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A Phase I Open-Label Study to Identify a Dosing Regimen of the Pan-AKT Inhibitor AZD5363 for Evaluation in Solid Tumors and in *PIK3CA*-Mutated Breast and Gynecologic Cancers

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Abstract

Purpose: This phase I, open-label study (Study 1, D3610C00001; NCT01226316) was the first-in-human evaluation of oral AZD5363, a selective pan-AKT inhibitor, in patients with advanced solid malignancies. The objectives were to investigate the safety, tolerability, and pharmacokinetics of AZD5363, define a recommended dosing schedule, and evaluate preliminary clinical activity.

Experimental Design: Patients were aged ≥18 years with World Health Organization (WHO) performance status of 0 to 1. Dose escalation was conducted within separate continuous and intermittent [4 days/week (4/7) or 2 days/week (2/7)] schedules with safety, pharmacokinetic, and pharmacodynamic analyses. Expansion cohorts of approximately 20 patients each explored AZD5363 activity in *PIK3CA*-mutant breast and gynecologic cancers.

Results: MTDs were 320, 480, and 640 mg for continuous ($n = 47$), 4/7 ($n = 21$), and 2/7 ($n = 22$) schedules, respectively. Dose-limiting toxicities were rash and diarrhea for continuous, hyperglycemia for 2/7, and none for 4/7. Common adverse events were diarrhea (78%) and nausea (49%) and, for Common Terminology Criteria for Adverse Events grade ≥3 events, hyperglycemia (20%). The recommended phase II dose (480 mg bid, 4/7 intermittent) was assessed in *PIK3CA*-mutant breast and gynecologic expansion cohorts: 46% and 56% of patients, respectively, showed a reduction in tumor size, with RECIST responses of 4% and 8%. These responses were less than the prespecified 20% response rate; therefore, the criteria to stop further recruitment to the *PIK3CA*-mutant cohort were met.

Conclusions: At the recommended phase II dose, AZD5363 was well tolerated and achieved plasma levels and robust target modulation in tumors. Proof-of-concept responses were observed in patients with *PIK3CA*-mutant cancers treated with AZD5363. *Clin Cancer Res; 24(9); 2050–9. ©2017 AACR.*

See related commentary by Costa and Bosch, p. 2029

Introduction

AKT is a serine/threonine protein kinase that functions as a key node in the PI3K/AKT network (also known as the PI3K/AKT/mTOR pathway), with a fundamental role in cell survival and proliferation (1). AKT is overexpressed or activated in a wide range of solid and hematologic malignancies, and aberrant AKT signaling is also associated with resistance to established cancer therapies, as well as advanced disease and/or poor prognosis (2).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Prior presentation: This study (Study 1, AstraZeneca study D3610C00001; NCT01226316) was presented in part at the 2014 Annual Meeting of the American Society of Clinical Oncology (ASCO; abstract number 2541) and at the 2015 ASCO Meeting (abstract number 2500).

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Translational Relevance

AZD5363 is a potent, selective inhibitor of AKT, a key node in the PI3K/AKT/mTOR pathway that is activated in a wide range of malignancies. *In vivo*, AZD5363 inhibited tumor growth in xenograft models. Preclinically, sensitivity to AZD5363 has been strongly related to the presence of *PIK3CA* mutations, which are relatively common in breast and gynecologic cancers. Our study, the first-in-human study of AZD5363, showed that at an identified recommended phase II dose, AZD5363 was well tolerated and achieved plasma levels and robust target modulation in tumors. The study is also the first report of a biomarker-stratified cohort (*PIK3CA* mutations in breast and gynecologic cancers) of patients treated with an AKT inhibitor. Results suggest that future efforts in developing this class of drugs for the treatment of solid tumors, including *PIK3CA*-mutated breast and gynecologic cancers, will need to be in combination with other anticancer drugs.

AZD5363 is a potent, selective inhibitor of the kinase activity of all three AKT isoforms (AKT1, -2, and -3; ref. 3). *In vitro*, AZD5363 inhibited tumor cell proliferation and phosphorylation of the AKT substrates PRAS40 and GSK3β, as well as the downstream pathway protein S6. *In vivo*, AZD5363 inhibited tumor growth in xenograft models and remained pharmacodynamically active for at least 24 hours (3). Preclinically, sensitivity to AZD5363 has been strongly related to the presence of *PIK3CA* mutations (4, 5), a trend that has also been observed with other inhibitors of the PI3K/AKT/mTOR pathway (6). *PIK3CA* mutations are found in breast and gynecologic cancers, and evaluation of AZD5363 in these settings is warranted (1).

We report the first-in-human study of AZD5363, which evaluated safety, pharmacokinetics (PK), and pharmacodynamics (PD) in three schedules and recommended a phase II dose for future development. We also report the first evaluation of an AKT inhibitor used as a single agent in a *PIK3CA*-mutated breast and ovarian cancer population.

Materials and Methods

Study design

This is a multipart, phase I, open-label, multicenter study of oral AZD5363 in patients with advanced solid malignancies (Study 1; NCT01226316; Supplementary Fig. S1). Parts A and B were, respectively, the dose-escalation and -expansion phases. Part C focused on an expansion cohort of patients with a *PIK3CA* tumor mutation. An additional expansion cohort of patients with an *AKT1* tumor mutation (part D) will be reported separately.

Patients

All patients were aged ≥18 years with a WHO performance status of 0 to 1 and minimum life expectancy of 12 weeks. Patients in Part C had advanced estrogen receptor–positive (ER^+) or human epidermal growth factor receptor 2–positive ($HER2^+$) breast cancer (based on local testing; ref. 7) or gynecologic (ovarian, cervical, or endometrial) cancer, with any *PIK3CA* mutation detected by local or central testing, and at least one measurable lesion. In addition for Part C, where known, other solid tumors (i.e., not breast or gynecologic) should be negative for mutations of *KRAS*, *NRAS*, *HRAS*, and *BRAF*. Key exclusion criteria are summarized in the Supplementary Material.

