Safety, Antitumor Activity, and Biomarker Analysis in a Phase I Trial of the Once-daily Wee1 Inhibitor Adavosertib (AZD1775) in Patients with Advanced Solid Tumors

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ABSTRACT

Purpose: The Wee1 kinase inhibitor adavosertib abrogates cell-cycle arrest, leading to cell death. Prior testing of twice-daily adavosertib in patients with advanced solid tumors determined the recommended phase II dose (RPh2D). Here, we report results for once-daily adavosertib.

Patients and Methods: A 3 + 3 dose-escalation design was used, with adavosertib given once daily on days 1 to 5 and 8 to 12 in 21-day cycles. Molecular biomarkers of Wee1 activity, including tyrosine 15–phosphorylated Cdk1/2 (pY15-Cdk), were assessed in paired tumor biopsies. Whole-exome sequencing and RNA sequencing of remaining tumor tissue identified potential predictive biomarkers.

Results: Among the 42 patients enrolled, the most common toxicities were gastrointestinal and hematologic; dose-limiting toxicities were grade 4 hematologic toxicity and grade 3 fatigue. The once-daily RPh2D was 300 mg. Six patients (14%) had confirmed partial responses: four ovarian, two endometrial. Adavosertib plasma exposures were similar to those from twice-daily dosing. On cycle 1 day 8 (pre-dose), tumor pY15-Cdk levels were higher than baseline in four of eight patients, suggesting target rebound during the day 5 to 8 dosing break. One patient who progressed rapidly had a tumor WEE1 mutation and potentially compensatory PKMYT1 overexpression. Baseline CCNE1 overexpression occurred in both of two responding patients, only one of whom had CCNE1 amplification, and in zero of three nonresponding patients.

Conclusions: We determined the once-daily adavosertib RPh2D and observed activity in patients with ovarian or endometrial carcinoma, including two with baseline CCNE1 mRNA overexpression. Future studies will determine whether CCNE1 overexpression is a predictive biomarker for adavosertib.

Introduction

Cell-cycle checkpoints enable cells to avoid replication catastrophe by allowing time prior to cell division for the repair of DNA damage and chromatin disruption. Cyclin-dependent kinases (Cdk) represent an essential family of serine/threonine kinases that regulate cellular entry into various phases of the cell cycle via their phosphorylation of specific partner cyclin proteins (1). Cdk1, in complex with cyclin B, regulates the G2–M checkpoint, which is essential in preventing cells with genomic DNA damage from proceeding into mitosis, while Cdk2 regulates the G1–S and intra-S-phase checkpoints via its interactions with cyclin E and cyclin A, respectively (1). Cdk1 (also known as CDC2) and Cdk2 are, in turn, regulated by the nuclear serine/threonine tyrosine kinase Wee1, which catalyzes the inhibitory phosphorylation of Cdk1 and Cdk2 at tyrosine residue 15 (Y15) in response to DNA damage. The resulting Y15-phosphorylated Cdk1 and 2 (pY15-Cdk) induce G2 or G1 cell-cycle arrest, respectively (2), and therefore serve as important biochemical markers of Weel enzymatic activity.

Genomic instability resulting from unrepaired DNA damage is one of the hallmarks of cancer. Tumors are often dependent on the G2–M checkpoint to prevent tumor cells with excessive DNA damage from entering into aberrant mitosis, which can lead to apoptosis via mitotic catastrophe (3); as such, targeting the G2–M checkpoint via inhibition of Wee1 kinase represents an attractive anticancer strategy (4). Furthermore, because Wee1 is also a key regulator of genome integrity via its suppression of Cdk1- and Cdk2-mediated DNA replication initiation during G1–S transition (5, 6), Wee1 kinase inhibition also enhances the DNA damage detected at the G2–M checkpoint. This multifaceted role of Wee1 renders this kinase a promising target for the development of oncology therapeutics.
Once-daily Adavosertib in Solid Tumors

Translational Relevance
Targeting multiple DNA damage response pathway components to induce synthetic lethality remains an important approach for anticancer therapy. Adavosertib (AZD1775) is a Wee1 kinase inhibitor that abrogates G1–S and G2–M cell-cycle arrest, leading to replication stress and, ultimately, tumor cell death. Adavosertib has substantial antitumor activity as a monotherapy when biomarker-based patient selection is performed. Here, we report a recommended phase II dose for once-daily adavosertib (300 mg daily on days 1–5 and 8–12 in 21-day cycles) that yields a pharmacokinetic profile comparable with that of the previously published twice-daily regimen. We identified tumor CCNE1 (cyclin E1) mRNA expression as a potential patient selection biomarker for response, particularly in ovarian and endometrial cancers. These results suggest that adavosertib monotherapy may hold promise in patients with advanced solid tumors when combined with biomarker selection using CCNE1 expression as a precision medicine approach to induce synthetic lethality.

Adavosertib (AZD1775, AstraZeneca) is a first-in-class, highly selective ATP-competitive small-molecule inhibitor of Wee1 kinase that directly prevents phosphorylation of Cdk2 and Cdk1 at Y15, thereby abrogating G1–S and G2–M cell-cycle arrest and resulting in premature S-phase entry and mitosis, enhanced DNA damage, and ultimately cell death (4–6). Adavosertib-induced DNA damage resulting from G1–S checkpoint disruption has been demonstrated in vitro and in patient tumors through detection of the DNA double-strand break marker serine 139–phosphorylated H2AX (γH2AX; refs. 2, 7). In vitro studies have also shown that adavosertib induces upregulation of the mitotic marker phosphorylated histone H3 (pH3) in some cell lines, evidence of G2–M checkpoint inhibition (7). The single-agent antitumor activity of adavosertib has been shown in multiple cancer cell lines (7–9) and in both p53-intact and p53-deficient human tumor xenograft models (7). Adavosertib potentiates the activity of other DNA damage–inducing agents in preclinical models (10–13), which has prompted several recent clinical trials (14–18). Adavosertib also acts as a radiosensitizer, enhancing radiation-induced DNA damage in vivo (19).

