

## Triplet therapy with palbociclib, taselesib and fulvestrant in *PIK3CA* mutant breast cancer and doublet palbociclib and taselesib in pathway mutant solid cancers

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## **ABSTRACT:**

Cyclin-dependent kinase-4/6 (CDK4/6) and phosphatidylinositol 3-kinase (PI3K) inhibitors synergise in *PIK3CA* mutant ER-positive HER2-negative breast cancer models. We conducted a phase Ib trial investigating safety and efficacy of doublet CDK4/6 inhibitor palbociclib plus selective PI3K inhibitor taselisib in advanced solid tumors, and triplet palbociclib plus taselisib plus fulvestrant in 25 patients with *PIK3CA* mutant, ER-positive HER2-negative advanced breast cancer. The triplet therapy response rate in *PIK3CA* mutant, ER-positive HER2-negative was 37.5% (95% CI 18.8-59.4). Durable disease control was observed in *PIK3CA* mutant ER-negative breast cancer and other solid tumors, with doublet therapy. Both combinations were well tolerated at pharmacodynamically active doses. In the triplet group, high baseline cyclin E1 expression associated with shorter progression-free survival (PFS) (HR 4.2, 95% CI 1.3-13.1, p=0.02). Early ctDNA dynamics demonstrated high on-treatment ctDNA association with shorter PFS (HR 5.2, 95% CI 1.4-19.4, p=0.04). Longitudinal plasma ctDNA sequencing provided genomic evolution evidence during triplet therapy.

## **STATEMENT OF SIGNIFICANCE:**

The triplet of palbociclib, taselisib, and fulvestrant has promising efficacy in patients with heavily pre-treated *PIK3CA* mutant ER-positive HER2-negative advanced breast cancer. A subset of patients with *PIK3CA* mutant triple negative breast cancer derived clinical benefit from palbociclib and taselisib doublet, suggesting a potential non-chemotherapy targeted approach for this population.

## INTRODUCTION:

Cyclin-dependent kinase-4/6 (CDK4/6) inhibitors in combination with endocrine therapy are widely approved for the treatment of advanced hormone receptor positive (HR+ve), HER2-negative (HER2-ve) metastatic breast cancer, both for patients relapsing on and off adjuvant endocrine therapy (1, 2). Yet despite high initial response rates, the majority of patients develop disease progression on treatment and a further subset of cancers are intrinsically resistant to CDK4/6 inhibitor and endocrine therapy combinations. Furthermore, translating the efficacy of CDK4/6 inhibitors to other solid tumors has been challenging. Identifying new treatment approaches to enhance the efficacy of CDK4/6 inhibitors is therefore a major priority.

In parallel to the development of CDK4/6 inhibitors, multiple agents against the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway have entered clinical practice, with the mTOR inhibitor everolimus being the first approved in combination with exemestane (3). The pan-PI3K inhibitor buparlisib modestly improved progression-free survival (PFS), albeit with a significant increase in toxicity (4). The more selective beta-sparing PI3K inhibitor taselisib improved PFS when combined with fulvestrant (5), and recently the alpha-selective PI3K inhibitor alpelisib in combination with fulvestrant significantly improved PFS for patients with *PIK3CA* mutant (*PIK3CA*-mt) HR+ve/HER2-ve cancers, was FDA approved and has become a standard of care (6).

Multiple preclinical studies have demonstrated synergy between PI3K and CDK4/6 inhibition in estrogen receptor positive (ER+ve) breast cancer (7, 8). PI3K inhibition increases ER transcriptional activity (9), which may be partly overcome by CDK4/6 inhibition. Conversely, CDK4/6 inhibition alone results in incomplete cell cycle arrest and early adaptation to CDK4/6 inhibition and so addition of PI3K inhibition leads to more profound cell cycle arrest (7, 8), also with induction of apoptosis observed (8). Combinations of CDK4/6 and PI3K inhibitors have demonstrated substantial efficacy *in vivo* (10-12). Furthermore, the triplet combination of CDK4/6 inhibitor, PI3K inhibition and ER degradation with fulvestrant ablates clonal growth when compared to the doublet combinations (8), also with activity confirmed *in vivo* (10). Combinations of PI3K and CDK4/6 inhibitors have also demonstrated antitumor potential in other cancer types, with activity observed in preclinical models of *PIK3CA*-mt triple negative breast cancer (TNBC) (11) and other solid tumors (13, 14).

This phase Ib trial investigated the activity of triplet therapy, with the combination of CDK4/6 inhibitor palbociclib plus PI3K inhibitor taselisib and fulvestrant in *PIK3CA*-mt ER+ve/HER2-ve advanced breast cancer, and doublet therapy of palbociclib plus taselisib in advanced solid tumors with pathway mutations. Detailed translational studies were conducted to

assess pharmacodynamic (PD) markers of PI3K inhibition, with potential biomarkers of sensitivity and resistance tested in both tissue and plasma ctDNA.

## RESULTS:

From March 2015 to November 2018, a total of 78 patients were enrolled between part A (escalation phase) and part B (expansion phase) (**Fig. 1A**), with main demographic characteristics detailed in **Table 1**. Patients had received a median of 3 prior lines of therapy for advanced disease, with a median of 2 prior chemotherapy lines for advanced disease. In part B1, all 25 patients had received prior aromatase inhibitor (AI), 6 of 25 (24%) patients had prior fulvestrant, and 10 of 25 (40%) patients received prior everolimus. A summary of mutation distribution as per enrolment can be also found in **Table 1**. Patients consented to provide both tissue and blood samples for pharmacokinetics (PK), pharmacodynamics (PD) and biomarker analyses, detailed in **Fig. 1B**.

### Dose escalation

Part A enrolled 20 patients with solid tumors in a 3+3 dose escalation trial design (**Supplementary Fig. S1A and S1B**). No dose limiting toxicities (DLTs) were observed at dose level 1 (N=4). At dose level 2 (taselisib 4mg with palbociclib 100mg, N=3), 2 patients experienced 3 DLTs of G3 mucositis, G3 hyperglycemia and G3 fatigue and the combination dose was deemed intolerable. There were no DLTs at dose level 3 (taselisib 2mg 3 week-on/1 week-off with palbociclib 125mg, N=7) and dose level 4 (taselisib 2mg continuous dosing with palbociclib 125mg, N=6). The RP2D was established at taselisib 2mg PO QD continuous dosing, plus palbociclib 100mg PO QD 3/1 for cycle 1, escalating to 125mg PO QD 3/1 in the absence of grade 3/4 myelosuppression.

### Pharmacokinetics and pharmacodynamics

Combination palbociclib and taselisib PK was consistent with single agent PK, suggesting no drug-drug interaction (**Supplementary Fig. S2A-C**). In part A, platelet-rich plasma (PRP) was analysed from all 20 patients at baseline and on-treatment for AKT and GSK3 $\beta$ . At the 2mg taselisib dose (at dose levels 3 and 4), there was decreased AKT and GSK3 $\beta$  phosphorylation at C1D1 4h, C1D15 6h and 8h (**Fig. 1C**). We noted an increase in phosphorylation of AKT and GSK3 $\beta$  at C1D8 pre-dose, which may indicate compensatory upregulation often seen in PI3K/AKT pathway inhibition, and may potentially suggest incomplete PI3K pathway inhibition. In part A, 5 patients had their tumor tissue samples analysed. Grouping all patients, there is a significant ~25% decrease in the ratio of phosphoSer780-Rb to total Rb at C1D15 pre-dose when compared to C1D1 pre-dose, indicating CDK4/6 signalling was modulated as predicted for palbociclib treatment (**Fig. 1D**).

