ESMO Open
Cancer Horizons

Genomic heterogeneity and efficacy of PI3K pathway inhibitors in patients with gynaecological cancer

Victor Rodriguez-Freixinos,[1] Fiorella Ruiz-Pace,[2] Lorena Fariñas-Madrid,[1] Ana Christina Garrido-Castro,[1,3] Guillermo Villacampa,[2] Paolo Nuciforo,[4] Ana Vivancos,[5] Rodrigo Dienstmann,[1,2] Ana Oaknin[1]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/esmoopen-2018-000444).

To cite: Rodriguez-Freixinos V, Ruiz-Pace F, Fariñas-Madrid L, *et al*. Genomic heterogeneity and efficacy of PI3K pathway inhibitors in patients with gynaecological cancer. *ESMO Open* 2019;**4**:e000444. doi:10.1136/esmoopen-2018-000444

Partial results of this study were previously presented at the American Society of Clinical Oncology 2017 Annual Meeting as a poster communication in the Gynecologic Cancer field.

Received 10 September 2018
Revised 23 November 2018
Accepted 24 November 2018

Published online
8 March 2019

For numbered affiliations see end of article.

Correspondence to
rdienstmann@vhio.net

ABSTRACT

Objectives Aberrant PI3K/AKT/mTOR activation is common in gynaecological malignancies. However, predictive biomarkers of response to PI3K pathway inhibitors (PAMi) have yet to be identified.

Methods We analysed the outcomes of patients with advanced gynaecological cancer with available genomic data, treated with PAMi as single agents or in combination in phase I clinical trials. Clinical relevance of the *PIK3CA* mutant allele fraction (MAF) was investigated. MAF of each variant was normalised for tumour purity in the sample (adjMAFs) to infer clonality of *PIK3CA* mutations, defined as clonal (≥0.4) or subclonal (<0.4).

Results A total of 50 patients with gynaecological cancer (24 ovarian; 15 endometrial; 11 cervical) with available targeted mutation profiling were selected. PAMi therapy was matched to *PIK3CA/PTEN* mutation in 30 patients (60%). The overall response rate, median time to progression (mTTP) and clinical benefit rate (CBR) of the entire population were 10% (N=5), 3.57 months (2.57–4.4) and 40% (N=18), respectively. Genotype-matched therapy did not lead to a favourable CBR (OR 0.91, p=1 (0.2–3.7)) or mTTP (3.57 months (2.6–4.4) vs 3.73 months (1.9–13.2); HR 1.41; p=0.29). We did not detect differences in mTTP according to therapy or *PIK3CA* codon mutation (HR 1.71, p=0.24). Overall, 41% of patients had a TTP ratio (TTP PAMi/TTP on immediately prior or subsequent palliative chemotherapy) ≥1.3, without statistically significant differences according to tumour type (p=0.39), molecular alteration status (p=0.13) or therapy (p=0.54). In univariate analysis, genotype-matched therapy in patients with *PIK3CA* clonal events was associated with improved mTTP (HR 3.6; p=0.03).

Conclusions Our study demonstrates that patients with advanced gynaecological cancer, refractory to standard therapies, achieved meaningful clinical benefit from PAMi. The impact of *PI3KCA* clonality on response to selected PAMi in patients with gynaecological cancer deserves further investigation.

Key questions

What is already known about this subject?

- The PI3K pathway is often dysregulated in gynaecological malignancies.
- The current understanding of molecular predictors of response to PI3K/AKT/mTOR inhibitors is limited.
- Clonal evolution of *PIK3CA* mutations has not been investigated as a predictive biomarker of response to PI3K/AKT/mTOR inhibitors in gynaecological malignancies.

What does this study add?

- Our study is one of the largest series reported of gynaecological tumours that have been prospectively analysed with next-generation sequencing through an institutional screening programme and treated with PI3K/AKT/mTOR inhibitors.
- Patients with advanced gynaecological cancer refractory to standard therapies achieved meaningful clinical benefit from PI3K/AKT/mTOR inhibitors.
- *PI3KCA* clonality impacted on response to selected PI3K/AKT/mTOR inhibitors in patients with gynaecological cancer.

How might this impact on clinical practice?

- Despite existing barriers that limit access to genotype-matched therapies for gynaecological cancers, and the complexity of PI3K pathway inhibition, our data suggest that estimating *PIK3CA* mutation clonality may be important to guide the selection of PI3K/AKT/mTOR inhibitors in patients with advanced gynaecological cancer.

INTRODUCTION

The PI3K pathway is often dysregulated in gynaecological malignancies,[1–4] and it has been assessed as a target for novel therapeutic strategies over the last decade. However, despite preliminary evidence of meaningful clinical benefit with PI3K/AKT/mTOR inhibitors (PAMi), particularly among patients with endometrial and ovarian cancer,[5] our current understanding of molecular predictors of response is limited. Research efforts are ongoing to further elucidate the mechanisms of response and resistance to these drugs. Preclinical studies suggest that activating oncogenic mutations in *PIK3CA* and/or loss of PTEN expression predict response to PAMi in gynaecological malignancies.[6–8] Conversely, mutations in mitogen-activated protein kinase (MAPK)

pathway, such as *KRAS, NRAS* and *BRAF*, might be associated with innate resistance to PAMi.[6–8] The integration of next-generation sequencing (NGS) platforms to both prescreening programmes and clinical trials has revealed a wide spectrum of clinically relevant genomic alterations in the PI3K and MAPK pathways, improving our understanding of the genomic drivers of gynaecological malignancies.[9] New insights in solid tumours are providing a deeper understanding of the PI3K pathway and its targetability. Development of second-generation drugs, such as isotype-specific PI3K inhibitors, has been associated with improved outcomes in patients with non-colorectal *PI3KCA*-mutated cancer treated in early-phase clinical trials.[10] Clonal evolution of *PIK3CA* mutations has also been investigated as a predictive biomarker of response to PAMi in patients with breast[11] or colorectal[12] cancer, but the results were not conclusive.

