Safety, anti-tumor activity, and biomarker analysis in a phase 1 trial of the once-daily Wee1 inhibitor adavosertib (AZD1775) in patients with advanced solid tumors

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Conflict of Interest Statement: L. Juwara, J. Ji, D. Wilsker, R.J. Kinders, R.E. Parchment, L. Chen, T.C. Chang, and B. Das reported payment from NIH/NCI (contract number 75N91019D00024) during the conduct of the study. Ganesh Mugundu was an employee of AstraZeneca at the time this study was conducted and declares AstraZeneca stock ownership. All other authors declare no potential conflicts of interest.
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 2 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 1-5 and 8-12 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 hematological; dose-limiting toxicities were grade 4 hematological toxicity and grade 3 fatigue. The once-daily RPh2D was 300 mg. Six patients (14%) had confirmed partial responses (PR): 4 ovarian, 2 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 4 of 8 patients, suggesting target rebound during the day 5-8 dosing break. One patient who progressed rapidly had a tumor \textit{WEE1} mutation and potentially compensatory \textit{PKMYT1} overexpression. Baseline \textit{CCNE1} overexpression occurred in both of 2 responding patients, only 1 of whom had \textit{CCNE1} amplification, and in 0 of 3 non-responding patients.

Conclusions: We determined the once-daily adavosertib RPh2D and observed activity in patients with ovarian or endometrial carcinoma, including 2 with baseline \textit{CCNE1} mRNA overexpression. Future studies will determine whether \textit{CCNE1} overexpression is a predictive biomarker for adavosertib.
TRANSLATIONAL RELEVANCE

Targeting multiple DNA damage response pathway components to induce synthetic lethality remains an important approach for anti-cancer 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 anti-tumor activity as monotherapy when biomarker-based patient selection is performed. Here, we report a recommended phase 2 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 to that of the previously published twice-daily regimen. We identified tumor \textit{CCNE1} (cyclin E1) mRNA expression as a potential patient selection biomarker for response, particularly in ovarian and endometrial cancers. These results suggest adavosertib monotherapy may hold promise in patients with advanced solid tumors when combined with biomarker selection using \textit{CCNE1} expression as a precision medicine approach to induce synthetic lethality.
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-Cdk1 and pY15-Cdk2) induce G2 or G1/S cell cycle arrest, respectively (2), and therefore serve as important biochemical markers of Wee1 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 anti-cancer 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.
Adavosertib (AZD1775, AstraZeneca, Cambridge, UK) 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) (2,7). In vitro studies have also shown that adavosertib induces upregulation of the mitotic marker phosphorylated histone H3 (pHH3) in some cell lines, evidence of G2/M checkpoint inhibition (7). The single-agent anti-tumor activity of adavosertib has been shown in multiple cancer cell lines (7-9) and in both TP53-intact and -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 (PK), preliminary anti-tumor 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-Cdk-1 and pY15-Cdk2 (referred to hereafter as pY15-Cdk1/2 or pY15-Cdk) and demonstrated that, 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 pHH3 and γH2AX also increased in 1 and 3 patients, respectively, at this time point (2). After PK 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 (QD)
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. 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, Maryland) 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 1-5 and 8-12 of each 21-day cycle, with escalations to 225, 250, 300, and 400 mg once daily on days 1-5 and 8-12 of each cycle. Patients unable to tolerate the starting dose level were reduced to 200 mg daily on days 1-3 and 8-10 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 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 pre-treatment and every 2 cycles thereafter (or every 3 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 PK analysis were collected prior to treatment initiation; on cycle 1 day 1 (C1D1) at 1–24 hours after dosing; and on C1D5 at 1–8 hours post-dose. Data from 2 patients who vomited within 1 hour of their first dose were excluded from analysis. In the fixed-dose expansion cohort, sparse PK sampling (C1D1 pre-dose and 2–4 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) (21) and colocalization of cleaved caspase-3 and γH2AX (22) as described in the Supplementary 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 9 patients who consented to genetic analyses. Details regarding sequencing and bioinformatic analyses can be found in the Supplementary Materials.
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 due to clinical deterioration from disease progression, 4 went off study due to disease progression, and 1 went off study due to worsening of a pre-existing fistula.