### Safety

The most frequently reported all-grade adverse events (AEs) across the 58 patients enrolled in the expansion cohorts (all treated with RP2D) were neutropenia (88%), thrombocytopenia (57%), anaemia (48%), fatigue (45%), leukopenia (36%) and diarrhoea (34%). The most common grade 3/4 AEs in the 58 expansion phase patients were neutropenia (53%) and leukopenia (21%), while the most common grade 3/4 treatment-related AEs were neutropenia (53%) and leukopenia (19%). A complete record of all adverse events reported in >10% of all patients across the trial can be found in **Table 2**. Overall, 30.8% (24/78 patients) had 35 serious adverse events (SAEs) of which 25.7% of these (9/35) were considered as definitely, probably or possibly related to treatment. There were no treatment-related deaths. Dose interruptions were required for taselisib in 45 of 78 patients (58%) and for palbociclib in 39 of 78 (50%) patients. In part B1, 1 of 25 patients (4%) required interruption for fulvestrant, and in part B3 4/7 (57.1%) for letrozole. Dose reductions for taselisib were required in 13 of 78 (17%) patients enrolled, 15 of 78 (19%) for palbociclib. There were delays in 28 of 78 patients (36%) for taselisib and in 41 of 78 (53%) for palbociclib. Treatment was discontinued in 54 of 77 patients (70%) following radiographical progression, 13 of 77 (17%) following clinical progression, 3 of 77 (4%) due to adverse events and 7 of 77 (9%) due to other factors.

### **Efficacy in dose expansions**

In part B1, 25 patients with *PIK3CA*-mt ER+ve HER2-ve advanced breast cancer were treated with the triplet of palbociclib plus taselisib plus fulvestrant, one patient was not assessable for the primary endpoint as had not completed first cycle of treatment. The confirmed objective response rate (ORR) was 37.5% (9/24, 95% CI 18.8-59.4, **Fig. 2A**). Clinical benefit rate (CBR) was 58.3% (14/24, 95% CI 36.6-77.9), with median PFS of 7.2 months (95% CI 3.9-9.9, **Fig. 2B**).

Part B2 recruited a total of 26 patients. Twelve patients with *PIK3CA*-mt ER-ve advanced breast cancer (9 TNBC and 3 HER2+ve) were treated with taselisib plus palbociclib, and 10 of them were evaluable for efficacy (8TNBC and 2 HER2+ve). The ORR was 10% (1/10, 95% CI 0.2-26.5), CBR 30% (3/10, 95% CI 6.7-65.2) and median PFS was 3.6 months (95% CI 1.7-5.6) (**Fig. 2C**). There were 14 patients with solid tumors of various subtypes treated in part B2; 4 *PIK3CA*-mt colorectal adenocarcinoma, 3 *PIK3CA*-mt ER+ve/HER2-ve breast cancer (included as part of the solid tumors subcohort), 1 *PIK3CA*-mt high grade serous ovarian cancer, 1 *PIK3CA*-mt clear cell ovarian carcinoma, 2 *PIK3CA*-mt non-small cell lung cancer (NSCLC), 1 *PIK3CA*-mt endometrial cancer, 1 *PIK3CA*-mt cervical cancer (adenosquamous carcinoma) and 1 *PIK3CG*-mt anaplastic oligodendroglioma. For the 12 evaluable solid tumors in part B2, ORR was 0% (0/12, 95% CI 0-26.5) and CBR 16.7%

(2/12, 95% CI 2.1-48.4) and median PFS of 3.8 months (95% CI 1.2-5.0) (**Supplementary Fig. S3A**).

Part B3 recruited 7 patients, 6 of whom were evaluable. CBR was 50% (3/6, 95% CI 11.8-88.1) and median PFS 8.6 months (95% CI, 1.6-24.2), ORR was not assessable (ORR 0% (0/6, 95% CI 0-45.9) as patients did not require measurable disease for B3.

### **Baseline prognostic/predictive biomarkers**

Overall, 57 archival formalin-fixed paraffin-embedded (FFPE) blocks (54 baseline + 3 on-progression) were retrieved and had tumor content for translational analysis (**Fig. 1B**). These consisted in 5 samples from the patients with breast cancer enrolled in part A (2 ER+ve/HER2-ve, 1 ER+ve/HER2+ve, 1 TNBC baseline + progression), 23 samples from patients in part B1 (all ER+ve/HER2-ve), 26 samples from patients coming from part B2 (8 baseline + 1 progression TNBC, 3 baseline + 1 progression ER-ve/HER2+ve breast cancer, 3 ER+ve/HER2-ve breast cancer, 4 colorectal cancer, 1 non-small cell lung carcinoma, 1 endometrial carcinoma, 1 cervical adenosquamous carcinoma, 1 high grade serous ovarian carcinoma, 1 clear cell ovarian carcinoma and 1 anaplastic oligodendroglioma), and 3 samples from patients in part B3. Biopsies had been taken a median of 4.67 years before first dosing of trial medication, 54% coming from metastatic and 46% from primary tumor specimens.

To assess tumor copy number, ultra-low whole genome sequencing (ulWGS) was conducted on baseline FFPE samples, with a mean coverage of 0.49X and mean 1.91 ploidy for 43 evaluable patients, 2 of them with paired on-progression samples (**Fig. 1B**). For patients with *PIK3CA*-mt ER+ve/HER2-ve breast cancer treated with the triplet combination in part B1 and tumor sequencing data, 6/17 patients (35%) had *CCND1* amplification, 9/17 (53%) *FGFR1* amplification and 2/17 (12%) cases of *CCNE1* amplification (**Fig. 3A**). There was no evident association found between copy number aberrations and efficacy, supported by further analysis of Cyclin D1 amplification by FISH assessment (**Supplementary Fig. S4A and S4B**). No association with efficacy was observed with copy number aberrations for patients with *PIK3CA*-mt ER- breast cancer or other solid tumors (**Supplementary Fig. S4C**). Paired baseline/progression CNV analysis for the two available patients showed mostly chromosomal stability, with evidence of homozygous deletion of RB1 in a patient with ER-ve/HER2+ breast cancer (**Supplementary Fig. S4D**).

Baseline tissue samples were analysed for cyclin D1 and cyclin E1 expression by immunohistochemistry. In the 42 archival FFPE tissue samples analysed for of cyclin D1, the median H-score was 140 (0-240), and there was no association with PFS in part B1 or in



ER-ve breast patients in part B2 (**Supplementary Fig. S4E and S4F**). In the 42 breast cancer samples with cyclin E1 immunostaining, the median percentage of tumor cells stained was 30% (0-90%) (**Fig. 3B**). In B1, patients with high cyclin E1 expression had worse PFS than patients with low cyclin E1 expression (median PFS 6.4 vs 10.4 months respectively, HR 4.2, 95% CI 1.3-13.1,  $p=0.02$ , log-rank test,  $N=20$ , **Fig. 3C**).

Within patients with *PIK3CA*-mt TNBC, 8 tumors were analysed by androgen receptor (AR) immunostaining. Median H-score was 140 (0-270) and median percentage of stained cells was 70% (0-90%). Overall, 6/7 evaluable patients (86%) had positivity for AR. The two patients with long-term clinical benefit, and a third additional patient achieving the only RECIST 1.1 confirmed PR in the TNBC subset, had high AR expression in their tumor, with median 270 vs 90 H-score for patients achieving CBR vs non-CBR, respectively ( $p=0.20$ , Mann-Whitney test) (**Fig. 3D**).

