A Small-Molecule Inhibitor of WEE1, AZD1775, Synergizes with Olaparib by Impairing Homologous Recombination and Enhancing DNA Damage and Apoptosis in Acute Leukemia


Abstract

Although some patients with acute leukemia have good prognoses, the prognosis of adult and pediatric patients who relapse or cannot tolerate standard chemotherapy is poor. Inhibition of WEE1 with AZD1775 has been shown to sensitize cancer cells to genotoxic chemotherapies, including cytarabine in acute myeloid leukemia (AML) and T-ALL. Inhibition of WEE1 impairs homologous recombination by indirectly inhibiting BRCA2. Thus, we sought to determine whether AZD1775 could sensitize cells to the PARP1/2 inhibitor olaparib. We found that combined treatment with AZD1775 and olaparib was synergistic in AML and ALL cells, and this combination impaired proliferative capacity upon drug withdrawal. AZD1775 impaired homologous recombination in olaparib-treated cells, resulting in enhanced DNA damage accumulation and apoptosis induction. This combination enhanced disease control and increased survival in a murine AML model. Furthermore, we demonstrated that combined treatment with AZD1775 and olaparib reduces proliferation and colony formation and increases apoptosis in AML patient samples. In aggregate, these studies raise the possibility of rational combinations of targeted agents for leukemia in patients for whom conventional chemotherapeutics may not be effective or well tolerated. Mol Cancer Ther; 16(10): 2058–68. ©2017 AACR.

Introduction

Acute leukemia is the most common form of pediatric cancer and a leading cause of cancer-related deaths in children. Although survival rates in children have improved, in part due to dose escalation and optimization of chemotherapy regimens, up to 50% of patients with acute myeloid leukemia (AML) and 20% of patients with acute lymphoblastic leukemia (ALL) relapse, and the prognosis for these patients is poor (1, 2). Outcomes for adult patients with acute leukemia are generally worse, particularly in AML, where incidence increases with advanced age (3). Because of its high toxicity, the use of chemotherapy is limited in patients over age 60, leaving few treatment options for many AML cases (4). Further dose escalation in pediatric patients or addition of nontargeted genotoxic agents is likely to increase toxicity while imparting minimal improvements in survival to these patients, necessitating the advent of novel therapeutic strategies (5).

One therapeutic strategy that has shown promise in BRCA1/2-mutated solid tumors is inhibition of PARP (6, 7). The PARP family includes 18 members, three of which are enzymes known to function in various DNA repair processes, including the recognition of single-strand breaks (SSB) in base excision repair (BER) (8). Impaired BER in PARP-inhibited cells results in accumulation of SSBs that are converted to double-strand breaks (DSB) upon collision with replication forks during S-phase of the cell cycle (7). PARP-inhibited cells rely on homologous recombination (HR) for DNA damage repair and survival, and cells possessing defects in the HR pathway are particularly susceptible to PARP inhibitors (4, 9–11).

Despite its promise in some solid tumors, the clinical application of PARP inhibitors in acute leukemia has been limited. This limited success is partly because mutations in DNA damage response (DDR)-associated genes are not common in acute leukemia, which is often driven by transcription factor mutations or gene fusions (4, 12, 13). However, pharmacologic impairment of the HR pathway could potentially sensitize acute leukemia cells to PARP inhibition. One approach to impairing the HR pathway is through inhibition of WEE1. WEE1 is a tyrosine kinase that regulates the cell cycle during the G2–M- and S-phases via inhibitory phosphorylation of CDK1 and CDK2, respectively. In response to DNA damage, CHK1 phosphorylates and activates WEE1 to promote cell-cycle arrest and DNA damage repair (14, 15). A small-molecule inhibitor of WEE1, AZD1775, has been shown to abrogate cell-cycle arrest and enhance DNA damage induction and apoptosis in cells exposed to genotoxic...
chemotherapies, such as cytarabine, gemcitabine, and cisplatin (16–19). In addition to its effects on the cell cycle, inhibition of WEE1 impairs HR-mediated repair through forced activation of CDK1 and subsequent inhibitory phosphorylation of BRCA2 (20). Therefore, we hypothesized that inhibition of WEE1 via AZD1775 would sensitize acute leukemia cells to the PARP inhibitor olaparib. Our data confirm that inhibition of WEE1 impairs HR in olaparib-treated cells. Combined inhibition of WEE1 and PARP1/2 results in a synergistic reduction in cellular proliferation in AML and ALL cell lines, likely through impaired HR and subsequent DNA damage accumulation and induction of apoptosis. This combination extended survival of mice with an MLL-rearranged murine AML. Finally, addition of AZD1775 to olaparib-treated AML patient samples enhanced apoptosis and reduced colony formation in methylcellulose. In aggregate, these studies indicate that combined treatment with olaparib and AZD1775 could be an effective treatment of acute leukemia.