Study objectives

The primary objective of Parts A and B was to investigate the safety and tolerability of oral AZD5363 and to define a recommended monotherapy dose and schedule for further clinical evaluation. Secondary objectives included PK evaluation of AZD5363 and preliminary assessment of antitumor activity. The objectives of Part C were to investigate safety, tolerability, PK, and antitumor activity of the defined AZD5363 dosing schedule in patients with ER^+ or $HER2^+$ breast cancer or gynecologic cancer harboring a *PIK3CA* mutation. Exploratory objectives of Study 1 overall included the characterization of the PD effects of AZD5363 in paired tumor biopsies and in platelet-rich plasma (PRP; Parts A and B).

Study design and treatment

Part A—dose escalation. Cohorts of 3 to 6 unselected patients received a single starting dose of 80 mg of AZD5363 and, after a 3- to 7-day washout, AZD5363 twice daily (bid). The dosing cycle was 21 days, excluding the run-in dose. Upon reaching a continuous dose considered appropriate by the Safety Review Committee (SRC), two intermittent dosing schedules were initiated in parallel: 4 days on, 3 days off every week (4/7) and 2 days on, 5 days off every week (2/7). Dose escalation continued for each schedule until a nontolerated dose was attained [≥2/6 dose-limiting toxicities (DLTs)] and an MTD was identified. Definitions of DLTs are provided in the Supplementary Material.

Part B—dose expansion. To confirm selection of the recommended dose for the schedules explored in Part A, the safety profile and tolerability were evaluated in up to 9 additional unselected patients. The SRC also reviewed safety and tolerability data on an ongoing basis.

Part C—expansion in PIK3CA-mutant patients. Two cohorts of patients with *PIK3CA*-mutant ER^+ or $HER2^+$ breast cancers (Cb cohort) or gynecologic cancers (Cg cohort) received the recommended dose and schedule identified from Parts A and B. Each cohort permitted a maximum of 120 patients; recruitment to each cohort was prospectively contingent on positive interim efficacy and safety data reviews after 20 and 40 patients had the opportunity to be followed for ≥12 weeks.

The trial (Study 1; NCT01226316) was performed in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice, and the AstraZeneca policy on bioethics (8). The local ethics committee or independent review board at each investigator site approved the protocol prior to study commencement. All patients provided written informed consent prior to study participation.

Assessments

Safety and tolerability were assessed by continual monitoring of adverse events (AE) according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Serial venous blood samples for PK assessment of AZD5363 in plasma were taken up to 48 hours after dose in Part A, and up to 1 week after the last day of weekly dosing in Part B. Evaluated PK parameters included area under the plasma concentration–time curve (AUC), maximum

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plasma concentration (C_{max}), time to C_{max} (t_{max}), and apparent terminal half-life ($t_{1/2}$).

Blood samples were obtained at scheduled time points to assess changes in PD biomarkers of AKT inhibition (such as p-GSK3β and pPRAS40) in PRP using solid-phase enzyme-linked immunosorbent Mesoscale Discovery multiplex assays. Paired tumor biopsies (pre- and on treatment) from consenting patients participating in this study and in a study of AZD5363 in Japanese patients (Study 4; NCT01353781; ref. 9) were pooled. Pooling provided an adequately sized cohort to assess proof of mechanism (PoM) for the measurement of changes in AKT pathway effectors, including p-AKT, p-PRAS40, p-GSK3β, and Foxo3a/Foxo1 localization, by immunohistochemistry (10). Details on the collection and analysis of PRP and tumor tissue samples, and mutation analyses in tissue and circulating free DNA (ctDNA), are described in the Supplementary Material.

To determine antitumor activity, tumor assessments were categorized based on RECIST version 1.1. Percentage change in tumor size was determined at each visit by the percentage change in the sum of the diameters of target lesions compared with baseline.

Statistical analysis

All patients who received ≥1 dose of AZD5363 were included in the safety analyses. Safety and tolerability were assessed in terms of AEs, serious AEs, deaths, laboratory data, vital signs, electrocardiogram (ECG) changes, left ventricular ejection fraction, and abnormalities of glucose metabolism. All patients who provided appropriate samples were assessed for PK and PD. Standard noncompartmental PK parameters were calculated using Phoenix WinNonlin version 6 software. Modeling and simulation were applied to emerging safety, PK, and PD data to provide an understanding of any dose exposure–response relationships, and to support dose-escalation and dosing-schedule decisions. Preclinical PK, PD, and efficacy data were used to define PoM thresholds for the reduction of phosphorylation of GSK3β and PRAS40 to provide confidence that on-target PoM was achieved at tolerable doses (described in the Supplementary Material).

The predefined formal trigger in Part C to stop the study for futility was four or fewer responses by RECIST once 20 patients in each cohort had the opportunity to be followed for 12 weeks, i.e., a ≤10% chance that the true proportion of RECIST responses was ≥40%. Antitumor activity was assessed by response rate, with two-sided Clopper–Pearson confidence intervals to provide probability statements of the efficacy signal.

Results

Dose escalation and expansion (Parts A and B)

Patients. A total of 90 patients were assigned to treatment; 47, 21, and 22 received AZD5363 in the continuous, 4/7, and 2/7 intermittent schedules, respectively. Patient demographic and baseline clinical characteristics are shown in Table 1. The most common cancers were rectal/colorectal (29%). By the time of the final analysis, all 90 patients had ceased study medication, most commonly for progression of the disease under investigation (64%).