Mutant allele fractions (MAFs), defined as the number of mutant reads divided by the total number of reads (coverage) at a specific genomic position, may influence prognosis and response to targeted therapies, including EGFR kinase inhibitors in *EGFR*-mutated lung cancer.[12–16] Although MAFs of driver genes are more likely to be clonal compared with MAFs of those that are not considered putative drivers, studies have shown that genes involved in the PI3K pathway, such as *PIK3CA*, have a higher proportion of subclonal events than those in the MAPK pathway.[17] Due to the strong influence of both tumour purity (fraction of neoplastic cells in the sample) and ploidy (either copy number gains or losses of wild-type/mutant alleles) on MAFs, the 'adjusted MAF' (adjMAF) for driver genes has been used to describe a clonal or subclonal distribution in individual tumour samples.[12]

Here, we investigated the feasibility and utility of conducting PI3K and MAPK pathway genomic characterisation in a cohort of patients with advanced gynaecological cancer enrolled on a prospective genomic profiling protocol at the Vall d'Hebron Institute of Oncology (VHIO). As an exploratory objective, we analysed the association of clinical and molecular markers, such as *PIK3CA*-mutated variants and clonality, with the magnitude of response and clinical benefit with genotype-matched PAMi in early phase I clinical trials.

MATERIALS AND METHODS

Patient population

Our study cohort included patients with advanced gynaecological malignancies who had experienced disease progression with standard regimens and provided consent for NGS at VHIO from March 2010 to November 2016 as part of a molecular prescreening programme (MPP) for early drug development trials. Patients who were opportunistically enrolled onto phase I clinical trials with PAMi, as a single-agent or in combination with other targeted drugs, were included in our study cohort. We excluded patients who received PAMi combined with cytotoxic drugs. Clinical data were retrospectively extracted from medical records by data curators from the Oncology Data Science (ODysSey) group.

Specimens

Archival tumour specimens were obtained from a biopsy or surgical resection of a primary or metastatic site of disease. Tumour area content from sections of formalin-fixed paraffin-embedded tissue was evaluated by a pathologist. A minimum tumour content of 20% was required in order to allow subclonal somatic mutation detection. Tumour purity was defined as the amount of sample occupied by cancer cells and not by surrounding stromal and immune/inflammatory cells. To minimise variability, the quantification of neoplastic cells was performed by an experienced pathologist (PN) in the same section used for sequencing, as recently recommended by other groups.[18]

Mutation analysis

NGS testing was performed at the VHIO Cancer Genomics Lab (UNE-EN ISO 15189:2013). Two molecular profiling assays were used over the study period. Between January 2010 and May 2014, mutation detection and quantification was performed using a multiplex mass spectrometry–based technology platform (massARRAY Sequenom), targeting hotspot mutations across 24 oncogenes, including frequent variants in *PIK3CA*, *AKT1*, *KRAS*, *NRAS* and *BRAF*). In June 2014, we transitioned to an amplicon-based NGS technology (MiSeq Illumina) platform assessing a total of 61 oncogenes and tumour suppressor genes, including frequently mutated exons of *PIK3CA*, *AKT1*, *PTEN*, *KRAS*, *NRAS* and *BRAF*. Average sequencing depth was 1000x allowing precise estimates for low MAFs (mutations were called at a minimum MAF of 3%). The calculated adjMAFs (MAF/tumour purity) of driver genes of interest were used to infer clonality of the events. A cut-off point of 0.4 was used taking the reference of the mean value of adjMAF in our population. We defined adjMAF ≥0.4 in *PIK3CA* as clonal and <0.4 as subclonal (online supplementary methods).

Clinical data collection and efficacy endpoints

Baseline patient and tumour characteristics, treatment regimen(s) and response were retrieved from medical records. Patients were categorised in two groups based on tumour mutational status: (1) PI3K-altered (*PIK3CA*, *AKT1* and/or *PTEN* mutations) cohort that received PAMi (genotype-matched); and (2) PI3K non-altered cohort that received PAMi. Response was assessed per Response Evaluation Criteria in Solid Tumours (RECIST) V.1.1. Time to progression (TTP) was defined as the time interval from the start of treatment to discontinuation due to disease progression or death, whichever occurred first (patients with permanent treatment discontinuation for toxicity without evidence of progressive disease were censored at the time of the last dose). Clinical benefit rate (CBR) was defined as the proportion of patients achieving complete response, partial response or stable disease >4

months. Median TTP on palliative chemotherapy given immediately before or after PAMi was estimated. The ratio of TTP on PAMi to TTP on chemotherapy was calculated and considered clinically meaningful if ≥1.3.[19]

Statistical analysis

A descriptive analysis of the variables included in the study was performed. Continuous variables were expressed as median and range or IQR, and categorical variables were expressed as absolute values and percentages. For the univariate analysis, Fisher's exact test was used for categorical variables and the Mann-Whitney U test for continuous variables. Survival analysis was calculated using the Kaplan-Meier method and log-rank test was used for statistical comparison. Multivariate Cox proportional-hazards models were used to obtain HRs with 95% CIs. All tests were two-sided, and a p value <0.05 was considered statistically significant. The data analyses were carried out using R V.3.2.3 statistical software and survival package.

RESULTS

Patient cohort

Between March 2010 and November 2016, a total of 264 patients with gynaecological cancer had available results from targeted NGS, including 152 patients with ovarian, 75 endometrial and 37 cervical cancer. Of these, 50 patients received PAMi treatment, regardless of PI3K pathway mutation status, of which 24 patients (48%) had ovarian cancer, 15 (30%) had endometrial and 11 (22%) had cervical carcinoma. Median age was 57 years (range, 30 to 70 years). Patients had received a median of two prior lines of systemic treatments (range, 1–6). Patient characteristics are detailed in table 1.

Molecular profiling

Of the PAMi-treated cohort, 32 patients (64%) had a sample tested using the massARRAY Sequenom platform and 18 (36%) were tested with the MiSeq Illumina platform. One or more somatic mutations were detected in 39 (78%) patients. Sequencing was mostly performed on samples derived from primary archival tissue (n=40; 80%) versus metastatic specimens (n=10; 20%). The prevalence of oncogene mutations in our cohort is depicted in figure 1A. The most frequently mutated gene was *PIK3CA* (52%), followed by *KRAS* (24%), *PTEN* (12%), *AKT1* (2%) and *NRAS* (2%). *PIK3CA* mutations were detected in 11 (46%) of 24 patients with ovarian cancer, 6 (40%) of 15 patients with endometrial cancer and 9 (82%) out of 11 patients with cervical cancer treated with PAMi. Mutations in exon 9 of *PIK3CA* were found in 17 (34%) patients and 9 (18%) patients had *PIK3CA* exon 20 mutations. The most frequent mutation was E545K (1633G>A) in nine (18%) patients, followed by H1047R (3140A>G) in eight (16%) patients and E542K (1624G>A) in four (8%) patients. Concomitant MAPK pathway (*KRAS*, *NRAS*) mutations and *PIK3CA* were identified in three (6%) patients (two with cervical cancer and one with endometrial cancer). *NRAS* mutation was detected in one (2%) patient and another patient had concomitant *KRAS/NRAS* mutations.

