original report

Dose Escalation Trial of the Wee1 Inhibitor Adavosertib (AZD1775) in Combination With Gemcitabine and Radiation for Patients With Locally Advanced Pancreatic Cancer

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abstract

PURPOSE AZD1775 (adavosertib) is an inhibitor of the Wee1 kinase. In this study, we built on our preclinical studies to evaluate the safety and efficacy of AZD1775 in combination with gemcitabine and radiation in patients with newly diagnosed locally advanced pancreatic cancer.

PATIENTS AND METHODS Thirty-four patients with locally advanced pancreatic cancer were enrolled with the intention to receive four 21-day cycles of gemcitabine (1,000 mg/m^2 days 1 and 8) with AZD1775 (once daily on days 1, 2, 8, and 9). Cycles 2 and 3 were administered concurrently with radiation, and cycles 5 to 8 were optional. AZD1775 was dose escalated using a time-to-event continual reassessment method on the basis of the rate of dose-limiting toxicities within the first 15 weeks of therapy. The primary objective was to determine the maximum tolerated dose of AZD1775 given in conjunction with gemcitabine and radiation. Secondary objectives were to estimate overall and progression-free survival and determine pharmacodynamic activity of AZD1775 in surrogate tissues.

RESULTS The recommended phase II dose of AZD1775 was 150 mg/d. Eight patients (24%) experienced a dose-limiting toxicity, most commonly anorexia, nausea, or fatigue. The median overall survival for all patients was 21.7 months (90% CI, 16.7 to 24.8 months), and the median progression-free survival was 9.4 months (90% CI, 8.0 to 9.9 months). Hair follicle biopsy samples demonstrated evidence of Wee1 inhibition with decreased phosphorylation of cyclin-dependent kinase 1 staining by immunohistochemistry after AZD1775 administration at the recommended phase II dose.

CONCLUSION AZD1775 in combination with gemcitabine and radiation therapy was well tolerated at a dose that produced target engagement in a surrogate tissue. The overall survival is substantially higher than prior results combining gemcitabine with radiation therapy and warrants additional investigation.

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INTRODUCTION

To improve outcomes for patients with locally advanced pancreatic cancer, better locoregional and systemic therapies are needed. Gemcitabine has been a backbone of systemic therapy for pancreatic cancer for more than a decade.[1,2] We have shown that the combination of gemcitabine and radiation is safe, well tolerated, and efficacious when using conformal radiation techniques.[3-5] An advantage of using full-dose chemotherapy with radiation therapy is that the patient is likely to receive a systemic benefit during the course of chemoradiation, which decreases the opportunity for occult metastatic disease to progress. Novel therapies that can increase local control while having systemic efficacy, therefore, may have the greatest potential to improve outcomes in patients with unresectable, nonmetastatic disease. On the basis of this concept, we searched for agents that sensitize tumor cells to gemcitabine for systemic disease control and to gemcitabine and radiation for local disease control.

In our search for ways to sensitize tumor cells to both gemcitabine and gemcitabine and radiation, we focused on drugs that alter the DNA damage response (DDR). The DDR consists of a network of molecules involved in cell cycle regulation and DNA repair.[6] Both gemcitabine and radiation activate the DDR in pancreatic cancer cells, which results in treatment resistance. Novel agents that disable this activation have the potential to sensitize to both chemotherapy and radiation treatment. Chk1 and Wee1 are key regulators of the intra-S-phase and G2 cell cycle checkpoints.[7,8]

ASSOCIATED CONTENT

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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These proteins regulate cell cycle arrest through inhibitory phosphorylations of cyclin-dependent kinases (phospho-CDKs). In addition, by affecting CDK activity, these proteins induce homologous recombination[9,10] and protect cells from replication stress related to depleted nucleotide pools and aberrant replication fork firing.[11-13] Pancreatic cancer cells may be selectively sensitive to DDR inhibitors because RAS mutations (present in most pancreatic cancers) are associated with increased replication stress through the depletion of nucleotide pools[14] and the slowing of replication fork activity.[15,16]