Safety and tolerability. The MTDs of the continuous, 4/7, and 2/7 schedules were 320, 480, and 640 mg bid, respectively (Supplementary Fig. S2). For the continuous dosing schedule, the 600 mg bid cohort was not tolerated: 2 of 2 patients experienced DLTs (one event of grade 3 rash and one of grade 4 rash). An intermediate dose level of 480 mg bid was, therefore, investigated; in this cohort, 4 of 6 patients experienced DLTs (three events of grade 3 rash and one of grade 3 diarrhea). At a further lower dose level of 320 mg bid, 0 of 12 patients experienced DLTs, and this dose was considered the MTD for the continuous schedule. In the 4/7 intermittent dosing schedule, no DLTs were observed in the 480 mg bid ($n = 11$) and 640 mg bid ($n = 10$) cohorts; however, based on the presence of chronic toxicities such as rash and diarrhea observed outside the first 21-day cycle DLT window, the lower dose of 480 mg bid was considered tolerable and appropriate for chronic use with 4/7 dosing. In the 2/7 intermittent dosing schedule, at 800 mg bid, 3 of 14 patients had DLTs (two events of grade 4 hyperglycemia and one of grade 3 hyperglycemia); again, considering observed chronic toxicities, a dose of 640 mg bid was explored. In this cohort, DLTs were observed in 1 of 8 patients (one event of grade 4 hyperglycemia), and the 640 mg bid dose was considered tolerable (Supplementary Fig. S2). All DLTs were reversible; no events of ketoacidosis were reported in patients with hyperglycemia. Two patients remained on AZD5363 for longer than 6 months (1 patient on the 480 mg bid 4/7 schedule and 1 patient on the 800 mg bid 2/7 schedule); the median duration of exposure was 44 days (range, 1–507).

Table 1. Patient demographics and baseline clinical characteristics in Parts A, B, and C

	Patients in Parts A and B ($n = 90$)	Patients in Part C ($n = 59$)
Mean age (SD), years	55.4 (10.8)	56.7 (12.7)
Male:female, *n*	51:39	2:57
Primary tumor location, *n* (%)		
Rectal/colorectal	26 (29)	NA
Pleura	7 (8)	NA
Lung	6 (7)	NA
Cervix	5 (6)	9 (15)
Colon	5 (6)	NA
Ovary	4 (4)	6 (10)
Breast	4 (4)	31 (53)
Uterus	0	10 (17)
Other[a]	33 (37)	3 (5)
WHO performance status, *n* (%)		
0	38 (42)	30 (51)
1	52 (58)	29 (43)
Prior anticancer regimens, *n* (%)		
0 regimens	0	1 (2)
1 regimen	12 (13)	6 (10)
2 regimens	27 (30)	11 (19)
≥3 regimens	51 (57)	41 (70)
Mean number of regimens (SD)	3.3 (2)	5.0 (3)

Abbreviation: NA, not available.
[a]Other includes cancers that occurred in 1 or 2 patients.

The most frequently reported AEs across all dosing schedules were gastrointestinal events (diarrhea, vomiting, nausea; Table 2). Grade ≥3 AEs were experienced by 56 patients (62%), most commonly hyperglycemia ($n = 18$; 20%), diarrhea ($n = 13$; 14%), and maculopapular rash ($n = 10$; 11%; Supplementary Table S5). Overall, 21 patients (23%) had an AE leading to discontinuation; the most common (≥2%) were diarrhea (8%), maculopapular rash (8%), and dehydration (2%). AEs leading to dose interruption or reduction were experienced by

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Table 2. AEs with frequency ≥15% irrespective of causality for Parts A and B in total or Part C in total

	Parts A and B												Part C
	Schedule 1 (continuous)						Schedule 2 (4/7)		Schedule 2 (2/7)				480 mg bid; 4/7
Number (%) of patients	80 mg bid (*n* = 5)	160 mg bid (*n* = 5)	240 mg bid (*n* = 6)	320 mg bid (*n* = 12)	400 mg bid (*n* = 11)	480 mg bid (*n* = 6)	600 mg bid (*n* = 2)	480 mg bid (*n* = 11)	640 mg bid (*n* = 10)	640 mg bid (*n* = 8)	800 mg bid (*n* = 14)	Total (*N* = 90)	(*n* = 59)
Patients with any AE	5 (100)	5 (100)	6 (100)	12 (100)	11 (100)	6 (100)	2 (100)	11 (100)	10 (100)	8 (100)	14 (100)	90 (100)	59 (100)
Diarrhea	1 (20)	1 (20)	4 (67)	11 (92)	11 (100)	6 (100)	2 (100)	9 (82)	9 (90)	4 (50)	12 (86)	70 (78)	47 (80)
Nausea	0 (0)	2 (40)	1 (17)	8 (67)	4 (36)	5 (83)	2 (100)	5 (45)	7 (70)	3 (38)	7 (50)	44 (49)	33 (56)
Vomiting	0 (0)	1 (20)	2 (33)	5 (42)	4 (36)	3 (50)	1 (50)	5 (45)	5 (50)	3 (38)	6 (43)	35 (39)	26 (44)
Fatigue	1 (20)	2 (40)	2 (33)	5 (42)	3 (27)	2 (33)	2 (100)	3 (27)	2 (20)	4 (50)	7 (50)	33 (37)	24 (41)
Decreased appetite	1 (20)	0 (0)	1 (17)	2 (17)	5 (45)	3 (50)	0 (0)	3 (27)	5 (50)	1 (13)	5 (36)	26 (29)	25 (42)
Hyperglycemia	2 (40)	1 (20)	0 (0)	0 (0)	4 (36)	3 (50)	1 (50)	4 (36)	4 (40)	2 (25)	5 (36)	26 (29)	24 (41)
Maculopapular rash	1 (20)	2 (40)	1 (17)	3 (25)	3 (27)	3 (50)	2 (100)	3 (27)	1 (10)	1 (13)	4 (29)	24 (27)	15 (25)
Constipation	1 (20)	0 (0)	2 (33)	4 (33)	4 (36)	0 (0)	0 (0)	1 (9)	1 (10)	2 (25)	2 (14)	17 (19)	10 (17)
Abdominal pain	0 (0)	0 (0)	1 (17)	5 (42)	2 (18)	2 (33)	0 (0)	2 (18)	0 (0)	3 (38)	0 (0)	15 (17)	16 (27)
Pyrexia	1 (20)	1 (20)	1 (17)	2 (17)	1 (9)	3 (50)	2 (100)	1 (9)	3 (30)	0 (0)	0 (0)	15 (17)	6 (10)
Headache	0 (0)	1 (20)	1 (17)	2 (17)	1 (9)	1 (17)	0 (0)	2 (18)	1 (10)	0 (0)	0 (0)	9 (10)	15 (25)
Anemia	0 (0)	0 (0)	1 (17)	0 (0)	1 (9)	0 (0)	0 (0)	2 (18)	1 (10)	2 (25)	0 (0)	7 (8)	12 (20)
Increased blood creatinine	0 (0)	1 (20)	0 (0)	1 (8)	3 (27)	0 (0)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	6 (7)	10 (17)
Proteinuria	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	13 (22)
Asthenia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	12 (20)
Hypomagnesemia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (15)