We have previously reported on the safety, pharmacokinetics, preliminary antitumor activity, and proof of mechanism for twice-daily adavosertib dosing (2). We found evidence of target engagement using our validated immunofluorescence microscopy assay to quantitate nuclear pY15-Cdk1 and pY15-Cdk2 (referred to hereafter as pY15-Cdk1/2) in patients with high baseline pY15-Cdk1/2 levels, adavosertib decreases pY15-Cdk1/2 at 2–5 hours after the fifth dose of twice-daily adavosertib. Nuclear pH3 and γH2AX also increased in one and three patients, respectively, at this timepoint (2). After pharmacokinetic analysis revealed sustained systemic drug concentrations for twice-daily adavosertib dosing (2), we opened an additional study arm to examine the safety and tolerability of once-daily dosing of adavosertib to improve patient medication adherence and to potentially attenuate toxicities in anticipation of improving the tolerability of future combination regimens. Here, in addition to presenting the safety, clinical activity, pharmacokinetics, and tumor pharmacodynamics of once-daily adavosertib, we also report potential biomarkers of response and resistance uncovered by hypothesis-generating genomic analysis of tumor specimens collected from a small subset of patients.

Patients and Methods

Patient eligibility criteria
This study enrolled patients age 18 years and older with histologically confirmed solid tumors for which all standard therapies had failed or for which standard therapies did not exist. Patients were required to meet normal organ function and other eligibility criteria as described in the Supplementary Materials and Methods. There were no tumor molecular inclusion criteria for this study of once-daily adavosertib.

Trial design
This study (NCT01748825) was conducted at the NIH Clinical Center (Bethesda, MD) under an NCI-sponsored investigational new drug application. The study was performed in accordance with the U.S. Common Rule and approved by the NIH Institutional Review Board; written informed consent was obtained from each participant. Following completion of the twice-daily adavosertib arm, we opened an arm to determine the MTD for once-daily adavosertib. A standard 3 + 3 design was used for dose escalation; the starting dose was 200 mg daily on days 1 to 5 and 8 to 12 of each 21-day cycle, with escalations to 225, 250, 300, and 400 mg once daily on days 1 to 5 and 8 to 12 of each cycle. Patients unable to tolerate the starting dose level were reduced to 200 mg daily on days 1 to 3 and 8 to 10 of each cycle.

Toxicities were graded using the NCI CTCAE version 4.0. Dose-limiting toxicity (DLT) and dose-reduction criteria are listed in the Supplementary Materials and Methods. Laboratory evaluations, physical examinations, and histories were performed before each treatment week for cycle 1 and at the start of each cycle thereafter. Tumor size was measured pretreatment and every two cycles thereafter (or every three cycles for patients on study for > 1 year) by radiography; RECIST 1.1 (20) was used for response classification. Twenty patients were enrolled on the MTD expansion cohort.

Pharmacokinetic analyses
In the dose-escalation cohort, blood specimens for pharmacokinetic analysis were collected prior to treatment initiation; on cycle 1 day 1 (C1D1) at 1 to 24 hours after dosing; and on C1D5 at 1 to 8 hours post-dose. Data from two patients who vomiting within 1 hour of their first dose were excluded from analysis. In the fixed-dose expansion cohort, sparse pharmacokinetic sampling (C1D1 pre-dose and 2 to 4 hours post-dose and C1D8 pre-dose) was conducted for comparison with tumor pharmacodynamic effects.

Pharmacodynamic analyses
Paired, 18-gauge core needle tumor biopsies were collected from expansion cohort patients on C1D1 and C1D8 prior to administering the daily dose. Biopsy specimens were evaluated for nuclear levels of pY15-Cdk1/2 (2), pH3, γH2AX, RAD51, and serine 343–phosphorylated Nbs1 (pNbs1; ref. 21) and colocalization of cleaved caspase-3 and γH2AX (22) as described in the Supplementary Materials and Methods.

Genomic and transcriptomic analyses
Tumor tissue specimens remaining after completion of pharmacodynamic analyses were delinked and subjected to whole-exome sequencing (WES) and RNA sequencing (RNA-seq) for nine patients who consented to genetic analyses. Details regarding sequencing and bioinformatic analyses can be found in the Supplementary Materials and Methods.
Results

Patient population and disposition

From August 2014 to October 2018, 42 patients were enrolled in the once-daily arm of this study (27 females, 15 males; Table 1). The median patient age for this arm was 64 (range, 26–83), and the median number of prior therapies was 5.5 (range, 0–15). The most common histology was ovarian carcinoma (10 patients; 24%).

In total, 35 patients were evaluable for objective response. Seven patients were not evaluable for response by RECIST 1.1 due to the worsening of clinical status after completing ≤ 1 cycle of therapy and prior to undergoing restaging CT scans. Two of these patients died because of clinical deterioration from disease progression, four went off study due to disease progression, and one went off study due to worsening of a preexisting fistula.