Materials and Methods

Cell culture

Jurkat, Molm13, MV4;11, REH, and OCI-AML3 cell lines were generous gifts from the laboratories of Drs. Douglas Graham (Pediatrics, Emory University, Atlanta, GA) and James DeGregori (Biochemistry & Molecular Genetics, University of Colorado, Aurora, CO). 32D cells were purchased from ATCC. Cell lines were DNA fingerprinted by multiplex PCR using the Profiler Plus or Identifier Kits (ABI) as described previously (21), and periodically tested for Mycoplasma by PCR. Cells were cultured in RPMI with 10% FBS and penicillin/streptomycin at 37°C in humidified air supplemented with 5% CO2 and maintained in culture for no longer than 2 months. WEHI3 conditioned media were added to the culture media at 10% as a source of IL3 for 32D cells. Isogenic cell lines were generated by transduction of target cells with murine stem cell virus expressing genes of interest, followed by an internal ribosome entry site and GFP (MSCV-iresGFP), as described previously (21). Primary AML samples were collected after informed consent with approval of the Colorado Multiple Institutional Review Board. Ficoll-separated mononuclear cells were cultured in IMDM supplemented with 20% BIT Serum Substitute, LDL, B-ME, penicillin/streptomycin, l-glutamine, IL3, Flt-3 ligand, and SCF. To assess colony-forming ability, primary samples were plated in Methocult H4434 Classic (Stemcell Technologies). Colonies were counted after 10 to 14 days of continuous drug exposure.

Chemotherapies, antibodies, and reagents

Olaparib and AZD1775 were provided by AstraZeneca. The chemical structure of AZD1775 has been described previously.
Figure 2.

AZD1775 enhances the antiproliferative effect of olaparib on leukemia cells. **A**, Relative numbers of viable cells treated with DMSO (vehicle control), olaparib (2 μmol/L), and/or AZD1775 (200 nmol/L) for 72 hours. Viable cell counts are normalized to cells receiving no treatment (NT). Results are shown as mean ± SEM from three independent experiments. †, P < 0.05; ‡, P < 0.01; ††, P < 0.001; †††, P < 0.0001. **B**, Live cell number of NT AML and ALL cell lines or treated with the indicated concentrations of DMSO (vehicle control), olaparib, and/or AZD1775 for 72 hours, removed from drug and cultured in fresh media for another 72 hours. Results are displayed as mean ± SEM from three independent experiments. Displayed P values were calculated by two-way ANOVA.
(22). Antibodies against phosphorylated CDK1 (Y15), total CDK1, γH2AX, tubulin, and caspase-3 were purchased from Cell Signaling Technology.

**Comet assays**

Formation of DNA DSBs was assessed using Comet Assay kits (Trevigen). Cells were collected after 72 hours of drug treatment and embedded in agarose on slides. Slides were subjected to electrophoresis in TAE buffer, followed by staining with SYBR Green (Trevigen) and visualization by fluorescent microscopy at ×4 magnification. CometScore software (TriTek) was used to calculate the olive moment, the product of the mean tail migration distance, and the fraction of total DNA in the tail, of a minimum of 50 cells per condition (23).

**Immunofluorescence**

Cells were treated with AZD1775 and/or olaparib for 48 hours in poly-D-lysine–coated chamber slides (Sigma-Aldrich). Cells were then fixed with 4% paraformaldehyde for 15 minutes at room temperature and permeabilized with 0.2% Triton X in PBS for 10 minutes. Cells were incubated in blocking solution (5% milk in 0.05% Triton X-PBS) for 30 minutes, followed by an overnight incubation in anti-Rad51 antibody (Cell Signaling Technology) at a dilution of 1:500. After several washes, Alexa Fluor 488–conjugated anti-rabbit antibody (1:500) was applied for 1 hour. ProLong Gold Antifade Mountant with DAPI (Life Technologies) was used for mounting. Images were acquired using an inverted epifluorescence microscope at ×100 magnification.

**Flow cytometry**

Cell viability was determined with the Guava EasyCytePlus (Millipore) by measuring cell counts with propidium iodide exclusion. Apoptosis was assessed using Guava Nexcel reagent according to the manufacturer’s protocol (Millipore). Cell-cycle analysis was performed using Guava Cell Cycle Reagent according to the manufacturer’s protocol (Millipore).