Our initial preclinical efforts to target the DDR focused on Chk1 inhibitors,[17,18] but we ultimately chose the Wee1 kinase inhibitor AZD1775 (adavosertib; AstraZeneca, Cambridge, United Kingdom), which targets the same pathway, for our clinical trial. Preclinical studies show that inhibition of Wee1 kinase by AZD1775 abrogates the G2 checkpoint (the only protective checkpoint in most cancer cells after chemotherapy or radiation therapy), which causes the cancer cells, but not the normal cells, to progress into mitosis before repairing the DNA damage and leads to cell death.[19,20] More importantly, Wee1/Chk1 inhibition induces high levels of replication stress in irradiated and chemotherapy-treated tumor cells.[12,21-23] Thus, AZD1775 might be particularly effective in pancreatic cancer, which already has high levels of baseline replication stress.

AZD1775 was previously assessed in a phase I dose escalation study in combination with gemcitabine alone. The maximum tolerated dose (MTD) of AZD1775 in that trial was 175 mg when given 2 days per week for 3 consecutive weeks in a 4-week cycle.[24] In addition, we have shown previously that the combination of full-dose gemcitabine (1,000 mg/m^2) and radiation is safe, well tolerated, and efficacious when using modern radiation planning techniques.[5] On the basis of the preclinical and clinical data, we designed a phase I dose escalation trial of AZD1775 in combination with gemcitabine and radiation in patients with previously untreated locally advanced pancreatic cancer. We included a pharmacodynamic end point derived from our preclinical studies that shows decreased phospho-CDK1 in hair follicles after checkpoint inhibition.[25]

PATIENTS AND METHODS

Study Design

Patients with locally advanced, unresectable adenocarcinoma of the pancreas were enrolled onto our clinical trial between March 2014 and April 2018. A local institutional review board and the appropriate regulatory agencies approved the protocol. All patients provided written informed consent before enrollment. The trial was conducted while following Good Clinical Practice guidelines and in accordance with the Declaration of Helsinki.

Patient Eligibility

The key eligibility criteria included a pathologically confirmed diagnosis of pancreatic adenocarcinoma determined to be a locally advanced/unresectable by an institutional multidisciplinary pancreas tumor board using National Comprehensive Cancer Network criteria and with no radiographic evidence of metastatic disease. Other eligibility criteria included an Eastern Cooperative Oncology Group performance status of 0 to 2 and adequate organ function on the basis of baseline laboratory values.

Treatment

Gemcitabine (1,000 mg/m^2) was administered intravenously over 30 minutes on days 1 and 8 of each 3-week cycle. AZD1775 was taken orally 3 to 4 hours and 24 hours after each gemcitabine infusion on days 1, 2, 8, and 9. The dose of AZD1775 was modified according to the time-to-event continual reassessment method (TITE-CRM).

Patients were treated with image-guided, intensity-modulated, or volumetric arc–modulated radiation therapy during cycles 2 and 3. The radiation therapy dose was 52.5 Gy in 25 fractions delivered five times per week. The radiation dose was chosen to be one dose level below the MTD found in our prior dose escalation study with gemcitabine and radiation therapy.[5] The target volumes included the primary pancreatic tumor in addition to radiographically enlarged regional lymph nodes when present. Breathing control techniques were used when possible. If the patient could not tolerate breath-hold, a four-dimensional computed tomography (CT) scan was performed to generate an internal target volume. Clinical target volumes were created with a 5-mm expansion on gross disease with an additional 5 mm for the planning target volume. Daily cone beam CT and/or kilovoltage x-ray imaging with fiducial markers were used for image guidance.

Patients had a 3-week treatment break after completion of radiation therapy followed by a fourth cycle of AZD1775 and gemcitabine during weeks 12 to 14. An additional four cycles (maximum eight cycles total) of gemcitabine and AZD1775 were permitted during weeks 15 to 26 for patients without evidence of progression. Patients were permitted to receive additional systemic therapy with standard regimens at the time of progression.

