AKT inhibition in solid tumors with *AKT1* mutations

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ABSTRACT

Background
AKT1 E17K mutations are oncogenic and occur in many cancers at a low prevalence. We performed a multi-histology basket study of AZD5363, an ATP-competitive pan-AKT kinase inhibitor, to determine the efficacy of AKT inhibition in AKT mutant cancers.

Methods
Fifty-eight patients with *AKT1*-mutant solid tumors were treated. Key end points included response rate, progression-free survival (PFS), and safety. Tumor biopsies and plasma cell-free DNA (cfDNA) were collected in the majority of patients with the goal of identifying predictive biomarkers of drug response.

Results
In heavily pretreated patients with *AKT1* E17K mutant tumors (median lines of systemic therapy=5), the median PFS was 5.5 (95% CI 2.9-6.9), 6.6 (1.5-8.3), and 4.2 (2.1-12.8) months in the ER+ breast, gynecologic, and other solid tumor cohorts, respectively. Imbalance of the *AKT1* E17K mutant allele, mostly frequently caused by loss of the remaining wildtype allele, was associated with longer PFS (hazard ratio [HR]=0.41, p=0.04), as was the presence of coincident PI3K pathway hotspot mutations (HR=0.21, p=0.045). Persistent declines in *AKT1* E17K in cfDNA were associated with improved PFS (HR=0.18, p=0.004) and response (p=0.025). Clinical benefit was not restricted to patients with detectable *AKT1* E17K in pretreatment cfDNA or patients with clonal AKT E17K mutations. The most common grade ≥3 adverse events were hyperglycemia (24%), diarrhea (17%) and rash (15.5%).

Conclusions
This study provides the first clinical data that *AKT1* E17K is a therapeutic target in human cancer. The genomic context of the *AKT1* E17K mutation further conditioned response to AZD5363. (Funded by AstraZeneca; ClinicalTrials.gov number, NCT01226316)

INTRODUCTION

Phosphoinositide 3-kinase (PI3K)/AKT is among the most frequently activated pathways in cancer.[1,2] Activation can occur through mutation of multiple signaling nodes including *PTEN*, *PIK3R1*, *PIK3CA*, *AKT*, and *mTOR*.[3-5] Clinical development of drugs targeting this pathway has focused primarily on inhibitors of PI3K isoforms and mTOR.[6-8] The AKT kinase family includes three structurally related serine-threonine kinases that serve as critical downstream effectors of PI3 kinase signaling. Large-scale genomic profiling of human cancers has identified gain-of-function mutations in *AKT1* in a broad range of tumor types with *AKT1* E17K being, by far, the most frequent hotspot.[9-14] This mutation promotes pathological localization of AKT1 to the plasma membrane, thereby stimulating constitutive downstream signaling.[15]

AKT inhibitors have been in clinical testing for several years, but have not been specifically tested in *AKT1*-mutant tumors. Testing these inhibitors in *AKT1*-mutant patients using traditional clinical trial designs is challenging because, unlike many other oncogenes, *AKT1* E17K is infrequent in all individual tumor lineages. To determine whether *AKT1*-mutant cancers are sensitive to direct AKT inhibition and whether tumor lineage influenced drug sensitivity, we performed a multi-cohort basket study of the orally administered pan-AKT inhibitor AZD5363[16] in patients with *AKT1*-mutant solid tumors. Tumor biopsies and analyses of tumor-derived DNA in plasma were performed to identify genomic determinants of drug response and to guide future combination studies.

METHODS

Study Oversight

The study was designed by AstraZeneca with the principal investigators and conducted in accordance with the provision of the Declaration of Helsinki and Good Clinical Practice guidelines. Institutional review boards at each center approved the protocol.

Patients

Eligible patients had histologically confirmed advanced solid tumors, refractory to standard therapies, no prior exposure to catalytic AKT inhibitors, and tumors harboring *AKT1* mutations but no known concurrent RAS/RAF mutations as determined by local tumor testing. Complete eligibility criteria are available in the Supplementary Appendix. Written informed consent was obtained for all participants.

Study Design, Treatment, and Endpoints

This was a multi-cohort basket study of patients with solid tumors harboring *AKT1* mutations. The phase I component of this study has been presented previously and defined the safety, optimal dose and schedule of AZD5363, and its efficacy in patients with *PIK3CA* mutations.[17,18] Here, patients were enrolled to one of three cohorts: estrogen receptor-positive (ER+) breast cancer, gynecologic cancers, and all other solid tumors and treated on a 21-day cycle of 480 mg AZD5363 twice daily for four days followed by three days off, repeated weekly. Key end points included investigator-assessed response according to RECIST version 1.1, PFS, and safety. In some cases, ER+ breast or endometrial cancer patients with progression on AZD5363 monotherapy were permitted to crossover to the combination of AZD5363 and fulvestrant, a selective estrogen receptor antagonist and degrader, at the approved dose regardless of prior fulvestrant exposure, based on preclinical data suggesting synergy with the combination.[19] Patient-level clinical data are available in **Table S1**.

