A Phase 1 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

Supplementary material

Supplementary methods

Study design

This is a multipart, Phase 1, open-label, multicenter study of oral AZD5363 in patients with advanced solid malignancies (Study 1; NCT01226316; Supplementary Figure 1).

Exclusion criteria

Key exclusion criteria included: clinically significant abnormalities of glucose metabolism (defined by a diagnosis of diabetes mellitus type I or II, baseline fasting [no calorific intake for at least 8 hours] glucose value of ≥7 mmol/L, glycated hemoglobin >8% [64 mmol/mol]); treatment with chemotherapy, immunotherapy, anticancer agents, cytochrome P450 (CYP) 3A4 inducers/inhibitors/substrates or CYP2D6 substrates, and nitrosourea or mitomycin C up to 6 weeks before study treatment; major surgery or radiotherapy within 4 weeks of study treatment initiation; any unresolved toxicities from prior therapy of Common Terminology Criteria for Adverse Events (CTCAE) grade >1 at the time of starting study treatment (with the exception of alopecia); spinal cord compression or brain metastases unless asymptomatic, treated, and stable and not requiring steroids; severe or uncontrolled systemic disease; pre-specified cardiac conditions; inadequate bone marrow reserve or organ function; and any gastrointestinal complications that would preclude adequate absorption of AZD5363.

Definitions for dose-limiting toxicities (DLTs)

A DLT was defined as any toxicity not attributable to the disease or disease-related processes under investigation, which included:

- Hematologic toxicity CTCAE grade ≥4 present for more than 4 days
- Non-hematologic toxicity CTCAE grade ≥3, including:
  - Infection, including febrile neutropenia

  - Confirmation of QTc prolongation (>500 ms) or QTc increase >60 ms from baseline
  - Grade ≥3 hyperglycemia (glucose >13.9 mmol/L) for more than 1 week, despite optimal intervention, which is not attributable to another comorbidity
  - Grade 4 hyperglycemia (glucose >27.8 mmol/L)
  - Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >10x upper limit of normal (ULN) and AZD5363 considered the most likely cause
  - AST or ALT >8xULN, in combination with doubling of bilirubin from baseline, and AZD5363 considered the most likely cause
- Any other toxicity that is greater than that at baseline, is clinically significant and/or unacceptable, does not respond to supportive care, and results in a disruption of dosing schedule of more than 14 days
- Any event, including significant dose reductions or omissions, judged to be a DLT by the Safety Review Committee.

A dose will be considered non-tolerated and dose escalation will cease if two or more of up to six evaluable patients experience a DLT at a dose level. Once the non-tolerated dose is defined, the maximum tolerated dose (MTD) will be confirmed at the previous dose level below the non-tolerated dose, or a dose between the non-tolerated dose and the last tolerated dose may be investigated. Six evaluable patients are required to determine the MTD. An evaluable patient is defined as a patient who has received AZD5363 and either has completed minimum safety evaluation requirements and received at least 75% of the specified dose during the first 21-day cycle, or has experienced a DLT during the first 21-day cycle.

Collection of platelet-rich plasma (PRP), tumor tissue, and circulating free DNA (ctDNA)

Methods of collection and processing of PRP samples have previously been described in Yap et al (1).

Consent for paired tumor biopsies was optional for patients with advanced solid tumors participating in this study (Study 1; NCT01226316) and from a study of AZD5363 in Japanese patients (Study 4; NCT01353781) (2). Sites were requested to obtain the tumor biopsy samples at baseline (prior to first AZD5363 dose) and on treatment (pre-dose on the last day of weekly dosing after 14 days' treatment [continuous dosing] or

pre-dose on the last day of weekly dosing in the second week of dosing [intermittent schedules]). For the analysis of AKT inhibition, no biopsy tissue was collected on the non-dosed days of the intermittent schedules. The day of on-treatment sampling in the 12 cases assessed for tumor proof of mechanism (PoM) ranged from Days 13 to 24 from the first day of the single dose and from Days 7 to 18 from the first day of the multiple-dose phase. The timing of collection of the on-treatment biopsy was recorded for six of 12 cases, and this was 7–14 hours after the last AZD5363 dose for four of six cases (Supplementary Table 1). Formalin-fixed paraffin-embedded (FFPE) samples were obtained from 29 patients across dosing schedules. Paired biopsies from 17 patients enrolled were considered non-evaluable as a result of either insufficient tumor tissue in one biopsy sample or absence of a sample; therefore, tumor paired biopsies from 12 patients (9 from Study 1 Parts A, B, C and 3 from Study 4) were pooled and used for PoM analysis.

Consent was given at pre-screening to provide 5–10 mL whole blood samples, which were processed for ctDNA analysis. Whole blood collected at baseline and at scheduled time points in 5–10 mL ethylenediaminetetraacetaic acid (EDTA) tubes was centrifuged in two steps to separate plasma from cells. In the first step, whole blood was processed within 1 hour into plasma by centrifugation at approximately 2000 G for 10 min using a pre-chilled centrifuge set to 4°C; the plasma was taken off by pipette and transferred to a 15 mL Falcon tube. In the second step, the plasma was centrifuged again at approximately 2000 G for 10 min using a pre-chilled centrifuge set to 4°C, taken off by pipette, aliquoted, and frozen at –80°C until extraction.

