

## WEE1 Kinase Targeting Combined with DNA-Damaging Cancer Therapy Catalyzes Mitotic Catastrophe

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### Abstract

WEE1 kinase is a key molecule in maintaining G<sub>2</sub>-cell-cycle checkpoint arrest for premitotic DNA repair. Whereas normal cells repair damaged DNA during G<sub>1</sub>-arrest, cancer cells often have a deficient G<sub>1</sub>-arrest and largely depend on G<sub>2</sub>-arrest. The molecular switch for the G<sub>2</sub>-M transition is held by WEE1 and is pushed forward by CDC25. WEE1 is overexpressed in various cancer types, including glioblastoma and breast cancer. Preclinical studies with cancer cell lines and animal models showed decreased cancer cell viability, reduced tumor burden, and improved survival after WEE1 inhibition by siRNA or small molecule inhibitors, which is enhanced by combination with conventional DNA-damaging therapy, such as radiotherapy and/or cytostatics. Mitotic catastrophe results from premature entry into mitosis with unrepaired lethal DNA damage. As such, cancer cells become sensitized to conventional therapy by WEE1 inhibition, in particular those with insufficient G<sub>1</sub>-arrest due to deficient p53 signaling, like glioblastoma cells. One WEE1 inhibitor has now reached clinical phase I studies. Dose-limiting toxicity consisted of hematologic events, nausea and/or vomiting, and fatigue. The combination of DNA-damaging cancer therapy with WEE1 inhibition seems to be a rational approach to push cancer cells in mitotic catastrophe. Its safety and efficacy are being evaluated in clinical studies. *Clin Cancer Res*; 17(13); 4200-7. ©2011 AACR.

### Background

#### Cell-cycle control by WEE1

Phosphorylation is critical for regulation of the cell cycle. Progress through the cell cycle is coordinated by cyclin-dependent kinases. In normal cells, several checkpoints ensure genomic integrity by repairing damaged DNA before cell-cycle progression, such as at the G<sub>1</sub>-S transition, S-phase, and G<sub>2</sub>-M transition. Entry into mitosis is triggered by the cyclin-dependent kinase CDK1, also known as CDC2, bound to cyclin B. The CDK1/cyclin B complex is also known as the maturation-promoting factor. CDK1 has a pivotal role in the human cell cycle; it is the only mammalian cyclin-dependent kinase out of 14 that is indispensable and that alone can drive the cell cycle (1, 2). CDK1 activity is balanced, on the one hand, by inactivating phosphorylation by the protein kinases WEE1 and myelin transcription factor 1 (MYT1) at

Y15 and T14, respectively, and on the other hand, by activating dephosphorylation by the protein phosphatase cell division cycle 25 homolog (CDC25; refs. 3-6).

WEE1 is a key player that serves as a mitotic inhibitor in the intricate network of kinases and phosphatases that regulate the G<sub>2</sub>-switchboard (Fig. 1; refs. 7-14). In essence, mitotic regulators can be distinguished that either push the cell forward into mitosis (depicted in orange as mitotic activators in Fig. 1) or hold the cell into G<sub>2</sub>-arrest (depicted in blue as mitotic inhibitors in Fig. 1). WEE1 and its complementary counterpart, CDC25, constitute the main switch for mitosis, which seems to be largely regulated by post-translational modification to ensure swift switching (15). This hot handle consists of double-activating feedback loops, so that activated CDK1 activates its activators (CDC25 and MastL) and inactivates its inactivators (WEE1 and MYT1) to push the cell cycle forward. The handle is withheld by 3 parallel CDK1-inactivating pathways: CHK1/WEE1/CDC25/CDK1, MYT1/CDK1, and PP2A/WEE1/CDC25.