Toxicity

Overall, gastrointestinal and hematological 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 hematological 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). Hematological 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.
and a 2% incidence each of grade 3 hypokalemia, hypomagnesemia, hyponatremia, or hypocalcemia.

In the dose escalation cohort, the first 2 patients (at the 200 mg dose level) experienced nausea and vomiting, prompting the addition of prophylactic anti-emetic treatment and accrual of a total of 6 patients at this dose level to ensure the safety of adavosertib with anti-emetic prophylaxis (Supplementary Table S1). The study design allowed for simultaneous enrollment at a given dose level, and at the 400 mg QD dose level, all 3 patients experienced a DLT during cycle 1: one patient experienced grade 3 fatigue; one patient was admitted with grade 4 pancytopenia complicated by 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 6 patients enrolled at the 300 mg QD dose level during dose escalation. Thus, 300 mg QD on days 1-5 and 8-12 of each 21-day cycle (with prophylactic anti-emetic coverage) was deemed to be the MTD and the recommended phase 2 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 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 2 patients out of the 3 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 4 patients out of the 26 enrolled at the MTD: 2 each with...
ovarian and endometrial cancers (Fig 1 A-C). These results suggest dose-dependent anti-tumor activity (Fisher’s Exact p-value = .05, 1-sided). The overall response rate for once-daily adavosertib (6 PRs among 42 enrolled patients; 14%; 90% confidence interval [6%, 26%]) was comparable to that observed for twice-daily adavosertib administration (2 PRs among 25 enrolled patients; 8%; 90% confidence interval [1%, 23%]) (2).

Twenty patients experienced a best response of stable disease (SD), including 2 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 4 (range 2-26), with 5 patients receiving ≥ 11 cycles of treatment (Fig 1 A). 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 amongst both responders and non-responders, 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 7, there were 2 with no available response information from their prior PARP inhibitor therapy. Two patients had a partial response to prior PARP inhibitory therapy, and both of these patients (1010042 and 1010043) also exhibited a partial response to adavosertib and had tumor BRCA1 loss-of-function mutations. Three patients did not respond to prior PARP inhibitor therapy, and of these, 2 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 partial response 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 partial response for over 1 year were diagnosed with high-grade serous ovarian carcinoma (HGSOC) and had 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 6 prior chemotherapies and 2 clinical trials, including a phase 2 trial of a poly (adenosine diphosphate-ribose polymerase) (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 prior to 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 non-target lesions.

**Pharmacokinetic analysis**

Once-daily adavosertib exhibited a largely dose-proportional PK profile ([Fig 2A](#), [Supplementary Table S4](#)). Plasma concentrations of adavosertib were higher on day 5 compared to 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 MTD
expansion cohort patients, the mean plasma concentration of adavosertib on C1D8, pre-dose was low, 41 ± 35 nM (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 80-90% accompanied by increased DNA damage response in tumor biopsies obtained 2-5 hours after the fifth dose, though 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. Based on the 11-hour plasma half-life of adavosertib, this 72-hour pause in dosing represents 6-7 drug half-lives; however, PK analysis of blood specimens collected pre-dose on C1D8 confirmed that plasma concentrations remained above the target concentration of 5 nM (the in vitro IC50) (7), and therefore, molecular target effects were expected.