### ctDNA dynamics as an efficacy surrogate

A total of 40 C1D1-C2D1 ctDNA pairs were analysed for early *PIK3CA* ctDNA dynamics, with 10 pairs excluded for failing quality control (methods). Comparing allele frequencies (AF) between C2D1 and C1D1, the median CDR<sub>28</sub> was 0.74 (range 0-3.46), decreased from baseline ( $p<0.01$ , Wilcoxon signed-rank test) (**Fig. 4A**). In the 16 evaluable patients with *PIK3CA*-mt ER+ve/HER2-ve breast cancer in part B1 receiving triplet therapy, on treatment ctDNA was suppressed ( $p<0.01$ , Wilcoxon signed-rank test) (**Fig. 4B**), with median CDR<sub>28</sub> AF 0.31 (0-3.46). Patients without suppressed CDR<sub>28</sub> ( $>0.1$ ) had worse PFS than patients with suppressed ( $<0.1$ ) CDR<sub>28</sub> (6.9 vs 15.2m respectively, HR 5.2, 95% CI 1.4-19.4,  $p=0.04$ , Log-rank test, **Fig. 4C**). Early changes in ctDNA by mutant copies/ml were consistent with analysis by AF (**Supplementary Fig. S5A-C**). Only two patients were found to derive complete ctDNA suppression (C1D1 positive and C2D1 under limit of detection), both in part B1. Patients with incomplete versus complete ctDNA suppression had worse PFS (6.9 vs 15.2m respectively, HR 5.1, 95% CI 1.3-20.6,  $p=0.02$ , Log-rank test, **Supplementary Fig. S5D**).

### Paired plasma sequencing to investigate evolution through therapy

To investigate genomic evolution through therapy, we performed paired error corrected ctDNA sequencing with a 200 cancer driver gene panel (detailed in methods) at C1D1-EOT in 23 patients, including 16 patients from part B1, 4 patients from B2 (1 *PIK3CA*-mt endometrium, 2 *PIK3CA*-mt ER+ve/HER2-ve breast and 1 *PIK3CA*-mt ER-ve/HER2+ breast), and 3 from B3 (2 *PIK3CA*-mt and 1 *PIK3CA* non-mutant ER+ve/HER2-ve breast).

From these, 20/23 (87%) had confirmed progression on their EOT sample. Median family coverage for all sequenced samples was 696X (11-1880).

Overall, 5 of 23 (22%) patients were found to have multiple *PIK3CA* mutations in any of the timepoints sequenced. Dynamics for *PIK3CA* mutations in the 4 patients with at least 1 read in each timepoint, clearly suggested the second *PIK3CA* mutations was subclonal in 2 patients (**Fig. 5A**). In part B1, 3 of 16 (19%) evaluable sequenced patients had multiple *PIK3CA* mutations. PFS was shorter for multiple vs single *PIK3CA* mutants (median PFS 2.7 vs 8.5m respectively HR 663.5, 95% CI 28.8-15286,  $p < 0.0001$ , Log-rank test) (**Fig. 5B**).

In total, 13 out of 23 (57%) patients had an *ESR1* mutation at any sequenced timepoint, with 3/13 (23%) patients harboring polyclonal mutations. Acquired Y537S (c.1610A>C) and Y537C (c.1610A>G) mutations were identified at EOT in two patients, with evidence of deselection of *ESR1* mutations in two other patients at EOT (**Fig. 5C**). In evaluable sequenced part B1 patients, 11/16 (69%) had an *ESR1* mutation. PFS was not significantly different for patients with *ESR1* mutant tumors compared to wild-type (HR 0.3, 95% CI 0.1-1.4,  $p = 0.14$ , Log-rank test), although analysis was underpowered (**Supplementary Fig. S6A**).

Overall, 5 of 23 (22%) patients were found to have *FAT1* mutations (2 with stop-gains, 3 with nonsynonymous) in any timepoint (**Fig. 5D**), with one patient acquiring a *FAT1* mutation at EOT that was not present at C1D1 (**Fig. 5E**). Of the two patients harbouring *FAT1* stop-gain mutations, one was an ER+/HER2-ve breast patient treated with the triplet that was censored after 5.5m for compliance, and the second one was an endometrial cancer treated with doublet that had early progression after only 1.1m of treatment. We did not find strong evidence for other potential genetic mechanisms of resistance in the paired sequencing.

## DISCUSSION

We provide the first study report of the safety and efficacy of the triplet combination therapy of CDK4/6, PI3K and ER signalling inhibitors in *PIK3CA* mutant ER-positive breast cancer, as well as the first data on combination palbociclib plus taselisib therapy in a range of different *PIK3CA* mutant solid tumors.

We observed a 37.5% response rate for the triplet combination in *PIK3CA* mutant ER-positive HER2-negative breast cancer. In a single arm study of a triplet combination, it is very hard to confidently differentiate the contributions of the components, although this response rate compares favourably with prior data on palbociclib and fulvestrant doublet. The PALOMA-3 registration trial reported a response rate of 15% overall, and 23% in patients with measurable disease (15). PALOMA-3 allowed for 1 line of chemotherapy in the advanced disease, and only 33% of patients treated had received prior chemotherapy. In the comparable part B1 of our study, 79% (19/24) evaluable patients had prior chemotherapy for advanced disease, and 50% (12/24) receiving 2 or more advanced chemotherapy lines. Also, PALOMA-3 excluded prior fulvestrant and/or everolimus, while B1 allowed both and 46% (11/24) evaluable patients had prior fulvestrant, everolimus or both. There is broad evidence that prior lines of chemotherapy treatment can heavily decrease response rates to subsequent lines of therapy for metastatic breast cancer patients, with responses rates reducing approximately by half in every subsequent line (16-18). Although it is possible that all efficacy in the triplet originated from palbociclib and fulvestrant, our response rate does suggest triplet efficacy. Current standard of care for *PIK3CA* mutant ER-positive HER2-negative breast cancer patients who do not relapse on AI, is sequential doublets of AI plus CDK4/6 followed by fulvestrant plus PI3 kinase inhibition with alpelisib at progression. In this setting, a triplet combination will need to be assessed against the sequential therapy approach. Therefore, we suggest that a triplet therapy is likely more relevant in the context of relapse on AI, where fulvestrant plus CDK4/6 inhibition is standard, and there is no clear role for subsequent alpelisib.

The safety profile observed in patients receiving palbociclib 125mg 3/1 in combination with taselisib 2mg daily is consistent with prior studies investigating palbociclib or taselisib in combination with fulvestrant. This triplet combination required a dose reduction in taselisib from the 4mg dose with fulvestrant, to 2mg in the triplet, and this may have compromised efficacy. It is possible that treatment delays may reduce efficacy of triplet combinations, and triplet combinations should likely be designed to limit the incidence of such delays. Regardless, there was still evidence of AKT pathway modulation in PRP taken at 4 hours post-dose on C1D1 and at 6-8h post-dose on C1D15, providing evidence of PI3K inhibition

by taselisib (**Fig. 1C**). Interestingly, there was evidence of increased AKT phosphorylation in PRP at 24h post dose (C1D8 pre-dose), suggesting a rebound of PI3K pathway activation. Our study provides clinical evidence for the activity of triplet therapy, but also suggests that further development of triplet combinations with alternative PI3K or AKT inhibitors may maximise the potential of such triplet therapy by optimising PI3K-AKT pathway suppression. In this study, we show that biomarkers of early disease progression on endocrine-CDK4/6 combinations also predicted for shorter PFS on triplet therapy. High cyclin E expression levels confer resistance to palbociclib in pre-clinical models (8) and high mRNA levels of cyclin E1 were found to confer relative resistance to palbociclib for patients enrolled in PALOMA-3 (19). Similarly high cyclin E1 associated with short PFS in this study (**Fig. 3C**).