WEE1 was identified through genetic studies of cell size control and cell-cycle progression in *Saccharomyces pombe* (16). This work founded Paul Nurse's Nobel prize in 2001. Subsequent work established WEE1 as an atypical tyrosine kinase that is considered part of the serine-threonine-specific family of protein kinases on the basis of its structure (5, 17). The human *WEE1* gene is located on 11p15.3-p15.1 (18), and the 646-amino acid protein contains 3 domains: an N-terminal regulatory domain, a central kinase domain, and a short C-terminal regulatory domain (19). The kinase

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domain seems to be strictly specific for phosphorylation of Y15 of CDK1 *in vivo* (3, 5, 8). *In vitro* kinase activity assays have shown that WEE1 is able to phosphorylate CDK1 and CDK2 complexed with cyclin A, B1, or E, but not with cyclin D1 (7). These experiments also showed that WEE1 cannot phosphorylate monomeric CDK1. Alternative substrates for WEE1 kinase have, however, not been systematically screened. Furthermore, WEE1 has a critical developmental role in mammals because WEE1<sup>-/-</sup> knockout mouse embryos die before embryonic day 4, and conditional WEE1 deletion results in growth defects and cell death because of DNA damage and chromosomal aneuploidy (20).

### Mitotic catastrophe as anticancer strategy

Cancer cells often have a deficient G<sub>1</sub>-checkpoint, for instance because of deficient p53 signaling, which can result in increased DNA damage at the G<sub>2</sub>-checkpoint compared with normal cells (21). Abrogation of the G<sub>2</sub>-arrest releases cells with unrepaired DNA damage into premature mitosis. This G<sub>2</sub>-abrogation can be brought about by pharmacotherapeutic manipulation, resulting in mitotic catastrophe and subsequent cell death through the apoptotic program when the extent of unrepaired DNA damage is sufficient (22–26). This abrogation of the G<sub>2</sub>-arrest seems to be a viable anticancer strategy in combination with DNA-damaging therapy. As WEE1 kinase gatekeeps the G<sub>2</sub>-arrest, the pharmacotherapeutic inhibition of WEE1 seems rational, but other explorable targets for G<sub>2</sub>-abrogation and mitotic catastrophe are ATR, MYT1, CHK1, Hsp90, and PP2A.

### WEE1 Inhibition

#### WEE1 as target for anticancer therapy

Kinases that have been proven to be useful targets in cancer therapy exert their driving action by mutation, protein fusion, or overexpression (27). Therefore, arguments for WEE1 as a candidate kinase cancer target can be derived from gene and protein expression in human cancers.

Several observations with increased WEE1 expression in human cancers have been reported. First, activity of WEE1 was found to be increased in samples of 20 patients with advanced hepatocellular carcinoma compared with non-cancerous cirrhotic tissue (28). Second, WEE1 protein was overexpressed in 35% of breast cancers, specifically in the luminal and HER2-positive subtypes, as determined by immunohistochemistry in 208 patient samples (29). Third, WEE1 expression correlated with patient survival in mantle cell lymphoma (30). Fourth, to establish expression profiles of the entire kinase gene family for a spectrum of human cancers, we did an *in silico* analysis of gene expression microarray data on the basis of 1,451 patient tumor samples and 479 normal tissue samples (31). WEE1 was on top of the list of kinases with substantial and frequent overexpression in glioblastoma. Other G<sub>2</sub>-M transition-related kinases that were overexpressed in glioblastoma include CDK1 and AURKA. WEE1 was also substantially overexpressed in other human cancers, such as colon and lung carcinoma, and seminoma.