Assessments

Disease assessments with CT or MRI were performed at baseline, every 6 weeks for 6 months, and then every 12 weeks until disease progression, death, or withdrawal. Adverse

events were graded by the investigator according to the CTCAE, version 4.0 until day 28 after discontinuation of study treatment.

Biomarker Studies
Tumor tissue samples and tumor-derived cell free (cf) DNA in plasma were collected for biomarker studies. Next-generation sequencing was performed utilizing both targeted and whole-exome sequencing on pre-treatment DNA from formalin-fixed paraffin embedded tumor and matched blood specimens (**Table S3**).[20,21] Droplet digital polymerase-chain-reaction analysis (ddPCR) using an allele-specific assay was performed on cfDNA from pre-treatment and longitudinally collected plasma samples. Complete sequencing and data analysis methods are described in the **Supplementary Appendix**.

Statistical Analysis
Interim analysis was planned following enrollment of 20 patients to each cohort. Efficacy and safety analyses included all patients receiving at least one dose of AZD5363 with the exception of one patient with an *AKT1*-wildtype tumor who was mistakenly enrolled and was thus excluded from the efficacy analyses. PFS was assessed using Kaplan-Meier methods. Statistical analyses were conducted on a data cut taken on May 31, 2016.

RESULTS

Patients, Efficacy, and Safety
Fifty-eight patients with *AKT1*-mutant solid tumors (52 E17K, 6 non-E17K) were treated (**Table 1**). Patients were heavily pretreated (median prior regimens=5). In total, 73% (38/52) of *AKT1* E17K patients achieved some regression of target lesions including confirmed partial responses according to RECIST in ER+ breast and endometrial cancers (n=4 and 2, respectively) as well as cervix cancer, triple negative breast cancer, and lung adenocarcinoma (n=1 each) (**Fig. 1**). Additional unconfirmed partial responses occurred in ER+ breast cancer (n=2), triple negative breast cancer (n=1), and anal adenocarcinoma (n=1). In *AKT1* non-E17K patients, tumor regressions not meeting response criteria were observed in two *AKT1* Q79K-mutant patients (prostate and ovarian), including one lasting 14 months. Median PFS (with 95% CI) in the *AKT1* E17K mutant ER+ breast, gynecologic, and other solid cancer cohorts was 5.5 months (2.9-6.9), 6.6 months (1.5-8.3), and 4.2 months (2.1-12.8), respectively. There was no apparent relationship between tumor type and likelihood of response. Six patients (5 ER+ breast and 1 endometrial) crossed over to AZD5363 plus fulvestrant after progression on AZD5363 monotherapy. None achieved an objective response, but one ER+ breast cancer patient who was previously fulvestrant-resistant had a durable tumor regression.

The most common grade ≥3 adverse events were hyperglycemia (24%), diarrhea (17%), and maculopapular rash (15.5%) (**Table 2**). Overall, 34% of patients required a dose reduction with diarrhea, maculopapular rash, and hyperglycemia being the most common indications. AZD5363 was permanently discontinued in 12% of patients due to adverse events. Drug-related serious adverse events occurred in 15.5% of patients and were consistent with the overall side-effect profile of AZD5363 (**Table S2**).

Non-Invasive Monitoring of Circulating Biomarker in cfDNA
As patients were enrolled on the basis of local archival tumor sequencing, we sought to determine the presence of *AKT1* E17K in cfDNA from plasma collected at the time of enrollment. Notably, *AKT1* E17K was detected in pre-treatment plasma by ddPCR in only 81.4% (35/43) of patients with evaluable samples (**Fig. 1**). Among patients with undetectable *AKT1* E17K in cfDNA (n=8), archival tumor was available for central sequencing in six patients and confirmed the presence of the E17K mutation in five. Two of these patients had partial responses and a third patient had a durable tumor regression lasting over 8 months. Broader analysis of plasma from these three patients using a capture-based cfDNA assay

also identified no tumor-derived mutations. The only patient where *AKT1* E17K could not be confirmed in either cfDNA or in archival tumor progressed rapidly.

To determine whether tumor-derived cfDNA could be utilized as an early surrogate of drug response and to explore the dynamics of the circulating biomarker under the selective pressure of AKT inhibition, longitudinal plasma samples were tested in 23 patients (**Fig. 2A**). A decrease in *AKT1* E17K mutant allele fraction of ≥50% from baseline during cycle 1 was observed in 95.5% (22/23) of patients but did not correlate with outcome (**Fig. 2B**). Conversely, persistent decreases maintained into cycle 2 were associated with longer PFS when compared with patients in whom cfDNA decreases were not achieved or did not persist (median PFS 5.6 versus 2.6 months, respectively, hazard ratio=0.18, p=0.004, **Fig. 2C**). Progression by cfDNA, defined as a rise in the circulating *AKT1* E17K mutant allele fraction of ≥50% above nadir, preceded radiographic progression in all but one patient by a median of 42 days (95% CI: 31-68 days) (**Fig. 2D**). Longitudinal profiling of cfDNA during treatment also captured fluctuations in disease burden, including re-sensitization to AZD5363 following addition of fulvestrant in one ER+ breast cancer patient. This fulvestrant-resistant patient achieved a durable tumor regression (-22%) lasting 8 months after crossing over to combined AZD5363 and fulvestrant therapy, the duration of which matched or exceeded that previously achieved with either agent alone (**Fig. 2E**). Broader next-generation sequencing of pretreatment cfDNA in this patient captured the complete mutational profile of genetically heterogeneous individual tumor sites (**Fig. 2F**).