29 (32%) and 21 (23%) patients, respectively. No AEs resulted in death, and 7 patients died as a result of the disease under investigation. All 90 patients had blood glucose levels above the upper limit of normal at some point following initiation of therapy with AZD5363—this developed within the first 2 weeks of multiple dosing in the majority of patients (77%). Grade 3 elevations (>13.9 mmol/L) were seen in 33 patients (37%). No other clinically important trends were observed in laboratory parameters, vital signs, physical findings, or ECG changes.

Pharmacokinetics and pharmacodynamics. Plasma concentrations of AZD5363 showed a median t_{max} of 2 hours (range, 0.5–6), with a terminal half-life of approximately 10 hours (range, 7–15) after the first dose. Exposure was approximately dose proportional for doses of 80 to 800 mg (Fig. 1A). Multiple-dose PK profiles are shown in Fig. 1B. The geometric mean PK exposures on day 4 of the 480 mg dose in the 4/7 schedule were: C_{max}, 1,426 ng/mL; minimum plasma concentration (C_{min}), 357 ng/mL; and AUC, 7,952 ng·h/mL. Intermittent dose schedules exceeded the predicted efficacious C_{min} estimated from xenograft tumors (Supplementary Material; ref. 11). The mean fraction of the AZD5363 dose excreted unchanged in urine ranged from 4% to 7%. Changes in PRAS40 and GSK3β phosphorylation in PRP occurred across multiple dose levels and precluded any definitive conclusion regarding a dose–response relationship. In patients treated with the recommended phase II dose and schedule (480 mg bid, 4/7 intermittent), a reduction of >30% in pPRAS40 and pGSK3β compared with baseline at 4 hours after the single dose of AZD5363 was observed (Fig. 2A), with return to baseline levels approximately 10 hours after treatment (Fig. 2B).

Additional observations indicating PD activity of AZD5363 included an increase in plasma and blood glucose, insulin, and C-peptide levels. In particular, blood glucose levels increased across all cohorts and peaked approximately 4 hours after each dose (Supplementary Fig. S5), returning toward predose baseline levels 8 hours after dose, with a clear dose–response relationship in terms of the magnitude of peak glucose levels observed (not shown).

Proof of target engagement in tumor tissue. Evaluable paired tumor biopsies from 12 patients [9 from the current study (Study 1; NCT01226316) and 3 from Study 4 (AstraZeneca study D3610C00004; NCT01353781; ref. 9)] who received a range of doses and schedules were evaluated to assess PoM. Over 50% inhibition of pPRAS40 was seen in 4 of 12 paired biopsies, and >30% decrease in pGSK3β was observed in 6 of 11 paired biopsies; 4 of 11 samples met both endpoints (Fig. 3A and B; Supplementary Table S1). Downregulation of PD biomarkers was observed >4 hours after dose, including with intermittent dosing (Supplementary Table S1). These data are consistent with the PD response achieved in BT474c xenografts grown in nude mice at a dose that resulted in significant tumor growth inhibition (Supplementary Fig. S4). AZD5363 treatment also increased phosphorylation levels of AKT (consistent with ATP competitive mechanism of action), inhibited phosphorylation of 4EBP1, and resulted in inhibition of Foxo nuclear translocation (Fig. 3A; ref. 10). In the 5 patients treated with the recommended phase II dose and schedule (480 mg bid, 4/7 intermittent), the average percentage decrease from baseline for pPRAS40 (59%) and pGSK3b (67%) exceeded the PD response required for preclinical efficacy (Fig. 3B).

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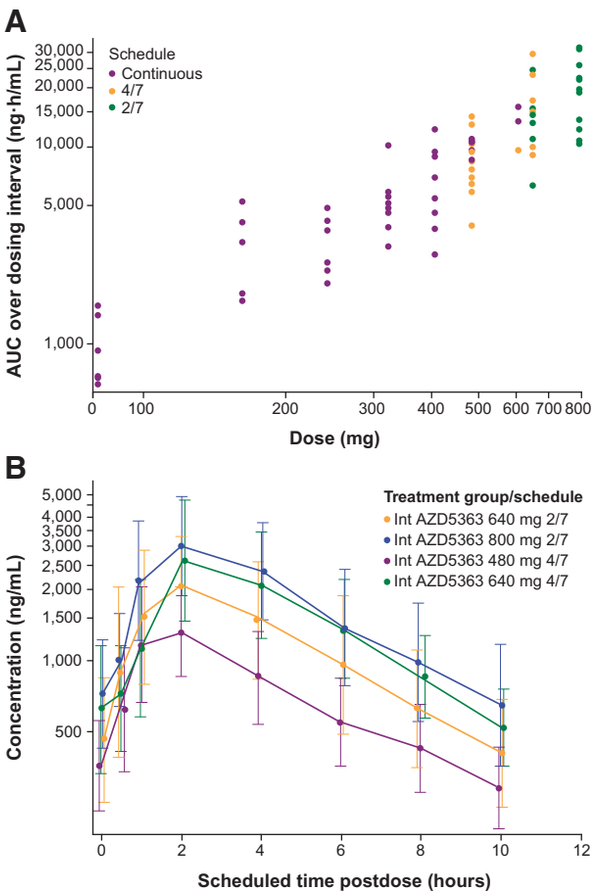

Figure 1.