Toxicity

Overall, gastrointestinal and hematologic toxicities were the most common adverse events attributed to adavosertib. Nausea, vomiting, or diarrhea each were experienced by > 60% patients enrolled, with nausea (81%) being the most common toxicity (Table 2); however, ≤12% of these gastrointestinal adverse events were ≥ grade 3. Of the hematologic toxicities, lymphopenia (71%), anemia (69%), and leukopenia (50%) were the most common any-grade events and also constituted the most common ≥ grade 3 adverse events (29%, 21%, and 21%, respectively). Hematologic toxicities were also the only grade 4 events: leukopenia (10%), neutropenia (12%), and thrombocytopenia (2%). The median durations until recovery for grade 3/4 hematologic toxicities were: lymphopenia, 18 days (range, 1–225 days); neutropenia, 5 days (1–26); leukopenia, 5 days (1–22); and thrombocytopenia, 6.5 days (1–21). Patients experienced several electrolyte imbalances, with the most common ≥ grade 3 event being hypophosphatemia (12%) of these gastrointestinal adverse events were grade 3).

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (female, male)</td>
<td>42 (27, 15)</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>64 (26–83)</td>
</tr>
<tr>
<td>ECOG score</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>Median number of prior therapies (range)</td>
<td>5.5 (0–15)</td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>10</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>5</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Alveolar soft part sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Leiomyoscarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Endometrial carcinosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Pleomorphic sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Synovial cell sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Uterine carcinosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Adverse events attributed to adavosertib.

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade 1–2 (%)</th>
<th>Grade 3 (%)</th>
<th>Grade 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>20 (47%)</td>
<td>9 (21%)</td>
<td>—</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>12 (29%)</td>
<td>5 (12%)</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>18 (43%)</td>
<td>12 (29%)</td>
<td>—</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>5 (12%)</td>
<td>4 (10%)</td>
<td>5 (12%)</td>
</tr>
<tr>
<td>Febrile neutropenia</td>
<td>—</td>
<td>1 (2%)</td>
<td>—</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>13 (31%)</td>
<td>5 (12%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>17 (40%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>25 (60%)</td>
<td>2 (5%)</td>
<td>—</td>
</tr>
<tr>
<td>Nausea</td>
<td>31 (74%)</td>
<td>3 (7%)</td>
<td>—</td>
</tr>
<tr>
<td>Vomiting</td>
<td>24 (57%)</td>
<td>5 (12%)</td>
<td>—</td>
</tr>
<tr>
<td>Electrolyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>3 (7%)</td>
<td>1 (2%)</td>
<td>—</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>7 (17%)</td>
<td>1 (2%)</td>
<td>—</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>10 (24%)</td>
<td>6 (14%)</td>
<td>—</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>5 (12%)</td>
<td>1 (2%)</td>
<td>—</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper respiratory infection</td>
<td>—</td>
<td>1 (2%)</td>
<td>—</td>
</tr>
<tr>
<td>Investigations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased</td>
<td>11 (26%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased</td>
<td>11 (26%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>19 (45%)</td>
<td>3 (7%)</td>
<td>—</td>
</tr>
<tr>
<td>Dehydration</td>
<td>5 (12%)</td>
<td>1 (2%)</td>
<td>—</td>
</tr>
<tr>
<td>Hypoalbuminemia</td>
<td>9 (21%)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Note: The percentage of patients experiencing each event is noted in parentheses (n = 42 patients).

In the dose-escalation cohort, the first two patients (at the 200-mg dose level) experienced nausea and vomiting, prompting the addition of prophylactic antiemetic treatment and accrual of a total of six patients at this dose level to ensure the safety of adavosertib with antiemetic prophylaxis (Supplementary Table S1). The study design allowed for simultaneous enrollment at a given dose level, and at the 400-mg once-daily dose level, all three patients experienced a DLT during cycle 1: one patient experienced grade 3 fatigue; one patient was admitted with grade 4 pancytopenia complicated by methicillin-resistant Staphylococcus aureus (MRSA) pneumonia and requiring a 2-week treatment delay and growth factor support; and one patient experienced grade 4 neutropenia and was unable to complete 10 days of treatment. No DLTs occurred at any other dose levels, including in the six patients enrolled at the 300-mg once-daily dose level during dose escalation. Thus, 300-mg once-daily on days 1 to 5 and 8 to 12 of each 21-day cycle (with prophylactic antiemetic coverage) was deemed to be the MTD and the recommended phase II dose. An additional 13 patients (the majority of whom were enrolled at the 300-mg dose level) underwent dose reductions due to non-DLT toxicities, which were largely hematological and nausea, vomiting, or fatigue (Supplementary Table S2).

Clinical activity

Six patients had a confirmed partial response (PR) (Fig. 1A); the median duration of response was 4.9 months (range, 4.3–23.3),
while the median time to response was 2.3 months (range, 1.3–13.7). The responding patients included two patients of the three enrolled at the highest dose level (400 mg; patients 1010042 and 1010043), both with ovarian cancer. Both of these patients were dose reduced due to DLTs and achieved the majority of their PR while receiving the MTD (300 mg). In addition, PRs were achieved by four patients of the 26 enrolled at the MTD: two each with ovarian and endometrial cancers (Fig. 1A–C). These results suggest dose-dependent antitumor activity (Fisher exact \( P \) value \( = 0.05 \), one-sided). The overall response rate for once-daily adavosertib \( [6 \text{ PRs among 42 enrolled patients; 14%; 90\% confidence interval (6\%–26\%)}] \) was comparable with that observed for twice-daily adavosertib administration [two PRs among 25 enrolled patients; 8%; 90\% confidence interval (1\%–23\%)] (2).