Median adjMAF of *PIK3CA* was 0.36 (IQR 0.25–0.49; range, 0.12–1.0), with 10 of 26 cases (38%) having potentially subclonal events (adjMAF <0.4). We explored differences in adjMAFs of *PIK3CA* according to histology and found no significant differences (p=0.81) (online supplementary figure 1).

Clinical trials and outcomes

Distribution of primary tumour type in the PI3K-altered cohort of 30 patients who received PAMi was 11 (37%) ovarian, 10 (33%) endometrial and 9 (30%) cervical cancers (figure 1B). Most therapies (80%) were given at recommended doses as part of the expansion cohorts of phase I clinical trials (table 1). Table 2 details genotype-matched (PI3K-altered cohort that received PAMi) and unmatched trials (PI3K non-altered cohort that received PAMi) across tumour types. Most matched trials in patients with ovarian and cervical cancer consisted of single-agent PI3K-alpha inhibitors as opposed to patients with endometrial cancer who mostly enrolled on trials investigating pan-PI3K/mTOR (table 2).

The overall response rate (ORR) for the overall cohort was 10% (5 partial response; 0 complete response), without significant differences between patients enrolled on genotype-matched and genotype-unmatched clinical trials (13% vs 5%, respectively; p=1). Responses were observed across different tumour types, including rare histologies such as granulosa cell or clear cell ovarian cancers (online supplementary table 1).

No differences in ORR were found among patients treated with combinations of agents compared with patients treated with single agent therapies (p=1). The CBR was 40%; 37% in the genotype-matched cohort and 39% in the unmatched cohort. Median TTP of PAMi-treated patients was 3.57 months (95% CI 2.57 to 4.4 months). The median TTP in patients with ovarian cancer was 4.2 months compared with 3.3 months in endometrial and 2.3 months in cervical cancer (HR ovarian vs others 0.59; p=0.096; figure 2A). There was no significant association between the presence of PI3K pathway gene mutations and either CBR (OR 0.91, p=1 (0.2–3.7)) or median TTP (3.57 months (95% CI 2.6 to 4.4) vs 3.73 months (95% CI 1.9 to 13.2); HR 1.41; p=0.29) (figure 2B). Genotype-matched treatment did not improve median TTP among any tumour type (online supplementary figure 2). Median TTP did not significantly differ according to *PIK3CA* codon mutation (exon 20: 4.40 months (95% CI 4.17 to NA); exon 9: 2.63 months (95% CI 1.63 to 8.83); HR 1.71, p=0.24) (online supplementary figure 3). Due to the interest in the development of isotype-specific PI3K inhibitors, we investigated the efficacy of p110α inhibitors. In the 26 patients treated with p110α inhibitors, no significant association was observed between CBR and *PIK3CA* mutational status (OR 0.36; p=0.56 (0.005–7.9)) (table 3).

Table 1 Patients' characteristics

Variables	All (N=50)	Genotype-matched (PI3K altered) (N=30)	Genotype-unmatched (PI3K non-altered) (N=20)	P value (univariate)
Median (range)	57 (30–70)	55 (30–70)	59 (34–68)	0.69
Subtype/histology				
Ovarian	24 (48%)	11 (37%)	13 (65%)	0.12
Serous papillary	9	1	8	
Clear cell	5	5	0	
Endometrioid	3	2	1	
Granulosa cell	3	2	1	
Mucinous	3	0	3	
Carcinosarcoma	1	1	0	
Endometrial	15 (30%)	10 (33%)	5 (25%)	
Endometrioid	13	9	4	
Serous papillary	2	1	1	
Cervical	11 (22%)	9 (30%)	2 (10%)	
Squamous	8	7	1	
Adenocarcinoma	3	2	1	
Prior metastatic lines				
Median (range)	2 (1–6)	2 (1–5)	2.5 (1–6)	0.69
≤2	29 (58%)	19 (63%)	10 (50%)	0.39
>2	21 (42%)	11 (37%)	10 (50%)	
Molecular alterations				
*PIK3CA**	20 (40%)	20 (67%)	0 (0)	
*PTEN**	4 (8%)	4 (13%)	0 (0)	
PIK3CA+PTEN†	2 (4%)	2 (7%)	0 (0)	
PIK3CA+AKT1†	1 (2%)	1 (3%)	0 (0)	
PIK3CA+KRAS/NRAS†	3 (6%)	3 (10%)	0 (0)	
*KRAS**	9 (18%)	0 (0)	9 (45%)	
Trial dose				
Escalation	10 (20%)	6 (20%)	4 (20%)	1
Expansion	40 (80%)	24 (80%)	16 (80%)	
Trial best response				
SD	28 (56%)	14 (47%)	14 (70%)	
PD	14 (28%)	10 (33%)	4 (20%)	
PR	5 (10%)	4 (13%)	1 (5%)	
Unknown	3 (6%)	2 (7%)	1 (5%)	
Trial reason for therapy discontinuation				
Progression	33 (66%)	22 (73%)	11 (55%)	
Toxicity	10 (20%)	3 (10%)	7 (35%)	
Other	7 (14%)	5 (17%)	2 (10%)	

*Alone.
†Co-mutation.
PD, progressive disease; PR, partial response; SD, stable disease.

Differences in TTP according to therapy were also assessed. We did not identify statistically significant differences in median TTP between PAMi combos, pan-PI3K/mTOR/AKT inhibitors and PI3K-alpha inhibitors (2.57 months, 3.73 months and 3.57 months, respectively; p=0.5) (online supplementary figure 4). We also