Study Assessments

Safety. Toxicity was assessed using the Common Terminology Criteria for Adverse Events (version 3.0). Toxicity was assessed on day 1 of each chemotherapy cycle, weekly during radiation therapy, and at each follow-up visit. Dose-limiting toxicities (DLTs) were limited to events that occurred during the first 105 days (four cycles) of study therapy that fit the protocol-specified criteria. Hematologic DLTs included any grade 4 to 5 event with the exception of grade 4 anemia, grade 4 leukopenia, grade 4 neutropenia

lasting for less than 7 days (unless the patient had a fever and/or received antibiotics), and grade 4 thrombocytopenia lasting for less than 4 days (unless a platelet transfusion was required). Nonhematologic DLTs included any grade 3 to 5 event with the exception of nausea, vomiting, alopecia, hypersensitivity reaction, hyperbilirubinemia as a result of biliary obstruction, or diarrhea that occurred in the setting of inadequate compliance and lasted for less than 48 hours.

Efficacy. Efficacy end points included overall survival (OS), progression-free survival (PFS), and freedom from local and distant progression since the time of study enrollment. CT scans were obtained at baseline; before cycle 4; and after the last cycle of study therapy, if optional study therapy was completed. During the follow-up interval, CT scans were obtained approximately every 3 months for 18 months and then every 4 to 6 months for another 18 months.

Pharmacodynamics. During the first or second cycle of treatment, consenting patients underwent two sequential punch biopsies of hair-bearing skin. The first biopsy occurred 3 hours after gemcitabine infusion but before AZD1775 administration on day 1, and the second biopsy occurred 3 to 5 hours after AZD1775 administration on day 1 or 2. Immunohistochemistry was performed on the formalin-fixed, paraffin-embedded samples to determine expression of phospho-CDK1 in replicating hair follicles.

Statistical Analyses

Trial design. The primary objective of this phase I trial was to determine the target dose and toxicity profile of AZD1775 when administered with gemcitabine and radiation in patients with unresectable pancreatic adenocarcinoma. Secondary objectives were to estimate the efficacy of this regimen and to determine whether Wee1 kinase is inhibited by AZD1775 in hair follicles.

Four dose levels of AZD1775 were evaluated with fixed doses of radiation and gemcitabine and with the lowest dose level consisting of a lower level of gemcitabine. The first patient was treated at dose level 0 (third level of five). The dose level for subsequent patients was assigned according to the TITE-CRM algorithm on the basis of the probability of DLT at each dose level,[26,27] which was continually updated using data from all enrolled patients. Patients with partial follow-up at the time of a new enrollment were weighted by the proportion of the observation period completed. New patients were assigned to the dose level estimated to have a probability closest to the target probability of 0.30 but not greater than 0.35. No skipping of dose level was allowed, and before escalation, at least one patient must have completed the full observation period (105 days) at the previous level without a DLT.

Analysis. All patients who received any study treatment were included in toxicity and efficacy analyses. A two-parameter logistic regression model was used to estimate the probability of DLT at each dose level. The Kaplan-Meier method was used to summarize OS, PFS, and freedom from distant and local progression since the time of study enrollment. SAS 9.2 software (SAS Institute, Cary, NC) was used for the analyses.

RESULTS

Patient Demographics and Clinical Characteristics

Between March 2014 and April 2018, 34 patients were enrolled (median age, 68 years; range, 44 to 78 years). The major demographic and clinical characteristics are listed in Table 1. Twenty-nine patients (85%) had stage cT4N0M0 disease, and five patients (15%) had stage cT4N1M0 disease. Median tumor size at the time of enrollment was 3.0 cm (range, 1.4 to 7.0 cm). Median baseline cancer antigen (CA) 19-9 was 370 U/mL (range, less than 2 to 20,492 U/mL).

Treatment Received

Thirty-four patients started study treatment, and 32 (94%) began cycle 2 and received radiation. Thirty patients (88%) completed the full course of radiation therapy to 52.5 Gy over an average duration of 38 days (range, 33 to 53 days). Twenty-five (84%) of the 32 patients who received radiation therapy missed less than 5 radiation treatment days. Twenty-six (76%) of the 34 patients in the study received at least four cycles of gemcitabine with AZD1775. Fifteen patients (44%) received the maximum number of eight cycles of gemcitabine with AZD1775.