Immunohistochemistry (IHC) analyses

Tumor biopsies were processed into FFPE blocks (fixed in 10% formalin for at least 24 hours and embedded in paraffin wax) prior to IHC analysis. Tumor tissue quality was assessed by hematoxylin and eosin (H&E) staining and pathology review, and biopsies with adequate tumor cell content were accepted for IHC assessment. Serial 4 µm sections were prepared fresh for IHC analysis and three non-contiguous sections were processed for each target protein/biomarker. Antibody-specific staining for phosphorylated AKT, PRAS40, GSK3β, S6, and 4EBP1 (see Supplementary Table 2 for antibody details) was assessed by a pathologist for each biomarker (0, negative; 1, weak; 2, moderate; 3, strong staining) and recorded as an H score (sum of [1 x weak] +

[2 x moderate] + [3 x strong] % tumor) based on the results from three sections for each antibody. H scores were reported for membrane, cytoplasmic, and nuclear compartments; a total H score of 300 was available for each compartment. Total H scores for pre- and post-treatment biopsies (membrane + cytoplasm + nuclear) were used to calculate the percentage reduction from baseline H score using the equation b−f/b*100 (b = H score at baseline, f = H score after treatment; average H scores from three slides used). A total H score of <10 was regarded as non-evaluable. This threshold was established from antibody evaluation/validation work assessing the performance of each antibody against a panel of tumor tissues. If an H score of <10 was recorded in any pre-dose sample, the paired samples for that patient were not evaluated. An H score of <10 in a post-dose sample was viewed as a positive result. For assessment of Foxo localization, an antibody that was specific for Foxo3a protein was used to assess the percentage positive stained nuclei at baseline and after treatment with AZD5363 (some minor cross-reactivity was noted with Foxo1 on Western blots; not shown). PTEN displayed both nuclear and cytoplasmic staining. For the determination of final PTEN status, the total H score was based on cytoplasmic and nuclear H score. PTEN was considered deficient when the H score was <10 and proficient when the H score was ≥10.

Molecular analyses

In Parts A and B, mutational analysis of archival tumor samples was performed using Sequenom™. In Part C, central testing of *PIK3CA* mutational status was carried out using the cobas *PIK3CA* mutation test, covering 17 hotspot mutations, in tissue and/or plasma ctDNA. Tissue and plasma samples were also tested with ddPCR, which covered four hotspot *PIK3CA* mutations (E545K and E542K in exon 9 and H1047L/R in exon 20) only. ctDNA was tested for baseline *ESR1* mutational status by droplet digital polymerase chain reaction (ddPCR). Details are described below. Assessment of serial ctDNA samples during treatment is not reported here, with the exception of the discontinuation sample tested from a small subset of patients (n=8).

Sequenom matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) technology

FFPE tumor samples from 67 patients (Parts A and B) were tested for the somatic point and complex mutations available in the OncoCarta Panel v1 test kit (Agena Bioscience, Hamburg, Germany) supplied by Sequenom (San Diego, CA, USA) (3, 4). DNA was

extracted from 2–4 x 5 µm sections from each tumor sample using a cobas extraction kit (Roche, Burgess Hill, UK). Elution volume was 50 µL (DNA samples from identical tumor samples were combined to give a total volume of 100 µL). DNA concentration was determined by spectrophotometry using a Nanodrop ND-1000 instrument (ThermoScientific, Wilmington, DE, USA). For the analysis, a minimum quantity of 250 ng of DNA was required.

Samples were analyzed using the OncoCarta Panel v1 kit for 238 mutations in 19 oncogenes (inclusive of *PIK3CA*, *KRAS*, and *AKT*) included in 24-plex groups. The recommended amount of DNA in each plex was 10 ng. The OncoCarta protocol supplied by Sequenom was followed and the resulting extension products were analyzed on a Sequenom MassArray 4 platform MALDI-TOF instrument.

cobas PIK3CA mutation test

DNA extraction from 2 x 5 µm sections of FFPE tissue was performed using the cobas DNA sample preparation kit (Roche) following the manufacturer's protocol and as previously described (5, 6). Mutation analysis was performed with the cobas *PIK3CA* mutation test (Roche) according to manufacturer's instructions and using the automated analysis software on the cobas z480 analyzer (5, 6). For plasma samples, DNA was extracted from 2 mL volumes using the cobas cell-free DNA (cfDNA) sample preparation kit (Roche) according to manufacturer's instructions. Mutation analysis was performed using the same cobas *PIK3CA* mutation test (Roche) and analysis of the data was performed by Roche using algorithms specific to ctDNA testing.

ddPCR analyses: ctDNA extraction

ctDNA was extracted from 2 mL plasma using the QIAamp circulating nucleic acid kit with the QIAvac 24 Plus vacuum manifold (Qiagen, Manchester, UK) according to the manufacturer's protocol. 50 µL buffer AVE was applied to each column and incubated for 5 min at room temperature prior to elution into Eppendorf LoBind microcentrifuge tubes. Samples were stored at –20^{o}C prior to ddPCR analysis.

ddPCR assays: PIK3CA and ESR1

ddPCR was performed using the QX200 AutoDG Droplet Digital PCR System (BioRad, Hercules, CA, USA) according to the manufacturer's protocol.

Custom assays were designed by IDT (Coralville, IA, USA), incorporating locked nucleic acid (LNA) bases into each probe to increase discrimination. Probes were from IDT and primers (SePOP desalted) from Eurogentec (Liège, Belgium; Supplementary Table 3). The four *PIK3CA* assays were run as a discriminatory multiplex assay and the four *ESR1* assays were run as single-plex assays. Multiplexing four assays was achieved by modifying both probe/primer concentrations and the concentration of FAM and HEX fluorescent labels. In any cases where the *PIK3CA* multiplex result was not conclusive, ddPCR was repeated with relevant single-plex assays.

5 µL of cfDNA or FFPE-extracted DNA was added to each 20 µL ddPCR reaction. Positive and negative controls were run in triplicate or quadruplicate on each plate. 150 bp gBlocks® (IDT) or genomic DNA (gDNA) reference standards (Horizon Discovery, Cambridge, UK) containing the mutation of interest were used as positive controls. Human gDNA (male) was used as wild-type control (Promega, Madison, WI, USA). Appropriate elution buffer and water served as negative controls. Thresholds were manually set for each sample using acceptance criteria defined during the optimization of each assay. QuantaSoft software (version 1.7.4; Bio-Rad, Hemel Hempstead, UK) was used to assign positive/negative droplets and convert counts into mutant copies/mL.

Supplementary results

Dose escalation and expansion (Parts A and B)

The MTDs of the continuous, 4 days on, 3 days off every week (4/7), and 2 days on, 5 days off every week (2/7) schedules were 320, 480, and 640 mg twice daily (bid), respectively (Supplementary Figure 2).