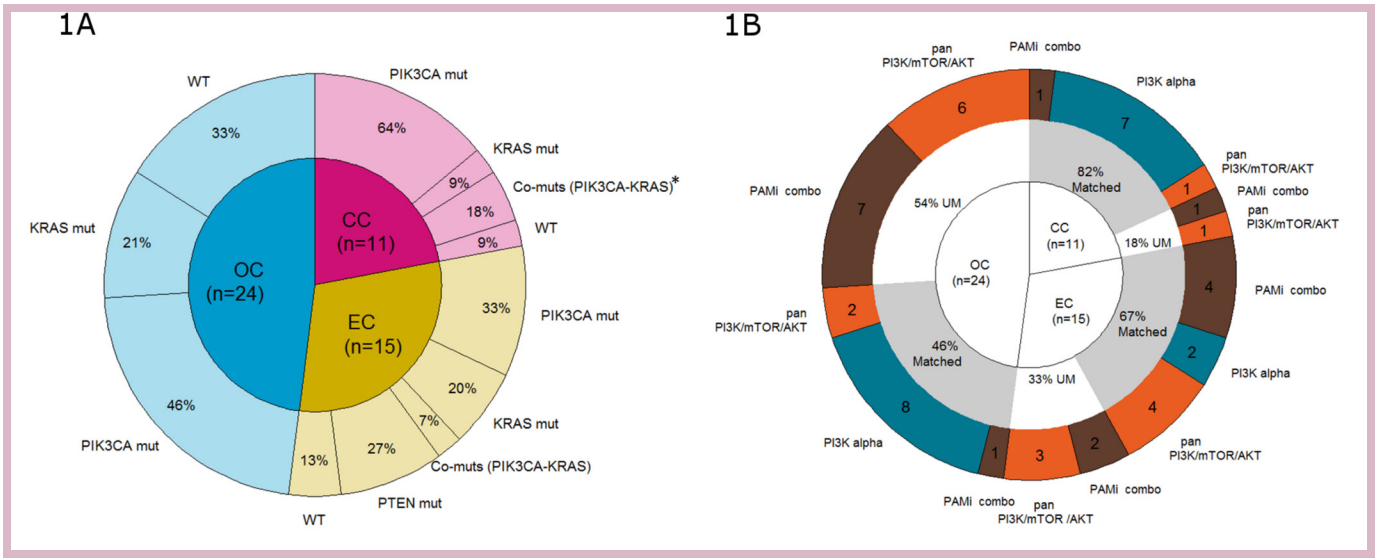

Figure 1 (A) Molecular alterations by primary tumour type. CC, cervical cancer; EC, endometrial cancer; mut, mutation; OC, ovarian cancer; WT, wild type for *PIK3CA*, *AKT1, PTEN*, *KRAS*, *NRAS* or *HRAS* mutations. *One of the patients also has NRAS mutation. (B) Distribution of PAMi treatments by primary tumour type. CC, cervical cancer; EC, endometrial cancer; OC: ovarian cancer; PAMi, PI3K/Akt/mTOR inhibitors; UM, genotype-unmatched.

investigated the potential impact of *PIK3CA* clonality on TTP with PAMi. Among patients with *PIK3CA* mutations, we found significant improvement of TTP with genotype-matched therapy in the presence of clonal events (4 months, 95% CI 3.5 to NA) compared with subclonal events (2.3 months, 95% CI 1.4 to NA; HR 3.6, p=0.03) (online supplementary figure 5).

Differences in TTP for genotype-matched and unmatched therapeutic trials compared with TTP on either the immediately prior or subsequent palliative chemotherapy (TTP PAMi:chemotherapy ratio) were also analysed in a total of 27 patients with available information (online supplementary table 2). Overall, 41% of patients obtained a TTP PAMi:chemotherapy ratio ≥1.3, without statistically significant differences according to tumour type (p=0.39), molecular alteration status (p=0.13), treatment regimen (p=0.54) or *PIK3CA* clonality (p=0.52) (figure 2C).

DISCUSSION

Despite diversity in the molecular landscape and patterns of clinical outcomes across gynaecological malignancies, activation of the PI3K/AKT/mTOR pathway is common to these tumours, suggesting that PAMi may have therapeutic relevance. However, clinical trials evaluating PAMi in advanced gynaecological malignancies have shown a modest therapeutic impact.[20] In addition, reliable biomarkers to guide patient selection for treatment with PAMi have yet to be identified. In our study, we demonstrate that patients with advanced gynaecological cancer, refractory to standard therapies, achieved meaningful clinical benefit with PAMi, regardless of the presence or absence of mutations in the PI3K/AKT/mTOR pathway.

Although the precision medicine era in cancer therapy is rapidly evolving, the integration of comprehensive genomic profiling in routine clinical practice has been less robust in gynaecological malignancies than in other tumour types such as melanoma and non-small cell lung cancer in which successful biomarker-based selection of treatment has been widely documented.[21–24] Nevertheless, over the past years, somatic tumour profiling has yielded important insights to tumour biology and has contributed to identify a wide variety of potentially targetable alterations, such as *BRCA1/BRCA2* mutations in patients with high-grade serous ovarian cancer[25 26] and potentially actionable PI3K/AKT/mTOR pathway alterations,[27–33] expanding therapeutic opportunities for patients with gynaecological cancer. To our knowledge, our study is one of the largest series reported to date of gynaecological tumours that have been prospectively analysed with NGS through an institutional screening programme and treated with PAMi.[34 35] We restricted targeted therapy to monotherapy or combinations with other targeted drugs, excluding combinations with chemotherapy in an attempt to avoid a confounding effect when studying the predictive value of PI3K pathway alterations for matched therapies. We were able to identify at least one somatic mutation in the majority (78%) of patients included in this cohort (*PIK3CA* in 52%, *PTEN* in 12% and *AKT1* in 2%), confirming the relevance of PI3K pathway alterations in gynaecological malignancies and our enrichment strategy in early clinical trials. This study provides valuable information regarding the impact of clinical and genomic heterogeneity on the efficacy of PAMi across multiple types of gynaecological tumours, including rare histologies such as clear cell, granulosa cell, mucinous or carcinosarcomas. We demonstrated a modest 10% ORR

Table 2 Characteristics of PAMi treatment by primary tumour type

Tumour primary	Target	Genotype-matched (PI3K altered) (N=30)	Genotype-unmatched (PI3K non-altered) (N=20)
Ovarian (N=24)			
PI3K alpha	PI3Kalpha	8 (33%)	0 (0)
Pan PI3K/ mTOR or AKT	PI3KmTOR	1 (4%)	3 (13%)
	PanPI3K	1 (4%)	0 (0)
	mTOR	0 (0)	1 (4%)
	AKT	0 (0)	2 (8%)
PAMi combos	PanPI3K+MEK	0 (0)	4 (17%)
	PI3Kalpha+MEK	0 (0)	2 (8%)
	PI3Kalpha+IGF1R	1 (4%)	1 (4%)
Endometrial (N=15)			
PI3K alpha	PI3Kalpha	2 (13%)	0 (0)
Pan PI3K/ mTOR or AKT	PI3KmTOR	0 (0)	3 (20%)
	PanPI3K	1 (7%)	0 (0)
	mTOR	2 (13%)	0 (0)
	PI3Kbeta	1 (7%)	0 (0)
PAMi combos	PI3Kalpha+FGFR	1 (7%)	0 (0)
	AKT+MEK	0 (0)	1 (7%)
	PI3Kalpha+SYK	0 (0)	1 (7%)
	PI3Kbeta+mTOR	1 (7%)	0 (0)
	PI3Kalpha+mTOR	2 (13%)	0 (0)
Cervical (N=11)			
PI3K alpha	PI3Kalpha	7 (64%)	0 (0)
Pan PI3K/ mTOR or AKT	PI3KmTOR	1 (9%)	1 (9%)
PAMi combos	panPI3K+MEK	0 (0)	1 (9%)
	PI3Kalpha+mTOR	1 (9%)	0 (0)