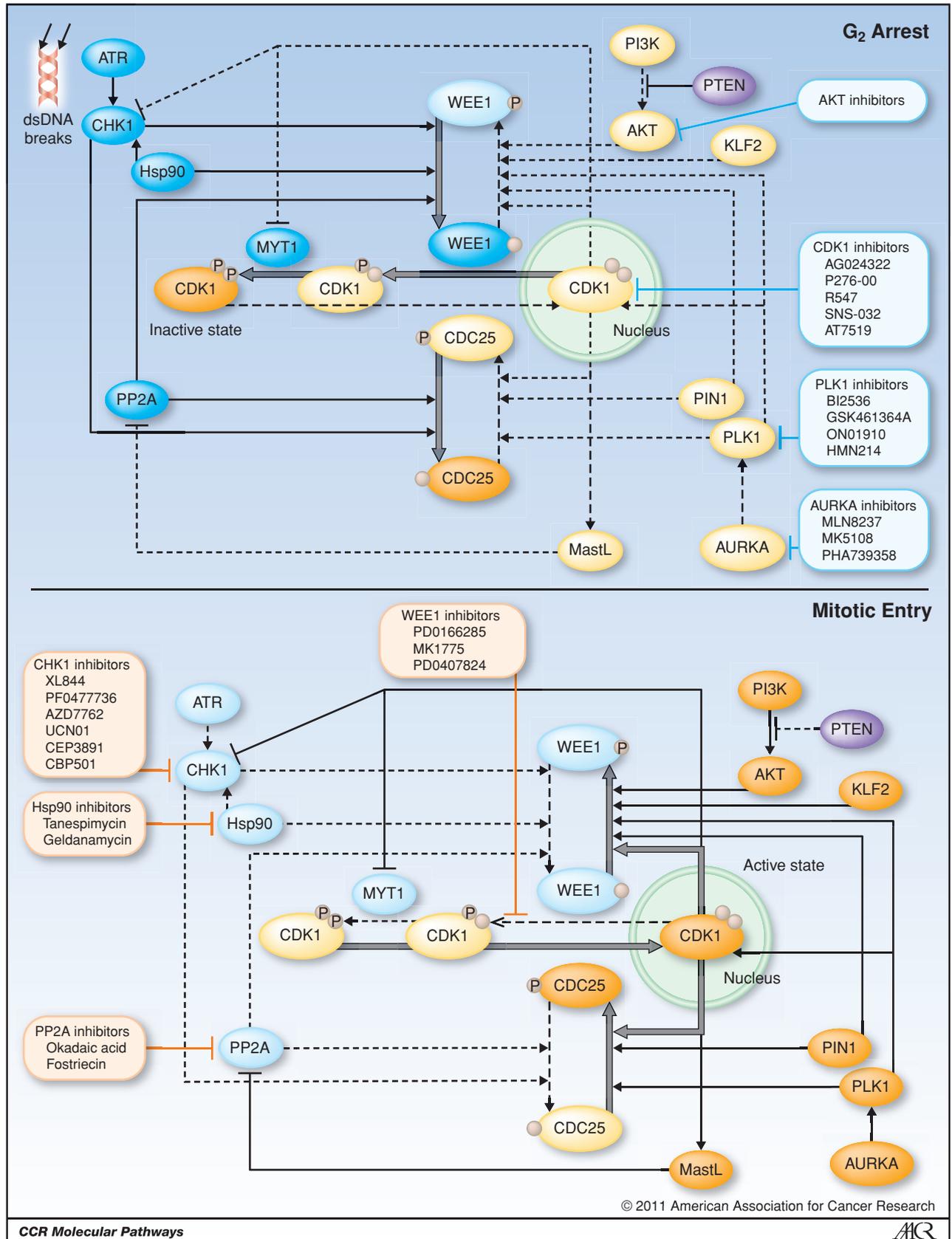
In contrast, a number of other authors have reported their observation of reported a lack of WEE1 expression in human cancer and normal tissues. First, the WEE1 gene was differentially underexpressed in colon cancer cell lines and tumor samples of 12 patients with nonmucinous and mucinous colon carcinoma, compared with nondysplastic colonic mucosa (32). Second, normal human prostate epithelial cells and prostate epithelium have low protein expression of WEE1, with increased susceptibility to unrepaired DNA damage in mitosis (33). Third, WEE1 protein was not detectable in 66% of tumor samples of 79 patients with non-small cell lung cancer (NSCLC; ref. 34). The WEE1 gene expression in tumor tissue was similar to that in normal tissue. Patients with WEE1-negative NSCLC had a higher recurrence rate and poorer survival.

