

# Molecular Pathways: Targeting the Protein Kinase Wee1 in Cancer

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## Abstract

Wee1 is a protein kinase that regulates the G<sub>2</sub> checkpoint and prevents entry into mitosis in response to DNA damage. Cyclin-dependent kinases (CDK) are a family of 14 serine/threonine protein kinases that coordinate the progression through the cell cycle. The Cdc2/cyclin B complex controls the progression from G<sub>2</sub> into mitosis. There are two mechanisms by which the G<sub>2</sub> checkpoint is initiated in response to DNA damage: phosphorylation of Cdc25c by CHK1 and of the Wee1 kinase, which phosphorylates Cdc2. Blockade at the G<sub>2</sub> checkpoint is especially important for p53-mutant cells because these tumors mainly rely on DNA repair at the G<sub>2</sub> checkpoint. AZD1775

(formerly MK-1775) is a small-molecule, pyrazol-pyrimidine derivative and potent and ATP-competitive specific inhibitor of the Wee1 kinase. Several preclinical and clinical studies demonstrated encouraging antitumor effects with manageable side effects of the combination of Wee1 inhibition and DNA-damaging agents. Promising combination schedules are being investigated at the moment, for example, combining PARP inhibition and Wee1 inhibition. Also, a weekly schedule with carboplatin and AZD1775 warrants investigation aimed at further improving the antitumor effect. *Clin Cancer Res*; 23(16): 4540–4. ©2017 AACR.

## Background

Wee1 is a protein kinase that regulates the G<sub>2</sub> checkpoint and prevents entry into mitosis in response to DNA damage (Fig. 1; ref. 1). The cell cycle is a highly controlled process. There are several mechanisms by which cells can modulate progression through the cell cycle in case of DNA damage or other factors that affect DNA replication. There are cell-cycle checkpoints that provide cells time to repair damaged DNA before transmission into mitosis (2). Cyclin-dependent kinases (CDK) are a family of 14 serine/threonine protein kinases that coordinate the progression through the cell cycle (3). The progression from G<sub>2</sub> into mitosis is controlled by the Cdc2/cyclin B complex, also known as CDK1/cyclin B. This complex is activated by dephosphorylation of tyrosine 15 (Tyr15) on Cdc2 by the phosphatase of Cdc25c (4). There are two mechanisms by which the G<sub>2</sub> checkpoint is initiated in response to DNA damage. First, there is the phosphorylation of Cdc25c by CHK1, which leads to its degradation (5). As a result, the activation of the Cdc2/cyclin B complex is prevented. Second, inactivation of the Cdc2/cyclin B complex takes place by phosphorylation of the Wee1 kinase (6). This blockade at the G<sub>2</sub> checkpoint is especially important for p53-mutant cells. p53 wild-type cells have the opportunity to arrest the cell cycle at the

G<sub>1</sub> checkpoint to repair damaged DNA. Cells with a defective p53 pathway rely mainly on DNA repair at the G<sub>2</sub> checkpoint (7). As p53-mutant cells rely on the G<sub>2</sub> checkpoint for DNA damage control, several small-molecule inhibitors of the G<sub>2</sub> checkpoint have been developed, which sensitize mostly p53-mutant tumor cells to DNA-damaging agents (8, 9). Ataxia telangiectasia-mutated (ATM) protein kinase or ataxia telangiectasia-related (ATR) protein kinase pathways are activated in the presence of DNA damage (1). ATM is activated by stress factors that result in double-strand breaks. ATM activates CHK2, resulting in phosphorylation of Cdc25c. Suppression of Cdc25c leads to inhibition of phosphorylation of the CDK1/cyclin B complex (10). ATR is activated by stress factors that result in single-strand breaks (11, 12). ATR plays a role in the activation and phosphorylation of CDK1. CDK1 phosphorylates Wee1 and Cdc25c, which results in activation of the Wee1 kinase and inactivation of the Cdc25c phosphatase activity. Next, Wee1 phosphorylates and inactivates the CDK1/cyclin B complex on tyrosine 15, resulting in cell-cycle arrest in G<sub>2</sub> and time for DNA damage repair. Overexpression of Wee1 has been reported in several cancer types, such as breast (luminal and HER2 positive; refs. 13, 14), ovarian (15), colorectal (16), gastric (17), malignant melanoma (18), and sarcoma (19). In ovarian cancer, melanoma, and glioma tumors, high expression of Wee1 is associated with poor outcome.