PAMi, PI3K/AKT/mTOR inhibitors.

and median TTP of 3.27 months in the overall cohort. Despite a heavily pretreated population lacking approved therapeutic options, the observed 40% CBR is clinically noteworthy. Sixty per cent of patients were enrolled onto genotype-matched trials, mostly PI3Kalpha inhibitors. However, none of the clinical outcomes that were assessed (ORR, TTP or CBR) were found to be associated with either the type of treatment (PAMi monotherapy vs combinations), tumour primary or *PIK3CA* mutational status. A key finding of our study is that 41% of patients achieved 30% increased TTP with PAMi compared with palliative chemotherapy given either immediately prior to or subsequently after experimental regimens, irrespective of their mutational status. Although a non-randomised comparison, this finding comprises an important and clinically meaningful metric in a cohort of patients with heavily pretreated gynaecological cancer with limited standard therapeutic options. Other studies found modest activity with single-agent mTOR inhibitors treatment, even in the setting of PI3K pathway activation, via mutation or amplification.[36–44] Single-agent PI3K inhibitors also failed to demonstrate meaningful impact on gynaecological malignancies and again, *PIK3CA* mutational status did not correlate with responses.[45–51] The *PIK3CA* mutant variant may also impact response to PAMi,[52] with *PIK3CA* H1047R mutations, which occur within the highly conserved kinase domain (exon 20), associated to increased likelihood of response to PAMi compared with other pathway aberrations.[52]

Comprehensive NGS testing may increase the number of patients with gynaecological cancer who are eligible for clinical trials assessing PAMi-based combinations. Several academic centres have demonstrated that targeted NGS can be used in routine clinical practice. However, despite the elevated proportion (75%–90%) of potentially actionable alterations detected, genomic profiling data are used as criteria for selection of a targeted therapy or clinical trial in only 5%–10% of cases.[34 35 53–62] Some reports have focused on the experience of PAMi among a cohort of patients with gynaecological cancer enrolled onto early phase I clinical trials. In a retrospective analysis of 140 patients with breast and gynaecological cancer treated with PAMi at the MD Anderson Cancer Center, a higher response rate to PAMi was observed in *PIK3CA*-mutated versus *PIK3CA*-wild-type tumours[5] and the majority of responses were seen in patients receiving combination therapies with PAMi and cytotoxic drugs. Another study in 55 patients with cervical cancer demonstrated that genotype-matched therapy with PAMi led to a favourable CBR at 6 months (53%) and significantly greater progression-free survival (PFS) than non-matched therapies.[35] Limited activity of single-agent PAMi, particularly mTOR inhibitors, has been reported in patients with tumours harbouring *KRAS* mutations.[63–65] In our study, of the four patients with *KRAS* mutations who were treated with single-agent PAMi, none derived clinical benefit. Additionally, we explored the impact of clonality of *PIK3CA* mutations on PAMi efficacy. *PIK3CA* clonal events, defined by adjMAF >0.4, were identified in 62% (100%–38%) of tumours, with a higher proportion in ovarian cancer. In univariate analysis, we found an association between clonal *PIK3CA* events and improved outcomes under PI3K inhibitors. Clonality of *AKT* E17K mutations in solid tumours, including gynaecological cancers, frequently exhibited selection against the remaining wild-type allele, by duplication of the mutant *AKT1* allele via copy-neutral loss of heterozygosity. Interestingly, this *AKT1* E17K allelic imbalance was shown to influence the response to AZD5363 (AKT inhibitor) (median PFS, 8.2 vs 4.1 months, respectively; HR 0.41; p=0.04).[66] Contradictory, clonality of *PIK3CA* mutations did not predict benefit with matched targeted agents in patients with colorectal cancer.[10] These results were partially explained by the limited activity in the *PIK3CA/KRAS* co-mutant populations,[10] known to confer primary resistance to