Multiple-dose PK of AZD5363 (day 8 of continuous schedule, day 4 of 4/7, and day 2 of 2/7 intermittent schedules). **A,** AUC dosimetry. **B,** Geometric mean (±SD) plasma concentration versus time. Abbreviation: Int, intermittent.

Recommended phase II dose. Based on preclinical models, the trough plasma concentrations achieved at the tolerable dose of 320 mg bid on a continuous schedule exceeded those required for xenograft activity (Supplementary Fig. S4). Further modeling has suggested that a 1.3- and 1.7-fold dose level over continuous dosing would be efficacious when administered on the 4/7 and 2/7 schedules, respectively (11). Based on the combination of tolerability profile, PK profile achieved, evidence of target engagement in normal and tumor tissue, and modeling predictions of efficacy, the dose of 480 mg bid on a 4/7 intermittent schedule was declared as the recommended phase II dose. The dose level of 640 mg bid 2/7 was also tolerable, achieved adequate PK exposure, showed evidence of target modulation in PRP (tumor biopsy data were not available in this schedule), and was predicted to be efficacious from modeling of preclinical data. This schedule could be of use in combination studies in the future.

Antitumor activity. There was limited evidence that AZD5363 induced tumor shrinkage in the unselected patient population in Parts A and B. A total of 27 (30%) and 6 (7%) patients achieved stable disease for ≥6 and ≥12 weeks, respectively. A *PIK3CA* mutation was detected in 12 of 67 patients with archival tumor tissue suitable for exploratory Sequenom analysis, of whom 8 of 12 received an AZD5363 dose ≥400 mg. In addition, 0 of 68 tumors harbored an *AKT1* E17K mutation, 29% (20/68) harbored a *RAS* mutation (18 *KRAS* and 2 *NRAS*), and 25% (3/12) of the *PIK3CA*-mutant tumors had concurrent *KRAS* mutation (Fig. 4A). In particular, the patient who achieved a RECIST partial response (*PIK3CA* E545K mutant) had cervical cancer with hepatic and lymph node metastases and was treated with 400 mg bid continuously.

Patients with tumors harboring mutations in *PIK3CA* (Part C)

At the final analysis, 31 patients with *PIK3CA*-mutant breast cancer (Cb cohort) and 28 patients with *PIK3CA*-mutant gynecologic cancer (Cg cohort) had received AZD5363, of whom 54 were included in the main tumor response analysis set. Of patients included in the analysis set (excluding 3 patients with no evaluable follow-up assessments), 12 of 26 (46%) and 14 of 25 (56%) showed a reduction in size of their tumors in the Cb and Cg cohorts, respectively (Fig. 4B). The corresponding confirmed RECIST responses at final analysis were 1/28 (4%) and 2/26 (8%), respectively. The observations at the interim assessment (scheduled when 20 patients had been dosed and had the opportunity to reach 12 weeks of treatment for each cohort) showed a RECIST response rate of ≤20% for a single agent and therefore met the criteria to stop further recruitment. Results of *PIK3CA* mutational analysis in tissue and ctDNA and other exploratory biomarkers (e.g., PTEN status, *ESR1* mutation status) are shown in Fig. 4A and Supplementary Table S4 and described in detail in the Supplementary Material.

The safety profile of AZD5363 in Part C, which is described in detail in the Supplementary Material, was consistent with the findings in Parts A and B.

Discussion

This first-in-human study assessed the safety and tolerability of AZD5363 and identified a recommended dosing schedule for further clinical evaluation. The study also explored single-agent activity of AZD5363 in populations of patients who had metastatic breast and gynecologic cancers with *PIK3CA* mutations.

DLTs of AZD5363 in the dose-escalation part of our study (Part A) were skin rash, diarrhea, and hyperglycemia. Whereas skin rash and diarrhea were predominant in the continuous schedule, hyperglycemia associated with the period of C_{max} was predominant in the intermittent 2/7 schedule, where highest AZD5363 exposures were achieved. The cases of skin rash and diarrhea were self-limiting and recovered once treatment stopped. These AEs have been noted in phase I studies of other AKT inhibitors, such as the allosteric inhibitor MK2206 or kinase inhibitors such as GSK2141795 and ipatasertib (GDC-0068; refs. 1, 12–17). Hyperglycemia development was acute and indicative of the inhibitory effect of AZD5363 on AKT, a key regulator of glucose transport and metabolism in peripheral tissues and the liver (17). No patients had ketotic or nonketotic hyperosmolar coma. However, patients with diabetes were excluded from our study, and it is not possible to rule out these complications in a diabetic population. A number of patients with hyperglycemia were treated with metformin according to a protocol-defined algorithm; however, the efficacy of this intervention requires further study.