Genomic correlates of response to AKT inhibition

To determine whether the genomic configuration of *AKT1* (number of mutant and wildtype copies) or co-incident tumor mutations influenced AZD5363 response, we performed whole exome or targeted sequencing of archival and fresh pre-treatment tumors in a subset of patients. In the 37 patients with adequate material for this analysis, 57% (21/37) exhibited allelic imbalance of the *AKT1* E17K mutation. Here, the frequency of the E17K allele was higher than expected for a heterozygous oncogenic mutation and higher than the allele frequency of other clonal somatic mutations in the corresponding tumors (**Fig. 3A**). This finding could not be explained by focal amplification of the E17K allele, which was present in only two tumors. To determine the etiology of this allelic imbalance, we performed allele-specific copy number analysis of the sequencing data, which revealed that 48% (10/21) of cases had copy-neutral loss-of-heterozygosity (CN-LOH). This duplication of the mutant *AKT1* allele with concomitant loss of the remaining wild-type copy ultimately resulted in two mutant *AKT1* E17K copies and no wildtype copies (**Fig.3B, Fig. S1-S2).** CN-LOH arose in molecular time shortly after acquisition of the E17K mutation and, in some patients, was followed by genomic gains of the locus. Notably, patients whose tumors exhibited allelic imbalance of *AKT1* E17K had a longer PFS than those without it with a median PFS of 8.2 versus 4.1 months, respectively (HR=0.41, p=0.04, **Fig. 3C**). In the study cohort, *AKT1* E17K allelic imbalance was associated with tumor lineage, arising more commonly in breast and gynecological cancers compared to all others enrolled (90 versus 10% respectively) (**Fig. 1**).

We also explored how clonality of the AKT1 E17K mutation influenced AZD5363 response. In total, 92% (34/37) of patients had clonal (present in all tumor cells) *AKT1* mutations (**Fig. 3A**). Two of the three patients with subclonal *AKT1* E17K mutations had rapid disease progression. The third patient, with ovarian granulosa cell cancer, had a mixed response with an overall tumor regression of 24% lasting 253 days (**Fig. 3D**). To understand the basis of this clinical benefit despite the presence of a subclonal *AKT1* E17K mutation, we sequenced 9 metastatic sites sampled prior to the initiation of AZD5363 treatment (**Fig. 3E**) and found that while the *AKT1* mutation was subclonal across the lesions, the resected right pelvic tumor that subsequently recurred and achieved the best response (-42.5%) had the highest cellular fraction (67% of cancer cells) of the *AKT1* E17K mutation (**Fig. 3E**). These results suggest that later acquisition of *AKT1* E17K driver mutations may not entirely preclude response to AZD5363.

Leveraging the broader-based sequenced we performed here, we explored whether particular co-mutations were associated with intrinsic sensitivity or resistance to AKT inhibition. Notably, five patients had coincident activating mutations in either up- or downstream effectors of PI3K/mTOR signaling. The presence of coincident PI3K pathway alterations was associated with improved PFS compared to those without (median not reached versus 4.3 months, HR=0.21, p=0.045). Importantly, concurrently mutated genes that would be expected to activate parallel signaling pathways did not necessarily preclude response to AZD5363. Two of five patients with loss-of-function *NF1* mutations (cervix and breast cancer) achieved durable partial responses, one of which also had a subclonal *FGFR3* S249C hotspot mutation. In a non-responding colorectal cancer patient, a subclonal *KRAS* A146T hotspot mutation not detected by local tumor profiling was identified in pretreatment cfDNA, a mutation which pre-clinically is associated with resistance to AZD5363.[16] Mutational hotspots in the ligand binding domain of *ESR1*, which are associated with acquired resistance to endocrine therapy and poor prognosis[22], were identified in metastatic tumor tissue or cfDNA in 35% (7/20) of ER+ breast cancer patients and were associated with a shorter median PFS compared to those without (p=0.02; **Fig. 1** and **Fig. S3**).

DISCUSSION

This study provides the first clinical evidence that *AKT1* E17K is a targetable oncogene in human cancer. Treatment with AZD5363 yielded durable responses and tumor regressions across a variety of tumor types harboring the mutation including breast (ER+ and triple negative), endometrial, cervix, and lung cancers.