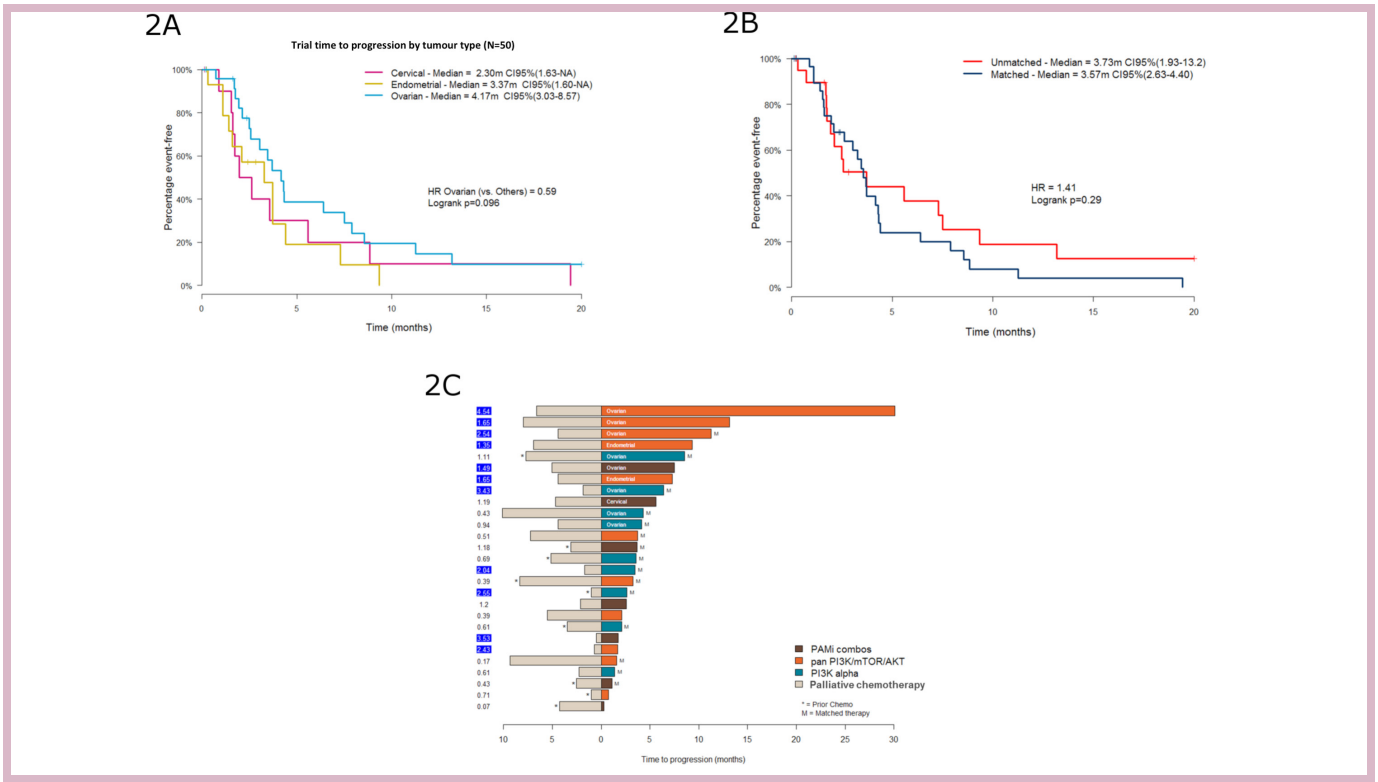

Figure 2 (A) Trial time to progression (TTP) by tumour type. (B) Trial TTP by genotype-matched therapy (N=50). (C) Differences in TTP for therapeutic trials compared with immediate prior (*) or posterior palliative chemotherapy. Patients achieving TTP PAMi:palliative chemotherapy ratio ≥1.3 are coloured in dark blue. Primary tumour site of patients achieving TTP >4 months with PAMi is shown. M, genotype-matched clinical trials; PI3K alpha, PI3K alpha inhibitor; PAMi, PI3K/Akt/mTOR inhibitors; pan PI3K/mTOR/AKT, PI3K/mTOR/AKT inhibitors.

PAMi as single agents.[67–71] The association between MAFs of driver genes identified through circulating tumour DNA (ctDNA) NGS and clinical outcomes with targeted therapy may potentially yield findings with broad implications. A recent report has evidenced clinical responses in patients with undetectable *AKT1* E17K in pretreatment ctDNA, which suggests that low tumour burden can negatively impact genomic screening strategies that rely on this technology for patient selection; however, serial ctDNA monitoring for *AKT1* mutations might be used as a surrogate marker of treatment response or disease progression.[66]

The identification of reliable biomarkers to guide appropriate patient selection for PAMi remains a significant challenge. Multiple mechanisms, not necessarily related to the intrinsic activation status of the PI3K pathway, have been attributed to the modest activity of PAMi demonstrated across a variety of solid tumours, including intrapathway or interpathway cross-talks or alternate signalling cascades, such as activation of the MAPK pathway following treatment with PAMi.[67–71] Despite existing barriers that limit access to genotype-matched therapies and clinical trials for gynaecological cancers, and the complexity of PI3K pathway inhibition, our data suggest promising activity of PAMi

Table 3 Clinical benefit obtained with PAMi

		Clinical benefit			
		No	Yes	OR (95% CI)	P value
PI3K pathway alterations (N=50)	No	11 (61%)	7 (39%)	0.91 (0.2 to 3.7)	1
	Yes	19 (63%)	11 (37%)		
PIK3CA status (N=50)	Wt	15 (68%)	7 (32%)	1.56 (0.4 to 6.2)	0.5
	Mut	15 (58%)	11 (42%)		
Patients treated with PI3Kalpha inhibitors, according to PIK3CA status (N=26)*	Wt†	1 (33%)	2 (67%)	0.36 (0.005 to 7.9)	0.56
	Mut	13 (59%)	9 (41%)		

*One *PIK3CA* wild-type patient not assessed for clinical benefit.
†Patients treated with PI3K alpha inhibitor in either monotherapy or combination.
Mut, mutation; Wt, wild type.

in patients with heavily pretreated gynaecological cancer, regardless of the presence of mutations in the pathway. Despite the small sample size and the lack of adjustment for copy-number analysis when estimating mutation clonality, *PIK3CA* clonal mutations may be important to guide the selection of targeted therapy in patients with advanced gynaecological cancer. This may become even more relevant when the dynamics of clonal evolution is taken into consideration, with consecutive liquid biopsies to assess mutation clonality. Furthermore, intratumoural heterogeneity has been proposed as the main cause of treatment failure and drug resistance in ovarian cancer and other primary cancer,[72] and we noted that in our study, 80% of analyses were performed in primary tumour samples raising the question whether tumour evolution and the degree of intratumoural heterogeneity might have impacted on our results. However, difficulties in collection of longitudinal samples from patients with cancer and the high costs of genomic profiling must be recognised. Additional correlative analyses from ongoing studies with PAMi and improved access to biomarker-matched ('basket') clinical trials are required to help elucidate the role of PAMi in gynaecological cancer and improve patient selection for these targeted therapies.

Author affiliations
[1]Medical Oncology Department, Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
[2]Oncology Data Science (ODySSey) Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
[3]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA
[4]Molecular Oncology Laboratory, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
[5]Cancer Genomics Laboratory, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Acknowledgements The authors acknowledge the VHIO for supporting research in gynaecological malignancies and the Cellex Foundation for providing research facilities and equipment.

Contributors VR-F, FR-P, GV, RD and AO collected, analysed and interpreted the data. RD, PN and AV were involved in molecular data generation and interpretation. VR-F, LF-M, ACG-C and AO were involved in clinical data generation and interpretation.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests AO is a consultant/advisory board member of AstraZeneca, Clovis, Tesaro, Roche and PharmaMar. RD reports personal fees from Roche, outside the submitted work.

Patient consent for publication Obtained.