Early *PIK3CA* dynamics in ctDNA have been shown to predict patient outcomes in both the PALOMA-3 study of fulvestrant and palbociclib (20) and the BEECH study of capivasertib and paclitaxel (21), both in the ER+ve/HER2-ve population. Here, we further validated the findings that early ctDNA dynamics could replace other assessments. In addition, patients with ER+ve/HER2-ve breast cancer with poor ctDNA suppression had worse PFS. Overall ctDNA suppression was less pronounced than previous analyses, with only 2 patients having absent ctDNA at C2D1. We hypothesise that this might reflect time of sampling, with the C2D1 samples in this study taken after at least 1 week-off palbociclib, whereas in our prior work on PALOMA-3 samples were taken at C1D15. It is likely that ctDNA rebound occurred during the week off palbociclib, and that some element of rebound in ctDNA levels was not fully suppressed by taselisib. In this study patients with double *PIK3CA* mutations had shorter PFS on triplet (**Fig. 5B**). This contrasts prior data suggesting that patients with multiple *PIK3CA* mutations had higher response rates on taselisib at a higher dose (22). Potentially this may reflect the lower dose of taselisib in the triplet, resulting in incomplete PI3 kinase inhibition in tumors with hyperactive multiple mutations.

ctDNA sequencing demonstrated ongoing cancer evolution during triplet combination therapy, with acquisition of a *FAT1* mutation in one patient, selection and loss of a second double *PIK3CA* mutation, and ongoing evolution of *ESR1* mutations. Our findings are consistent with prior observations that loss of the *FAT1* tumor suppressor promotes CDK4/6 resistance through CDK6 overexpression via Hippo pathway (23) and that *ESR1* Y537S and Y537C are acquired through fulvestrant therapy (24). In this study, only 1 of 23 (4.3%) patients was found to have an *RB1* mutation, and this was already present at C1D1, matching previous observation in the PALOMA-3 trial that *RB1* acquisition on palbociclib is a rather uncommon mechanism of resistance (24). We did not identify any further mechanisms of resistance with plasma sequencing, including no evidence of acquired *PTEN* mutations to suggest bypass PI3K $\beta$  activation (25). This potentially suggests that biomarkers of CDK4/6

inhibitor resistance may also predict relative resistance to triplet combinations, identifying an important priority for future triplet studies. However, we do note that more potent PI3K inhibition may generate different results.

We acknowledge a number of limitations in our study. Firstly, our patient population is too small to make any robust conclusions in antitumor efficacy for the palbociclib and taselesib doublet combination. However, although only one confirmed RECIST 1.1 response was observed in a patient with *PIK3CA* mutant TNBC, a subset of patients with TNBC potentially characterized by high AR expression had durable disease control, which correlates with *in vitro* studies showing higher sensitivity to palbociclib in LAR TNBC (11). Interestingly, 6 of 7 patients with *PIK3CA* mutant TNBC in our study had some degree of IHC expression for AR, a finding that adds evidence to TNBC LAR subtypes being enriched for *PIK3CA* mutations (26, 27). Previous studies have demonstrated the potential of non-chemotherapy approaches for selected TNBC subtypes, with the potential for antiandrogen therapy in TNBC with AR expression (28, 29). Our study suggests that targeted non-chemotherapy combinations could have activity in subtypes of biologically distinct subgroups, and further clinical studies are warranted. We also note that our biomarker analysis are limited by small numbers, and are hypothesis generating, although observations on cyclin E1 IHC (19), AR IHC in TNBC (26, 27), and ctDNA dynamics (20, 21) are all supported by prior evidence.

In conclusion, our study demonstrates safe and manageable toxicity in solid tumors for the combination of palbociclib and taselesib with or without the addition of endocrine therapy. Our data suggest promising efficacy for the fulvestrant triplet combination in previously treated patients with *PIK3CA* mutant ER+ve/HER2-ve breast cancer. Our study further supports high expression of cyclin E1 as a poor prognostic marker on palbociclib combinations and reinforce the evidence of early ctDNA changes predicting treatment efficacy. These findings provide preliminary proof-of-concept that support future combination approaches involving both PI3K and cyclin-dependent pathway inhibitors [NCT03065062, NCT01872260, NCT02088684, NCT04191499].

## METHODS:

### Trial design and patients:

The phase Ib study to assess the safety, tolerability and activity of the PI3K inhibitor taselisib (GDC-0032), in combination with palbociclib, with the subsequent addition of fulvestrant in *PIK3CA*-mutant breast cancers or letrozole in advanced breast cancers (PIPA) trial (NCT02389842) was an open-label non-randomized trial sponsored by the Royal Marsden NHS Foundation Trust (RMH) and The Institute of Cancer Research (ICR). Primary objectives were to identify a recommended phase 2 dose (RP2D) by establishing a combination maximum tolerated dose (MTD) for palbociclib plus taselisib, define the adverse event profile for the doublet combination with or without fulvestrant or letrozole, and to assess the efficacy of the triplet palbociclib, taselisib and fulvestrant in a cohort of patients with *PIK3CA*-mt advanced ER+ve/HER2-ve breast cancer. Secondary objectives were to determine preliminary efficacy in patients with other solid tumors including a cohort of patients with *PIK3CA*-mt ER-ve advanced breast cancer, pharmacokinetics (PK), pharmacodynamic (PD) characterization in tumor biopsies and platelet-rich plasma (PRP), and mechanisms of antitumor response and resistance by analysing tumor biopsy and circulating tumor DNA (ctDNA) samples.

Patients with previously treated advanced solid tumors enriched for but not requiring *PIK3CA* mutations were included in part A (escalation phase) and treated at different dose levels in a 3+3 dose escalation trial design (**Supplementary Fig. S1A and S1B**). Subsequent patients were recruited into part B expansion cohorts (**Fig. 1A**), Part B1 (triplet therapy) included patients with *PIK3CA*-mt, ER+ve/HER2-ve advanced breast cancer who received at least one prior line of endocrine therapy and up to two lines of chemotherapy (initially open to any number of prior lines of chemotherapy, but this was amended after 15 patients were recruited). Prior exposure to fulvestrant and/or everolimus was permitted but prior exposure to CDK4/6 inhibitors was not. Part B2 (doublet therapy) was composed of patients with *PIK3CA*-mt, ER-ve (whether HER2+ve or -ve) advanced breast cancer or any advanced solid tumor with mutations leading to a hyperactivated PI3K/AKT/mTOR pathway. Patients with ER-ve/HER2+ve were eligible if they had exposure to at least two prior lines of anti-HER2 therapy (one prior line permitted if no further HER2 directed therapy was available locally), while patients with TNBC and solid tumors were eligible with a minimum of one prior line of chemotherapy exposure. Part B3 included patients with ER+ve/HER2-ve advanced breast cancer without regard for *PIK3CA* status to assess the safety of palbociclib plus taselisib plus letrozole.

*PIK3CA* mutations testing was performed by an accredited laboratory on archival or fresh tumor samples or circulating tumor DNA in plasma. Patients in parts A, B2 and B3 required measurable disease assessed by RECIST 1.1 or evaluable disease. For patients in part B1, measurable disease was mandatory. Written informed consent was obtained from the patients. The study was approved by an institutional review board and conducted in accordance with the Declaration of Helsinki.

### **Treatment:**

For dose expansions, palbociclib was administered at 100mg PO QD 3-weeks-on, 1-week-off (3/1) for cycle 1, escalating to 125mg PO QD 3/1 from cycle 2 in absence of grade 3/4 myelosuppression, plus taselisib 2mg PO QD, plus fulvestrant 500mg IM or letrozole as per standard schedule. Treatment was given until patient asked to be withdrawn from the trial, evidence of disease progression or unacceptable toxicity. In parts B1 and B3, pre-/peri-menopausal women had ovarian suppression with the luteinizing hormone-releasing hormone (LHRH) agonist goserelin.