Twenty patients experienced a best response of stable disease (SD), including two with unconfirmed PRs who went off study due to adverse events and progressive disease, respectively. The median number of treatment cycles for these 20 patients was four (range, 2–26), with five patients receiving \( \geq 11 \) cycles of treatment (Fig. 1A). Among the patients with SD, mesothelioma (4/19 patients) was the most common histology; the median duration of SD was 1.6 months (range, 1.2–10.7 months).

Examination of response data and existing targeted next-generation sequencing (NGS) clinical reports available for a subset of patients prior to enrollment revealed that \( BRCA1/2 \) and \( TP53 \) mutations were noted among both responders and nonresponders, and not all responders harbored these mutations (Fig. 1A). One responding patient (1010064) had neither \( BRCA1/2 \) nor \( TP53 \) tumor mutations but had a microsatellite instability-high tumor and had progressed on immune checkpoint inhibitor prior to enrollment.

Seven of the 10 patients with ovarian cancer on this study had undergone prior PARP inhibitor therapy (Supplementary Table S3). Of these seven, there were two with no available response information from their prior PARP inhibitor therapy. Two patients had a partial response (PR) to prior PARP inhibitory therapy, and both of these patients (1010042 and 1010043) also exhibited a PR to adavosertib and had tumor \( BRCA1 \) loss-of-function mutations. Three patients did not respond to prior PARP inhibitor therapy, and of these, two also had a best response of progressive disease on our adavosertib trial: patient 1010048, who had tumor \( TP53 \) and \( NF1 \) mutations and \( MYC \) amplification, and patient 1010052, who had no clinical NGS report available. One patient did not respond to prior PARP inhibitor therapy but did have a PR to adavosertib on our study; this patient had a tumor that had no \( BRCA1/2 \) mutations but instead had a deleterious \( TP53 \) mutation, \( AKT3 \) amplification, and \( CCNE1 \) amplification (Supplementary Table S3).

Both patients who maintained a PR for over 1 year were diagnosed with high-grade serous ovarian carcinoma (HGSOC) and had

Figure 1. Patient response to once-daily adavosertib. A, The number of treatment cycles completed is shown for each patient on study for at least one cycle, along with the corresponding starting dose level. Patients experiencing a PR are indicated, as are the \( BRCA1/BRCA2 \) and \( TP53 \) status of each patient who had results available from previously conducted genetic sequencing. B, Target lesion response over time for patients evaluable for objective response. Each line represents a single patient; lines are colored by best response as indicated. The horizontal dotted red line indicates a \( \geq 30\% \) decrease in the size of target lesion compared with baseline. Both patients on study for \( >12 \) months with a durable PR had HGSOC. C, Best target lesion response in patients evaluable for objective response. Colors represent the indicated tumor histologies. The dashed pink line indicates a \( \geq 30\% \) decrease in target lesion size. Asterisks denote the two patients for whom target lesion size decreased by \( \geq 30\% \) but who did not have confirmed PRs.
undergone extensive prior therapy. After initial diagnosis, patient 1010042 had a total abdominal hysterectomy with bilateral salpingo-oophorectomy and subsequent adjuvant carboplatin/paclitaxel; at the time of enrollment on this study, she had progressed on at least six prior chemotherapies and two clinical trials, including a phase II trial of a PARP inhibitor. Patient 1010057 was initially diagnosed with stage III C ovarian carcinoma and underwent surgery and adjuvant chemotherapy approximately 2 years before starting adavosertib; her disease was platinum refractory, leading to enrollment on a trial of avelumab-entinostat for 8 months before progression and subsequent enrollment on this study. After achieving a PR at 12 weeks after the start of adavosertib and maintaining the PR for nearly 2 years, she discontinued adavosertib due to an increase in nontarget lesions.

Pharmacokinetic analysis

Once-daily adavosertib exhibited a largely dose-proportional pharmacokinetic profile (Fig. 2A; Supplementary Table S4). Plasma concentrations of adavosertib were higher on day 5 compared with day 1, consistent with the data for twice-daily adavosertib and the approximately 11-hour half-life for this agent (2). Also consistent with this half-life, among the 16 patients in the MTD expansion cohort, the mean plasma concentration of adavosertib on C1D8, pre-dose was low, 41 ± 35 nmol/L (Fig. 2B). Relative to the MTD for the twice-daily regimen, that for once-daily adavosertib yielded comparable plasma exposures (Supplementary Fig. S1).

Pharmacodynamic analyses

Results from the twice-daily arm of this study demonstrated adavosertib-mediated Wee1 kinase suppression (pY15-Cdk1/2 reduction) of 80% to 90% accompanied by increased DNA damage response (DDR) in tumor biopsies obtained 2 to 5 hours after the fifth dose, although in approximately half of the cases, baseline nuclear pY15-Cdk levels were too low to quantify a pharmacodynamic effect. Given the importance of extensive, deep kinase suppression in achieving maximum tumor control (23), we examined whether adavosertib-induced Wee1 target suppression and effects on DNA damage, mitosis, and apoptosis remained detectable after the pause in dosing between day 5 and day 8 despite substantial drug clearance. On the basis of the 11-hour plasma half-life of adavosertib, this 72-hour pause in dosing represents 6 to 7 drug half-lives; however, pharmacokinetic analysis of...
blood specimens collected pre-dose on C1D8 confirmed that plasma concentrations remained above the target concentration of 5 nmol/L (the in vitro IC50 ref. 7), and therefore, molecular target effects were expected.