By comparing pharmacodynamic biomarker levels in tumor biopsy pairs obtained at baseline (pre-treatment 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 pH3), DNA damage response (increased nuclear γH2AX, pNbs1, and RAD51) (21), and apoptosis (increased colocalization of cleaved caspase-3/γH2AX) (22). Among the 8 patients with evaluable biopsy pairs, 3 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 (HGSOC, 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 2 of these 3 patient 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 (\(\gamma\)H2AX and/or pNbs1), indicating that protracted target inhibition at this time point continues to be associated with DNA damage response (Fig 2B). Importantly, \(\gamma\)H2AX increases were due to DNA damage response rather than DNA double-strand breaks from apoptosis (DNA “laddering”), as evidenced by the absence of colocalized cleaved caspase-3 in \(\gamma\)H2AX-positive cells (22) (data not shown).

The 5 remaining patients with evaluable biopsy pairs exhibited baseline nuclear pY15-Cdk levels too low to measure a drug-induced decrease (\(\leq 2.2\%\) NAP). However, in 4 of these patients, adavosertib treatment caused statistically significant increases in nuclear pY15-Cdk (192%-1094% increases; \(p < 0.0001\); Fig 2B). Three of these 4 patients achieved partial responses to adavosertib. Among these 5 patients with low baseline levels of nuclear pY15-Cdk, none demonstrated biologically significant DDR biomarker induction (\(\geq 4\%\) NAP (21)) at C1D8 relative to baseline, and one responding patient, 1010051, had significantly lower % NAP \(\gamma\)H2AX at C1D8 relative to baseline (\(p < 0.05\)).

None of the 8 patients evaluable for pharmacodynamic response showed changes in tumor levels of nuclear RAD51, and nuclear pH3 levels were generally unchanged between
baseline and C1D8 (data not shown). One patient with a low baseline pH3 level (1010056, best response of PD) exhibited a small but statistically significant increase in pH3 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 9 patients, of which, 4 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 DNA damage response (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 3 responding patients (patients B, C, and F). Consistent with the prior NGS report data, we observed tumor \( TP53 \) mutations in both responding and non-responding 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 2 patients with a best response of progressive disease, one (patient D) had breast carcinoma with a tumor splice site mutation in the adavosertib target \textit{WEE1} (Fig 3A; Supplementary Table S5). This mutation, a 4-base pair 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) (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 2 kinases known to phosphorylate Cdk1: Wee2 and Myt1, also known as \textit{PKMYT1} (28). In examining baseline tumor RNA expression for patient D and the 4 others (2 PR; 2 SD) for whom pre-treatment RNA-seq data were available, we found that patient D, along with the 2 responding patients (F and B) had slightly (patients D and B) or substantially (F) reduced expression of \textit{WEE1} relative to the average expression across these 5 patients (Fig 3B). \textit{WEE2} expression was negligible in all patients, consistent with prior studies demonstrating that \textit{WEE2} expression is restricted to germ cells (28). However, patient D, along with patient G (best response of SD), exhibited upregulation of baseline \textit{PKMYT1} expression relative to the other patient tumors. Adavosertib does not substantially inhibit \textit{PKMYT1} (29), suggesting that \textit{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) vs. non-responders (patients D, E, and G) (Fig 3B). The low expression of \textit{WEE1} and \textit{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 2 responding patients had elevated tumor RNA expression of Schlafen family member 11, SLFN11, a predictive 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 to the 3 non-responding patients, patients B and F exhibited higher baseline expression of CCNE1, which encodes cyclin E1—the Cdk-2 partner protein that regulates the G1/S checkpoint. CCNE1 copy number amplification 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 2 responding patients with high baseline CCNE1 expression did not exhibit CCNE1 CNA (patient F; Fig 3A-B), suggesting that baseline CCNE1 gene expression, rather than CCNE1 amplification alone, may have value in predicting response to adavosertib.