### **Pharmacokinetics (PK) and Pharmacodynamics (PD):**

Collection of blood samples for PK analysis in part A and part B is illustrated in **Fig. 1B** and plasma analysed with fully validated analytical methods. Pharmacokinetic parameters were derived from non-compartmental analysis (Phoenix 64). PD analysis (pSer473 and total AKT, and pSer9 and total GSK3 $\beta$ ) was performed in PRP collected in part A on Cycle 1 Day 1 (pre-dose and 4 hours post dose), Cycle 1 Day 8 (pre-dose), Cycle 1 Day 15 (6 and 8 hours post-dose) and Cycle 2 Day 15 (2 hours post-dose), as previously reported (30). Ratios of phosphorylated biomarker as percentage of C1D1 pre-dose normalised to total biomarker as percentage of C1D1 pre-dose and platelet counts were performed. Optional snap frozen tumor tissue samples for PD were lysed and analysed for phosphorylated Ser780 and total Rb on C1D1 (pre-dose/baseline), C1D15 (pre-dose) and at disease progression using ICR validated assays on the MesoScale Discovery (MSD<sup>®</sup>) technology platform .

### **Archival tissue for predictive biomarkers:**

All patients provided an archived formalin-fixed paraffin-embedded (FFPE) sample from either the primary or a metastatic location (**Fig. 1B**). Cyclin D1 IHC staining used specific rabbit monoclonal antibody SP4 clone (ThermoScientific, catalogue number RM-9104-S1) and reported as H-score. Cyclin D1 FISH staining used Vysis CCND1/CEP11 FISH Probe Kit (Abbott Molecular, catalogue number 03N88020) and reported as counted ratio CCND1/CEP11. Cyclin E1 IHC used specific mouse monoclonal antibody HE12 (Abcam,

catalogue number ab3927) and scored as percentage of invasive nuclei stained. For patients with TNBC, androgen receptor (AR) IHC staining was performed using the specific mouse monoclonal antibody AR441 clone and scored as percentage of invasive nuclei stained.

DNA extraction from FFPE was performed using QIAGEN AllPrep DNA/RNA FFPE Kit using MiniColumns (Qiagen catalogue number: 80234) and quantified using an RNaseP assay for ddPCR as previously described (31).

#### **Blood processing for ctDNA:**

Blood samples were collected in EDTA or STRECK tubes before each cycle of treatment (~28 days) from C1D1 to the end of treatment (EOT) (**Fig. 1B**). Plasma and buffy coat were separated by centrifugation for 20 minutes at 1,600g at room temperature at the ICR, and stored immediately at -80°C until nucleic acids extraction. EDTA tubes were processed within 2 hour following venipuncture and STRECK tubes within 48-72 hours. Plasma DNA was extracted from 4ml of sample (for C1D1-EOT pairs) or 2ml (for C2D1) using the MagMAX Cell-Free DNA Isolation Kit (Thermo Scientific, Catalogue Number A29319) on a KingFisher™ Flex Purification System per manufacturer's instructions.

#### **Next Generation Sequencing:**

For ultra-low passage whole-genome sequencing (ulWGS) on tissue DNA (**Fig. 1B**), libraries were prepared from 5-50ng input using Illumina's KAPA HyperPlus Kit with Library Amplification (Cat No. 07962428001) and quantified with Illumina's Library Quantification Kit ABI Prism™ qPCR Master Mix (Cat No. 07960204001) as per manufacturer's instructions, and sequenced on a NovaSeq 6000, using SP 300 cycles reagents to a target depth of 0.8X. Data were analysed for copy number variations (CNV) by alignment with reference hg19 genome using BWA (RRID:SCR\_010910), duplicate removal with Picard (RRID:SCR\_006525, <http://broadinstitute.github.io/picard/>), purity assessment and copy number calling used ichorCNA (32) with settings for high tumor fraction to get a thresholded adjusted copy number call (amplification/gain/neutral/heterozygous deletion/homozygous deletion) with adjusted values plotted for each sample. Copy number evaluations were limited to tumors with at least 20% tumor purity.

For paired C1D1 and end of treatment (EOT) plasma sequencing (**Fig. 1B**), an in-house targeted error-corrected capture panel targeting 200 cancer driver genes (RMH200, 1.2Mb size) was used (**Supplementary Table S1**). Briefly, IDT xGen® Dual Index UMI adapters were ligated with KAPA HyperPlus Kit, and sequenced to target depth 20,000X on a NovaSeq 6000 Sequencing System (Illumina). Samples were aligned using BWA (33) then annotated and combined into UMI consensus families using fgbio



(<http://fulcrumgenomics.github.io/fgbio/>). 16 plasma samples from healthy donors were sequenced to generate an error model using a modified pileup pipeline (34). Variant calls were generated using VarDict (35) and pileup (35). 4 alternative families of size 3 or more reads were required to call a mutation (5 alternative families for an indel) removing mutations with significant strand bias. All calls were manually curated with Integrative Genomics Viewer (IGV) (36), reported as allele frequency (AF) and annotated using annovar (37). All sequencing was performed on the NovaSeq 6000 (Illumina). Functional predictions of *FAT1* mutations were assessed using SIFT (RRID:SCR\_012813) (38), MutationAssessor (RRID:SCR\_005762) (39) and PROVEAN (RRID:SCR\_002182) (40).

### **ctDNA ratio for *PIK3CA* mutations:**

A circulating tumor DNA ratio (CDR) for *PIK3CA* mutations between C1D1-C2D1 (CDR<sub>28</sub> – day 28) was performed by droplet digital PCR (ddPCR) (**Fig. 1B**). Singleplex *PIK3CA* assays were used for common mutations E542K (c.1624 G>A), E545K (c.1633 G>A), H1047R (c.3140 A>G), and H1047L (c.3140 A>T) located in exons 9 and 20, as previously described (20). For patients with multiple *PIK3CA* mutations, the mutation in exon 9 or 20 were used for CDR analysis. The CDR<sub>28</sub> was calculated as both the *PIK3CA* mutant copies per ml and the allele frequency at C2D1 relative to C1D1.

### **Statistical analysis**

Part B1 followed a two-stage minimax Simon's design, with an unacceptable response rate of 0.1 and an acceptable response rate of 0.3 (with a one-sided alpha=0.05 and beta=0.2). 15 patients were to be treated in stage one and if two or more responses observed 25 patients in total were recruited with 6 responses in total required to infer efficacy. Part B2 was exploratory and intended to allocate up to 38 patients. Data for this part is presented separate for ER-ve breast cancer patients and other solid tumors. Part B3 planned to recruit 6 patients reflecting a 3+3 design. Safety analyses were performed in all patients receiving any study medication at any dose level. All efficacy and biomarker studies were conducted in the response evaluable population, which includes any enrolled patient meeting all eligibility criteria, receiving at least one cycle of trial medication and having baseline assessment of disease. Primary efficacy endpoint was confirmed objective response rate (ORR) by RECIST 1.1. Clinical benefit rate (CBR) was defined as complete response (CR), partial response (PR) or stable disease (SD) lasting  $\geq 24$  weeks. Progression free survival (PFS) was defined as the time from first dose (C1D1) until progression by RECIST 1.1 or death from any cause. For ORR, CBR and PFS analysis, 95% confidence intervals (CIs) were estimated. Log-rank test was used to test associations with PFS, with hazard ratios calculated using the Mantel-Haenszel method. For CDR<sub>28</sub>, non-parametric Wilcoxon's matched-pairs signed-rank test

was used to detect differences between C2D1 and C1D1 concentration. A p value <0.05 was considered for statistical significance in all analysis. Statistics were performed using GraphPad PRISM® version 8.2.1 and Stata® version 15.