## Clinical-Translational Advances

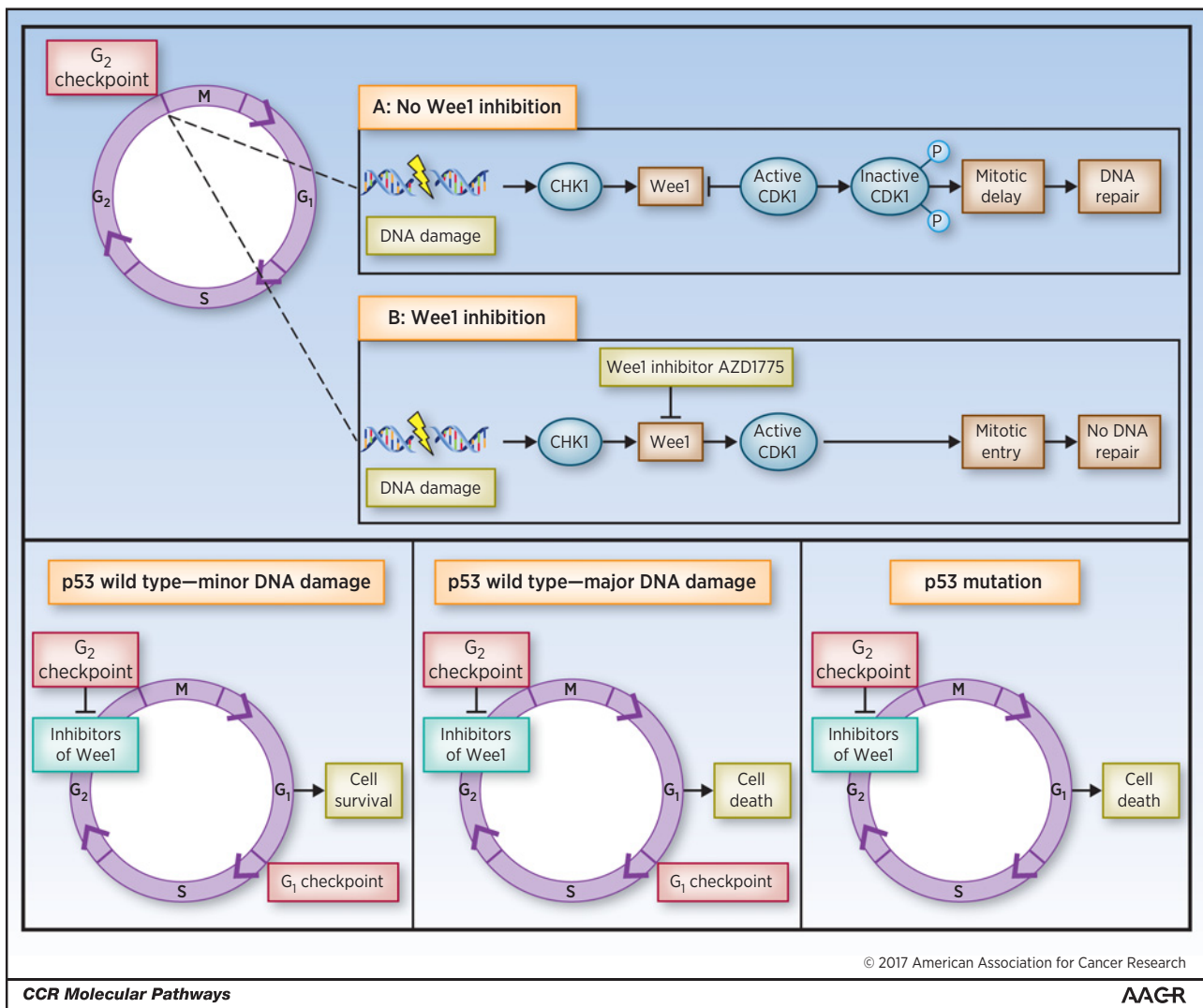
AZD1775 (formerly MK-1775), a pyrazol-pyrimidine derivative, is a potent and ATP-competitive specific small-molecule inhibitor (20, 21) of the Wee1 kinase. An IC<sub>50</sub> of 5.2 nmol/L has been reported (20). *In vivo*, AZD1775 has a relatively short terminal half-life ( $t_{1/2}$ ), ranging from 9 to 12 hours. Results of a study by Cuneo and colleagues demonstrated that sensitization to radiation by Wee1 inhibition occurred in both p53-mutant and wild-type cells. They showed that in two cell lines with TP53-null