After 6 months of study treatment, two patients underwent tumor resection and remained cancer free at the time of

TABLE 1. Patient Characteristics

Baseline Characteristic	No.	%
Sex		
Female	15	44
Male	19	56
ECOG performance status		
0	13	38
1	19	56
2	2	6
Age, years		
70-80	10	29
60-70	13	38
50-60	8	24
≤ 50	3	9
Stage		
T4N0M0	29	85
T4N1M0	5	15
Tumor size, cm		
2	3	9
2-4	24	71
4	7	21

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

TABLE 2. Dose Escalation Results

Dose Level*	AZD1775 Dose (mg)	Gemcitabine Dose (mg/m²)	Patients Enrolled	Patients With DLT	Description of DLT for Each Patient
−1	100	1,000	1	0	
0	125	1,000	13	2	ALT/AST elevation (grade 3) Anorexia/nausea (grade 3)
1	150	1,000	9	2	Anorexia/nausea (grade 3) Altered mental status (grade 3)
2	175	1,000	11	4	Fatigue (grade 3) Abdominal pain (grade 3) Fatigue (grade 3) Neutropenia/thrombocytopenia (grade 3)

Abbreviation: DLT, dose-limiting toxicity.
*Time-to-event continual reassessment method dose escalation.

data analysis. Of the remaining patients, 15 (44%) received salvage chemotherapy at the time of progression (five received gemcitabine plus nab-paclitaxel; three received fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX); and one received infusional fluorouracil, leucovorin, and oxaliplatin.

DLTs and Significant Adverse Events

Of the 34 patients enrolled in the study, eight (24%) experienced a DLT (one patient experienced two DLTs). DLTs included anorexia/nausea (n = 2), fatigue (n = 2), abdominal pain (n = 1), altered mental status (n = 1), liver enzyme elevation (n = 1), and neutropenic fever/thrombocytopenia (n = 1; Table 2). The estimated DLT rate from the two-parameter logistic regression model at 150 mg (dose level 1) was 0.24 and at 175 mg (dose level 2), 0.38 (Fig 1). Because the target DLT rate was 0.30, dose level 1 with 150 mg of AZD1775 was selected as the recommended phase II dose.

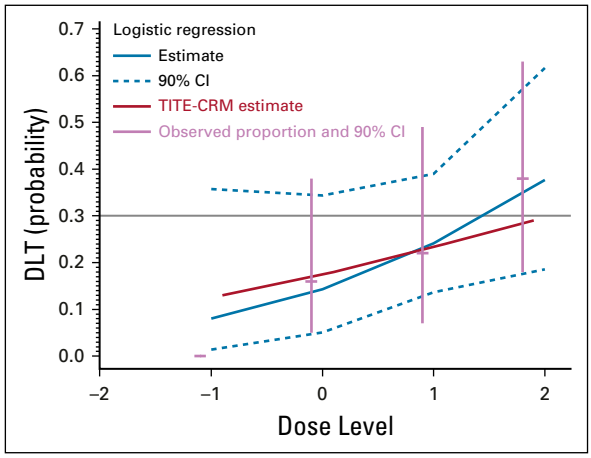

FIG 1. Estimates of dose-limiting toxicity (DLT) rates using the time-to-event continual reassessment method (TITE-CRM) and a two-parameter logistic regression model with 90% CI at each dose level of the Wee1 inhibitor AZD1775. The target DLT rate was 0.30. Dose level 1 (150 mg AZD1775) was determined to be the maximum tolerated dose and recommended phase II dose.

Eighteen (53%) of the 34 patients enrolled in the study experienced a grade 3 or 4 treatment-related adverse event (Table 3). Overall, 27 grade 3 or 4 serious adverse events were reported, with four patients experiencing more than one. There were no treatment-related deaths in the study.