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**TABLES.**

**Table 1.**

		A (N=20)	B1 (N=25)	B2 ER-ve (N=12)	B2 Solid tumors (N=14)	B3 (N=7)	Total (N=78)	
Median age, years (IQR)		66.2 (53.7-70.5)	57.8 (51.2-65.3)	59.5 (52.2-65.6)	63.4 (52.8-68.4)	56.2 (52.6-61.1)	59.9 (52.4-68.4)	
Performance status, n (%)	PS 0	5 (25)	14 (56)	9 (75)	2 (14)	4 (57)	34 (44)	
	PS1	15 (75)	11 (44)	3 (25)	12 (86)	3 (43)	44 (56)	
Gender, n (%)	Male	10 (50)	0 (0)	0 (0)	4 (29)	0 (0)	14 (18)	
	Female	10 (50)	25 (100)	12 (100)	10 (71)	7(100)	64 (82)	
Median prior lines of systemic therapy for advanced disease (range min-max)		3 (1-10)	3 (1-9)	2 (1-10)	3 (2-8)	2 (1-4)	3 (1-10)	
Median prior lines of cytotoxic chemotherapy for advanced disease (range min-max)		2 (0-5)	1 (0-7)	2 (1-6)	3 (0-7)	0 (0-2)	2 (0-7)	
Prior cytotoxic chemotherapy, n (%)	Yes	19 (95)	25/25 (100)	12/12 (100)	13 (93)	7/7 (100)	76 (97)	
	No	1 (5)	0/25 (0)	0/12 (0)	1 (7)	0/7 (0)	2 (3)	
Prior ET, n (%)	Yes	NA	25/25 (100)	5/12 (42)	NA	7/7 (100)	37 (84)*	
	No	NA	0/25 (0)	7/12 (58)	NA	0/7 (0)	7 (16)*	
Prior tamoxifen, n (%)	Yes	NA	20/25 (80)	3/12 (25)	NA	7/7 (100)	30 (68)*	
	No	NA	5/25 (20)	9/12 (75)	NA	0/7 (0)	14 (32)*	
Prior AI, n (%)	Yes	NA	25/25 (100)	5/12 (42)	NA	7/7 (100)	37 (84)*	
	No	NA	0/25 (0)	7/12 (58)	NA	0/7 (0)	7 (26)*	
Prior fulvestrant, n (%)	Yes	NA	6/25 (24)	0/12 (0)	NA	1/7 (14)	7 (16)*	
	No	NA	19/25 (76)	12/12 (100)	NA	6/7 (86)	37 (84)*	
Prior everolimus, n (%)	Yes	NA	10/25 (40)	0/12 (0)	NA	3/7 (43)	13 (30)*	
	No	NA	15/25 (60)	12/12 (100)	NA	4/7 (57)	31 (70)*	
Mutations as per enrolment, n (%)	PIK3CA Single	H1047R	0 (0)	10 (40)	3 (25)	2 (14)	0 (0)	15 (19)
		H1047L	0 (0)	2 (8)	0 (0)	0 (0)	0 (0)	2 (3)
		E542K	0 (0)	5 (20)	2 (17)	3 (22)	0 (0)	10 (13)
		E545K	0 (0)	6 (24)	2 (17)	1 (7)	0 (0)	9 (12)
		Others	1 (5)	0 (0)	2 (17)	6 (43)	0 (0)	9 (12)
		Unknown	5 (25)	0 (0)	1 (8)	1 (7)	1 (14)	8 (10)
	PIK3CA Multiple	H1047R + D350G	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)
		E542K + N347K	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)
		E545K + p.*1069Wext*4 (Nonstop extension)	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	1 (1)
		H1047R + I1058L + I1058F	0 (0)	0 (0)	1 (8)	0 (0)	0 (0)	1 (1)
	Others non-PIK3CA		10 (50)	0 (0)	0 (0)	1 (7)	0 (0)	11 (14)
No mutation known		4 (20)	0 (0)	0 (0)	0 (0)	6 (86)	10 (13)	

NA= Not applicable, not breast-exclusive cohorts

\* Calculated from breast cancer patients exclusive

**Table 2.**

All adverse events reported >10%	Escalation		Expansion								Total (n=78)	Total %	G3-4 %
	A (n=20)		B1 (n=25)		B2 ER-ve (n=12)		B2 solid tumors (n=14)		B3 (n=7)				
	G.1-G.2	G.3-G.4	G.1-G.2	G.3-G.4	G.1-G.2	G.3-G.4	G.1-G.2	G.3-G.4	G.1-G.2	G.3-G.4			
Neutropenia	12	6	8	15	4	7	6	4	2	5	69	88.5	47.4
Thrombocytopenia	10	4	10	1	7	0	8	0	7	0	47	60.3	6.4
Anaemia	11	3	11	1	4	0	7	1	4	0	42	53.8	6.4
Fatigue	10	1	11	0	4	0	5	0	6	0	37	47.4	1.3
Mucositis oral	4	1	10	1	6	0	3	1	3	1	30	38.5	5.1
Diarrhoea	9	1	8	1	3	1	2	0	5	0	30	38.5	3.8
Nausea	9	0	10	0	2	0	5	0	0	0	26	33.3	0.0
Leukopenia	2	1	3	7	5	1	0	1	1	3	24	30.8	16.7
Rash	6	2	6	2	3	1	0	1	2	0	23	29.5	7.7
Abdominal pain	5	0	8	1	1	0	6	0	1	1	23	29.5	2.6
ALT Elevation	5	1	6	2	1	0	1	2	3	0	21	26.9	6.4
Mucosal inflammation	10	0	3	0	2	1	2	0	0	0	18	23.1	1.3
Cough	2	0	10	0	3	0	1	0	2	0	18	23.1	0.0
Constipation	4	0	5	0	2	0	4	0	2	0	17	21.8	0.0
AST Elevation	6	0	4	2	0	0	3	0	0	1	16	20.5	3.8
Headache	2	1	5	0	3	0	3	0	2	0	16	20.5	1.3
Vomiting	4	0	3	2	1	0	3	1	1	0	15	19.2	3.8
Hypokalaemia	2	1	2	1	0	0	6	1	1	0	14	17.9	3.8
Decreased appetite	8	0	6	0	0	0	0	0	0	0	14	17.9	0.0
Dyspnoea	2	0	4	4	1	0	0	0	1	0	12	15.4	5.1
Musculoskeletal pain	3	0	2	1	2	0	1	0	2	0	11	14.1	1.3
Oropharyngeal pain	1	0	4	0	1	0	0	0	5	0	11	14.1	0.0
Alkaline phosphatase increased	3	0	2	0	1	0	1	1	1	0	9	11.5	1.3
Dyspepsia	1	0	7	0	1	0	0	0	0	0	9	11.5	0.0
Pain in extremity	2	0	4	0	0	0	0	0	3	0	9	11.5	0.0
Rhinitis	0	0	4	0	2	0	0	0	3	0	9	11.5	0.0
Upper respiratory tract infection	4	0	1	0	1	0	0	0	3	0	9	11.5	0.0
Gamma-glutamyltransferase increased	0	2	2	2	0	1	0	1	0	0	8	10.3	7.7
Hyponatraemia	1	3	0	1	0	1	1	1	0	0	8	10.3	7.7
Abdominal distension	2	0	2	1	0	0	2	0	1	0	8	10.3	1.3
Pyrexia	4	0	3	0	0	0	0	0	1	0	8	10.3	0.0
Urinary tract infection	1	0	2	0	0	0	2	0	3	0	8	10.3	0.0