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doi: 10.1158/1078-0432.CCR-17-0520

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**Figure 1.**

Involvement of Wee1 in G<sub>2</sub>-M regulation and possible consequences of Wee1 inhibition in p53-mutant cells upon exposure to DNA damage.

and TP53-mutant cells, there was an increase in histone H3 phosphorylation, indicative of G<sub>2</sub> checkpoint abrogation, leading to early mitosis. In addition, in TP53 wild-type cells that were treated with AZD1775, there was a minimal effect on histone H3 phosphorylation. This was probably related to the fact that these cells are able to arrest at the G<sub>1</sub> checkpoint (22). However, this study shows no proof for the functionality of the entire p53 pathway. Also, other studies that have demonstrated activity of Wee1 inhibition in p53 wild-type tumors did not show the functionality of the whole p53 pathway. Another possible explanation why p53 wild-type tumors could benefit from Wee1 inhibition is the existence of an alternative mechanism for the synthetic lethality caused by p53 mutation and Wee1 inhibition. Kato and colleagues described that aberrations in the CDKN2A locus, frequently present in diverse tumor types, can contribute to dysregulation of the G<sub>1</sub>-M checkpoint that could lead to synthetic lethality when combined with Wee1 inhibition (23). *In vitro* studies showed that simultaneous treatment of a Wee1 inhibitor

and, for example, gemcitabine, or Wee1 inhibition followed by gemcitabine resulted in increased cell death. In comparison, sequential treatment with first gemcitabine followed by Wee1 inhibition increased cell death to a greater extent. These data suggest that optimal treatment is sequential administration of first the DNA-damaging agent, followed by the Wee1 inhibitor. This makes sense because the mechanism of action of the DNA-damaging agents is induction of DNA damage. When followed by Wee1 inhibition, this will lead to not (fully) repaired DNA due to lack of cell-cycle arrest. The antitumor effect of AZD1775 in combination with carboplatin was investigated in a rat xenograft model with human cervical adenocarcinoma cells. AZD1775 (doses of 10, 20, and 30 mg/kg) was administered 24 hours after carboplatin (50 mg/kg). The AZD1775 dose dependently enhanced the antitumor effect of carboplatin in these tumor models (20). These antitumor effects were also found in clinical studies investigating combination therapies with DNA-damaging agents and Wee1 inhibition. However, dosing schedules as used in