Another approach to identify kinase cancer targets is RNA interference (RNAi) screening. A screening with silencing of 89 tyrosine kinases in breast cancer cells, representing estrogen, progesterone, and HER2-negative subtypes, identified WEE1 as a potential target for breast cancer therapy (35). In another RNAi screening, cell viability was determined after targeting nearly all kinases in breast, lung, and cervical cancer cells, and WEE1 was identified as a potential therapeutic target (29).

Taken together, WEE1 can be considered to be a tumor suppressor, the loss of which emanates from normal prostate epithelium and, possibly, colon and lung epithelial cells susceptible to genetic aberrations and cancerous transformation. On the other hand, cancer cells with genomic instability, typically those with deficient p53 signaling, depend on WEE1 for survival of mitosis. Under these circumstances, WEE1 can be considered a cancer-conserving oncogene, inhibition of which holds potential as an effective sensitizer in combination with DNA-damaging therapy.

#### Selectivity of WEE1 inhibition

A number of small molecule compounds can inhibit WEE1. These compounds are based on pyrimidine and pyrrolo-carbazole derivatives, and their working mechanism aims to abolish CDK1 phosphorylation at Y15 (5, 36–39). PD0166285 is a pyrido-pyrimidine derivative that is a potent but nonselective inhibitor of WEE1 [half maximal inhibitory concentration (IC<sub>50</sub>) of 24 nmol/L]. Other kinase targets of PD0166285 are c-Src, MYT1, epidermal growth factor receptor (EGFR), fibroblast growth factor receptor 1 (FGFR1), CHK1, and platelet-derived growth factor receptor β (PDGFRβ; IC<sub>50</sub> values of 8, 34, 35, 39, 72, and 85 nmol/L, respectively; refs. 40–42). PD0407824 is a pyrrolo-carbazole derivative that is a less potent but more selective inhibitor of WEE1 (IC<sub>50</sub> of 97 nmol/L), but also of CHK1 (IC<sub>50</sub> value of 47 nmol/L; refs. 5, 37). Other kinase targets, such as AKT, CDK4, FGFR, PDGFR, and c-Src, are inhibited only at much higher concentrations (IC<sub>50</sub> values >3,000 nmol/L). Other potent pyrrolo-carbazole derivatives that inhibit WEE1 include WEE1 inhibitor II and 4-(2-phenyl)-9-hydroxypyrrolo[3,4-c]-carbazole-1,3-(2H,6H)-dione (PHCD; refs. 29, 35, 37).



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MK1775 is a pyrazolo-pyrimidine derivative that is a potent and more selective inhibitor of WEE1 (IC<sub>50</sub> of 5 nmol/L). Other kinase targets of MK1775 consist of Yamaguchi sarcoma viral oncogene homolog 1 [(YES1) IC<sub>50</sub> of 14 nmol/L], and 7 unspecified others out of 223 kinases tested with an IC<sub>50</sub> of more than 500 nmol/L (43, 44).