By comparing pharmacodynamic biomarker levels in tumor biopsy pairs obtained at baseline (pretreatment on C1D1) and C1D8, we assessed multiple molecular drug responses (Fig. 2B), including Wee1 inhibition (decreased nuclear pY15-Cdk1/2, entry into mitosis (prevalence of nuclear pHH3), DDR (increased nuclear γH2AX, pNbs1, and RAD51; ref. 21), and apoptosis (increased colocalization of cleaved caspase-3/γH2AX; ref. 22). Among the eight patients with evaluable biopsy pairs, three exhibited baseline nuclear pY15-Cdk levels sufficiently high to measure a statistically significant decrease upon adavosertib treatment: patients 1010064 (endometrial carcinoma, best response of PR), 1010065 (ER+ breast carcinoma, best response of PD), and 1010066 (HGGOC, best response of PD) exhibited baseline % nuclear area positive (NAP) pY15-Cdk values of 7.8%, 8.1%, and 22.8%, respectively (Fig. 2B). On C1D8, nuclear pY15-Cdk was significantly reduced relative to baseline in two of these three patients’ tumors: by 35% and 62%, respectively, in patients 1010064 and 1010066 (P < 0.0001); patient 1010065 exhibited a small, but not statistically significant, reduction in nuclear pY15-Cdk (7%) on C1D8 (Fig. 2B; Supplementary Fig. S2). Both patients showing significantly decreased pY15-Cdk at C1D8 also exhibited significant increases in levels of DNA repair markers (γH2AX and/or pNbs1), indicating that protracted target inhibition at this timepoint continues to be associated with DDR (Fig. 2B). Importantly, γH2AX increases were due to DDR rather than DNA double-strand breaks from apoptosis (DNA “laddering”), as evidenced by the absence of colocalized cleaved caspase-3 in γH2AX-positive cells (ref. 22; data not shown).

The five remaining patients with evaluable biopsy pairs exhibited baseline nuclear pY15-Cdk levels too low to measure a drug-induced decrease (≤2.2% NAP). However, in four of these patients, adavosertib treatment caused statistically significant increases in nuclear pY15-Cdk (192%-1094% increases; P < 0.0001; Fig. 2B). Three of these four patients achieved a PR to adavosertib. Among these five patients with low baseline levels of nuclear pY15-Cdk, none demonstrated biologically significant DDR biomarker induction (≥4% NAP (21)) at C1D8 relative to baseline, and one responding patient, 1010051, had significantly lower % NAP γH2AX at C1D8 relative to baseline (P < 0.05).

None of the eight patients evaluable for pharmacodynamic response showed changes in tumor levels of nuclear RAD51, and nuclear pHH3 levels were generally unchanged between baseline and C1D8 (data not shown). One patient with a low baseline pHH3 level (1010056, best response of PD) exhibited a small but statistically significant increase in pHH3 positivity from baseline to C1D8 (from 1.1% to 4.1% cells positive, respectively; P < 0.0001).

Genomic and transcriptomic analyses

To identify tumor molecular features potentially associated with response to once-daily adavosertib—and that could be further explored in subsequent studies—we performed exploratory WES and RNA-seq analyses on the flash-frozen baseline and/or on-treatment tumor tissue collected on this study that remained after completion of pharmacodynamic analyses. For patients who consented to this hypothesis-generating sequencing analysis, tumor specimens were delinked from identifiable patient information; delinked patients are represented by letters. Tumor tissue for genomic analysis was obtained from nine patients, of which, four had paired baseline/on-treatment specimens. We analyzed the resulting WES data for mutations and copy-number variations (CNVs) in genes previously shown to be predictive of response to adavosertib and/or associated with cell cycle and DDR (24–26). Genomic alterations detected in baseline tumor specimens were generally consistent with those found in C1D8 specimens for the 4 patients with paired specimens (patients A, D, F, and G), though the degree of CNV varied slightly for some genes within each pair (Fig. 3A; Supplementary Table S5). We did not observe any obvious mutational signatures specific to the three responding patients (patients B, C, and F). Consistent with the prior NGS report data, we observed tumor TP53 mutations in both responding and nonresponding patients. Tumor BRCA1/2 mutations were not found in any of the responding patients but were detected in patients with a best response of progressive disease or for whom response was not assessable.

Among the two patients with a best response of progressive disease, one (patient D) had breast carcinoma with a tumor splice site mutation in the adavosertib target WEE1 (Fig. 3A; Supplementary Table S5). This mutation, a 4-bp deletion at the end of exon 10 and extending into the subsequent splice donor site (11–9608401–AAAGT–A), is strongly predicted to disrupt Wee1 function according to the Genome Aggregation Database (gnomAD; ref. 27). We hypothesized that the resulting reduction in levels of active Wee1 kinase may have led to compensatory cell-cycle checkpoint signaling that rendered this patient resistant to adavosertib, such as upregulation of the other two kinases known to phosphorylate Cdk1: Wee2 and Myt1, also known as PKMYT1 (28). In examining baseline tumor RNA expression for patient D and the four others (two PR; two SD) for whom pretreatment RNA-seq data were available, we found that patient D, along with the two responding patients (F and B) had slightly (patients D and B) or substantially (F) reduced expression of WEE1 relative to the average expression across these five patients (Fig. 3B). WEE2 expression was negligible in all patients, consistent with prior studies demonstrating that WEE2 expression is restricted to germ cells (28). However, patient D, along with patient G (best response of SD), exhibited upregulation of baseline PKMYT1 expression relative to the other patient tumors. Adavosertib does not substantially inhibit PKMYT1 (29), suggesting that PKMYT1 upregulation could potentially account for the intrinsic resistance to adavosertib in patient D.