Efficacy

Median follow-up for all patients was 15 months at the time of data analysis. Median OS for the 34 patients enrolled in the study was 21.7 months (90% CI, 16.7 to 24.8 months; Fig 2). Median PFS was 9.4 months (90% CI, 8.0 to 9.9 months; Fig 3). For the 20 patients treated at dose level 1 or 2, median OS was 22.5 months (90% CI, 10.3 to 25.3 months), and median PFS was 9.7 months (90% CI, 5.8 to 11.0 months). First sites of failure for patients who received study therapy included distant metastasis in 17 (50%) and local progression in seven (21%). Two patients who progressed locally only received one cycle of study therapy, and two others had stabilization of their primary disease on subsequent imaging studies. Only three isolated local failures occurred. The 12-month freedom from local failure was 68% for all patients. For patients treated with dose level 1 or 2, the 12-month freedom from local failure was 84%. Twenty-six of the patients who received radiation therapy in the study had an elevated CA19-9 at baseline. CA19-9 values decreased for all these patients after therapy (at the 4-month time interval) and dropped by more than 50% from baseline in 22 (85%) of the 26 patients.

Pharmacodynamic Assessment

Two sequential skin punch biopsy samples were obtained from 20 consenting patients. The first biopsy occurred after gemcitabine but before AZD1775 and was expected to show a gemcitabine-induced increase in phospho-CDK1 per our preclinical studies. The second biopsy occurred 3 to 4 hours after AZD1775; if AZD1775 were active, the initial phospho-CDK1 signal should be suppressed. Immunohistochemistry demonstrated decreased hair follicle phospho-CDK1 staining after AZD1775 in 16 (80%) of the 20 patients, which indicated target inhibition (P = .004; Fig 4).

TABLE 3. Significant Adverse Events

Adverse Event	No.	%
Patients with a serious adverse events	18	53
Total events	27	
GI		
Abdominal pain	1	3
ALT/AST elevation	1	3
Anorexia, nausea/vomiting	3	9
Cholangitis	2	6
Colitis	2	6
Diverticulitis	1	3
GI bleed	1	3
Cardiac		
Myocardial infarction	1	3
Hematologic		
Febrile neutropenia	4	12
Septic shock	2	6
Pulmonary		
Pneumonia	1	3
Pulmonary embolus	1	3
Constitutional		
Fatigue	3	9
Fever	3	9
Other		
Altered mental status	1	3

DISCUSSION

Successful treatment of locally advanced pancreatic cancer requires control of gross local disease in addition to microscopic metastatic disease. Our preclinical studies suggested that a DDR inhibitor could improve local control by sensitizing the primary tumor to gemcitabine and radiation and systemic disease control by sensitizing microscopic disease to gemcitabine.[17,18,20] The primary goal of the current study was to establish the recommended phase II dose of AZD1775 when combined with gemcitabine and gemcitabine and radiation. In the process of establishing this dose, our data also suggested that this combination improves both local and systemic disease control, with an OS of 22 months, a distant metastasis–free survival of 10 months, and few isolated local failures.

The current study is the first, to our knowledge, to examine the combination of a Wee1 inhibitor and radiation therapy in patients with pancreatic cancer. In combination with gemcitabine alone, the MTD for AZD1775 was previously determined to be 175 mg.[24] In our study, the MTD of AZD1775 with gemcitabine and concurrent radiation therapy was slightly lower at 150 mg. This difference could be due to the addition of radiation therapy or the long DLT interval (105 days), during which we assessed toxicity, compared with the single cycle used in the prior dose escalation study. At this dose level, we saw decreased phospho-CDK1 in hair follicles, which suggests that 150 mg AZD1775 is a biologically effective dose level and is appropriate for future studies.

Locally advanced pancreatic cancer is sometimes treated with chemotherapy alone. The LAP07 study demonstrated no survival benefit from chemoradiation therapy in patients who did not progress on systemic therapy.[28] Despite these findings, local control is still important because up to 30% of patients may die as a result of locoregional progression.[29] In our study, the combination of AZD1775 with gemcitabine and radiation produced an OS result of 22 months. This number compares favorably with that of patients treated in LAP07 (11.9 to 13.6 months since enrollment), which had similar eligibility criteria and used gemcitabine.[28] The favorable survival observed in our study is potentially related to the sensitization of both local and distant disease by AZD1775.