The breadth and depth of pretreatment sequencing data available allowed us to explore how different facets of these patients' tumors further conditioned response to AKT inhibition. We unexpectedly found that tumors harboring *AKT1* E17K mutations frequently exhibit selection against the remaining wildtype allele, most often due to duplication of the mutant allele via CN-LOH, resulting in allelic imbalance. This genomic configuration, surprising for an oncogene, appears to be both allele and lineage-specific as it was enriched in *AKT1* E17K-mutant breast and endometrial cancers, but not observed in other tumor lineages or affecting other driver mutations involving the PI3K and MAPK pathways (**Fig. S2**). This *AKT1* E17K allelic imbalance was associated with a statistically and clinically significant improvement in PFS. This finding suggests that classifying genomic biomarkers as simply present or absent may overlook additional informative factors, such as genomic configuration, that are relevant to patient selection and lineage dependence. Similarly, we found that while two patients with tumors bearing subclonal *AKT1* mutations did not respond to AZD5363, one granulosa cell cancer patient with extensive intratumoral heterogeneity had durable tumor regression at disease sites harboring the highest cellular fraction of *AKT1* E17K. This finding suggests that limiting targeted therapy to patients only with clonal *AKT1* mutations may not be entirely appropriate.

Surprisingly, we identified five patients whose tumors harbored activating mutations in other effectors of PI3K/mTOR signaling in addition to *AKT1* E17K, a finding we confirmed in 12.5% of *AKT1* mutant patients from an independent genomic dataset (**Fig. S4**). Again, the statistically and clinically significant longer PFS observed in these dual mutant patients, argues that rather than implying functional redundancy, coincident mutations in effectors of the same pathway may result in distinct signaling phenotypes with important therapeutic implications. Further biologic investigation of whether such coincident drivers further sensitize tumors to PI3K pathway inhibition is warranted.

The analysis of cfDNA within the context of this early-phase study also yielded several findings with broad implications. Importantly, we observed responses in patients with

undetectable *AKT1* E17K in pretreatment cfDNA. Our findings emphasize how low tumor burden and insufficient shedding of cfDNA into plasma can impact detection of actionable biomarkers in plasma and has downstream implications for genomic screening strategies that rely on this technology for patient selection. We also demonstrate how cfDNA can be used to detect intratumoral heterogeneity unappreciated by single site tissue biopsies and how serial monitoring cfDNA for *AKT1* mutations can serve as a surrogate for response and progression.

Although E17K is the most common *AKT1* mutation and was the focus of this study, other activating mutations in *AKT1-3* have been identified.[23] Among these, *AKT1* Q79K is the second most recurrent hotspot mutation after E17K (**Fig. S4**). Of the patients with non-E17K-mutations in this study, only those with *AKT1* Q79K demonstrated tumor regressions. Looking beyond *AKT1* E17K mutations to other mutant alleles in all three AKT isoforms, might therefore broaden the population of *AKT*-mutant patients that could benefit from AKT inhibitors.

Despite the promising progression free survival achieved with AZD5363 in patients with heavily pretreated *AKT1* E17K mutant breast and gynecologic cancers, the observed response rate was lower than with therapies targeting *EGFR*, *ALK*, *ROS1*, and *BRAF*.[24-26] Realizing the full potential of AZD5363 in *AKT1*-mutant cancers may require drug combinations. Overall, the strongest signal of activity was observed in ER+ breast cancer as well as endometrial cancers of the subtype associated with sensitivity to anti-estrogens. We also observed re-sensitization to AZD5363 following re-introduction of fulvestrant in an *ESR1*-mutant patient who had previously demonstrated resistance to both therapies, a finding consistent with preclinical data demonstrating reciprocal feedback between ER and downstream PI3K/AKT signaling.[19,27] Taken together, these data provide a strong rationale for combining AKT inhibition with anti-estrogen therapy in estrogen-dependent *AKT1*-mutant cancers.

In summary, we demonstrate that mutant *AKT1* is a rational therapeutic target for AZD5363 in diverse cancers. Unlike prior basket studies that sought to expand the indication of an FDA-approved drug previously studied extensively using traditional trial designs[28], we show that a drug can be successfully studied in a mutation-specific context even when the mutation is consistently rare across all populations. By incorporating comprehensive tissue- and plasma-based correlative studies, we elucidate the multifaceted genomic basis of response in a manner that facilitated simultaneous translational genomic discoveries and clinical hypothesis validation to inform future studies.

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Previous Presentations
Part of this work was previously presented at the NCI-EORTC-AACR International Conference on Molecular Targets and Cancer Therapeutics 2015 and ASCO Annual Meeting 2016

FIGURE LEGENDS

Figure 1: Integrated treatment outcome and genomics of *AKT1* E17K solid tumors. Data are shown for 58 patients evaluable for response and grouped by membership in the *AKT1* E17K-mutant breast, gynecological, and other solid tumor cohorts followed by those patients with non-E17K *AKT1* mutations. From top to bottom: best change from baseline in the target lesion diameter according to RECIST version 1.1 (gradient arrows reflect not evaluable); duration of therapy (days) and cross over to combination AZD5363 and fulvestrant; and genomic annotation from pre-treatment tumor tissue or cfDNA sequencing. All genes in the mutational heatmap, including *AKT1*, reflect results from pre-treatment centrally-determined genomic data rather than local testing. Twelve patients enrolled lacked genomic data (track: Genomic Data). Individual mutations are shown as annotated in the accompanying legend and subclonality was determined as described in the **Supplementary Appendix**. Individual annotation tracks annotate the cancer type, best response, the detection of *AKT1* E17K by ddPCR in baseline plasma samples, and the existence of pre-treatment genomic data from either tumor tissue or cfDNA sequencing.