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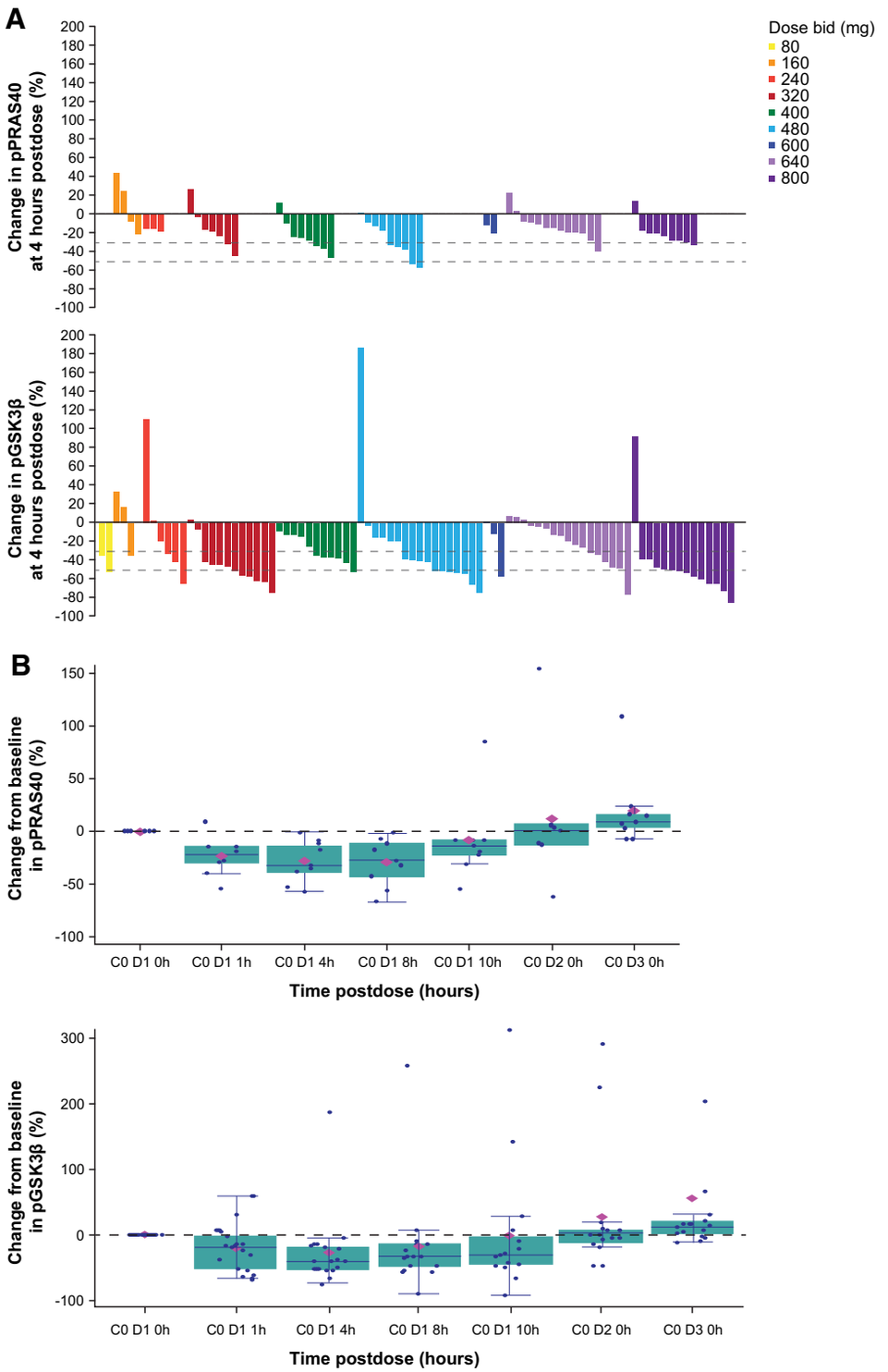

Figure 2.

Assessment of PD markers in PRP and paired tumor biopsies following treatment with AZD5363. **A,** Percentage change from baseline in pThr246 PRAS40 and pSer9 GSK3β markers in PRP at 4 hours after dose. **B,** Temporal change in pPRAS40 and pGSK3β in PRP. In **A** and **B**, percentage change in pPRAS40 and pGSK3β in PRP after a single dose of AZD5363 is shown. Data shown are from Parts A and B only (PD population) and where a result is available for one biomarker or the other. **A,** Data for each biomarker are ranked in order of descending percentage change, and ranking is conducted for each biomarker separately. X indicates missing data. **B,** Percentage change from baseline in PD markers at various time points after 480 mg single dose (includes all dosing schedules). Samples failing quality control or evaluated as of poor quality, as well as one outlier value for pGSK3β at cycle 0, day 3, 0 hours after dose, were excluded. Horizontal line, median; diamond, mean; box, quartiles 1 to 3; whiskers extend from the quartiles to the most extreme observation within 1.5xIQR. Outliers (>1.5xIQR) are individually displayed. Abbreviations: C, cycle; D, day; IQR, interquartile range.

The trough concentrations predicted to provide efficacy based on preclinical modeling were exceeded in patients receiving intermittent schedules. PD analyses in PRP showed levels of target inhibition that were consistent with PoM in pre- and postbiopsies from 12 patients. Owing to the limited sample size, no formal statistical testing relating to the PD changes in tumor tissue was done. Collectively, the toxicity, PK, and PD data, critical aspects of the pharmacologic audit trail (18), have been used to select the dose of 480 mg bid intermittent 4/7 for Part C expansion and as the