preclinical research cannot be extrapolated directly to clinical trials. The dose used in *in vitro* studies is often high in comparison with the safe dose used in clinical trials; 50 mg/kg carboplatin in preclinical data would suggest an ultrahigh dose of approximately 3,500 mg carboplatin for an average person. Hirai and colleagues first reported increased sensitivity to various antitumor agents by coadministration of AZD1775 in an ovarian cancer cell line with *TP53* mutation (20). This study and following studies demonstrated that AZD1775 induces cell death and sensitizes p53-defective tumor cells in response to radiotherapy and to gemcitabine, carboplatin, and cisplatin (20, 21, 24, 25). Kim and colleagues investigated the role of Wee1 in gastric cancer. They conducted a combination treatment with AZD1775 and 5-fluorouracil (5-FU) and paclitaxel in gastric cancer cells and established a mouse model. The cells were treated with AZD1775 alone, 5-FU or paclitaxel alone, or a combination of AZD1775 and 5-FU or paclitaxel. This study demonstrated that AZD1775 treatment alone is effective in reducing gastric tumor size, but combination therapy further reduced growth of the gastric tumors (17). Leijen and colleagues performed a phase II study of AZD1775 combined with carboplatin in patients with p53-mutated ovarian cancer. They included 24 patients and achieved an overall response rate of 43% [95% confidence interval (CI), 22–66], including one patient with a prolonged partial response. Patients were platinum refractory or resistant, with progression within 3 months after the end of first-line standard carboplatin–paclitaxel therapy. Leijen and colleagues showed evidence that AZD1775 enhances carboplatin efficacy in *TP53*-mutated tumors (22). Other studies demonstrated that sensitization of tumor cells also occurred in p53 wild-type tumor cells (26). AZD1775 demonstrated antitumor activity as a single agent in both p53 wild-type as well as in p53-mutant tumors. However, in the wild-type cells, integrity of the entire p53 pathway was not demonstrated. Further studies are warranted to demonstrate if and to what extent Wee1 inhibition is dependent on p53 mutation status and p53 pathway integrity. Hirai and colleagues investigated several dosing schedules of AZD1775 in combination with 5-FU or capecitabine. They tested once weekly, twice weekly, and five times weekly schedules. However, all schedules resulted in enhancement of the antitumor effect of 5-FU, whereby both the twice weekly and the five times weekly schedules seemed to be more effective than the once weekly schedule (27). The relatively short half-life, ranging from 9 to 11 hours, as well as the results of preclinical data suggest that dosing multiple times per week would lead to enhanced antitumor effect. As a result, the 2.5-day treatment was introduced.

Combination therapy of DNA-damaging agents with Wee1 inhibition shows promising results. The question is whether a Wee1 inhibitor could be administered as single dose to achieve (comparable with combination therapy) antitumor activity as well. Krealing and colleagues showed that AZD1775 is effective as monotherapy in sarcoma cells independent of the p53 status. Although there was no p53 mutation, it was not clear whether the functionality of the entire p53 pathway was undisturbed. They found a similar level of cell death in cells with a defective p53 system, p53-null cells, and p53 wild-type cells. Guertin and colleagues, who conducted a preclinical evaluation of AZD1775 as single-agent anticancer therapy, found similar results. In the applied non-small cell lung cancer xenograft model, treatment with AZD1775 resulted in a decrease of approximately 50%