To further explore the frequency of tumor CCNE1 CNA and its association with CCNE1 mRNA overexpression, we analyzed TCGA and MSK-IMPACT datasets using cBioPortal (www.cbiportal.org; Supplementary Fig S3). In the MSK-IMPACT 2017 dataset (34), CCNE1 CNA was identified in 1.8% of patients (194 of 10336), 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 prevalence of CCNE1 overexpression with and without CNA to determine whether selecting for patients with CCNE1 CNA is likely to also capture patients with CCNE1 overexpression. In the TCGA ovarian carcinoma 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 \textit{CCNE1} overexpression (29/69, 42\% of the specimens with \textit{CCNE1} aberrations), only \textit{CCNE1} CNA (17/69, 24.6\%), or both (23/69, 33.3\%; \textbf{Supplementary Fig S4B}). Together, these data demonstrate that a substantial subset of ovarian and endometrial tumors may exhibit \textit{CCNE1} mRNA overexpression in the absence of \textit{CCNE1} CNA and that \textit{CCNE1} CNA is not necessarily indicative of \textit{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 5 patients with baseline RNA expression data, 3 (1 patient each with ovarian, endometrial, and NSCLC) had baseline tumor gene expression patterns strongly indicative of an IFN-\(\gamma\)-dominant tumor immune microenvironment, including both responding patients (B and F) who also had tumor \textit{CCNE1} CNA (\textbf{Fig 3C}). The remaining 2 patients, both with breast cancer, had 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 IFN-\(\gamma\) 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 \textit{CCNE1} overexpression and an IFN-\(\gamma\)-dominant tumor immune microenvironment, we sought to explore further the relationship between these two molecular features using the TCGA dataset. Focusing on ovarian and endometrial carcinomas, we assessed whether \textit{CCNE1} expression was elevated in IFN-\(\gamma\)-dominant tumors compared to 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 IFN-γ–dominant microenvironment compared to those with any other immune profile (Fig 3D-E).
DISCUSSION

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

The anti-tumor activity measured for once-daily adavosertib (6 PRs among 42 patients; 14%) was comparable to that for twice-daily adavosertib (2 PRs among 25 patients; 8%) (2), as was the plasma exposure. The 6 patients with a PR included 4 of the 26 receiving the MTD (300 mg) and 2 of the 3 patients enrolled on the 400 mg dose level, suggesting dose-dependent anti-tumor 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, 5 patients had a best response of SD for ≥ 9 months: one patient each with mesothelioma, hepatocellular carcinoma, and prostate cancer, and 2 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, partial responses occurred only at the MTD dose level (225 mg twice daily x 5 doses for 2
weeks, in 3-week cycles). Furthermore, in a previous phase 1 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 mg or 225 mg, but not at 25 mg (39). 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-Cdk and increased levels of nuclear γH2AX measured at 2-5 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 anti-tumor activity (23,40,41), 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-Cdk1/2 levels. Of the 3 patients with high baseline pY15-Cdk, 2 exhibited significantly reduced C1D8 levels of nuclear pY15-Cdk (by 35% and 62%); in contrast, for the twice-daily arm, nuclear pY15-Cdk levels were reduced by 84% and 90% at 2-5 hours after dose 5 in 2 of 2 patients (2). Given the similar plasma adavosertib exposures for the two regimens, the
reduced drug effect on molecular target measured 72 hours after the day 5 dose is consistent with partial recovery of Wee1 enzymatic activity following the dosing interval between days 5 and 8, when adavosertib is cleared (2). Importantly, variations in clinical activity and tumor pharmacodynamic response were not associated with pre-dose plasma adavosertib concentrations on C1D8, suggesting that other factors may account for the observed interpatient variabilities. We also found that adavosertib treatment increased nuclear pY15-Cdk levels at C1D8 in 4 of the 5 patients with low baseline levels (including in 3 patients who responded to adavosertib), suggesting target rebound during the dosing break between days 5 and 8. This is consistent with results from previous studies demonstrating target activity rebound to greater-than-baseline levels for other kinase inhibitors, such as those targeting MET, mTOR, PI3, and EGFR kinases (42-44).

While results from some such studies indicate that target rebound is associated with therapeutic resistance (43,44), preclinical data from EGFR-mutant lung cancer models show that erlotinib induces higher post-treatment phospho-EGFR levels in vitro and enhanced antitumor activity in vivo when administered as high-dose intermittent pulses rather than a continuous low-dose treatment (42). Further exploration of the relationship between target recovery and antitumor activity for adavosertib could be explored in future preclinical studies.