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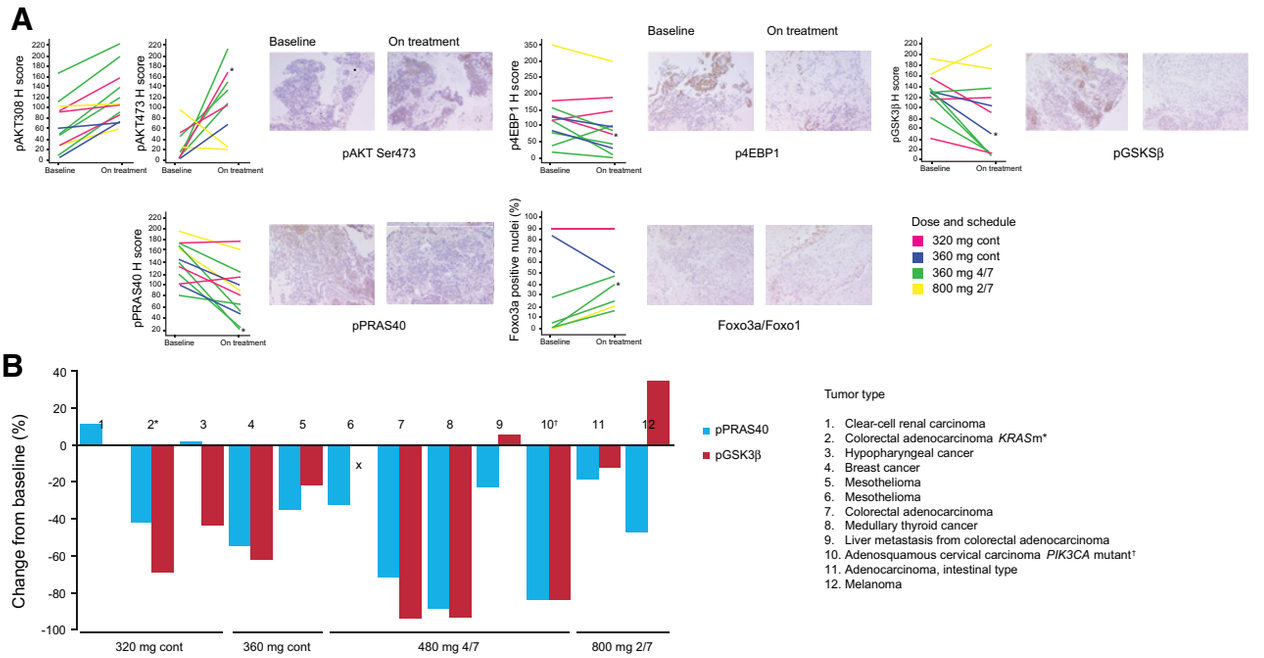

Figure 3.

A, Comprehensive assessment of PD activity of AZD5363 in paired tumor biopsies from 12 patients by immunohistochemistry. **B,** PoM on tumor paired biopsies for pPRAS40 and pGSK3b as proximal indicators of AZD5363 target engagement and therefore selected as the key PoM markers. **A,** Total H scores for evaluable pairs are shown for each biomarker (average of 3 nonconsecutive tissue sections; details in the Supplementary Material). For Foxo3a/Foxo, the percentage of positive nuclei is shown. Asterisks indicate the patient for whom representative staining images are shown. **B,** The percentage change is based on the average H score for individual biomarkers in baseline and on-treatment biopsies from three nonconsecutive tissue sections. Each pair of bars represents data from an individual patient; tumor type is indicated in the table (right). X indicates missing data. *, *KRAS*-mutant colorectal cancer; †, *PIK3CA* E545K-mutant cervical cancer (this patient was enrolled in Study 1 Part C). Abbreviation: cont, continuous.

recommended phase II dose and schedule of AZD5363 monotherapy.

Proof-of-principle responses were observed in the dose-escalation phase of our study (e.g., *PIK3CA* E545K-mutant cervical cancer). Within the *PIK3CA* expansion cohorts, a number of patients showed regression of their tumors (46% in breast cancer and 56% in gynecologic cancers; Fig. 4B). However, the RECIST response rates in the two expansion cohorts of patients with *PIK3CA* mutations in ER^+ breast cancer and gynecologic cancers were modest (4% and 8%, respectively). Several considerations should be made. *PIK3CA* mutational status can "change" upon disease recurrence (Supplementary Material and Supplementary Fig. S3), reflecting intratumoral heterogeneity and clonal selection (19, 20), and studies on the role of *PIK3CA* as a predictive biomarker of PI3K pathway inhibitors have not been conclusive (21). Molecular analyses using different platforms revealed interesting differences of *PIK3CA* mutations seen in the archival tumor tissue and ctDNA at baseline. Results have been detailed in the Supplementary Material and Supplementary Table S4. To our knowledge, this is the first report of the evaluation of an AKT inhibitor in dedicated *PIK3CA*-mutation–positive breast and gynecologic cancers as a single agent. For example, *PIK3CA* mutations were not a requirement while evaluating the AKT inhibitor perifosine, which underwent phase II trials in breast cancer and showed 0/18 responses (22), or the allosteric AKT inhibitor MK2206 in breast cancer (1/20 responses; ref. 23) and endometrial cancer (0/18 responses; ref. 24). More recently, the phase I trial of ipatasertib in solid tumors reported no RECIST responses, although there were minor degrees of tumor regression (16), and no trials evaluating the drug as a single agent in breast or gynecologic cancers are reported. Encouraging early response rates of the use of AZD5363 (6/21, 28%) with the schedules recommended in this study have been reported (25). Further efforts to improve outcomes by combining AZD5363 with fulvestrant in *AKT*-mutant breast cancer are ongoing.

Rewiring of signal transduction pathways and clonal evolution are critical mechanisms of resistance, and combination therapy is almost inevitably necessary (26, 27). For example, the approved PI3K pathway (mTOR) inhibitor everolimus had modest clinical efficacy when used as a single agent in breast cancer (28). We consider the tumor shrinkage caused by AZD5363 as a single agent in a significant number of patients in the *PIK3CA*-mutant cohorts to be an encouraging proof of concept and the basis for evaluation of the drug in combination therapy. Our phase I study has optimized multiple intermittent regimens in order to provide flexibility in the use of such a novel agent in combination with multiple standard-of-care or experimental agents (29).