Safety and tolerability – Part C

The safety profile of AZD5363 in Part C was consistent with the findings in Parts A and B. The most common adverse events (AEs; irrespective of causality) in the 59 patients harboring mutations in *PIK3CA* were diarrhea (n=47; 80%), nausea (n=33; 56%), vomiting (n=26; 44%), decreased appetite (n=25; 42%), hyperglycemia (n=24; 41%), and fatigue (n=24, 41%; Table 2). AEs of CTCAE grade ≥3 (irrespective of causality) were experienced by 40 patients (68%), the most common of which were hyperglycemia (n=12; 20%), diarrhea (n=7; 12%), and maculopapular rash (n=6; 10%; Table 3). All 59 patients (100%) experienced at least one AE that was considered by the investigator to

be causally related to AZD5363. The most common treatment-related (as assessed by the investigator) AEs of CTCAE grade ≥3 were hyperglycemia (n=12; 20%), maculopapular rash (n=6; 10%), and diarrhea (n=5; 8%).

Thirteen patients (22%) discontinued treatment as a result of an AE (erythematous rash [n=2], acute coronary syndrome, acute kidney injury, asthenia, atrial flutter, blood creatinine increased, cellulitis, decreased appetite, diarrhea, drug hypersensitivity, hematemesis, upper respiratory tract bacterial infection, troponin increased, ventricular tachycardia, vomiting [all n=1]; some patients experienced more than one AE). Four AEs resulted in the death of four patients: pulmonary embolism (female aged 38 years, with onset on Day 164), hematemesis (female aged 59 years, with onset on Day 165), cellulitis (female aged 52 years, with onset on Day 35), and acute coronary syndrome (female, aged 64 years, onset on Day 502). None of the deaths were considered treatment related.

Molecular analyses – Part C

Results of *PIK3CA* mutation testing on tissue and plasma (ctDNA) by local testing, central testing, and ddPCR, as well as other exploratory biomarkers, are shown in Supplementary Table 4. Forty-eight of 59 patients (81%) were enrolled based on the detection of a *PIK3CA* mutation by local testing on tumor tissue; 11 patients were enrolled based on central testing (cobas® PCR) by tumor tissue and/or plasma. Overall, in 89% (40/45) of cases enrolled following local testing and with tissue samples evaluable for further testing, a *PIK3CA* mutation was confirmed by cobas PCR and/or ddPCR (Supplementary Table 4).

PIK3CA mutational status can 'change' upon disease recurrence, reflecting intratumoral heterogeneity and clonal selection (7, 8). In Part C, 98% (58/59) of patients enrolled on the basis of local or central tumor testing (one patient was enrolled based on plasma central testing). The time interval between tissue collection and first dose was variable (up to 22.8 and 6.8 years for breast and gynecologic cancer patients, respectively; not shown), and in 67% (39/58) of these patients, the tissue provided for central testing was from the primary tumor. A *PIK3CA* mutation was 'not detected' in baseline plasma by central testing or ddPCR from 12/31 (39%) patients with samples available for analysis. Although biological and/or technical reasons (ie no propensity of the tumor cells to shed in the circulation and/or limit of detection of the specific analytical method) cannot be

excluded, this suggests potential true loss of mutations during tumor evolution in these patients. Notably, both archival tissue and plasma were available for molecular testing and the median time lapse between tissue and plasma collection was 2.08 years (range 0–22.56 years; Supplementary Table 4). The comprehensive mutational landscape of the *PIK3CA*-mutant tumors was not investigated; however, assessment of hotspot *ESR1* mutations (a major mechanism of acquired resistance to aromatase inhibitors) in baseline plasma demonstrated the presence of an *ESR1* mutation in 5/19 (26%) evaluable Cb patients (2 D538G, 2 Y537S, 1 Y537C), and in one of these five cases, our ddPCR analysis suggested clonal evolution and loss of *PIK3CA* mutation (Supplementary
Figure 3). As expected, none of the 12 patients with gynecologic cancer had a detectable *ESR1* mutation in baseline ctDNA.

Archival tumor tissue from 37 patients (19 Cb and 18 Cg) was evaluable for *PTEN* status by IHC. *PTEN* expression was proficient in 32 cases and deficient in five cases (two Cb, three Cg; Figure 4B and Supplementary Table 4).

Pharmacodynamic (PD) biomarkers and pharmacokinetic/pharmacodynamic modeling

Preclinical activity of AZD5363

In preclinical models *in vitro* and *in vivo*, AZD5363 was consistently shown to reduce the phosphorylation of AKT-specific target sites in GSK3β (Ser 9) and PRAS40 (Thr 246) and to lead to an increase in phosphorylated AKT at both T308 and S473 sites (9). The impact on protein phosphorylation correlated with AZD5363 plasma concentration and was dose dependent up to the MTD. A dose-dependent increase in blood glucose levels related to the pharmacologic inhibition of AKT was also reported in preclinical models (9).

In tumor xenograft models, continuous bid oral dosing of 75–150 mg/kg resulted in >60% tumor growth inhibition compared with controls and, in some tumor models, tumor regression was seen at ~100 mg/kg bid (9). In BT474c xenografts, 100 mg/kg bid inhibited tumor growth by ~80%, and the maximum well-tolerated dose of 200 mg/kg bid completely inhibited tumor growth. Tumor growth inhibition was achieved at plasma concentrations of AZD5363 in mice that were below the minimum plasma concentration (C_{min}) achieved in humans following continuous dosing of 320 mg bid (Supplementary

Figure 4A). The latter was 191 ng/mL, equivalent to 0.099 μM free concentration (fraction unbound 0.223; AstraZeneca data on file).

Preclinical pharmacodynamics

The PD response of AKT substrates PRAS40 and GSK3β following a single AZD5363 100 or 300 mg/kg dose was compared over a 24-hour period in mice bearing BT474c tumors (Supplementary Figure 4B). Phospho-PRAS40 (pPRAS40) was reduced by 90% over the first 2 hours after dosing and by ~70% 24 hours after a 300 mg dose. pPRAS40 levels had almost completely recovered by 24 hours after a single AZD5363 100 mg/kg dose. In contrast, phospho-GSK3β (pGSK3β) was reduced by >50% over the first 8 hours after a 300 mg dose and by 30% at 24 hours; pGSK3β was reduced by >30% 24 hours after a single 100 mg/kg dose.