Ethics approval All clinical trials were conducted in accordance with the guidelines of the VHIO Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Levine DA, Bogomolniy F, Yee CJ, *et al*. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. *Clin Cancer Res* 2005;11:2875–8.
2. Janku F, Tsimberidou AM, Garrido-Laguna I, *et al*. PIK3CA mutations in patients with advanced cancers treated with PI3K/Akt/mTOR axis inhibitors. *Mol Cancer Ther* 2011;10:558–65.
3. Miyake T, Yoshino K, Enomoto T, *et al*. PIK3CA gene mutations and amplifications in uterine cancers, identified by methods that avoid confounding by PIK3CA pseudogene sequences. *Cancer Lett* 2008;261:120–6.
4. Hayes MP, Wang H, Espinal-Witter R, *et al*. PIK3CA and PTEN mutations in uterine endometrioid carcinoma and complex atypical hyperplasia. *Clin Cancer Res* 2006;12:5932–5.
5. Janku F, Wheler JJ, Westin SN, *et al*. PI3K/AKT/mTOR inhibitors in patients with breast and gynecologic malignancies harboring *PIK3CA* mutations. *JCO* 2012;30:777–82.
6. Ihle NT, Lemos R, Wipf P, *et al*. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer Res* 2009;69:143–50.
7. Di Nicolantonio F, Arena S, Tabernero J, *et al*. Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. *J Clin Invest* 2010;120:2858–66.
8. Engelman JA, Chen L, Tan X, *et al*. Effective use of PI3K and MEK inhibitors to treat mutant KRAS G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 2008;14:1351–6.
9. Evans T, Matulonis U. Next-generation sequencing: role in gynecologic cancers. *J Natl Compr Canc Netw* 2016;14:1165–73.
10. Liberal JM, Dienstmann R, *et al*. Matching degree between PI3K/Akt/mTOR (PAM) pathway mutations (mut) and therapy (TTX) as predictor of clinical benefit (ClinBen) in early trials. *J Clin Oncol* 2016;34.
11. Oliveira M, Dienstmann R, Bellet M, *et al*. Clonality of PIK3CA mutations (mut) and efficacy of PI3K/Akt/mTOR inhibitors (PAMi) in patients (PTS) with metastatic breast cancer (MBC). *JCO* 2016;34(suppl 15).
12. Dienstmann R, Elez E, Argiles G, *et al*. Analysis of mutant allele fractions in driver genes in colorectal cancer-biological and clinical insights. *Mol Oncol* 2017;11:1263–72.
13. Azuara D, Santos C, Lopez-Doriga A, *et al*. Nanofluidic digital PCR and extended genotyping of Ras and BRAF for improved selection of metastatic colorectal cancer patients for anti-EGFR therapies. *Mol Cancer Ther* 2016;15:1106–12.
14. Laurent-Puig P, Pekin D, Normand C, *et al*. Clinical relevance of KRAS-mutated subclones detected with picodroplet digital PCR in advanced colorectal cancer treated with anti-EGFR therapy. *Clinical Cancer Research* 2015;21:1087–97.
15. Ono A, Kenmotsu H, Watanabe M, *et al*. Mutant allele frequency predicts the efficacy of EGFR-TKIs in lung adenocarcinoma harboring the L858R mutation. *Ann Oncol* 2014;25:1948–53.
16. Zhou Q, Zhang X-C, Chen Z-H, *et al*. Relative abundance of *EGFR* mutations predicts benefit from gefitinib treatment for advanced non–small-cell lung cancer. *JCO* 2011;29:3316–21.
17. McGranahan N, Favero F, de Bruin EC, *et al*. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci Transl Med* 2015;7.
18. Lhermitte B, Egele C, Weingertner N, *et al*. Adequately defining tumor cell proportion in tissue samples for molecular testing improves interobserver reproducibility of its assessment. *Virchows Arch* 2017;470:21–7.
19. Von Hoff DD, Stephenson JJ, Rosen P, *et al*. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *JCO* 2010;28:4877–83.
20. Bregar AJ, Growdon WB. Emerging strategies for targeting PI3K in gynecologic cancer. *Gynecol Oncol* 2016;140:333–44.
21. Shaw AT, Kim DW, Nakagawa K, *et al*. Crizotinib versus chemotherapy in advanced *ALK*-positive lung cancer. *N Engl J Med* 2013;368:2385–94.
22. Long GV, Stroyakovskiy D, Gogas H, *et al*. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* 2014;371:1877–88.
23. Long GV, Weber JS, Infante JR, *et al*. Overall survival and durable responses in patients with *BRAF* V600–mutant metastatic melanoma receiving dabrafenib combined with trametinib. *JCO* 2016;34:871–8.
24. Smith I, Procter M, Gelber RD, *et al*. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* 2007;369:29–36.
25. Kaufman B, Shapira-Frommer R, Schmutzler RK, *et al*. Olaparib monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation. *JCO* 2015;33:244–50.