More recent reports have shown encouraging survival results in patients with locally advanced disease treated with FOLFIRINOX[30] and gemcitabine plus nab-paclitaxel.[31] When our trial was designed, single-agent gemcitabine was standard therapy for this patient population. Although 15 patients in our study received additional chemotherapy upon progression, only three received FOLFIRINOX and five gemcitabine plus nab-paclitaxel, which suggests that our encouraging survival numbers are not the result of highly efficacious salvage chemotherapy. Given the benefit of these newer regimens in patients with locally advanced[30,31] and metastatic disease,[32,33] we propose that in future trials with the Wee1 inhibitor AZD1775, patients first be treated with FOLOFIRINOX or gemcitabine plus nab-paclitaxel followed by treatment with AZD1775 concurrently with gemcitabine and radiation therapy.

Many alternative radiation fractionation schedules have been tested in patients with locally advanced pancreatic

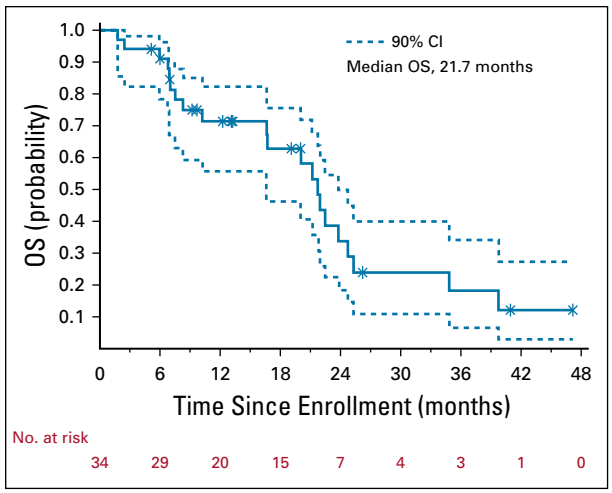

FIG 2. Overall survival (OS) with 90% CI for all patients enrolled in the trial calculated from the time of study enrollment to the date of death. (*) Indicates censor.

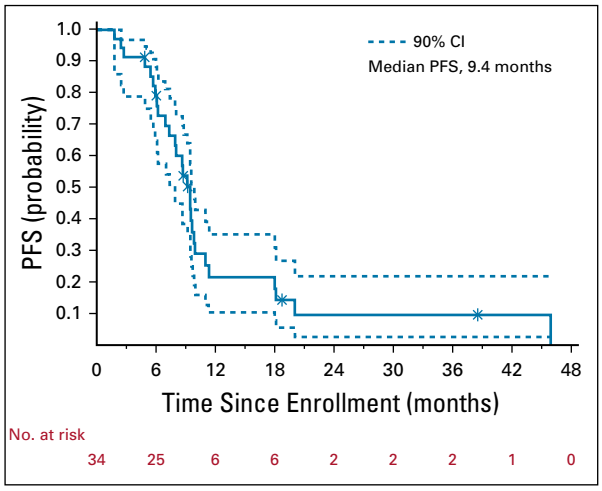

FIG 3. Progression-free survival (PFS) with 90% CI for all patients enrolled in the trial calculated from the time of study enrollment to the date of progression.

cancer. Stereotactic body radiation therapy (SBRT) involves high doses per treatment delivered over three to five fractions. Results with SBRT have demonstrated promising local control; however, the studies reported have shown limited long-term survival with these approaches. For example, a phase II trial by Herman et al[34] showed a median survival of 13.9 months, with 1-year local control of 78%. Other groups have focused on delivering a high dose per fraction over 15 daily treatments using highly conformal techniques. A study by Krishnan et al[35] showed a median OS of 15.3 months and median time to locoregional recurrence of 11.2 months with this approach. In our study, we used similar radiation planning techniques as are performed in SBRT, including respiratory motion management, image guidance, and intensity-modulated treatment delivery. An advantage of using standard fractionation is that portions of the tumor adjacent to bowel do not need to be underdosed to meet tolerance limits. In addition, the use of a tumor-sensitizing agent allows for preferential dose enhancement in the tumor compared with normal tissue.