We also detected an adavosertib-induced DNA damage response (nuclear pNbs1 and γH2AX) without a pHH3 response in patients with both high and low baseline nuclear pY15-Cdk at approximately 72 hours post–dose 5—indicative of compromised DNA replication integrity as a result of Cdk2-mediated progression through S-phase; the
lack of appreciable pHH3 response suggests that protracted inhibition of Cdk1 signaling is not required for adavosertib clinical activity.

We utilized the remaining biopsy specimens from 9 patients for hypothesis-generating genomic and transcriptomic analyses to identify molecular characteristics potentially predictive of adavosertib response or resistance. Response was independent of BRCA1/2 or TP53 mutation status, as determined both by our WES data and previously generated clinical NGS reports. Though a complete analysis was precluded by the lack of such data for all patients, and the overall sample size was small, our observation of BRCA1/2- and TP53-independent adavosertib activity is corroborated by prior clinical and preclinical findings (2,7,14,45).

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 copy number amplification. CCNE1 CNA was previously found to be potentially predictive of response to the combination of adavosertib and chemotherapy in a phase 2 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 (46), and an ongoing tissue-agnostic phase 2 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 chromatin remodeling required for DNA replication, and regulating centrosome duplication integrity (47-49), 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 (48,49), 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 (50) 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 (51). Going forward, additional use of proteomic, transcriptomic, and metabolomic strategies to identify predictive biomarkers may be important for improving response rates to targeted agents (51).

Finally, both of the responding patients with CCNE1-overexpressing tumors also had an IFN-γ–dominant tumor immune 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 IFN-γ–dominant tumor microenvironment may occur, at least in part, due to CCNE1 overexpression and the resulting accumulated DNA damage accompanying G1/S checkpoint dysregulation (52).
Corroborating our findings regarding the potential relationship between a IFN-γ–dominant tumor immune microenvironment and adavosertib response, data from a recent phase 2 study of adavosertib combined with cisplatin in patients with metastatic triple-negative breast cancer demonstrated that upregulation of IFN-γ pathway gene expression was associated with clinical benefit amongst the 24 patient tumors examined (53). Together, these data may provide justification for trials evaluating immune checkpoint inhibitors combined with Wee1 inhibitors in CCNE1-overexpressing patients with ovarian and endometrial cancers, and to this end, an ongoing phase 1 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 lysis (54), suggesting that combinations with NK cell– or IL-15–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 present study and others—to have activity. This includes the aforementioned tissue-agnostic phase 2 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 2 study of adavosertib combined with carboplatin in patients with platinum-resistant/refractory, p53-deficient tumors yielded an overall response rate (ORR) of 43% (55). 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 pharmacological 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.
ACKNOWLEDGEMENTS

The authors wish to thank all patients and their families for participating in this study. The authors also thank Donna Ketchum and Angie B. Dull for expert digital microscopy and quantitative image analyses of γH2AX/pNbs1 and pY15Cdk1/2, respectively; Anna Lee Fong and Luis Romero for performing whole-exome sequencing and RNA sequencing, respectively; and Lindsay Dutko and Shahanawaz Jiwani for tumor macro-dissection. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.
REFERENCES


TABLES

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (female, male)</td>
<td>42 (27, 15)</td>
</tr>
<tr>
<td>Median Age, y (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>
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<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>Leiomyosarcoma</td>
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</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>
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<tr>
<td>Pleomorphic sarcoma</td>
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</tr>
<tr>
<td>Synovial cell sarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Uterine carcinosarcoma</td>
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</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td>1</td>
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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><strong>Hematological</strong></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><strong>Gastrointestinal</strong></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><strong>Electrolyte</strong></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>Hyponatremia</td>
<td>8 (19%)</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><strong>Infection</strong></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><strong>Investigations</strong></td>
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<td></td>
</tr>
<tr>
<td>Alanine aminotransferase increased</td>
<td>11 (26%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aspartate aminotransferase increased</td>
<td>11 (26%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Other</strong></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>