A tolerated continuous bid dose of 100 mg/kg in mice resulted in significant xenograft tumor growth inhibition (9). At 2–4 hours, a reduction of >50% was observed in tumor tissue for both pPRAS40 and pGSK3β, and at 8 hours, the inhibition was >50% and >30%, respectively (Supplementary Figure 4C).

Preclinical comparison of continuous and intermittent dosing

Modeling of data from BT474c tumor xenograft studies was used to predict intermittent dosing schedules that would deliver equivalent efficacy from the 4/7 and 2/7 schedules. Dose equivalents of 1.3x (4/7) and 1.7x (2/7) were predicted to deliver equivalent tumor growth inhibition to continuous bid dosing (10). The observed free trough concentration in patients on the intermittent 480 mg 4/7 schedule (0.186 μM) exceeded that for continuous dosing by 1.9-fold. Intermittent dosing was shown to deliver increased and prolonged reduction of pPRAS40 and lower AKT hyperphosphorylation compared with continuous dosing. End-of-study pPRAS40 levels were significantly reduced by 2 hours after

100 mg/kg continuous dosing but returned to control levels after 8 hours (Supplementary Figure 4D). A 4/7 dose of 130 mg/kg maintained ~50% reduction in pPRAS40 at 8 hours, and 170 mg/kg 2/7 resulted in >80% reduction at 8 hours and ~30% reduction 120 hours after dosing.

References

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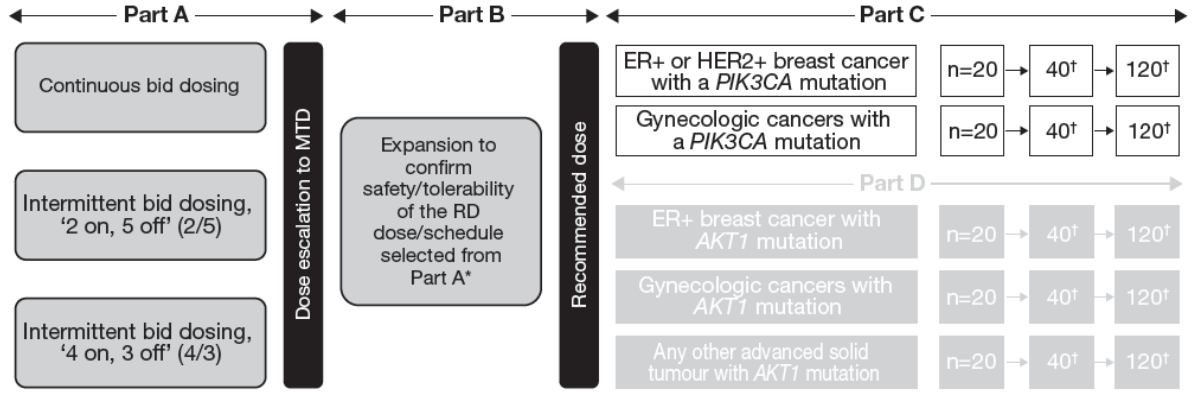
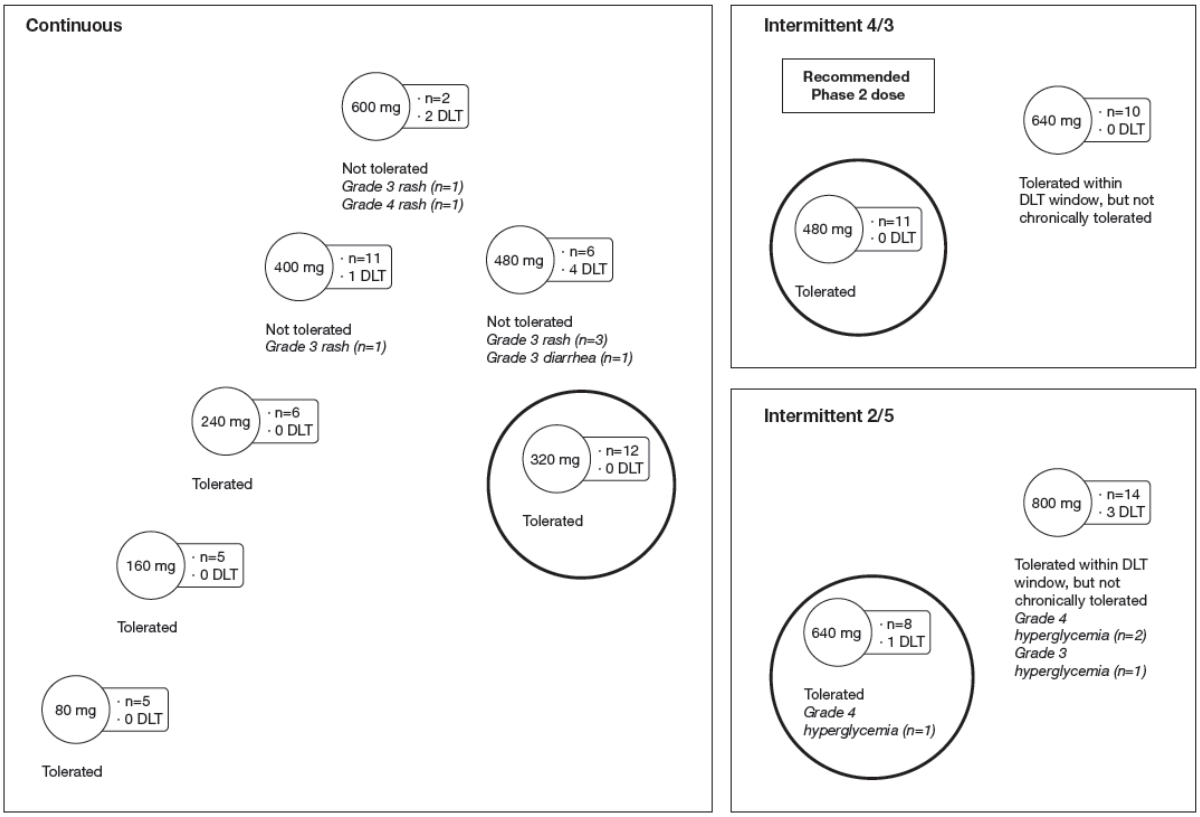
Supplementary Figure 1. Study 1 (NCT01226316) design