In further examining baseline expression levels of genes associated with cell-cycle regulation and/or response to adavosertib (24, 25), we found that most of the genes examined were not differentially expressed in responders (patients B and F) versus nonresponders (patients D, E, and G; Fig. 3B). The low expression of WEE1 and PKMYT1 in patients B and F may have partially contributed to their response to adavosertib, as lower target levels increase the drug-to-target ratio. In addition, these two responding patients had elevated tumor RNA expression of Schlafen family member 11, SLFN11, a predictable marker of response to DNA damage–inducing agents that promotes cell death by irreversibly blocking DNA replication in response to DNA damage (30–32). Finally, in comparison with the three nonresponding patients, patients B and F exhibited higher baseline expression of CCNE1, which encodes cyclin E1—the Cdk2 partner protein that regulates the G1–S checkpoint. CCNE1 copy-number amplification (CNA) was shown to be potentially associated with response to an adavosertib-chemotherapy combination in patients with platinum-resistant ovarian cancer (33). However, in our dataset, one of the two responding patients with high baseline CCNE1 expression did not exhibit CCNE1 CNA (patient F; Fig. 3A and B), suggesting that baseline CCNE1 gene expression, rather than CCNE1 amplification alone, may have value in predicting response to adavosertib.

Clin Cancer Res; 27(14) July 15, 2021 3839
Figure 3.
Tumor molecular analysis of patients treated with once-daily adavosertib. Pre- (C1D1) and/or on-treatment (C1D8) tumor tissue for exploratory genomic analysis was obtained from nine patients. Prior to genomic analyses, patient specimens were delinked from patient names and medical records, retaining only limited clinical information; each delinked patient is designated by a unique letter. Patient diagnoses are indicated, and best responses to adavosertib are shown in parentheses: PD, progressive disease; PR, partial response; SD, stable disease.

A, OncoPlot showing mutations (top) and CNVs (bottom) in genes associated with cell cycle and DNA repair. Mutation types and CNV values are noted by colored boxes, as indicated. In addition to the genes shown, no mutations or CNVs were found in the remaining DNA repair– and cell cycle–associated genes that were analyzed: ATM, PALB2, RAD51C, RAD51D, FANC, MLH1, CHEK1, CHEK2, DNA-PK, PARP, AXL, and LKB1. Specific variants identified, and the predicted functional consequences of each, are noted in Supplementary Table S5.

B, Baseline expression of genes implicated in Wee1 kinase inhibitor response. Unsupervised consensus clustering was performed on baseline (C1D1) gene expression data for the 4,000 genes with the greatest variability in expression, and relative expression levels are shown for a panel of genes demonstrated to affect Wee1 kinase inhibitor activity (24, 25). For both (B) and (C), data are shown for the 5 patients with available baseline tissue for sequencing analyses: br, breast; en, endometrial; ns, non–small cell lung cancer; ov, ovarian. Below the heatmap of mRNA expression, patients with tumor CCNE1 CNA (>2 and <5 copies) are denoted by red boxes.

C, Baseline tumor immune microenvironment phenotype classifications. Gene expression data were analyzed using the CRI AATlas Portal Tumor Microenvironment tool, which assesses the degree to which each tumor gene expression profile matches each of six identified tumor microenvironment gene expression signatures (36). Colors indicate signature scores, which range from 0 (white) to 1 (red); red represents the highest probability of the given subtype and white, the lowest. In ovarian (D) and endometrial (E) cancer TCGA datasets, CCNE1 RNA expression levels are significantly elevated in IFNγ-dominant tumors relative to tumors with other immune microenvironment phenotypes. Significance levels from two-sided unpaired t tests are indicated by asterisks (*, P < 0.05; **, P < 0.01; ***, P < 0.001).
To further explore the frequency of tumor CCNE1 mRNA overexpression, we analyzed The Cancer Genome Atlas (TCGA) and MSK-IMPACT datasets using cBioPortal (https://www.cbioportal.org/; Supplementary Fig. S3). In the MSK-IMPACT 2017 dataset (34), CCNE1 mRNA was identified in 1.8% of patients (194/10,336), with the highest incidence found in ovarian (10.3%) and endometrial carcinomas (8.7%). In separate ovarian and endometrial carcinoma TCGA Pan-Cancer Atlas cohorts, we evaluated the prevalence of CCNE1 expression with and without CNA to determine whether selecting for patients with CCNE1 mRNA overexpression was likely to also capture patients with CCNE1 overexpression. In the ovarian carcinoma TCGA cohort, 60 of 201 specimens (30%) had either CCNE1 mRNA overexpression, CCNE1 CNA, or both; of these, 21 (35%) had both CCNE1 mRNA overexpression and CCNE1 CNA, while 22 (36.7%) had CCNE1 CNA but no CCNE1 overexpression, and 17 (28.3%) had CCNE1 overexpression but no CNA (Supplementary Fig. S4A). Similarly, for the endometrial carcinoma TCGA cohort, 14% of the total specimens (69/507) had either only CCNE1 overexpression (29/69, 42% of the specimens with CCNE1 aberrations), only CCNE1 CNA (17/69, 24.6%), or both (23/69, 33.3%; Supplementary Fig. S4B). Together, these data demonstrate that a substantial subset of ovarian and endometrial tumors may exhibit CCNE1 mRNA overexpression in the absence of CCNE1 CNA and that CCNE1 CNA is not necessarily indicative of CCNE1 overexpression.