Figure 2: Non-invasive monitoring of treatment response in cfDNA.
a. Imaging at baseline and 6 weeks after treatment initiation indicate a response (in red) to AZD5363 in an E17K-mutant ER-positive HER2-negative breast cancer that is confirmed molecularly with an initial decrease in, and persistently low levels of, the *AKT1* E17K burden in cfDNA. **b.** Tumor burden, indicated by a >50% decrease in *AKT1* E17K mutant allele fraction in circulating cfDNA, was evident in all but one patient (95.5%) by day 11 of cycle 1 of treatment, but did not correlate with outcome as measured by a duration on therapy of greater than 12 weeks (left). Data are shown for 23 patients with longitudinal cfDNA samples collected throughout treatment and who were positive for *AKT1* E17K by ddPCR at baseline. **c.** A decline of circulating *AKT1* E17K of >50% at day 21 compared to pretreatment was correlated with response to AKT inhibition (HR=0.1603; p-value = 0.00194, log-rank). **d.** In evaluable patients (see **Supplementary Appendix**), cfDNA progression (rise of *AKT1* E17K allele fraction of >50% above nadir) preceded radiographic progression by a median of 42 days (range: 0-113 days) Each line is a patient, all cfDNA collection time-points (grey dots) are shown normalized to the date of RECIST progression and the gray arrow is the start of therapy. Green filled circles correspond to the time-point of cfDNA progression as defined above and the red line indicates median lead time of cfDNA progression relative to radiological progression (green box is the 95% CI of lead times). The bottom-most patient had a radiological progression without *AKT1* E17K rise in cfDNA. **e.** Shown are multi-lesion tissue and cfDNA sequencing results (left) and the longitudinal profile of circulating *AKT1* E17K and *ESR1* D538G over the course of AZD5363 monotherapy as well as AZD5363 and fulvestrant combination therapy (right). Following initiation of combination therapy, the patient achieved a decline in circulating *AKT1* E17K and *ESR1* D538G, blood tumor markers, and a minor radiographic response lasting 8 months.

Figure 3: Clonality of the sensitizing biomarker.
a. For 37 patients with sufficient baseline sequencing data, *AKT1* E17K mutant allele frequency is shown (orange filled circle) as is the median allele frequency of all somatic mutations detected in each patient (horizontal line; vertical line is the median absolute deviation) from pre-treatment tumor tissue or cfDNA sequencing. Patients with focal amplification of *AKT1* E17K are indicated as red triangles, while those possessing subclonal *AKT1* E17K are shown as blue triangles. Patients are grouped as having a heterozygous *AKT1* E17K (left) and those possessing high mutant allele fraction (right). **b.** Schematic of the acquisition of AKT1 E17K (red line) mutant allele imbalance in this study cohort, beginning from a heterozygous mutation in a diploid genome and chromosome 14 (leftmost; maternal and paternal chromosomes are indicated). Allelic imbalance in the form of CN-LOH that duplicates the mutant allele (top) and can be followed by other serial genetic changes including genomic gains and whole-genome duplication (WGD) or either heterozygous loss

of the WT copy (bottom left) or whole-chromosome or more focal gains of the mutant allele (see also **Fig. S1-2**). **c.** *AKT1* E17K mutant allele imbalance by any of the mechanisms described in panel (b) is associated with improved PFS in response to AKT inhibition (median PFS of 8.2 versus 4.1 months, respectively; HR = 0.41, p=0.04). **d.** A patient with an ovarian granulosa cell tumor received AZD5363 for 8 months and achieved a best response of 24% tumor regression (right pelvic tumor regression shown, yellow), a notable response that was far greater than would have been predicted on the basis of the frequency of the sensitizing *AKT1* mutation. **e.** Sequencing of eight metastatic sites sampled prior to therapy revealed that whereas the earliest arising lesions were clonal (*FOXL2* and *TERT*), the *AKT1* mutation was variably subclonal across the lesions and was present at highest cellular fraction (67%, subclonal) in the right pelvic tumor that achieved the best response to AZD5363 therapy (labeled E in panel d). **f.** The presence of coincident activating mutations in either up- or downstream effectors of PI3K/mTOR signaling in AKT1 E17K-mutant tumors was associated with improved PFS (median not reached versus 4.3 months without such lesions, HR=0.21, p=0.045).

FIGURES

Figure 1: Integrated treatment outcome and genomics.