**β-Galactosidase staining**

Cells were treated with AZD1775 and/or olaparib for 48 hours. After drug treatment, cells were placed in drug-free media and cultured in poly-D-lysine–coated chamber slides (Sigma-Aldrich) for an additional 96 hours. β-Galactosidase staining was performed using the Senescence β-Galactosidase Staining Kit according to the manufacturer’s protocol (Cell Signaling Technology).

**Animal experiments**

Female 6-week-old C57BL/6j mice were purchased from The Jackson Laboratory and housed in sterile microisolators in the Center for Comparative Medicine at the University of Colorado Anschutz Medical Campus (Aurora, CO). Five mice per treatment group were used for each of the two experiments for a total of 10 mice per treatment group. A total of 5 × 10³ luciferase-tagged AML cells were injected by tail vein injection into unirradiated recipients to induce leukemia (24). Beginning 3 days later, mice were treated 5 days per week with olaparib 50 mg/kg and/or AZD1775 80 mg/kg once per day by oral gavage. 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 (Denver, CO).

**Statistical analysis**

Data analysis and graphing was performed using GraphPad Prism 5 (GraphPad Software). Unless otherwise indicated, graphs represent the mean from a minimum of three biological replicate experiments, and error bars portray the SEM. One-way ANOVA was used to compare three or more samples with a single variable. Two-way ANOVA was used to compare three or more samples with two variables. The Bonferroni correction was applied to determine significance between any two conditions. Combination index (CI) values were calculated using the equation of Chou and Talalay with CalcuSyn (Biosoft; ref. 25). CI values were classified as CI >1.1, antagonism; CI = 0.9–1.1, additive; CI = 0.85–0.9, slight synergism; CI = 0.7–0.85, moderate synergism; CI = 0.3–0.7, synergism; CI = 0.1–0.3, strong synergism; and CI < 0.1, very strong synergism (26). The Mantel–Cox (log-rank) test was used to determine significant differences in survival.

**Results**

AZD1775 impairs HR

Cells treated with PARP inhibitors require an intact HR pathway to prevent DNA damage accumulation. Inhibition of WEE1 impairs HR by promoting increased CDK1 activity and subsequent inhibitory phosphorylation of BRCA2 (20). Thus, we sought to determine whether inhibition of WEE1 could reduce HR in acute leukemia cells treated with the PARP1/2 inhibitor olaparib. To assess this, we selected five cell lines representing three subtypes of acute leukemia. MV4;11 and Molm13 are AML cells lines that possess MLL rearrangements and FLT3 internal tandem duplications, while OCI-AML3 cells harbor mutations in NPM1 and DNMT3A. Jurkat is a T-cell ALL line with a mutated TP53 gene, and REH is a B-cell ALL cell line that harbors an ETV6-RUNX1 fusion.

First, we examined the antiproliferative effect of olaparib and AZD1775 as single agents in short-term liquid culture. Both olaparib and AZD1775 had similar single-agent activity in MV4;11, Molm13, Jurkat, and REH cells (Supplementary Fig. S1). In light of the inhibitory effect of olaparib on these cell lines in this short-term assay, we next addressed whether they were competent in HR. Query of the Cancer Cell Line Encyclopedia revealed heterozygous mutations in HR pathway genes, BRCA2 (Molm13 and REH) and NBN (MV4;11), although the extent to which these mutations impair HR is unknown. Thus, we sought to determine whether these cell lines had intact HR in a functional assay. PARP1 deficiency or inhibition enhances the accumulation of Rad51 foci in cells in which the HR machinery is functional (27). In response to olaparib treatment, each cell line displayed a significant increase in Rad51 foci formation, indicating these cell lines are capable of activating the HR pathway (Supplementary Fig. S2). After demonstrating an increase in Rad51 foci in response to olaparib treatment, we sought to determine whether addition of AZD1775 could impair activation of the HR pathway. Indeed, when MV4;11 cells were treated with AZD1775 and olaparib, we observed reduced Rad51 foci compared with cells treated with olaparib alone (Fig. 1a,b). Thus, inhibition of
Figure 3.
Combined AZD1775 and olaparib treatment enhances DNA damage accumulation, apoptosis induction, and senescence. 

A, MV4;11 cells were treated with DMSO (vehicle control), olaparib (2 μmol/L), and/or AZD1775 (200 nmol/L) for 72 hours and analyzed via Comet assay. Images are representative from one of three independent experiments. 