Given that the tumor immune microenvironment may influence DNA damage repair processes (35), we used baseline RNA-seq data to classify each patient tumor immune subtype, as previously defined using TCGA gene expression data (36), and to determine whether any baseline tumor immune subtypes are associated with response to adavosertib. Of the five patients with baseline RNA expression data, three [one patient each with ovarian, endometrial, and non–small cell lung cancer (NSCLC)] had baseline tumor gene expression patterns strongly indicative of an IFNγ-dominant tumor immune microenvironment, including both responding patients (B and F) who also had tumor CCNE1 CNA (Fig. 3C). The remaining two patients, both with breast cancer, had baseline tumor immune phenotypes characterized as inflammatory (patient F; best response of SD) or lymphocyte depleted (patient D; best response of PD). The responses in patients with IFNγ-dominant tumors are particularly interesting given the generally poor prognosis of patients with this tumor immune subtype (36).

Finally, because both responding patients in our small pilot analysis had tumors characterized by both CCNE1 overexpression and an IFNγ-dominant tumor immune microenvironment, we sought to explore further the relationship between these two molecular features using TCGA datasets. Focusing on ovarian and endometrial carcinomas, we assessed whether CCNE1 expression was elevated in IFNγ-dominant tumors compared with the other tumor immune subtypes. For the 272 TCGA ovarian and 186 endometrial carcinoma specimens examined, mean CCNE1 expression was significantly higher for tumors with an IFNγ-dominant microenvironment compared with those with any other immune profile (Fig. 3D and E).

Discussion

Building on our previous evaluation of twice-daily adavosertib (2), we were able to demonstrate the safety and preliminary antitumor activity for once-daily dosing of this Wee1 inhibitor in patients with advanced solid tumors. The MTD was 300 mg once daily on days 1 to 5 and 8 to 12 of each 21-day cycle. The DLTs were grade 4 hematologic toxicity and grade 3 fatigue. Grade 4 adverse events were exclusively hematologic. The predominance of gastrointestinal and hematologic toxicities for twice-daily adavosertib has also been observed in other recent Phase I trials of adavosertib combinations (14, 15). Overall, once-daily dosing of adavosertib had an adverse event profile comparable with that of the twice-daily dosing regimen.

The antitumor activity measured for once-daily adavosertib (six PRs among 42 patients; 14%) was comparable with that for twice-daily adavosertib (two PRs among 25 patients; 8%; ref. 2), as was the plasma exposure. The six patients with a PR included four of the 26 receiving the MTD (300 mg) and two of the three patients enrolled on the 400-mg dose level, suggesting dose-dependent antitumor activity. All responses observed in this study occurred in patients with endometrial or ovarian cancer, consistent with the previous findings in these tumor types (2, 37, 38). In addition, five patients had a best response of SD for ≥ 9 months: one patient each with mesothelioma, hepatocellular carcinoma, and prostate cancer, and two patients with leiomyosarcoma.

The apparent dose-dependent activity of once-daily adavosertib suggests a potentially narrow therapeutic index for this agent and is corroborated by our prior findings for the twice-daily adavosertib regimen (2). In the latter dataset, PRs occurred only at the MTD dose (225 mg twice daily × 5 doses for 2 weeks, in 3-week cycles). Furthermore, in a previous phase I study of adavosertib–chemotherapy combinations, pharmacodynamic data from surrogate tissue (hair follicles from skin biopsies) demonstrated significant target suppression (phospho-Cdk1 attenuation) for chemotherapy combinations with adavosertib administered twice daily at either 200 or 225 mg, but not at 25 mg (14). Together, these data indicate that the therapeutic index for adavosertib may be quite narrow, necessitating careful dose-escalation designs for future combination studies.

Inhibition of Wee1 kinase activity directly prevents Y15 phosphorylation within Cdk1 and Cdk2, resulting in premature S-phase and mitotic entry, enhanced DNA damage, and ultimately cell death (4–6). We previously demonstrated clinical proof of mechanism for twice-daily adavosertib based on reduced tumor levels of nuclear pY15-Wee1 and increased levels of nuclear γH2AX measured at 2 to 5 hours after the fifth adavosertib dose (2). We have shown with other targeted agents that >90% continuous inhibition of molecular target enzymatic activity is necessary for maximizing antitumor activity (23, 39, 40), and the once-daily adavosertib regimen provided an opportunity to evaluate the persistence of adavosertib pharmacodynamic effects in tumor at 72 hours after the fifth dose (i.e., pre-dose on C1D8).

Consistent with the twice-daily adavosertib results (2), patient tumors in the once-daily arm segregated into two groups based on baseline nuclear pY15-Cdk1 and Cdk2, resulting in premature S-phase and mitotic entry, enhanced DNA damage, and ultimately cell death (4–6). We previously demonstrated clinical proof of mechanism for twice-daily adavosertib based on reduced tumor levels of nuclear pY15-Cdk and increased levels of nuclear γH2AX measured at 2 to 5 hours after the fifth adavosertib dose (2). We have shown with other targeted agents that >90% continuous inhibition of molecular target enzymatic activity is necessary for maximizing antitumor activity (23, 39, 40), and the once-daily adavosertib regimen provided an opportunity to evaluate the persistence of adavosertib pharmacodynamic effects in tumor at 72 hours after the fifth dose (i.e., pre-dose on C1D8).
Takebe et al.