### Preclinical observations

*In vitro*, several cancer cell lines have been exposed to WEE1 inhibition, which resulted in inhibition of outgrowth of cancer cells. First, inhibition of WEE1 by PD0166285 at nanomolar concentrations showed effective G<sub>2</sub>-abrogation when combined with irradiation in various cancer cell lines, including ovarian, colon, cervical, lung, and hepatocellular carcinoma (42, 45, 46). The efficiency of G<sub>2</sub>-abrogation by PD0166285 seemed to correlate with the functional status of p53. Second, in the wild-type p53 B16 melanoma cell line PD0166285, monotherapy showed an antiproliferative effect at micromolar concentration with an early G<sub>1</sub>-arrest and an inhibition of microtubule stabilization, but no cell death (47). Third, knockdown of WEE1 in cervical cancer cells, but not in normal human epithelial cells, in combination with adriamycin treatment, induced G<sub>2</sub>-abrogation and resulted in apoptosis (48). Fourth, WEE1 inhibition by MK1775 at nanomolar concentrations in combination with gemcitabine induced premature mitotic entry and cell death in p53-deficient colon and lung carcinoma cells. MK1775-treated ovarian carcinoma cells expressing short hairpin RNA (shRNA) against p53 were much more sensitive to gemcitabine, carboplatin, or cisplatin than the wild-type p53 cells (44). Fifth, the same group reported that MK1775 also enhanced the cytotoxic effects of 5-fluorouracil (5-FU) at nanomolar concentrations in p53-deficient colon cancer cells and pancreatic cancer cells, but not in wild-type p53 colon cancer cells (49). Furthermore, combination therapy with other cytostatics, such as pemetrexed, doxorubicin, camptothecin, and mitomycin C, provided similar cytotoxic effects. Sixth, knockdown of WEE1 by siRNA reduced viability of breast cancer cells, but not of normal mammary epithelial cells (35). WEE1 inhibition by WEE1 inhibitor II, at micromolar concentrations as monotherapy, reduced cell viability, increased DNA damage, and induced apoptosis in various breast cancer cells that represent estrogen-receptor positive, HER-amplified, and triple-negative subtypes, but not in normal mammary epithelial cells and fibroblasts (35). Remarkably, WEE1 expression levels in these cell lines did not correlate

with sensitivity to inhibitor. Seventh, WEE1 kinase inhibitor PHCD reduced viability and induced apoptosis in breast and cervical cancer cells with high WEE1 gene expression, but not in prostate carcinoma cells and normal mammary epithelium with low levels of WEE1 gene expression (29). Eighth, silencing of WEE1 by siRNA sensitized stathmin-transfected breast cancer cells to paclitaxel and vinblastine (50). Finally, we have shown profound cell death in several glioblastoma cell lines and primary glioblastoma cell cultures after PD0166285 exposure at micromolar concentrations, most notably in combination with DNA-damaging therapy such as irradiation or the alkylating cytostatic temozolomide, but not in normal human fibroblasts and astrocytes (31). These effects in glioblastoma cells were paralleled by siRNA WEE1 silencing and did not correlate with p53 status. We observed similar cell death in glioblastoma stem-like cell populations, both as CD133-positive fraction and when grown as neurospheres.

*In vivo*, WEE1 inhibition has resulted in tumor growth reduction in several studies using xenograft animal models. First, nude rats subcutaneously bearing colon carcinoma WiDr xenografts were administered MK1775 orally at a dose of 5 to 20 mg/kg in combination with gemcitabine and showed reduced tumor growth without animal toxicity (44). Similar results were obtained when combining MK1775 with carboplatin for cervical cancer HeLa-luc xenografts and with cisplatin for ovarian carcinoma TOV21G-shp53 xenografts. In this study, inhibition of CDK1 phosphorylation, at Y15 as downstream substrate, and of pHH2, to monitor mitotic entry, were established as potential surrogate markers in skin hair follicles for monitoring pharmacotherapeutic effects in tumor tissue. In another study by the same group, a gene expression profile was obtained from skin samples of rats with xenografts of WiDR cells after combination therapy of gemcitabine and MK1775, which was consistent with WEE1 inhibitor sensitivity (43). Effects of MK1775 monotherapy were only moderate. Second, the same group showed similar tumor growth reduction for MK1775 in combination with 5-FU or capecitabine in nude rats with colon carcinoma WiDr xenografts or breast cancer MX1 xenografts, again without toxicity (49). Third, the same group also observed marked tumor regression in p53-deficient primary pancreatic cancer subcutaneous xenografts in mice after combination therapy with MK1775 and gemcitabine, compared with monotherapy and control, but not in wild-type p53 xenografts (51). Fourth, in mice harboring intracranial xenografts of