## TABLE LEGENDS

**Table 1.** Demographic characteristics and mutation distribution of enrolled patients.

**Table 2.** All adverse events reported for >10% patients with grading.

## FIGURE LEGENDS

### Figure 1. Design of the PIPA trial with pharmacodynamic analysis

- A) Consort diagram for clinical trial with final number of recruited patients Part A= escalation phase, part B= expansion phase. mBC= metastatic breast cancer.
- B) Study overview and samples for translational research. ulWGS= ultra-low whole genome sequencing, IHC= immunohistochemistry, FISH= fluorescence in situ hybridization, PK= pharmacokinetics, PD= pharmacodynamics, ctDNA= circulating tumor DNA.
- C) Pharmacodynamic analysis in platelet-rich plasma for pSer473: total AKT ratio (left) and pSer9: total GSK3 $\beta$  ratio (right) at taselisib 2mg dose levels 3 (black) & 4 (red). \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , paired t-test.
- D) Pharmacodynamic analysis in tumor lysate for pSer780 Rb:total Rb ratio for dose levels 1, 2 & 4). \* $p < 0.05$ , paired t-test.

### Figure 2. Efficacy of triplet therapy of palbociclib, taselisib and fulvestrant in *PIK3CA* mutant ER+HER2- and doublet palbociclib and taselisib in ER- advanced breast cancer

- A) Waterfall plot with best percentage of tumor change from baseline sum of the longest diameter in target lesions and best confirmed overall response for evaluable patients in part B1 (N=24). SLD= Sum of the longest diameter. Best overall response colour code: green= partial response (PR), yellow= stable disease (SD), red= progressive disease (PD). Below a schematic view of prior relevant treatment and *PIK3CA* single vs double mutation as per screening.
- B) Kaplan-Meier curve representing progression-free survival (PFS) for evaluable patients in part B1 (N=24). Median PFS 7.2 months (95% CI 3.9-9.9).
- C) Left: Waterfall plot with percentage of tumor change and best overall response for evaluable ER-ve breast patients in part B2 (N=10). Below a schematic view of phenotypic classification (TNBC vs ER-ve/HER2+ve) and single vs multiple *PIK3CA* mutation as per enrolment. Right: Kaplan-Meier curve representing progression-free survival (PFS) for evaluable for ER-ve breast patients in part B2 (N=10). Median PFS 3.6 months (95% CI 1.7-5.6).

### Figure 3. Baseline biomarker analysis on tumor tissue

- A) Ultra-low whole genome sequencing (ulWGS) for baseline CNV of all *PIK3CA*-mt ER+ve/HER2-ve breast patients in part B1 with baseline tumor sequencing (N=17), reported as thresholded adjusted copy number call (amplification/gain/neutral/heterozygous deletion/homozygous deletion). On the right best tumor change and progression-free survival (PFS) per patient.
- B) Examples of cyclin E immunohistochemistry staining (left to right: high, moderate and low expression).
- C) Kaplan-Meier curve representing progression-free survival (PFS) for patients stained with cyclin E in part B1 (N=21), split by above/below median cyclin E percentage of invasive nuclei stained. HR 4.2 (95% CI 1.3-13.1),  $p = 0.02$ , Log-rank test.
- D) H-score for androgen receptor (AR) expression distribution in available baseline tumors of TNBC patients available (N=7). Median 270 H-score for patients achieving CBR (N=3) vs 90 H-score for patients without CBR (N=4),  $p = 0.20$ , Mann-Whitney test.

#### Figure 4. Circulating tumor DNA dynamics as a surrogate of efficacy

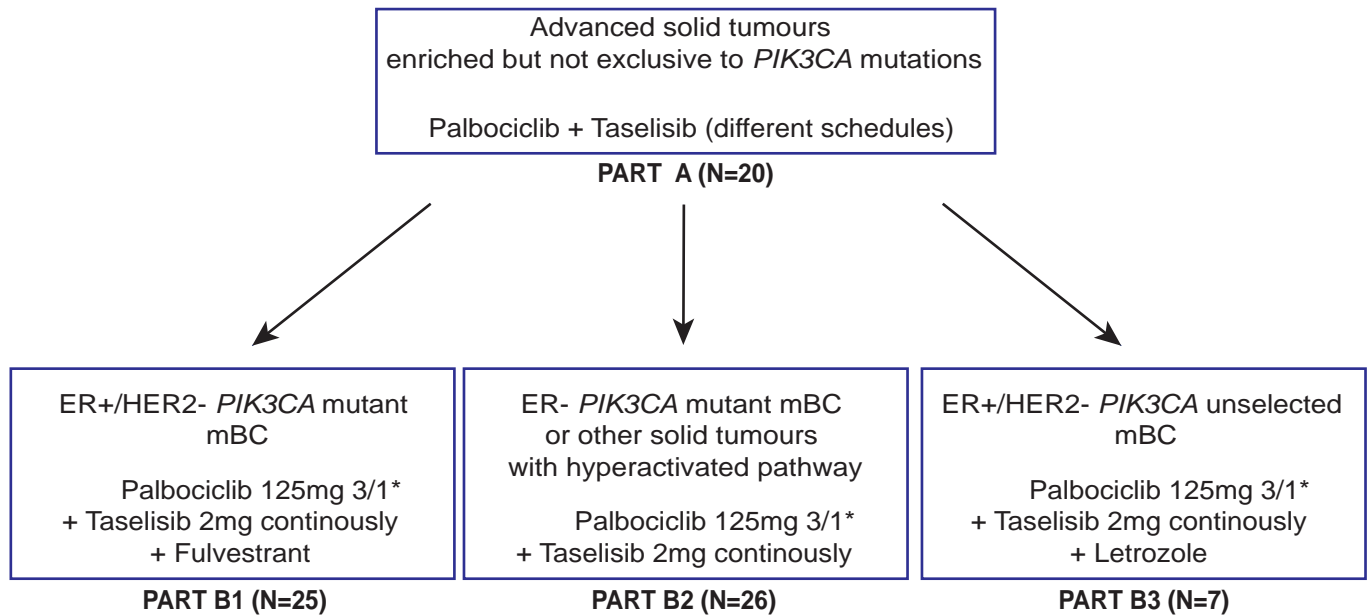
- A) Early *PIK3CA* mutation dynamics comparing C1D1 to C2D1 allele frequency for the whole population analysed passing predefined quality control for longitudinal analysis (N=30).  $P < 0.01$ , Wilcoxon signed-rank test (paired).
- B) C1D1 to C2D1 *PIK3CA* allele frequency for part B1 patients (N=16).  $p < 0.01$ , Wilcoxon signed-rank test (paired).
- C) Kaplan-Meier curve representing progression-free survival (PFS) for patients in part B1 stratified above/below 0.1 CDR28 allele frequency. Median PFS 6.9m vs 15.2m, respectively. HR 5.2 (95% CI 1.4-19.4),  $p = 0.04$ , Log-rank test.

#### Figure 5. Paired ctDNA sequencing to study evolution through triplet therapy.

- A) Double *PIK3CA* mutation allele frequencies dynamics for patients with at least 1 read in both timepoints (N=4) represented in a log<sub>2</sub> scale. C1D1= Cycle 1 Day 1, EOT= End of treatment.
- B) Kaplan-Meier curves for patients in part B1 stratified as double vs single *PIK3CA* mutant (N=16). Median PFS 2.7 vs 8.5m respectively.  $p < 0.0001$ , Log-rank test
- C) *ESR1* mutation allele frequencies dynamics with frequent acquisition and loss of *ESR1* mutations. Evidence of a second Y537S and Y537C acquisition, loss of T182P in a double mutant patient and loss of E380Q in a single mutant patient.
- D) Lollipop diagram for predicted truncating *FAT1* mutations.
- E) *FAT1* mutation allele frequencies dynamics represented in a log<sub>2</sub> scale. PIPA20 had an acquisition.