Our tumor RNA-seq analysis for this small group of patients revealed high relative baseline expression of CCNE1 in 2 patients who responded to adavosertib (B and F), though only one (B) had CCNE1 CNA. CCNE1 CNA was previously found to be potentially predictive of response to the combination of adavosertib and chemotherapy in a phase II study of patients with platinum-resistant ovarian cancer (33). In addition, CCNE1 overexpression was shown to sensitize triple-negative breast cancer models to Wee1 kinase inhibition (45), and an ongoing tissue-agnostic phase II trial (NCT03253679) is evaluating adavosertib activity in patients with CCNE1-amplified tumors. Given the function of cyclin E1 in promoting transition through the G1–S checkpoint, initiating the chromatid remodeling required for DNA replication, and regulating centrosome duplication integrity (46–48), it is unsurprising that Wee1 inhibition by adavosertib may promote cell death in CCNE1-overexpressing tumors via exacerbation of cell-cycle dysregulation at both the G1–S and G2–M checkpoints. Loss of p53 has been shown to enhance the aberrant centrosome amplification resulting from cyclin E overexpression (47, 48), and the TP53 deletions in both of the responding patients with CCNE1-overexpressing tumors may have further contributed to their response to adavosertib.

The discrepancies between CCNE1 CNA and overexpression in our small dataset were confirmed in our evaluation of TCGA data for ovarian and endometrial carcinomas. Lack of correlation between genomic alterations and respective gene expression levels due to transcriptomic silencing (49) may partially explain the modest efficacy of some targeted therapies in large precision medicine trials, such as NCI-MATCH and ASCO TAPUR, which rely heavily on DNA-based targeted NGS to identify actionable alterations (50). Going forward, additional use of proteomic, transcriptomic, and metabolomic strategies to identify predictive biomarkers may be important for improving response rates to targeted agents (50).

Finally, both of the responding patients with CCNE1-overexpressing tumors also had an IFNγ-dominant tumor microenvironment, as classified by an RNA expression-based algorithm devised by Thorsson and colleagues (36). Their study found that patients with this tumor immune subtype had relatively poor prognosis despite high tumor lymphocyte infiltration and active CD8 T-cell expression signatures; the authors attributed this unexpected finding to the higher cellular proliferation index of this tumor subtype and/or an underlying immune editing mechanism that promotes immune escape (36). In the context of our results, it is also possible that the IFNγ-dominant tumor microenvironment may occur, at least in part, due to CCNE1 overexpression and the resulting accumulated DNA damage accompanying G1–S checkpoint dysregulation (51). Corroborating our findings regarding the potential relationship between a IFNγ-dominant tumor immune microenvironment and adavosertib response, data from a recent phase II study of adavosertib combined with cisplatin in patients with metastatic triple-negative breast cancer demonstrated that upregulation of IFNγ pathway gene expression was associated with clinical benefit among the 24 patient tumors examined (52). Together, these data may provide justification for trials evaluating immune checkpoint inhibitors combined with Wee1 inhibitors in patients with CCNE1-overexpressing ovarian and endometrial cancers, and to this end, an ongoing phase I trial is assessing safety and tolerability for the combination of adavosertib and the anti-PD-L1 agent durvalumab (NCT02617277). More recently, preclinical data demonstrated that adavosertib sensitizes murine head-and-neck carcinoma cells to granzyme-dependent natural killer (NK)-cell cytolysis (53), suggesting that combinations with NK cell-based or IL15-based therapies may also hold promise.

Future clinical development plans for adavosertib may include additional monotherapy or combination therapy studies in patient populations with known tumor molecular aberrations and/or in the ovarian and endometrial histologies in which this agent has been shown—through the current study and others—to have activity. This includes the aforementioned tissue-agnostic phase II study of adavosertib monotherapy in patients with CCNE1-amplified tumors (NCT03253679), which could potentially be expanded upon in future studies to explore the use of CCNE1 expression, rather than amplification, as a patient selection marker. In addition, an ongoing study of adavosertib monotherapy in patients with recurrent uterine serous carcinoma (NCT03668340) has demonstrated a promising preliminary overall response rate of 29% in this patient population, for which over 90% of tumors have TP53 mutations (37). In the ovarian carcinoma space, a prior phase II study of adavosertib combined with carboplatin in patients with platinum-resistant/refractory, p53-deficient tumors yielded an overall response rate (ORR) of 43% (54). A recent investigation of this combination in patients with platinum-resistant ovarian carcinoma, regardless of tumor p53 status, demonstrated an ORR of 67% (33). Future adavosertib monotherapy or combination studies using CCNE1-based selection strategies in patients with ovarian or endometrial carcinomas may yield improved response rates and may provide opportunities for adequately powered comparisons between CCNE1 amplification and CCNE1 expression in predicting response to adavosertib.

Together, our results demonstrate the safety, tolerability, and preliminary clinical activity of once-daily adavosertib and provide a pharmacologic basis for these findings. Our genomic analysis results suggest tumor molecular characteristics potentially associated with response and resistance that may aid in improving patient selection for future adavosertib trials.
Once-daily Adavosertib in Solid Tumors

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