**Figure 1.** Mitotic activators (orange) and inactivators (blue) regulate the transition from G<sub>2</sub>-checkpoint arrest to mitosis. CDK1 is deactivated by WEE1 kinase phosphorylation and is activated by CDC25 phosphatase dephosphorylation. Three parallel inactivating pathways maintain G<sub>2</sub>-checkpoint arrest: CHK1/WEE1/CDC25/CDK1, MYT1/CDK1, and PP2A/WEE1/CDC25. Double-positive feedback loops, with activated CDK1 activating its activators and inactivating its inactivators, trigger mitosis. Mitosis is positively regulated by PI3K/AKT, AURKA/PLK1, MASTL/PP2A, Kruppel-like factor 2 (KLF2), and PIN1. Pharmacotherapeutic manipulation with inhibitors of AKT, CDK1, PLK1, or AURKA stagnates the cell cycle at the G<sub>2</sub>-M transition. Inhibitors of WEE1, CHK1, Hsp90, or PP2A trigger mitosis. The WEE1 kinase is controlled by several mechanisms. WEE1 activity is enhanced through phosphorylation by CHK1 and binding to 14-3-3 peptides (73-75). Other positive regulators of WEE1 are PP2A and Hsp90 (76, 77). At the onset of mitosis, WEE1 must be downregulated rapidly to activate CDK1. Therefore, phosphorylation of WEE1 by CDK1 and PLK1 at S53 and S123 creates phosphodegrons that signal the ubiquitination of WEE1 by CDC34 and proteasome-dependent degradation by the F-box proteins β-trcp-1 and Tome-1 (11, 78-80). Furthermore, phosphorylation of WEE1 at S642 by AKT1 creates a 14-3-30 peptide-binding site that ports WEE1 from the nucleus and decreases its level of activity (12). WEE1 expression levels are also negatively regulated by KLF2 (81). Moreover, the circadian clockwork controls WEE1 as well (82). dsDNA, double-stranded DNA.

glioblastoma U251 cells, which were transduced with shRNA against *WEE1*, we observed a reduced tumor burden and survival advantage when combined with irradiation (31). A comparable survival advantage was shown for combination therapy of PD0166285 and irradiation in the same glioblastoma model. Repeated experiments with highly invasive E98 glioblastoma xenografts again showed a similar therapeutic response for combination therapy of E98 transduced with shRNA against *WEE1* or PD0166285, with irradiation in comparison with irradiation only or control shRNA. No toxicity was observed in these animals.

These *in vitro* and *in vivo* results provide solid arguments that *WEE1* inhibition combined with DNA-damaging therapy, either irradiation or cytostatics, results in efficient cell death in various human cancer types by mitotic catastrophe. Cells with intact  $G_1$ -checkpoint arrest, such as normal cells or cancer cells with intact p53 signaling, are less dependent on the  $G_2$ -checkpoint arrest and are, therefore, not as sensitive to *WEE1* inhibition. These effects are unlikely due to off-target effects of the evaluated inhibitors, because knockdown of *WEE1* yielded similar results. Furthermore, it is encouraging that adverse events were not observed in rat or mouse even at high doses, although toxicity studies in animals have not yet been published.