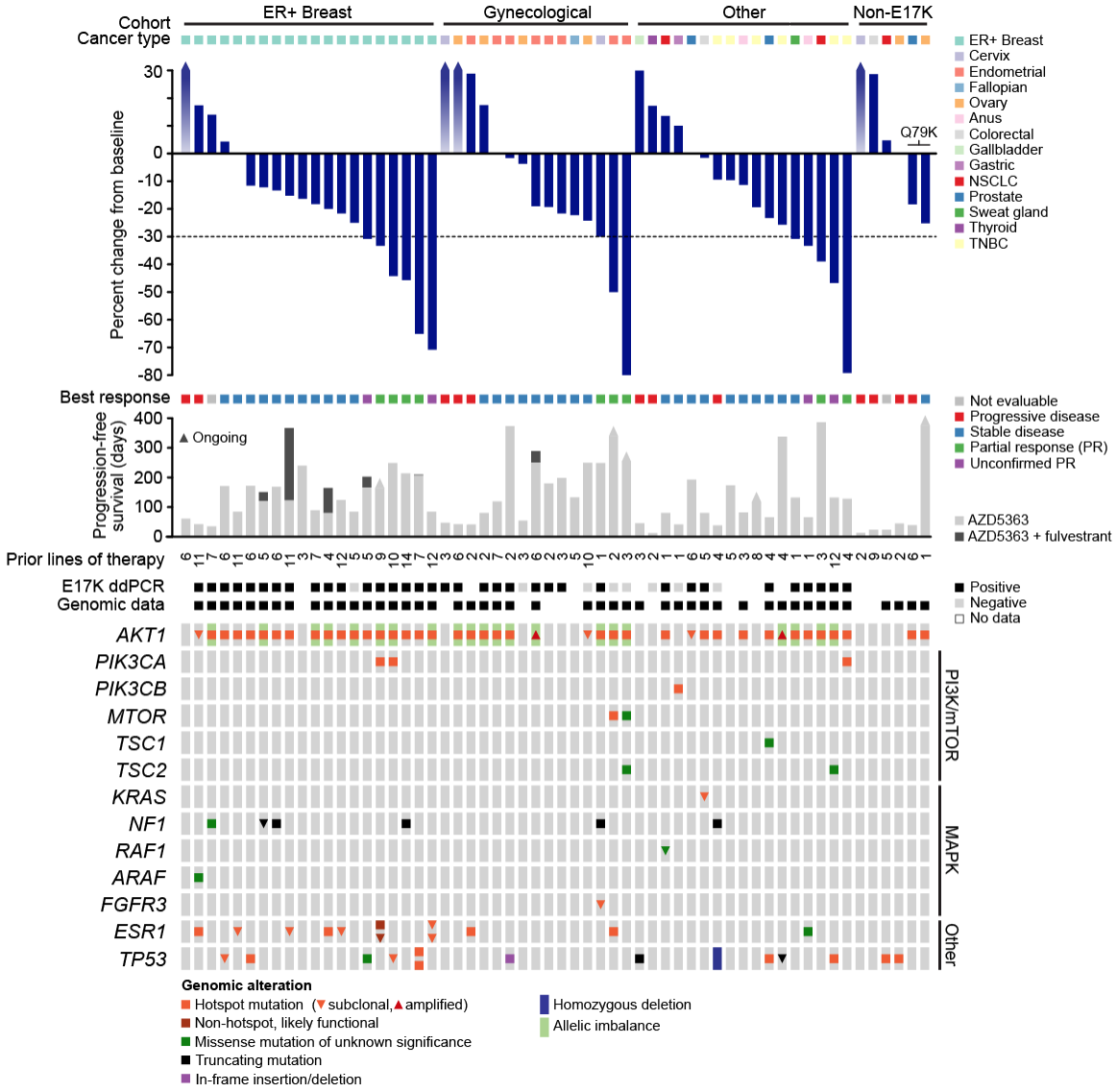
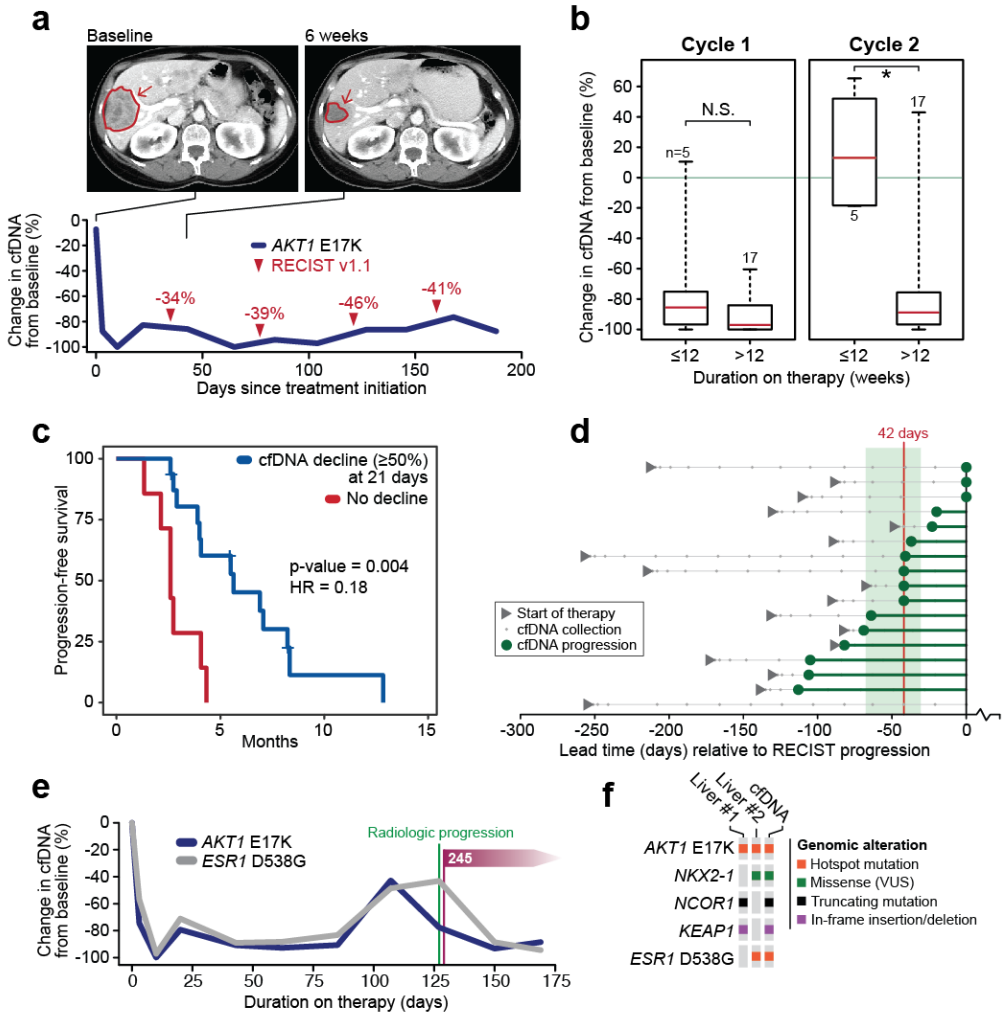