B, Olive moment values normalized to MV4;11 cells receiving no treatment (NT). (Continued on the following page.)
WE1 impairs HR in olaparib-treated leukemia cells, consistent with previous findings in breast cancer cells (20).

AZD1775 synergizes with olaparib in acute leukemia cell lines and reduces proliferative capacity upon drug withdrawal

As AZD1775 impaired HR in cells treated with olaparib, we sought to determine whether cells treated with AZD1775 and olaparib demonstrated reduced viability compared with cells treated with olaparib alone. At doses achievable in the plasma of patients (28), AZD1775 potentiated the reduction in proliferation induced by olaparib in four of five cell lines (Fig. 2A; Supplementary Fig. S3). To better model a clinical setting in which cancer cells are exposed to varying drug concentrations over time, we examined a wider range of drug combinations in MV4;11 cells. Using Chou and Talalay median effects analysis, we determined that 18 of the 20 combinations analyzed were synergistic (CI < 1.0), with higher concentrations of olaparib and AZD1775 resulting in greater levels of synergy (Table 1).

Table 1. Combination index values for MV4;11 cells treated with olaparib and AZD1775 for 72 hours

<table>
<thead>
<tr>
<th>Olaparib (μmol/L)</th>
<th>AZD1775 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>0.25</td>
<td>0.787</td>
</tr>
<tr>
<td>0.5</td>
<td>0.867</td>
</tr>
<tr>
<td>1.0</td>
<td>0.976</td>
</tr>
<tr>
<td>2.0</td>
<td>1.028</td>
</tr>
</tbody>
</table>

AZD1775 enhances DNA damage, apoptosis, and senescence induced by olaparib

As cells with impaired PARP activity require HR to resolve SSA that occur spontaneously, we hypothesized that impaired HR induced by WEE1 inhibition would result in accumulation of DNA damage in cells treated with olaparib and AZD1775 (7). To test this hypothesis, we used the alkaline comet assay to visualize SSA in cells treated with olaparib and/or AZD1775. AZD1775 enhanced DNA damage in olaparib-treated cells as evidenced by the prevalence of distinct comet tails (Fig. 3A). Indeed, the addition of AZD1775 enhanced the average olive moment by approximately 10-fold compared with olaparib alone (Fig. 3B). This increase in DNA damage was confirmed by increased γH2AX in cells with both PARP and WEE1 inhibition (Fig. 3C).

To determine whether addition of AZD1775 to olaparib induced more apoptosis compared with olaparib alone, we measured apoptosis by Annexin V staining after 72 hours of treatment. The addition of AZD1775 to olaparib led to significantly more apoptosis than with either agent alone (Fig. 3D). We also observed an increase in cleaved caspase-3 accompanied by reduced pro-caspase-3 in MV4;11 cells treated with olaparib and AZD1775, further suggesting increased apoptosis in cells treated with both PARP and WEE1 inhibitors (Fig. 3E).

As cells treated with olaparib and AZD1775 fail to recover proliferative capacity upon drug removal (Fig. 2B), we sought to determine whether the drug combination induced senescence in...
surviving cells. To test this, Molm13 and REH cells were treated with AZD1775 and/or olaparib for 48 hours. After drug treatment, cells were replated in drug-free media for an additional 96 hours, then fixed and stained for β-galactosidase. In Molm13, we observed a 6-fold increase in β-galactosidase–positive cells in cells treated with olaparib and AZD1775 compared with cells treated with olaparib alone (Fig. 3F). REH cells treated with the drug combination displayed an 8-fold increase in β-galactosidase–positive cells compared with cells treated with only olaparib (Fig. 3G).

Our laboratory and others have previously demonstrated that AZD1775 enhances the efficacy of some genotoxic agents by abrogating the cell-cycle arrest that occurs in cells treated with chemotherapy alone (16, 32). Therefore, we examined whether changes in the cell cycle occur in cells treated with olaparib and AZD1775. Jurkat cells treated with olaparib display a G2–M phase arrest, and this is abrogated with addition of AZD1775 (Supplementary Fig. S5A and S5C). In addition, Molm13 cells treated with the drug combination have a decreased percentage of cells in the G2–M phase, although olaparib alone does not induce a G2–M phase arrest in this cell line (Supplementary Fig. S5A). However, we did not observe similar changes in other cell lines tested, indicating that altered cell-cycle progression is not a unifying mechanism responsible for the combinatorial activity of olaparib and AZD1775 (Supplementary Fig. S5A and S5B). Together, these results indicate that combined inhibition of WEE1 and PARP1/2 enhances accumulation of DNA DSBs, apoptosis, and senescence, independent of cell-cycle effects of the drugs.