This study has some limitations. First, this was a single arm, single institution trial, so selection bias was possible. However, the patients treated in this study resemble those treated in our prior studies[3-5] but with substantially better outcomes. In addition, our pharmacodynamic end point was in a surrogate tissue (hair follicles). Although we are uncertain that the effect seen in hair follicles also was present within the tumor, the dose-response relationship we saw in local control suggests that AZD1775 was efficacious in controlling the primary tumor.

In conclusion, the combination of AZD1775, gemcitabine, and conformal image-guided radiation therapy was tolerable and efficacious, especially with regard to freedom from local progression and survival. Using a TITE-CRM design, we determined the recommended phase II dose of AZD1775 to be 150 mg, which produced target engagement in hair follicles. Although preliminary, these results warrant additional investigation.

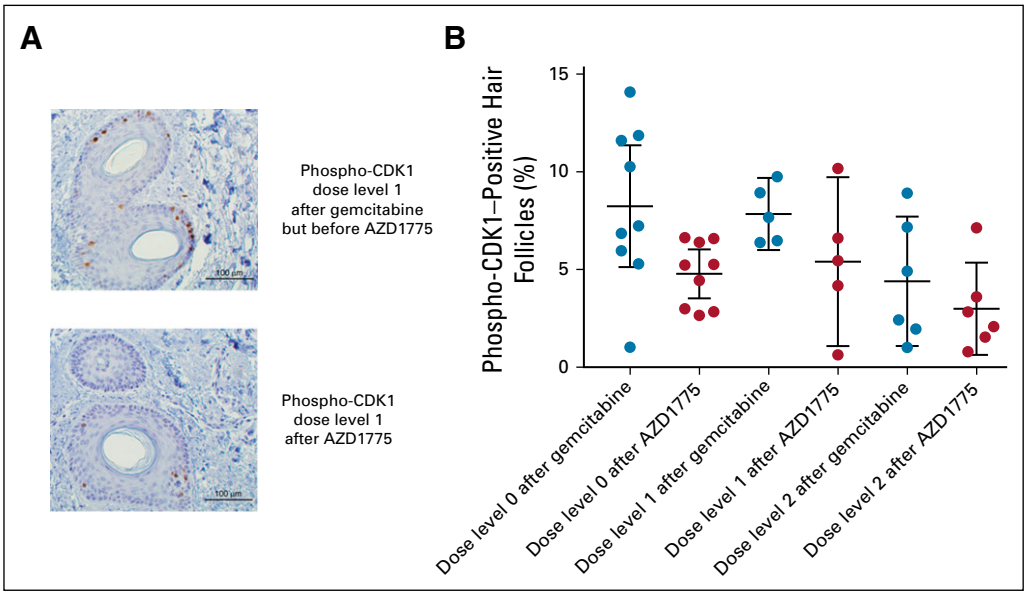

FIG 4. Pharmacodynamics of AZD1775 in surrogate tissues. Sequential punch biopsy samples of hair-bearing skin were obtained after gemcitabine infusion but before AZD1775 administration and after AZD1775 administration. Immunohistochemistry on paraffin-embedded specimens was performed using anti–phosphorylation of cyclin-dependent kinase 1 (phospho-CDK1) antibody. (A) Representative images of phospho-CDK1 staining of hair follicles before and after AZD1775 in a patient treated in this study. (B) Mean percentage of phospho-CDK1–positive hair follicles with 95% CI before and after AZD1775 for each dose level.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI https://doi.org/10.1200/JCO.19.00730.

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Manuscript writing: All authors

Final approval of manuscript: All authors

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Dose Escalation Trial of the Wee1 Inhibitor Adavosertib (AZD1775) in Combination With Gemcitabine and Radiation for Patients With Locally Advanced Pancreatic Cancer

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