhibition, while those above/right indicate antagonism. CI values are provided in Supplemental Figure 2.

Figure 2. MK1775 abrogates the S-phase checkpoint and augments apoptosis induced by cytarabine

HL60 and OCI-AML3 cells were treated at the indicated doses of cytarabine and MK1775 (nM). **A.** After 24 hours cells were harvested and protein lysates subject to Western blotting for P-CDK2 (Y) and actin. Relative phosphorylation as compared to DMSO treated cells from at least 2 independent experiments was determined using ImageJ software and is depicted graphically. **B.** After 24 hours cells were harvested, fixed and permeabilized, stained and analyzed by flow cytometry for cell cycle distribution. Representative histograms are shown. The mean percentage of cells in S-phase from 3 independent experiments is depicted graphically (* $P < 0.05$; ANOVA with Bonferroni post-test). **C.** After 48 hours cells were harvested, stained for Annexin V and with 7-AAD, and analyzed by flow cytometry. Cells that were Annexin V⁺/7-AAD^{neg} were considered in early apoptosis, and those Annexin V⁺/7-AAD⁺ in late apoptosis.

Figure 3. Treatment with MK1775 does not sensitize AML cells to doxorubicin

A. Cells were treated with doxorubicin and/or MK1775 at the indicated doses for 72 hours, then counted by flow cytometry and PI exclusion. The number of live cells relative to control cells is depicted. **B.** Normalized isobolograms of CI values of data from panel B. Individual data points indicate different combinations of doxorubicin and MK1775. CI values are provided in Supplemental Figure 4.

Figure 4. Treatment of AML cells with cytarabine and MK1775 is synergistic independent of p53 function in an isogenic model

A. OCI-AML3 cells were transduced with a dominant negative form of p53 (DDp53) or the control vector (MiG) and sorted for GFP expression. Transduced cells were treated with nutlin-3a at the indicated doses for 48 hours then counted by flow cytometry and PI exclusion. The number of live cells, relative to DMSO treated, is depicted. Cells were also treated with doxorubicin at the indicated doses and the expression of p21 was assessed by western blot (insets). **B.** MiG and DD-p53 cells were treated with cytarabine or doxorubicin, with or without MK1775 for 72 hours and then counted by flow cytometry. The number of live cells relative to untreated is depicted. P=Non-significant (ANOVA). **C.** OCI-AML3 cells were transduced with vectors expressing non-silencing (shNS) or p53-silencing (shp53) shRNA and selected in puromycin. Cells were then treated for 72 hours with cytarabine and/or MK1775 and counted. The number of live cells, relative to DMSO treated, is depicted. CI Values are provided in Supplemental Figure 4.

Figure 5. MK1775 sensitizes lung cancer cells to cytarabine and pemetrexed independent of p53 function

A. A549 cells were treated with nutlin-3a for 72 hours, then counted by flow cytometry and PI exclusion. The number of live cells, relative to untreated cells is depicted. **B.** A549 cells were treated with cytarabine and/or MK1775 for 72 hours and counted by flow cytometry. The number of live cells relative to untreated is depicted. **C.** A549 cells were treated with pemetrexed and/or MK1775 for 72 hours and counted by flow cytometry and the number of live cells relative to untreated is depicted. **D.** A549 cells were transduced with MiG or DDp53 and sorted for GFP. Cells were then treated with nutlin-3a at the indicated concentrations. The number of live cells, relative to DMSO treated is depicted. **E.** Transduced A549 cells were treated with pemetrexed (PMTX) and/or MK1775 at the indicated concentration for 72 hours and counted by flow cytometry. The number of live cells relative to untreated is depicted. P=Non-significant (ANOVA).

Figure 6. MK1775 and cytarabine is well tolerated and more effective than cytarabine alone in vivo

A. Mice without leukemia were treated with the cytarabine and/or MK1775, as described in the text. Weights and complete blood counts were obtained on day 22. **B.** Mice were injected with luciferase expressing AML cells. On day 5 after injection, recipients were divided into groups with equal leukemia burden ($n=5/\text{group}$), as determined by measuring luciferase expression, and treatment initiated with vehicle controls (UT), MK1775, cytarabine (ARA-C) or both (A+M). Leukemia burden as measured by luciferase expression was measured twice per week. Data are representative of 2 independent experiments. **C.** Mice with leukemia were euthanized when ill appearing. Kaplan-Meier curves depict the percent of surviving mice over time. Data are compiled from 2 independent experiments ($n=10/\text{group}$).