## Clinical-Translational Advances

### Clinical studies with *WEE1* inhibitor

Clinical studies are currently at an early stage with preliminary phase I results for MK1775. The other inhibitors, PD0166285 and PD0407824, have not been tested in patients. Preliminary results of an ongoing phase I study (NCT00648648) of oral MK1775 as monotherapy and in combination with gemcitabine, cisplatin, or carboplatin in 91 patients with advanced solid cancer, excluding central nervous system malignancies, were reported with good tolerance and strong target engagement (52). Adverse events were observed in 20 patients and mainly consisted of hematologic events, nausea and/or vomiting, and fatigue. Patients on monotherapy of oral MK1775 did not present dose-limiting toxicity up to 1,300 mg. The maximum tolerated dose in combination therapy varied between 200 to 325 mg of MK1775. Four phase I and II studies with MK1775 are currently active: combined with carboplatin in ovarian cancer (NCT01164995, phase II); combined with gemcitabine, cisplatin, or carboplatin in advanced solid tumors (NCT00648648, phase I); combined with 5-FU or 5-FU-cisplatin in advanced solid tumors (NCT01047007, phase I); and combined with topotecan-cisplatin in cervical cancer (NCT01076400, phase I and II).

### Clinical studies with other therapeutic targets for $G_2$ -checkpoint abrogation

Alternative therapeutic targets for mitotic catastrophe by  $G_2$ -abrogation with clinical data are CHK1 and Hsp90 (53). These are also discussed here.

CHK1 kinase activates *WEE1* and inactivates CDC25 and, therefore, seems to be a suitable alternative therapeutic

target. The current CHK1 inhibitors are not selective and vary in potency to inhibit CHK2 activity; this is reflected by the off-target effects presumably responsible for sensitization of wild-type p53 cells to CHK1 inhibition in preclinical studies. Clinical trials in patients with advanced solid tumors and lymphoma with the nonselective CHK1 inhibitor UCN-01 (also inhibiting CHK2, *WEE1*, and AKT), either as monotherapy or in combination with cisplatin, carboplatin, topotecan, or irinotecan, showed dose-limiting toxicity consisting of hyperglycemia with resultant metabolic acidosis, pulmonary dysfunction, nausea, vomiting, hypotension, and arrhythmia. At maximal tolerated doses, biological responses were detected in biopsies with inhibition of CHK1 activity, but without clinical responses (54–58). The CHK1 and CHK2 inhibitor XL844 was evaluated in combination with gemcitabine in 2 phase I trials in patients with chronic lymphocytic leukemia, advanced solid tumor, or lymphoma, with unreported results (NCT00475917 and NCT00234481). The CHK1 and CHK2 inhibitor AZD7762 has also been tested in phase I trials combined with gemcitabine or irinotecan in patients with advanced solid tumors (NCT00413686, NCT00473616, and NCT00937664), with unreported results. For further details, we refer to a recent review (59).

Hsp90 is a cytoplasmic molecular chaperone that activates several client proteins including *WEE1* and CHK1 (53). The Hsp90 inhibitor tanespimycin (17AAG) results in nonspecific CHK1 and *WEE1* depletion, inducing p53-dependent  $G_2$ -checkpoint abrogation (53). Clinical trials of tanespimycin monotherapy showed a lack of response activity in metastatic prostate cancer, renal cell cancer, and melanoma and showed some activity in relapsed multiple myeloma with acceptable toxicity (60–64). Combination therapy with sorafenib, paclitaxel, and trastuzumab seems to be well tolerated (63, 65, 66). For further details, we refer the reader to a recent review (67).

### Role of *WEE1* in glioblastoma

*WEE1* kinase inhibition seems to be of particular interest in glioblastoma therapy, on the basis of several arguments. First, the resistance of glioblastoma to radiotherapy is due to preferential checkpoint response and repair of DNA damage (68). Second, spontaneous epigenetically silenced MGMT, a DNA-repair protein that dealkylates temozolomide-alkylated DNA, is related with a more favorable response to chemo-irradiation therapy in patients with glioblastoma (69). This finding argues in favor of combining DNA-damaging chemoradiotherapy with *WEE1* inhibition to prevent DNA damage repair. Third, the genomic alterations of glioblastoma are characterized by profound chromosomal instability (70), a constitutively activated phosphoinositide 3-kinase (PI3K)/AKT pathway in 88%, and deficient p53 signaling in 87% of glioblastomas (71). These alterations bring about a profound  $G_2$ -checkpoint dependency for glioblastoma. Fourth, we showed that *WEE1* is the top-ranking overexpressed kinase in glioblastoma, that *WEE1* expression in glioblastoma correlates with patient survival inhibition, and that inhibition of

WEE1 by siRNA or small molecular compound results in efficient cell death and survival benefit in animal models (31).