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

**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 1 cycle, along with the corresponding starting dose level. Patients experiencing a partial response (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 ≥ 30% decrease in the size of target lesion compared to baseline. Both patients on study for >12 months with a durable PR had high-grade serous ovarian carcinoma. C) Best target lesion response in patients evaluable for objective response. Colors represent the indicated tumor histologies. The dashed pink line indicates a ≥ 30% decrease in target lesion size. Asterisks denote the 2 patients for whom target lesion size decreased by ≥ 30% but who did not have confirmed partial responses.

**Figure 2.** Pharmacokinetic and pharmacodynamic effects of once-daily adavosertib. A) Mean adavosertib plasma concentrations following once-daily dosing on days 1-5 and 8-12 of each cycle. Mean values for each dose level are shown for escalation cohort patients. Error bars indicate standard deviation, and the number of patients in each group is shown. B) Modulation of tumor Wee1 kinase activity and DNA damage response markers following the 72-hour break in daily adavosertib dosing in patients with tumors assessable for baseline pY15-Cdk. Percent nuclear area positive (NAP) values for tyrosine 15–phosphorylated Wee1 targets Cdk1 and Cdk2 (pY15-Cdk; black) or for the DNA damage response markers γH2AX (blue) or pNbs1 (green) are shown for tumor biopsies collected prior to treatment (open circles) and prior to the cycle 1 day 8 dose, following the break in dosing on days 6 and 7 (closed circles). Patients are grouped according to baseline pY15-Cdk levels. Patient responses and number of treatment cycles completed are shown above the graph, as are trough (cycle 1 day 8, pre-dose) adavosertib plasma concentrations. Patients who went off study for clinical progression.
prior to restaging are designated as having a best response of progressive disease.
Asterisks indicate significant differences between mean C1D1 and C1D8 values
according to nonparametric Mann-Whitney tests (*p<0.05; **p<0.01; ***p<0.001;
****p<0.0001). †Though statistically significant, these γH2AX and pNbs1 responses are
not biologically significant, as the C1D8 levels do not rise above the previously
established baseline range of 0-4% NAP.(21)

**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 9 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 copy number
variations (CNV; 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 copy number variations were found in the remaining DNA
repair– and cell cycle–associated genes that were analyzed: ATM, PALB2, RAD51C,
RAD51d, FANC, MLH11, CHEK1, CHEK2, DNA-PK, PARP, AXL, and LKB1. Specific
variants identified, and the predicted functional consequences of each, are noted in
Supplementary Table S5. NSCLC, non-small cell lung cancer; cholang,
cholangiocarcinoma; STS, soft tissue sarcoma. B) Baseline expression of genes
implicated in Wee1 kinase inhibitor response. Unsupervised consensus clustering was
performed on baseline (C1D1) gene expression data for the 4000 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 copy number amplification (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 iAtlas
Portal Tumor Microenvironment tool, which assesses the degree to which each tumor gene expression profile matches each of 6 identified tumor microenvironment gene expression signatures. 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. D and E) In ovarian (D) and endometrial (E) cancers within the TCGA dataset, CCNE1 RNA expression levels are significantly elevated IFN-γ dominant tumors relative to tumors with other immune microenvironment phenotypes. Significance levels from 2-sided unpaired t-tests are indicated by asterisks (*p < 0.05; **p < 0.01; ***p < 0.001).
Figure 1. Patient response to once-daily adavosertib

A

Patient Number

B

Best response for target lesion (%)

Time (weeks)

400 mg QD
300 mg QD (MTD)
250 mg QD
225 mg QD
200 mg QD
PR partial response
B BRCA1/2 mut.
O BRCA1/2 WT
T TP53 mut.
□ TP53 WT

C

Change from Baseline (%)

Patient Number

Unconfirmed PR
**Figure 2.** Pharmacokinetic and pharmacodynamic effects of once-daily adavosertib

A

- **400 mg QD** ($n = 3$)
- **300 mg QD** ($n = 6$; MTD)
- **250 mg QD** ($n = 3$)
- **225 mg QD** ($n = 3$)
- **200 mg QD** ($n = 4$)

Adavosertib plasma concentration (nM) vs. Time (hr), day 1 and day 5.