Combined AZD1775 and olaparib treatment enhances survival of mice with murine AML

We then asked whether AZD1775 and olaparib treatment could slow leukemia progression in vivo. For these studies, we selected a murine MLL-ENL, FLT3-ITD+ AML cell line that responds to combined treatment with olaparib and AZD1775 in vitro (Supplementary Fig. S6). The murine AML cells were injected via tail vein into mice as described previously (21). Single-agent treatment with either olaparib or AZD1775 failed to enhance survival compared with vehicle controls (olaparib or AZD1775 median survival, 17 days; vehicle median survival, 14 days; Fig. 4C). However, the combination treatment slowed leukemia progression as measured by luciferase expression and extended median survival to 31 days (Fig. 4A–C). Mouse weights and complete blood counts were assessed 7 days after treatment initiation to evaluate toxicity. Mice receiving combination treatment had reduced hemoglobin levels compared with mice treated with vehicle control or olaparib; however, there was no change in weight, red blood cell counts, white blood cell counts, or platelet levels with either single-agent or combination treatment (Supplementary Fig. S7). Two of the 10 mice treated with AZD1775 and olaparib were euthanized due to ill appearance, although no evidence of leukemia was observed at the time of sacrifice.
AZD1775 Synergizes with Olaparib in Acute Leukemia

Figure 5.
AZD1775 and olaparib reduce proliferation and colony formation and induce apoptosis in AML patient samples. A and B, AML patient samples were treated with DMSO (vehicle control), olaparib (2 μmol/L), and/or AZD1775 (200 nmol/L) for 72 hours and analyzed for cell viability (A) and apoptosis induction (B). Results are displayed as mean ± SEM of two technical replicates. C, Quantification of colonies formed in methylcellulose after continuous exposure to DMSO (vehicle control), olaparib (2 μmol/L), and/or AZD1775 (200 nmol/L) for 10 to 14 days. Results are normalized to cells receiving no treatment (NT) and are displayed as mean ± SEM of three technical replicates. D, Representative images of AML sample 7293 colonies formed after 10 days of continuous exposure to DMSO (vehicle control), olaparib (2 μmol/L), and/or AZD1775 (200 nmol/L). E, Patient sample AML 10-5-10 was treated with DMSO (vehicle control), olaparib (2 μmol/L), and/or AZD1775 (nmol/L) for 48 hours, after which protein lysates were subjected to Western blotting using antibodies specific to γH2AX and actin.
AZD1775 enhances apoptosis induction and reduces colony formation in AML patient samples

We next examined the effects of AZD1775 and olaparib on cell viability, apoptosis induction, and colony formation on AML patient samples. Samples with baseline apoptosis greater than 40% 72 hours after plating or that did not form colonies under no treatment conditions were excluded from analysis (Supplementary Fig. S8). Patient sample AML 10-5-10 harbors a FLT3–ITD mutation (33), but genetic information for the other patient samples was not available. Exposure to the drug combination resulted in a reduction in viable cell numbers, an increase in apoptotic cells, and a decline in colony formation (Fig. 5A–C). Further supporting reduced colony-forming ability, the colonies that did form in cells exposed continuously to AZD1775 and olaparib were smaller than those in cells exposed to olaparib (Fig. 5D). Similar to our observations in cell lines, combined treatment with olaparib and AZD1775 enhanced DNA damage as evidenced by elevated γH2AX (Fig. 5E). Thus, combined olaparib and AZD1775 treatment could be an effective therapeutic approach for acute leukemia.

Discussion

Although improvements to chemotherapy regimens have increased survival of patients with acute leukemia, outcomes remain poor for patients that relapse and for elderly patients that cannot tolerate standard treatment (34, 35). Thus, novel treatment options are necessary to improve outcomes in these patient populations. In this study, we have demonstrated that the rational combination of AZD1775 and olaparib inhibits proliferation and enhances apoptosis in AML and ALL cell lines and AML patient samples. This combination also significantly prolonged survival of mice with murine AML.

