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 (CBB) of the entire population were 10% (N=5), 3.57 months (2.57–4.4) and 40% (N=15), 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 PIK3CA clonality on response to selected PAMi in patients with gynaecological cancer deserves further investigation.

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.5–8 Conversely, mutations in mitogen-activated protein kinase (MAPK) pathways have been assessed as a target for novel therapeutic strategies over the last decade.

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

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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.
► PIK3CA 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.
pathway, such as KRAS, NRAS and BRAF, might be associated with innate resistance to PAMi. 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. 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 PIK3CA-mutated cancer treated in early-phase clinical trials. Clonal evolution of PIK3CA mutations has also been investigated as a predictive biomarker of response to PAMi in patients with breast or colorectal 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. 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. 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.

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.

**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 oncoproteins, 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 oncoproteins 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.  

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%)                                     |                     |

*D 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
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. 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. 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 and potentially actionable PI3K/AKT/mTOR pathway alterations, 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. 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 AKTI 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
with limited standard therapeutic options. Other studies found modest activity with single-agent mTOR inhibitors, even in the setting of PI3K pathway activation, via mutation or amplification. Single-agent PI3K inhibitors also failed to demonstrate meaningful impact on gynaecological malignancies and again, PIK3CA mutational status did not correlate with responses. The PIK3CA mutant variant may also impact response to PAMi, 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.

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. 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 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.

Limited activity of single-agent PAMi, particularly mTOR inhibitors, has been reported in patients with tumours harbouring KRAS mutations. 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). Contradictory, clonality of PIK3CA mutations did not predict benefit with matched targeted agents in patients with colorectal cancer. These results were partially explained by the limited activity in the PIK3CA/KRAS co-mutant populations, known to confer primary resistance to

### 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%)                                  |
| 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.
PAMi as single agents.\textsuperscript{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.\textsuperscript{66}

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 \( \geq 1.3 \) 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.

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.\textsuperscript{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 | 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,\(^\text{72}\) 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.

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**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, GW, RD and AO collected, analysed and interpreted the data. RD, FN and AW were involved in molecular data generation and interpretation. VR-F, LF-M, AGS-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.

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