B

<table>
<thead>
<tr>
<th>AZD1775 plasma concentration (nM), C1D8 pre-dose</th>
<th>% Nuclear Area Positive</th>
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<tbody>
<tr>
<td>54.7</td>
<td>25 (0)</td>
</tr>
<tr>
<td>17.2</td>
<td>25 (1)</td>
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<tr>
<td>14.9</td>
<td>25 (8)</td>
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<tr>
<td>92.7</td>
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<tr>
<td>11.8</td>
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<tr>
<td>17.8</td>
<td>25 (3)</td>
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<tr>
<td>114.1</td>
<td>25 (8)</td>
</tr>
<tr>
<td>74.3</td>
<td>25 (8)</td>
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</table>

**Patient number**

- **High baseline pY15Cdk**
  - pY15Cdk pre-treatment
  - γH2AX pre-treatment
  - pNbs1 pre-treatment
- **Low baseline pY15Cdk**
  - pY15Cdk C1D8 pre-dose
  - γH2AX C1D8 pre-dose
  - pNbs1 C1D8 pre-dose
Figure 3. Tumor molecular analysis of patients treated with once-daily adavosertib

<table>
<thead>
<tr>
<th></th>
<th>ovarian</th>
<th>breast</th>
<th>endometrial</th>
<th>NSCLC</th>
<th>cholang</th>
<th>STS</th>
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<tr>
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<td>C1D1</td>
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<tr>
<td>Pt C (PR)</td>
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<tr>
<td>Pt D (PD)</td>
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<tr>
<td>Pt E (SD)</td>
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<tr>
<td>Pt F (PR)</td>
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</tr>
<tr>
<td>Pt G (SD)</td>
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<tr>
<td>Pt H (PD)</td>
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</tr>
</tbody>
</table>

**Missense mutation** | **Splice site mutation** | **Frameshift mutation (deletion)** | **Nonsense mutation** | **Multiple mutation types** | **CNV (>2 and <5 copies)** | **CNV (>5 copies)**

B

Color Key

-1.5 0 1 Row Z-Score

Pt E (SD) Pt G (SD) Pt D (PD) Pt F (PR) Pt B (PR)

Br NSCLC Br En Ov

MLH1 WEE1 AXL BRCA2 CDK2 MYC PALB2 RB1 ARFRP1 PAXIP1 RAD18 RAD51D RAD51C TP53

SLFN11 PARP1 SETD2 ATRX ATR PIK3CA FANCA CHEK1 CHEK2 AKT3 K Ras RN F43 CDC25A PKNMY1 PKMT2 CCNE1 CNA

C

Color Key

Wound healing IFN-γ dominant Inflammatory Lymphocyte depleted Immunologically quiet TGF-β dominant

D

TCGA Ovarian Cancers

CCNE1 RNA expression

** Wound healing IFN-γ dominant Inflamm. Lymphocyte depleted

E

TCGA Endometrial Cancers

CCNE1 RNA expression

** Wound healing IFN-γ dominant Inflamm. Lymphocyte depleted

Immune subtype

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Clinical Cancer Research

Safety, anti-tumor activity, and biomarker analysis in a phase 1 trial of the once-daily Wee1 inhibitor adavosertib (AZD1775) in patients with advanced solid tumors

Naoko Takebe, Abdul Rafeh Naqash, Geraldine O'Sullivan Coyne, et al.

Clin Cancer Res  Published OnlineFirst April 16, 2021.

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