Figure 2: Non-invasive monitoring of treatment response in cfDNA.



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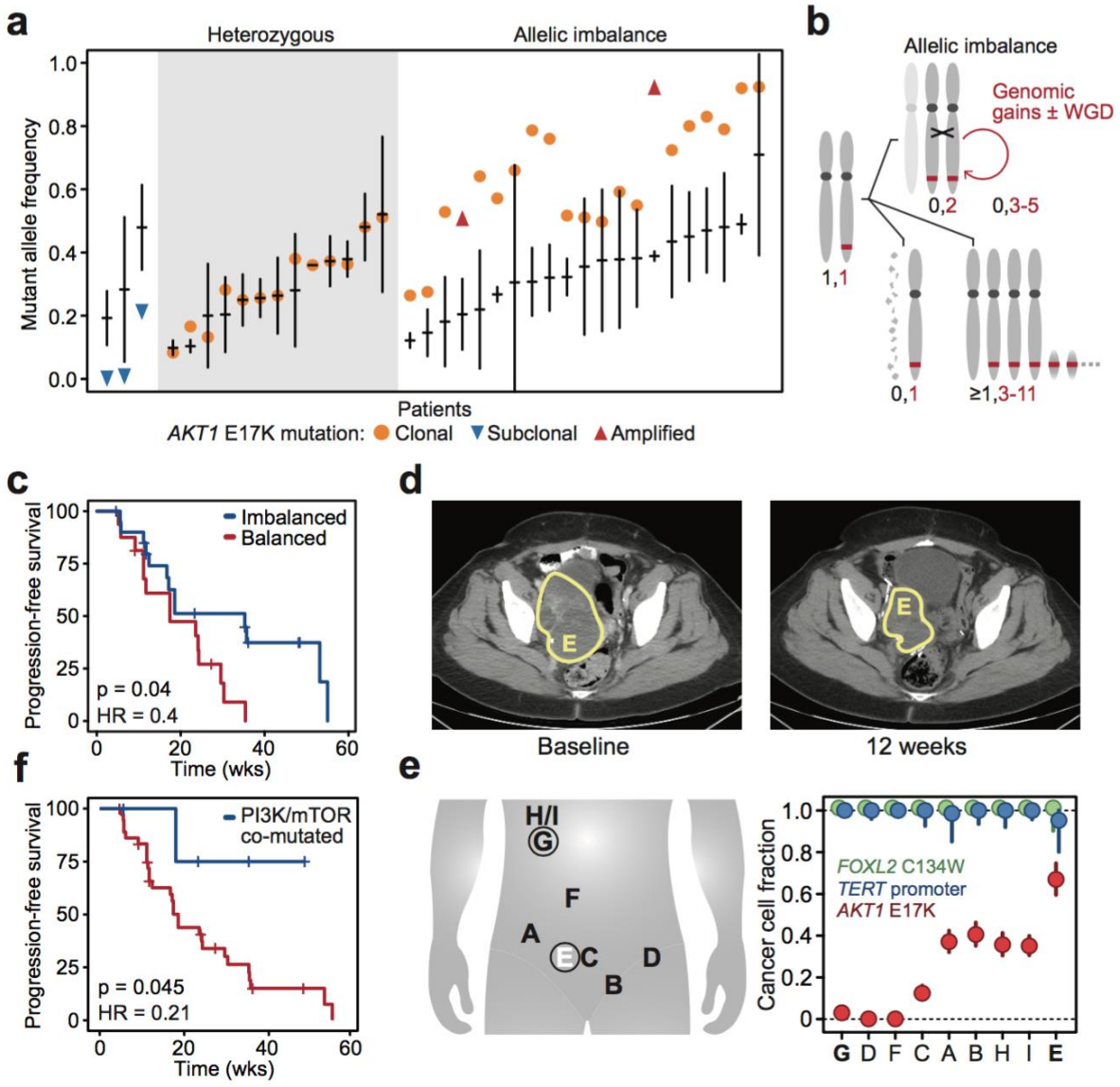

TABLES

Table 1: Patient demographics and baseline disease characteristics.