Combination of AKT inhibitors with chemotherapy is hypothesized to abrogate antiapoptotic effects of activation of AKT following treatment with chemotherapeutic agents such as cisplatin and paclitaxel. Combinations of AKT inhibitors with targeted agents include combinations with MEK inhibitors to overcome feedback signaling loops, combinations with PARP

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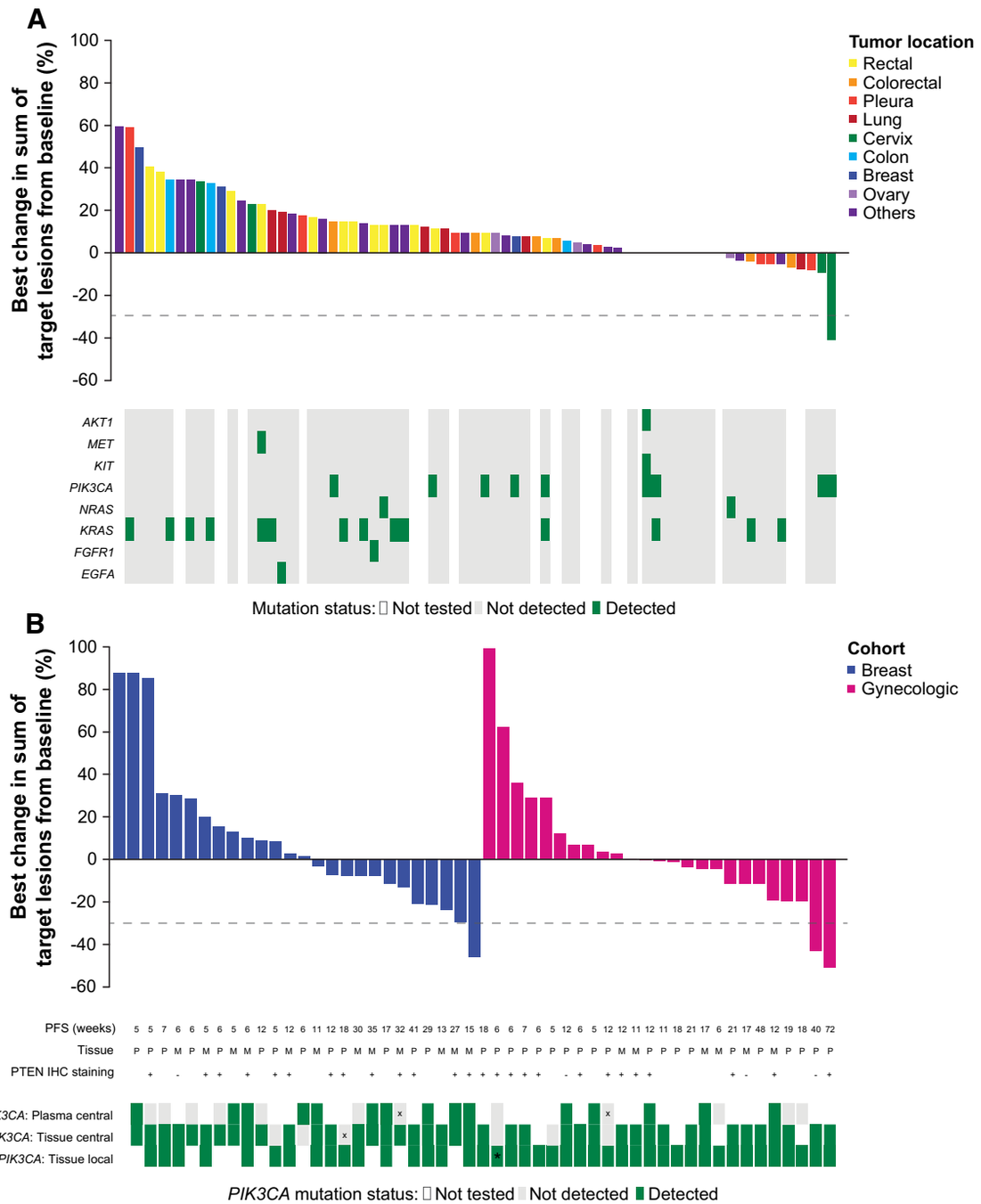

Figure 4.

Waterfall plots of best percentage change in target lesion size from baseline in patients from (**A**) Parts A and B and (**B**) Part C, with associated molecular data. **A,** Exploratory mutation analysis by Sequenom in patients who provided suitable archival tumor tissue, who had baseline tumor assessment, and for whom best percentage change could be calculated. Mutations of potential interest in relationship to modulation of response to AZD5363 or known oncogenic drivers are shown. The *AKT1* mutation detected in 1 patient was Q43X. **B,** Progression-free survival (PFS), *PIK3CA*-mutation status by local testing (tissue), central testing (cobas PCR, tissue and plasma), and PTEN status (immunohistochemistry, tissue) are shown. Only patients with baseline tumor assessment and measurable disease and for whom best percentage change could be calculated are included in the plot. The type of tissue used for central and exploratory assessment is indicated (M = metastatic, P = primary). Additional exploratory data on *PIK3CA* and *ESR1* mutation testing in ctDNA by ddPCR are described in Supplementary Table S4 (including time lapse between tissue biopsy and plasma collection) and in the Supplementary Material.

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inhibitors to reduce effective homologous recombination, and combinations with hormonal agents such as fulvestrant and abiraterone in estrogen- and androgen-driven breast and prostate cancer, respectively (1).

To conclude, our research identified an optimal dose and schedule for use in subsequent multiple phase II studies evaluating AZD5363, e.g., AZD5363 in combination with chemotherapy (NCT02423603, NCT01625286) or hormonal therapy (NCT02077569) in breast cancer, with olaparib in ovarian cancer (NCT02338622), and with enzalutamide in prostate cancer (NCT02525068). Results of these trials are now awaited.

Disclosure of Potential Conflicts of Interest

U. Banerji reports receiving commercial research grants from AstraZeneca. E. J. Dean is currently an employee of AstraZeneca. J.A. Pérez-Fidalgo reports receiving speakers bureau honoraria from AstraZeneca, Pfizer, and Roche. S.N. Westin is a consultant/advisory board member for AstraZeneca, Clovis, and Medivation. P. Kabos is a consultant/advisory board member for Eli Lilly. C. Corcoran, M. Cullberg, B.R. Davies, P. Elvin, and M. Pass hold ownership interest (including patents) in AstraZeneca. J.H.M. Schellens holds ownership interest (including patents) in Modra Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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Clinical Cancer Research

A Phase I Open-Label Study to Identify a Dosing Regimen of the Pan-AKT Inhibitor AZD5363 for Evaluation in Solid Tumors and in *PIK3CA*-Mutated Breast and Gynecologic Cancers

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