Although olaparib has proven to be an effective treatment in BRCA1/2–mutant malignancies, the expansion of this drug to cancers lacking defects in the HR pathway has been limited (6, 7, 36, 37). Here, we demonstrate that inhibition of WEE1, a known regulator of HR, sensitizes BRCA1/2 wild-type and mutant cell lines to olaparib. The doses of AZD1775 that we used in vitro are comparable with the IC50 of this compound in cell-based assays in which phosphorylation of CDK is inhibited, and lower than achieved in vivo in an early-phase clinical trial (38). Similarly, the concentrations of olaparib used in vitro can be achieved in vivo (39), although they are higher than is necessary to inhibit poly-(ADP)-ribosylation in vitro (40). We have demonstrated that inhibition of WEE1 impairs HR in olaparib-treated cells. The precise mechanisms of cell death due to PARP inhibition in the context of impaired HR remain unresolved, with at least four models proposed (8). These models share the common endpoint of unresolved and accumulated DNA damage and apoptosis, which our data support. Although the impairment of HR may contribute to the observed sensitization, WEE1 has multiple effects on cell cycle and DNA damage repair pathways through its regulation of CDK1 and CDK2 that could also contribute to the sensitization to olaparib. Indeed, WEE1 inhibition results in stalled replication forks that require processing to avoid collapse, and PARP is required for recruitment of DDR proteins to stalled forks for processing and repair, suggesting that failure to repair stalled replication forks could also contribute to the observed combinatorial activity of AZD1775 and olaparib (41, 42).

Early work describing WEE1 inhibition as an effective chemosensitizing agent suggested combinations with WEE1 inhibitors were only effective in cells lacking functional P53 (19, 43–45). However, previous work from our laboratory has demonstrated that inhibition of WEE1 sensitizes AML and lung cancer cells to antimetabolite chemotherapeutics independent of P53 functionality (21). Likewise, this work demonstrates that AZD1775 synergizes with olaparib in both TP53 wild-type (MV4;11, Molm13, REH) and mutant (Jurkat) cell lines. Furthermore, in an isogenic model, inhibition of endogenous P53 failed to enhance the combinatorial effect of AZD1775 and olaparib, suggesting that sensitivity to this combination is independent of P53 functionality in this context. Although this combination appears to be effective regardless of TP53 status, it is still likely to display selectivity for cancer cells as a number of malignancies, including AML, have increased expression of WEE1 compared with noncancerous controls (16, 46–48). As noted, AZD1775 did not significantly enhance the combinatorial effect of olaparib in all cell lines; thus, this combination is likely to be effective in some, but not all, acute leukemia cells. Further work is required to identify biomarkers to predict sensitivity to this combination.

In addition to our in vitro data, we have demonstrated that combined AZD1775 and olaparib treatment enhanced the survival of mice injected with a murine AML cell line. Notably, this cell line possesses an MLL–ENL fusion that has been shown to mediate resistance to PARP inhibitors via HOXA9–mediated upregulation of DDR genes (4, 24). Thus, this combination could provide a novel treatment option for MLL-rearranged leukemias, which are frequently resistant to standard chemotherapy. It should be noted that 2 of the 10 mice treated with the combination died with no evidence of leukemia. Although mouse weights and complete blood counts suggest minimal drug toxicity after 7 days of treatment, future studies are necessary to assess cumulative toxicity with prolonged treatment. Although optimizations to dosing and the timing of treatments may be necessary, this combination is not expected to be particularly toxic, as both olaparib and AZD1775 have been used in multiple clinical trials with reasonable toxicity profiles (6, 38). Indeed, early results from a phase Ib clinical trial combining olaparib with AZD1775 for the treatment of refractory solid tumors suggest no dose-limiting toxicities (49).

In summary, this report describes a novel synergistic combination, AZD1775 and olaparib, for the treatment of acute leukemia. We have demonstrated that pharmacologic inhibition of WEE1 has potential to broaden the application of PARP inhibitors beyond the current use in BRCA1/2–mutant cancers. Taken together, these data provide support for clinical trials testing AZD1775 and olaparib for acute leukemia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: T.B. Garcia, C.C. Porter
Development of methodology: T.B. Garcia, C.C. Porter
AZD1775 Synergizes with Olaparib in Acute Leukemia

Grant Support
This work was supported by grants from the NIH (to C.C. Porter; CA172385), the University of Colorado Cancer Center (CA046344), and the CJl Medical Scientist Training Program (GM000847).

The costs of publication of this article were deferred in part by the page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 7, 2016; revised May 22, 2017; accepted June 16, 2017; published OnlineFirst June 27, 2017.

References
A Small-Molecule Inhibitor of WEE1, AZD1775, Synergizes with Olaparib by Impairing Homologous Recombination and Enhancing DNA Damage and Apoptosis in Acute Leukemia