26. Swisher EM, Lin KK, Oza AM, *et al*. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2017;18:75–87.
27. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature* 2011;474:609–15.
28. Carpten JD, Faber AL, Horn C, *et al*. A transforming mutation in the pleckstrin homology domain of Akt1 in cancer. *Nature* 2007;448:439–44.
29. Kuo K-T, Mao T-L, Jones S, *et al*. Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. *Am J Pathol* 2009;174:1597–601.
30. Hashiguchi Y, Tsuda H, Inoue T, *et al*. PTEN expression in clear cell adenocarcinoma of the ovary. *Gynecol Oncol* 2006;101:71–5.
31. Kandoth C, Schultz N, Cancer Genome Atlas Research Network, *et al*. Integrated genomic characterization of endometrial carcinoma. *Nature* 2013;497:67–73.
32. The Cancer Genome Atlas Research Network. Integrated genomic and molecular characterization of cervical cancer. *Nature* 2017;543:378–84.
33. Wright AA, Howitt BE, Myers AP, *et al*. Oncogenic mutations in cervical cancer: genomic differences between adenocarcinomas and squamous cell carcinomas of the cervix. *Cancer* 2013;119:3776–83.
34. Boland GM, Piha-Paul SA, Subbiah V, *et al*. Clinical next generation sequencing to identify actionable aberrations in a phase I program. *Oncotarget* 2015;6:20099–110.
35. Hou M-M, Liu X, Wheler J, *et al*. Targeted PI3K/Akt/mTOR therapy for metastatic carcinomas of the cervix: a phase I clinical experience. *Oncotarget* 2014;5:11168–79.
36. Oza AM, Elit L, Tsao M-S, *et al*. Phase II study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the NCIC Clinical Trials Group. *JCO* 2011;29:3278–85.
37. Fleming GF, Filiaci VL, Marzullo B, *et al*. Temsirolimus with or without megestrol acetate and tamoxifen for endometrial cancer: a Gynecologic Oncology Group study. *Gynecol Oncol* 2014;132:585–92.
38. Slomovitz BM, Lu KH, Johnston T, *et al*. A phase 2 study of the oral mammalian target of rapamycin inhibitor, everolimus, in patients with recurrent endometrial carcinoma. *Cancer* 2010;116:5415–9.
39. Ray-Coquard I, Favier L, Weber B, *et al*. Everolimus as second- or third-line treatment of advanced endometrial cancer: ENDORAD, a phase II trial of GINECO. *Br J Cancer* 2013;108:1771–7.
40. Colombo N, McMeekin DS, Schwartz PE, *et al*. Ridaforolimus as a single agent in advanced endometrial cancer: results of a single-arm, phase 2 trial. *Br J Cancer* 2013;108:1021–6.
41. Tsoref D, Welch S, Lau S, *et al*. Phase II study of oral ridaforolimus in women with recurrent or metastatic endometrial cancer. *Gynecol Oncol* 2014;135:184–9.
42. Oza AM, Pignata S, Poveda A, *et al*. Randomized phase II trial of ridaforolimus in advanced endometrial carcinoma. *J Clin Oncol* 2015;33:3582.
43. Alvarez EA, Brady WE, Walker JL, *et al*. Phase II trial of combination bevacizumab and temsirolimus in the treatment of recurrent or persistent endometrial carcinoma: a Gynecologic Oncology Group study. *Gynecol Oncol* 2013;129:22–7.
44. Mackay HJ, Eisenhauer EA, Kamel-Reid S, *et al*. Molecular determinants of outcome with mammalian target of rapamycin inhibition in endometrial cancer. *Cancer* 2014;120:603–10.
45. Matulonis U, Vergote I, Backes F, *et al*. Phase II study of the PI3K inhibitor pilaralisib (SAR245408; XL147) in patients with advanced or recurrent endometrial carcinoma. *Gynecol Oncol* 2015;136:246–53.
46. Makker V, Recio FO, Ma L, *et al*. A multicenter, single-arm, open-label, phase 2 study of apitolisib (GDC-0980) for the treatment of recurrent or persistent endometrial carcinoma (maggie study). *Cancer* 2016;122:3528.
57. Myers AP, Broaddus RR, Makker V, *et al*. Phase II, two-stage, two-arm PIK3CA mutation stratified trial of MK-2206 in recurrent endometrial cancer. *J Clin Oncol* 2013;31.
48. Fu S, Hennessy BT, Ng CS, *et al*. Perifosine plus docetaxel in patients with platinum and taxane resistant or refractory high-grade epithelial ovarian cancer. *Gynecol Oncol* 2012;126:47–53.
49. Behbakht K, Sill MW, Darcy KM, *et al*. Phase II trial of the mTOR inhibitor, temsirolimus and evaluation of circulating tumor cells and tumor biomarkers in persistent and recurrent epithelial ovarian and primary peritoneal malignancies: a Gynecologic Oncology Group study. *Gynecol Oncol* 2011;123:19–26.
50. Temkin SM, Yamada SD, Fleming GF. A phase I study of weekly temsirolimus and topotecan in the treatment of advanced and/or recurrent gynecologic malignancies. *Gynecol Oncol* 2010;117:473–6.
51. Tinker AV, Ellard S, Welch S, *et al*. Phase II study of temsirolimus (CCI-779) in women with recurrent, unresectable, locally advanced or metastatic carcinoma of the cervix. A trial of the NCIC Clinical Trials Group (NCIC CTG IND 199). *Gynecol Oncol* 2013;130:269–74.
52. Janku F, Wheler JJ, Naing A, *et al*. PIK3CA mutation H1047R is associated with response to PI3K/Akt/mTOR signaling pathway inhibitors in early-phase clinical trials. *Cancer Res* 2013;73:276–84.
53. Johnson DB, Dahlman KH, Knol J, *et al*. Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. *Oncologist* 2014;19:616–22.
54. Schwaederle M, Daniels GA, Piccioni DE, *et al*. On the road to precision cancer medicine: analysis of genomic biomarker actionability in 439 patients. *Mol Cancer Ther* 2015;14:1488–94.
55. Meric-Bernstam F, Brusco L, Shaw K, *et al*. Feasibility of large-scale genomic testing to facilitate enrollment onto genomically matched clinical trials. *JCO* 2015;33:2753–62.
56. Jameson GS, Petricoin EF, Sachdev J, *et al*. A pilot study utilizing multi-omic molecular profiling to find potential targets and select individualized treatments for patients with previously treated metastatic breast cancer. *Breast Cancer Res Treat* 2014;147:579–88.
57. Gray SW, Hicks-Courant K, Cronin A, *et al*. Physicians' attitudes about multiplex tumor genomic testing. *JCO* 2014;32:1317–23.
58. Tsimberidou A-M, Iskander NG, Hong DS, *et al*. Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. *Clin Cancer Res* 2012;18:6373–83.
59. Le Tourneau C, Delord J-P, Gonçalves A, *et al*. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol* 2015;16:1324–34.
60. Sholl LM, Do K, Shivdasani P, *et al*. Institutional implementation of clinical tumor profiling on an unselected cancer population. *JCI Insight* 2016;1:e87062.
61. Zehir A, Benayed R, Shah RH, *et al*. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017;23:703–13.
62. Stockley TL, Oza AM, Berman HK, *et al*. Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: the Princess Margaret IMPACT/COMPACT trial. *Genome Med* 2016;8.
63. Meyer LA, Slomovitz BM, Djordjevic B, *et al*. The search continues: looking for predictive biomarkers for response to mammalian target of rapamycin inhibition in endometrial cancer. *Int J Gynecol Cancer* 2014;24:713–7.
64. Hassan B, Akcakanat A, Sangai T, *et al*. Catalytic mTOR inhibitors can overcome intrinsic and acquired resistance to allosteric mTOR inhibitors. *Oncotarget* 2014;5:8544–57.
65. Carew JS, Kelly KR, Nawrocki ST. Mechanisms of mTOR inhibitor resistance in cancer therapy. *Targ Oncol* 2011;6:17–27.
66. Hyman DM, Smyth LM, Donoghue MTA, *et al*. AKT inhibition in solid tumors with *AKT1* mutations. *JCO* 2017;35:2251–9.
67. Carracedo A, Pandolfi PP. The PTEN–PI3K pathway: of feedbacks and cross-talks. *Oncogene* 2008;27:5527–41.
68. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. *JCO* 2010;28:1075–83.
69. O'Reilly KE, Rojo F, She Q-B, *et al*. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006;66:1500–8.
70. Bosch A, Li Z, Bergamaschi A, *et al*. PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor–positive breast cancer. *Sci Transl Med* 2015;7.
71. Ibrahim YH, García-García C, Serra V, *et al*. PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. *Cancer Discov* 2012;2:1036–47.
72. Kim S, Han Y, Kim SI, *et al*. Tumor evolution and chemoresistance in ovarian cancer. *NPJ Precision Onc* 2018;2.