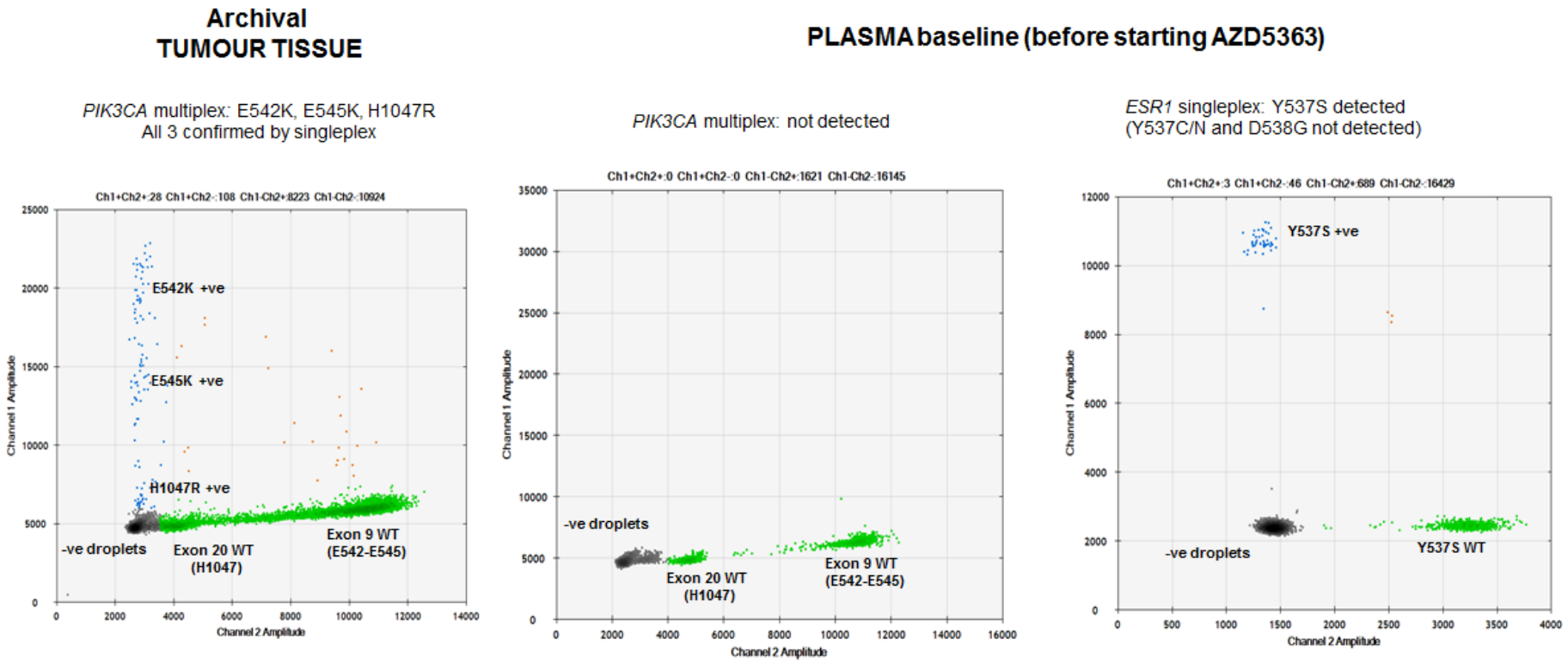


Parts A, B, and C are reported in this manuscript. *AZD5363 480 mg bid in a 4/7 schedule was selected; †Recruited only if a response was observed in the previous set of patients

Supplementary Figure 2. Dose escalation and DLTs in Part A, with MTDs of the continuous, 4/7, and 2/7 schedules being 320, 480, and 640 mg bid, respectively



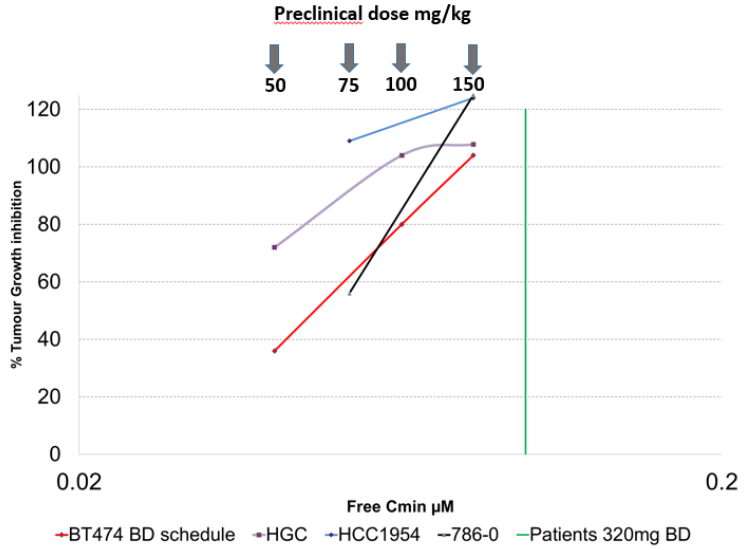
Supplementary Figure 3. Exploratory mutation analyses (ddPCR) in archival tissue and plasma (ctDNA) samples from a breast cancer patient who had progressive disease as best response (Cb cohort)