compared with the initial tumor volume. These antitumor effects were also observed in additional xenograft models (28). Do and colleagues conducted a phase I study of single-agent AZD1775 in patients with refractory solid tumors. The dosing schedule was based on previous combination trials with AZD1775 and chemotherapeutic agents. The MTD was found to be 225 mg twice a day over 2.5 days per week for 2 weeks per 21-day cycle. Toxicities at this dose level were manageable and mainly grade 1 to 2 according to the Common Terminology Criteria for Adverse Events (CTCAE). There were two partial responses shown in patients with a BRCA mutation. Min and colleagues found that PARP binding to Chk1 at stalled replication forks is needed for S-phase checkpoint activation (29). PARP inhibitors such as olaparib play a pivotal role in the repair of DNA single-strand breaks. *BRCA 1/2*-mutant or other homologous recombination-deficient cancers lack the ability to properly repair double-strand breaks. The combination of a PARP inhibitor and an impaired homologous recombination system, for example, by *BRCA 1/2* mutation, leads to synthetic lethality. Radiosensitization with PARP inhibition is more effective in cells with double-strand break repair-defective tumors. Wee1 indirectly inhibits homologous recombination repair (HRR). Karnak and colleagues performed a study to test the combination of AZD1775 and olaparib in pancreatic cancer as a radiosensitizing strategy. They found indeed that the combination produced significantly more radiation damage in pancreatic cells than inhibition of Wee1 or PARP alone. They showed that Wee1 inhibition leads to inhibition of HRR and abrogation of the G<sub>2</sub> checkpoint when combined with olaparib. Further investigation in clinical trials of this combination is warranted (30). Another strategy for combination therapy could be weekly carboplatin and AZD1775. Increasing evidence shows that administration of a platinum compound in a "dose-dense" schedule, which includes more frequent scheduling with increased dose intensity, could lead to improvement of antitumor effect and decreased resistance (31). With more frequent carboplatin administration, AZD1775 also should be administered more frequently to achieve a sequential administration of both. There is no evidence yet, if lower and more frequent dosing of Wee1 would lead to effective exposure in the tumor. A pharmacokinetic and pharmacodynamic study of weekly carboplatin and Wee1 should be performed to investigate the systemic exposure to both drugs. Secondly, the safety and preliminary antitumor activity could be studied. Currently, there are several clinical trials ongoing with AZD1775 in different tumor types. AZD1775 is being combined with irinotecan, radiotherapy, docetaxel, cytarabine, and other anticancer drugs and is being studied in colorectal cancer, lung cancer, acute myeloid leukemia, and pancreatic cancer (ClinicalTrials.gov, last visit January 27, 2017). An optimal dose-schedule biomarker has not been found yet. Another promising biomarker would be a marker to test the p53 pathway in its entirety. Not only p53-mutant cells but also cells with phenotypical loss of p53 function could be found. As a result, Wee1 inhibition could be applied wider, and more patients could have benefit from this treatment.

## Toxicity of Wee1 Inhibitors

The toxicity profile of AZD1775 was first studied in rats and showed that the majority of organs that were affected were proliferation-dependent organs, such as lymphoid and

hematopoietic organs and the gastrointestinal tract. By the end of a 2-week or longer recovery period, there was evidence of reversibility. On the basis of these studies and the histomorphologic examination of the bone marrow, it is expected that hematologic changes will fully recover. Leijen and colleagues found that monotherapy given as single dose was well tolerated, and the MTD was not reached. The most common adverse events in the combination part (with gemcitabine, cisplatin, or carboplatin) were fatigue, nausea, vomiting, diarrhea, and hematologic toxicity (25). The subsequent phase II study with AZD1775 combined with carboplatin in patients with *TP53*-mutant platinum-refractory or resistant ovarian cancer also demonstrated manageable toxicity. The most common adverse events were overall manageable (CTCAE grade 1–2) and were mostly fatigue (87%), nausea (78%), thrombocytopenia (70%), diarrhea (70%), and vomiting (48%; ref. 32). On the basis of the safety data from six completed clinical trials and preliminary data from ongoing studies, mainly hematologic disorders should be observed closely. Next, this promising combination will be applied to other *TP53*-mutant tumor

types. It is of interest to test the activity and safety of a schedule intensive weekly carboplatin–AZD1775 combination, with the aim of further increasing the efficacy of the combination.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** J.J.J. Geenen, J.H.M. Schellens

**Development of methodology:** J.J.J. Geenen, J.H.M. Schellens

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** J.J.J. Geenen, J.H.M. Schellens

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** J.J.J. Geenen, J.H.M. Schellens

**Writing, review, and/or revision of the manuscript:** J.J.J. Geenen, J.H.M. Schellens

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** J.J.J. Geenen, J.H.M. Schellens

Received February 21, 2017; accepted April 19, 2017; published OnlineFirst April 25, 2017.

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*Clin Cancer Res* 2017;23:4540-4544. Published OnlineFirst April 25, 2017.

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