### Future Perspectives

Interestingly, pushing the cell cycle forward into mitosis may be a more effective therapeutic strategy than stagnation of the cell cycle by halting mitosis. Many kinase inhibition strategies aim to interrupt the replicative cycle of cancer cells, including inhibitors of EGFR, PDGFR, and AKT. These inhibitors, so far, have not shown relevant patient responses in glioblastoma therapy (72). Preclinical studies indicate that timely pushing of the cancer cell cycle can be more efficient than interrupting cell proliferation (31). Explorations to push cancer cells in mitotic catastrophe, by optimal combination therapy in patients, hold potential to turn the tide.

The best therapeutic target to induce mitotic catastrophe by G<sub>2</sub>-abrogation remains elusive, be it ATR, MYT1, CHK1, Hsp90, PP2A, WEE1, or other unknown G<sub>2</sub>-M transition targets or combinations thereof. This topic is one of the focuses of future work. Another focus is to improve the selectivity of WEE1 inhibitors. Current inhibitors suffer from a lack of selectivity. Because WEE1 seems to have a strictly specific function, off-target effects due to nonselective effects of the available inhibitors are not required for adequate G<sub>2</sub>-abrogation and are, therefore, considered undesirable. Increased WEE1 selectivity would likely account for a more beneficial toxicity profile, although MK1775 monotherapy already showed minimal toxicity. Another focus is to verify alternative biomarkers for WEE1 engagement at the tumor site. So far, the phosphorylation status of the downstream substrate CDK1, phosphorylation of HH2 to monitor mitotic entry, and a gene expression profile that correlates with WEE1 inhibitor sensitivity have been postulated (43, 44). The obvious incentive is to provide a reproducible, reliable, and accurate representation of target engagement using minimal sample tissue with maximal ease. Furthermore, the optimal timing of treatment with the DNA-damaging component and the

G<sub>2</sub>-abrogating component is of critical importance. In experiments so far, these issues have not been addressed.

Some concern can be raised when assuming that a subpopulation of cancer cells with sublethal DNA damage can survive forced mitosis, despite genomic instability and treatment-induced DNA damage, with viable daughter cancer cells with accelerated clonal evolution as a consequence. These offspring cells could, theoretically, be more resistant to the combination therapy by natural selection. However, it is more likely that DNA damage is a stochastic process, so that these offspring cells are also led to mitotic catastrophe by acquiring additional DNA damage that becomes lethal in the following cell cycles. Another concern is that normal cells are exposed to combination therapy that may deregulate these cells by inhibiting the tumor suppressor function of WEE1. At this point, no experimental suggestions exist for this phenomenon, presumably because of an adequate G<sub>1</sub>-checkpoint response.

### Conclusions

WEE1 kinase, as gatekeeper of the G<sub>2</sub>-checkpoint arrest, holds potential as a therapeutic target to manipulate entry into mitosis of cancer cells. Compelling preclinical data indicate that targeting WEE1 induces a catastrophic mitosis because of premature mitotic entry with unrepaired lethal DNA damage. This strategy selectively targets cells that depend on G<sub>2</sub>-checkpoint arrest, in which cancer cells with deficient p53 signaling contrast with normal cells. The combination of DNA-damaging treatment with subsequent WEE1 inhibition-induced release into mitotic catastrophe is an attractive paradigm that justifies current evaluation of safety and efficacy in clinical studies, but it is only at its infancy.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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