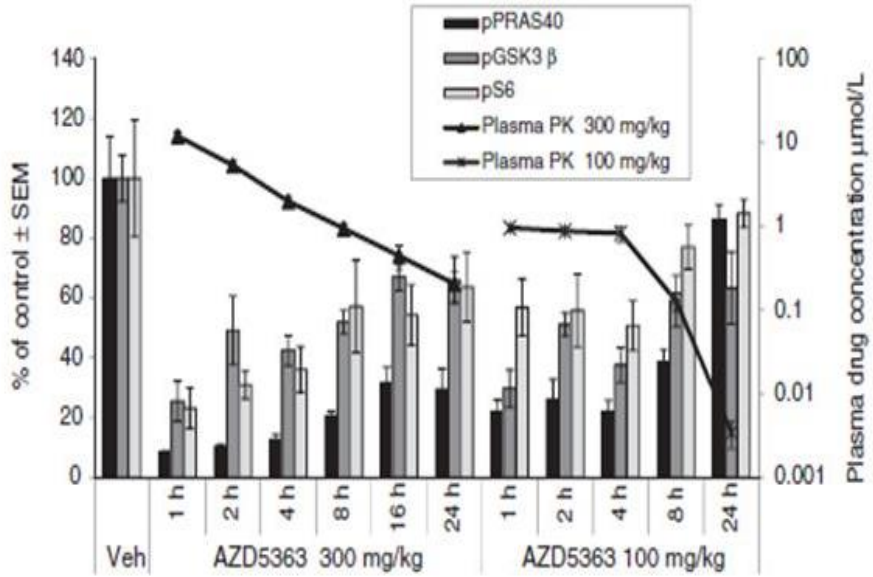
The primary tumor collected 22.8 years before the first dose of AZD5363 harbored polyclonal *PI3KCA* mutations (individual mutations are labeled in the multiplex plot, all confirmed in single-plex assays), which were not detected in baseline plasma collected at study enrollment, before starting AZD5363. In the same plasma sample, an *ESR1* Y537S mutation was detected, suggesting that the lack of *PIK3CA* mutation in plasma is likely due to loss of the mutation during tumor evolution rather than low tumor shedding in the circulation

Supplementary Figure 4. A) Tumour growth inhibition in preclinical tumor xenograft models (BT474c, HGC-27, HCC1954 and 786.0) treated with AZD5363 bid po at the indicated doses. B) AZD5363 PD in BT474c tumor xenografts. C) AZD5363 PD at steady state in BT474c xenograft tumors. D) Time course of pPRAS40 and pAkt S473 in BT474c tumor xenograft tissue following the final dose of AZD5363 after 3 weeks' continuous (100 mg/kg bid) or intermittent dosing (130 mg/kg bid 4/7 and 170 mg/kg bid 2/7)

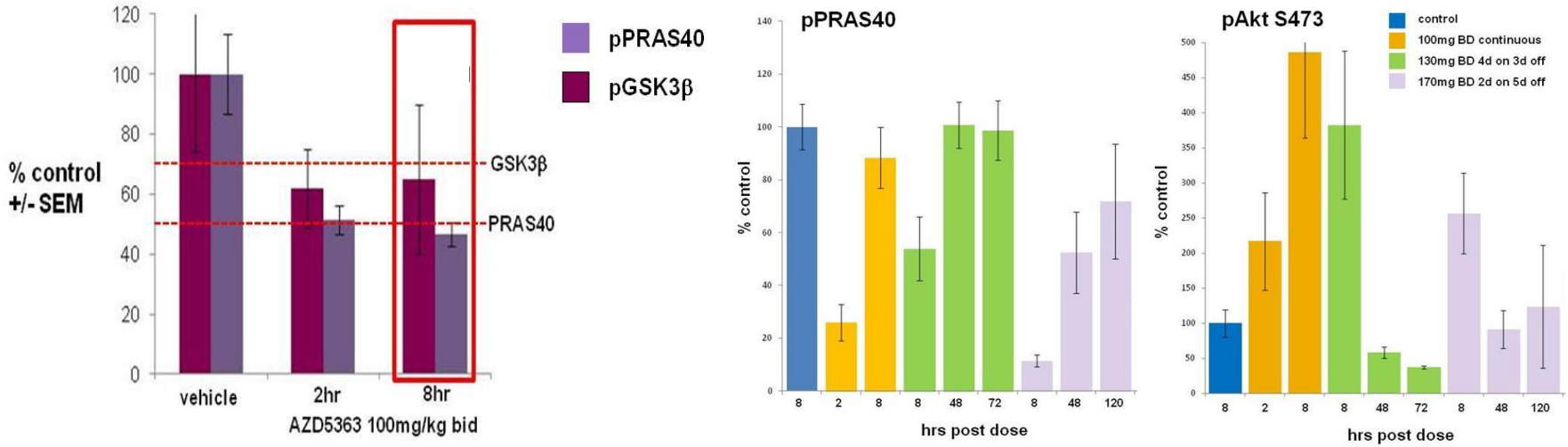
A)



B)