	Patient cohort			
	ER+ Breast	**Gynecological**	**Other**	**Total**
N	20	18	20	58
Median age (range), years	57 (38–71)	63 (46–71)	57 (31–77)	59 (31–77)
Gender, n (%)				
Male	0	-	5 (25)	5 (8.6)
Female	20 (100)	18 (100)	15 (75)	53 (91.4)
WHO performance status[a], n (%)				
0	10 (50)	9 (50)	8 (40)	27 (46.6)
1	10 (50)	9 (50)	12 (60)	31 (53.4)
Primary tumor site, n (%)				
ER+ Breast	20 (100)	-		20 (34.5)
Triple Negative Breast			6 (30)	6 (6.2)
Uterus	-	8 (44.4)	-	8 (13.8)
Ovary and fallopian tubes	-	7 (38.9)	-	7 (12)
Cervix	-	3 (16.7)	-	3 (5.2)
Lung	-	-	3 (15)	3 (5.2)
Prostate	-	-	3 (15)	3 (5.2)
Colon	-	-	2 (10)	2 (3.4)
Other	-	-	6 (30)[b]	6 (10.3)
Median Prior Lines of Systemic Therapy (range)	7 (3-14)	2.5 (1-10)	4 (1-12)	5 (1-14)
AKT1 mutation status[c], n (%)				
E17K	20 (100)	14 (77.8)	17 (85)	52 (89.7)
Other[d]	0	3 (16.7)	3 (15)	5(8.6)
Not detected	0	1 (5.6)	0	1 (1.7)

All patients in the full analysis set received at least one dose of AZD5363. [a] 0, fully active; 1, restricted in physically strenuous activity. [b] Bladder (n=1), stomach (n=1), thyroid (n=1), other (n=3). [c] Determined by local laboratories at baseline. [d] F35L, Q79K (x2), T34N, and V201A. WHO, World Health Organization.

Table 2: Adverse events. Shown here are the adverse events occurring in greater than 10% of patients overall, and of grade ≥3 severity occurring in two or more patients.

	Patients (N=58)	
AE, n (%)	**All grades**	**Grade ≥3**
Any AE (irrespective of causality)	58‡ (100)	41 (70.7)
Any AE (causally related)*	53 (91.4)	30 (51.7)
AE by preferred term (irrespective of causality)		
Diarrhea	45 (77.6)	10 (17.2)
Nausea	30 (51.7)	1 (1.7)
Fatigue	23 (39.7)	2 (3.4)
Vomiting	23 (39.7)	2 (3.4)
Hyperglycemia	22 (37.9)	14 (24.1)
Rash maculopapular	18 (31)	9 (15.5)
Abdominal pain	14 (24.1)	1 (1.7)
Decreased appetite	14 (24.1)	0
Pyrexia	11 (19)	0
Dizziness	10 (17.2)	1 (1.7)
Back pain	9 (15.5)	0
Cough	9 (15.5)	0
Dry mouth	9 (15.5)	0
Headache	9 (15.5)	1 (1.7)
Pain in extremity	9 (15.5)	3 (5.2)
Aspartate aminotransferase increased	8 (13.8)	2 (3.4)
Alanine aminotransferase increased	5 (8.6)	3 (5.2)
Edema peripheral	8 (13.8)	0
Stomatitis	8 (13.8)	1 (1.7)
Constipation	7 (12.1)	0
Hypertension	7 (12.1)	2 (3.4)
Nasal congestion	7 (12.1)	0
Pruritus	7 (12.1)	1 (1.7)
Blood creatinine increased	6 (10.3)	1 (1.7)
Dry skin	6 (10.3)	0
Hypokalemia	6 (10.3)	0
Micturition urgency	6 (10.3)	0
Myalgia	6 (10.3)	0
Urinary tract infection	5 (8.6)	2 (3.4)
Blood alkaline phosphatase increased	4 (6.9)	2 (3.4)
Dehydration	3 (5.2)	2 (3.4)
Sepsis	2 (3.4)	2 (3.4)
Small intestinal obstruction	2 (3.4)	2 (3.4)

All patients in the safety analysis set received at least one dose of AZD5363. A patient can have one or more preferred terms reported. Table includes AEs with an onset date on or after the date of first dose and up to and including 28 days following the date of last dose of study medication. At the time of data-cut, six patients (10.3%) had un-coded AEs, out of which two patients (3.4%) had un-coded AEs of grade 3 severity. *As assessed by the investigator. AE, adverse event. ‡Includes one patient mistakenly enrolled without an *AKT1* mutation, included in safety but not efficacy analysis set.

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