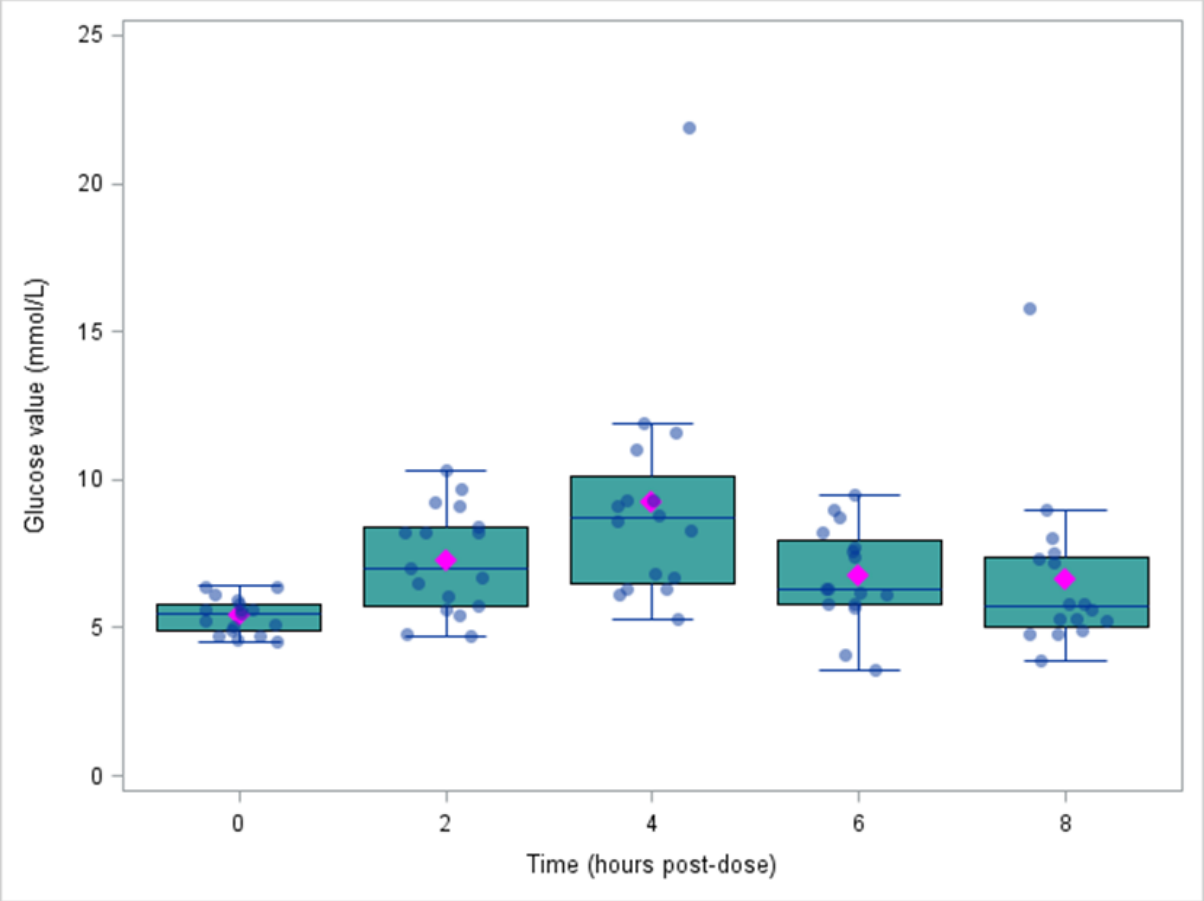


C) **D)**



A) The plasma free C_{min} for clinical doses of 320 mg bid continuous and intermittent schedules (480 mg and 640 mg bid) are indicated. B) Mean percentage pPRAS40, pGSK3β, and pS6 compared with vehicle-treated controls is shown relative to plasma drug concentration after a single 100 mg/kg or 300 mg/kg po dose. Reproduced from Davies BR et al. Mol Cancer Ther 2012;11:873–87. C) Mean percentage inhibition of pPRAS40 and pGSK3β was >50% and >30% (respectively; PoM threshold as indicated by dotted lines) at 2 and 8 hours after the final dose (boxed area) following 28 days' continuous dosing with AZD5363 100 mg/kg bid po. After 28 days' treatment, tumor growth inhibition was 80% compared with vehicle-treated controls. C) and D) Adapted from Elvin P et al. J Clin Oncol 2014;32(5s):abst 2541. po, by mouth

Supplementary Figure 5. Glucose (non-fasting/random) values over time after 480 mg single-dose AZD5363 (all dosing schedules)



Data from patients enrolled in Parts A and B only (safety population) are shown. Mean was taken when duplicate values were present at post-baseline time points. Fasting values are excluded. Horizontal line, median; diamond, mean; box, quartile 1 to quartile 3; whiskers extend from the quartiles to the most extreme observation within 1.5x the interquartile range (IQR). Outliers (>1.5xIQR) are individually displayed

Supplementary Table 1. Percentage change from baseline for PoM biomarkers for 12 evaluable patients

		AZD5363 dose (mg bid) and schedule											
		320 cont	**320[a] cont**	**320 cont**	**360 cont**	**360 cont**	**480 4/7**	**480 4/7**	**480 7/7**	**480 4/7**	**480[b] 4/7**	**800 2/7**	**800 2/7**
Timing of on-treatment biopsy	Days after first (single) dose	13	14	21	16	24	17	15	17	14	–	13	14
	Days after start of multiple dosing	7	8	17	10	18	10	9	10	9	10	8	9
	Time after last dose	14 h 0 min	11 h 30 min	NA	NA	NA	10 h 15 min	1 h 45 min	7 h 10 min	4 h 10 min	NA	NA	NA
Biomarkers (% change from baseline)	pPRAS40	11.5	–40.0	1.9	–53.8	–31.8	–30.2	–70.7	–88.1	–20.8	–83.1	–16.9	–46.0
	pGSK3β	0.0	–68.0	–42.6	–61.0	–20.5	–	–93.2	–93.9	5.1	–84.0	–10.3	34.7
	Foxo3a	0.0	–	–85.5	–64.5	–	–	–70.4	30.9	13.8	46.3	–32.3	–
	tAKT	27.7	–8.1	100.0	–59.9	–38.5	[c]	–34.5	–63.2	[c]	[c]	250.0	–91.2
	pAKT473	93.8	2350.0	–	1950.0	–	–	966.7	4033.3	192.6	780.0	–73.7	–14.3
	pAKT308	14.3	69.6	225.0	16.2	1366.7	1020.0	76.5	34.0	180.0	157.1	8.7	125.0
	tS6	0.6	0.0	–31.1	0.0	–2.2	–9.4	0.0	2.3	0.0	12.5	18.4	–9.0
	pS6	–11.5	0.0	23.5	9.1	–60.0	–37.5	–77.8	–60.9	–67.4	59.4	200.0	–65.6
	t4EBP1	–6.7	–12.8	–68.3	–80.0	–20.5	35.1	–27.9	–64.9	12.1	52.1	–	9.7
	p4EBP1	27.9	–44.9	5.7	–62.5	–25.0	–48.9	–95.7	–91.7	185.7	–50.0	–	–15.1

The percentage change reported in the table is based on the average H score for individual biomarkers in baseline and on-treatment biopsies from three non-consecutive tissue sections. [a]*KRAS*-mutant colorectal cancer; results for pPRAS40 and pGSK3β are plotted in Figure 3B; [b]*PIK3CA* E545K mutant cervical cancer (this patient was enrolled in Part C); [c]For these patients, total AKT staining was equivocal and could not be reliably interpreted, although pAkt308/473 was clearly present in both pre- and on-treatment samples. –, missing data; cont, continuous; NA, not available

Supplementary Table 2. Antibodies and conditions for IHC analysis

Target protein	Catalogue code	Antigen retrieval	Dilution	Detection
pPRAS40	CST 2997	10 mM EDTA pH8 (10x), 2 min, 110°C	1/200	Rabbit Vector Elite ABC Kit (PK-6101)
pGSK3β	CST 9323	Dako S2367 pH 9, 2 min, 110°C	1/40	Rabbit Envision Kit, Dako K4011
Foxo3a/Foxo1	CST 2497	10 mM EDTA pH8 (10x), 2 min, 110°C	1/40	Rabbit Envision Kit, Dako K4011
Akt	In-house AZN-11P	10 mM EDTA pH8 (10x), 2 min, 110°C	1/200	Rabbit Envision Kit, Dako K4011
pAkt473	Dako M3628	Dako S2367 pH 9, 2 min, 110°C	1/40	Rabbit Envision Kit, Dako K4011
pAkt308	CST 2965	Dako S2367 pH 9, 2 min, 110°C	1/50	Rabbit Envision Kit, Dako K4011
S6	CST 2217	10 mM EDTA pH8 (10x), 2 min, 110°C	1/200	Rabbit Envision Kit, Dako K4011
pS6	CST 4857	10 mM EDTA pH8 (10x), 2 min, 110°C	1/100	Rabbit Envision Kit, Dako K4011
4EBP1	CST 9644	10 mM EDTA pH8 (10x), 2 min, 110°C	1/5000	Rabbit Envision Kit, Dako K4011
p4EBP1	CST 2855	10 mM EDTA pH8 (10x), 2 min, 110°C	1/500	Rabbit Envision Kit, Dako K4011
PTEN	CST 9559	Dako S1699 pH 6, 5 min, 110°C	0.4 µg/mL[a]	Rabbit Vector Elite ABC Kit (PK-6101)

Antigenic stability studies were carried out for pGSK3β, pPRAS40, Foxo3a/Foxo1, and pS6. IHC was assessed using an Aperio slide-scanning microscope. The length of time after sectioning prior to staining up to 28 days had no effect on the total positive pixel output or the distribution pattern of positive pixels (assessment by Aperio Positive Pixel algorithm). Wax covering of the slides did not result in any differences in staining properties and room temperature storage without wax covering was used throughout the study. [a]Protein concentration

Supplementary Table 3. ddPCR primers and probes

ddPCR primers	
Name	**Sequence**
ESR1 forward	AAAGGCATGGAGCATCTGTA
ESR1 reverse	CTAGTGGGCGCATGTAGG
PIK3CA exon 9 forward	GACAAAGAACAGCTCAAAGCAA
PIK3CA exon 9 reverse	GCACTTACCTGTGACTCCATAG
PIK3CA exon 20 forward	TCGAAAGACCCTAGCCTTAGA
PIK3CA exon 20 reverse	TGTGTGGAAGATCCAATCCAT
ddPCR probes	
Name	**Sequence**
ESR1 D538G WT	5HEX/ TC+TAT+G+A+CCT+G+CT 3IABkFQ/
ESR1 D538G mutant	56-FAM/ AT+G+G+CCTG+CT 3IABkFQ/
ESR1 Y537C/N/S WT	5HEX/ CCCT+C+T+A+TGA+CCT 3IABkFQ/
ESR1 Y537C mutant	56-FAM/ TC+T+G+TGA+C+CT 3IABkFQ/
ESR1 Y537N mutant	56-FAM/ CC+CCT+C+A+ATG+ACC 3IABkFQ/
ESR1 Y537S mutant	56-FAM/ CC+CTC+T+C+TGACCT 3IABkFQ/
PIK3CA E542K/E545K WT	5HEX/TCT+G+AAATCACT+G+AGC 3IABkFQ/
PIK3CA H1047R/L WT	5HEX/ TG+CA+C+A+T+CAT+GG 3IABkFQ/
PIK3CA E542K mutant	56-FAM/C+T+A+AAATCAC+T+G+AGC 3IABkFQ/
PIK3CA E545K mutant	56-FAM/C+T+G+AAATCAC+T+A+AGC 3IABkFQ/
PIK3CA H1047R mutant	56-FAM/TG+CA+C+G+TCA+TG 3IABkFQ/
PIK3CA H1047L mutant	56-FAM/TG+CA+C+T+T+CA+TGG 3IABkFQ/

WT, wild type

Supplementary Table 4. Tables with results of molecular analyses of Part C patients (*PIK3CA* and *ESR1* mutation status in tissue and ctDNA, PTEN status in archival tissue)

Suppl Table 4.xlsx

Supplementary Table 5. AEs of CTCAE grade ≥3 (irrespective of causality) in Parts A and B (frequency >3% total) and Part C (frequency >5% total)

	Parts A and B											Part C
	Continuous							Intermittent 4/7		Intermittent 2/7		
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)	480 mg bid; 4/7 (n=59)
Hyperglycemia	0 (0)	0 (0)	0 (0)	0 (0)	1 (9)	2 (33)	0 (0)	4 (36)	4 (40)	2 (25)	5 (36)	18 (20)	12 (20)
Diarrhea	0 (0)	0 (0)	1 (17)	0 (0)	3 (27)	3 (50)	1 (50)	2 (18)	2 (20)	0 (0)	1 (7)	13 (14)	7 (12)
Maculopapular rash	0 (0)	0 (0)	0 (0)	2 (17)	2 (18)	3 (50)	2 (100)	0 (0)	0 (0)	0 (0)	1 (7)	10 (11)	6 (10)
Abdominal pain	0 (0)	0 (0)	0 (0)	1 (8)	1 (9)	1 (17)	0 (0)	0 (0)	0 (0)	1 (13)	0 (0)	4 (4)	0
Fatigue	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	1 (17)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)	4 (4)	4 (7)
Vomiting	0 (0)	0 (0)	0 (0)	1 (8)	1 (9)	0 (0)	0 (0)	0 (0)	1 (10)	1 (13)	0 (0)	4 (4)	3 (5)
Back pain	1 (20)	0 (0)	0 (0)	0 (0)	1 (9)	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	0 (0)	3 (3)	0
Blood bilirubin increased	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	0 (0)	3 (3)	0
Nausea	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	1 (9)	1 (10)	0 (0)	0 (0)	3 (3)	0
Hypokalemia	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (7)
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)	3 (5)
Proteinuria	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5)