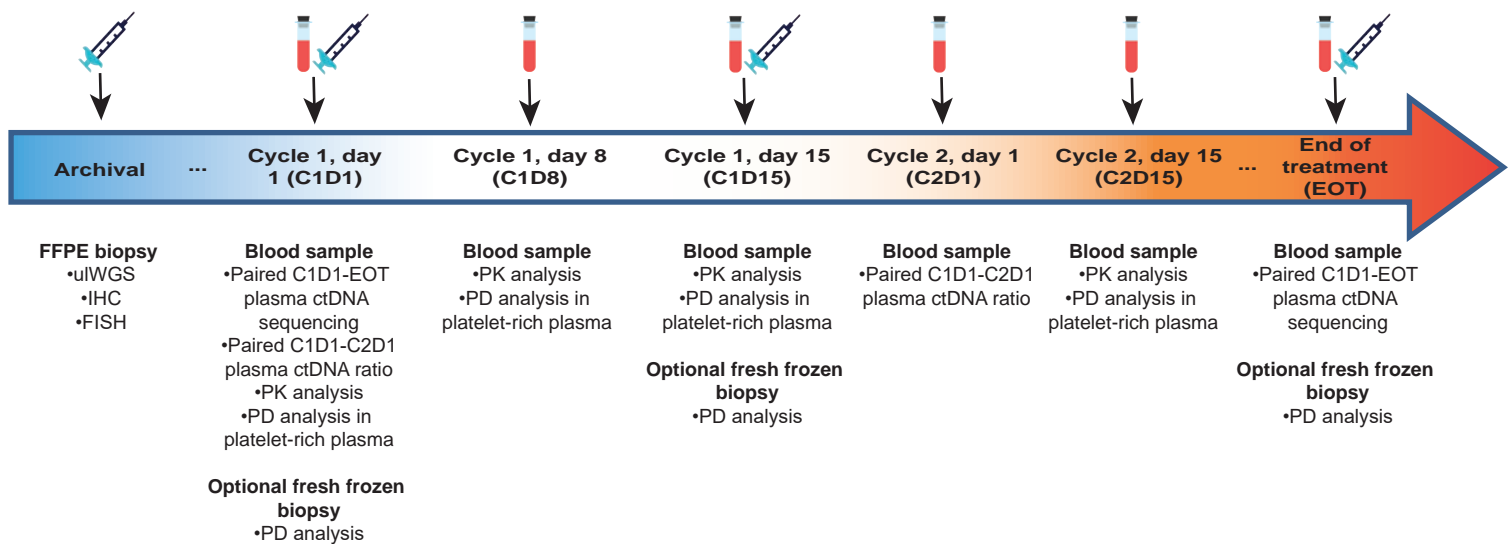
Fig. 1

A

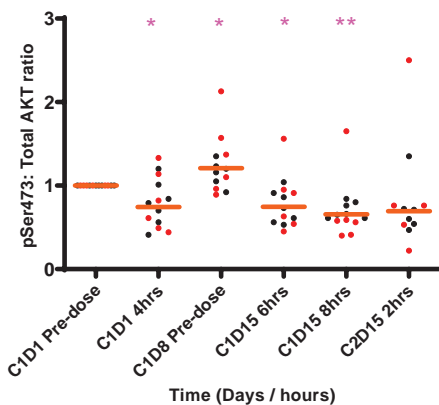


\*Following a safety review committee recommendation, in dose expansion (part B) palbociclib was administered at 100mg OD 3/1 for cycle 1, escalating to 125mg OD 3/1 in the absence of myelosuppression.

B



C



D

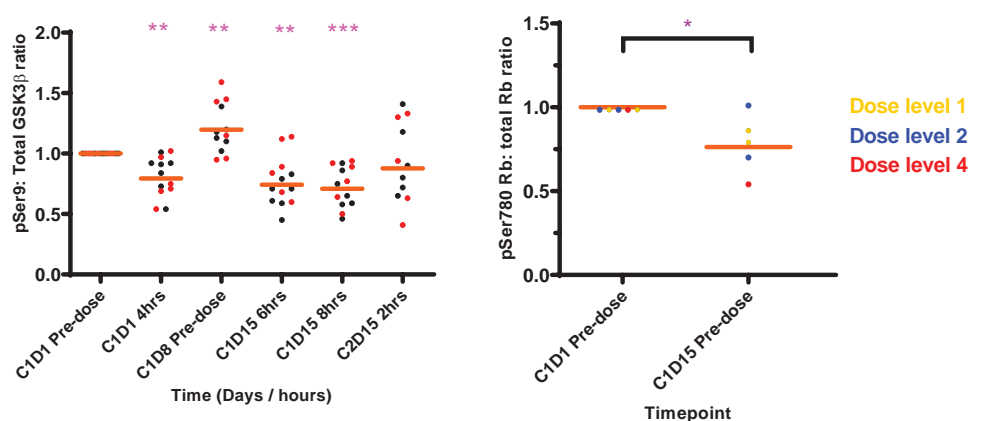
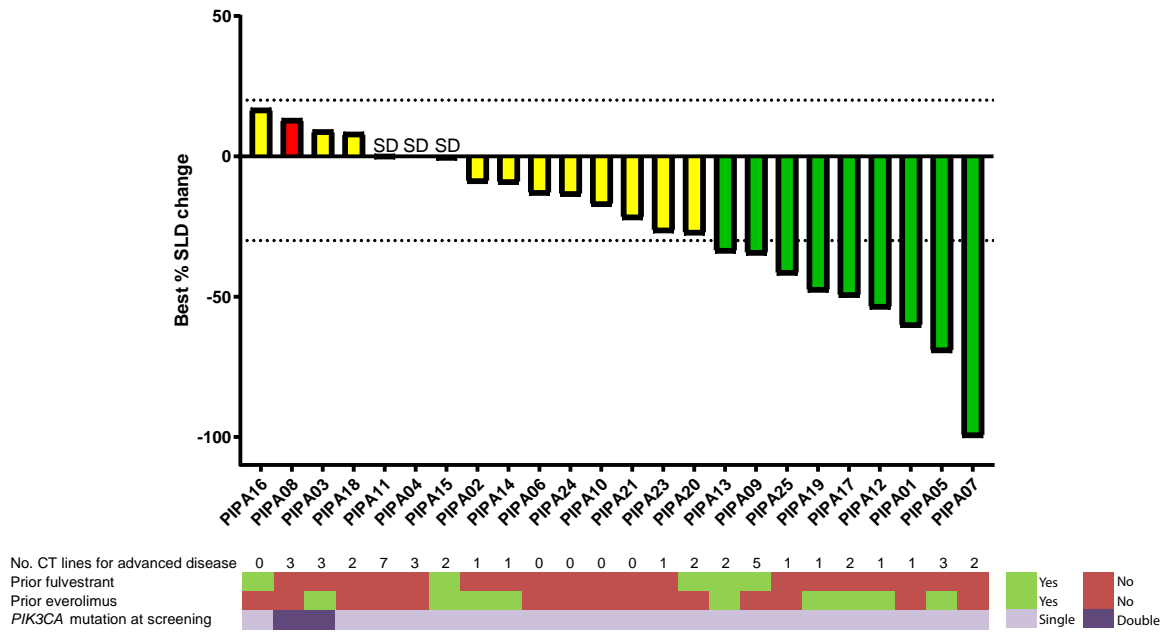
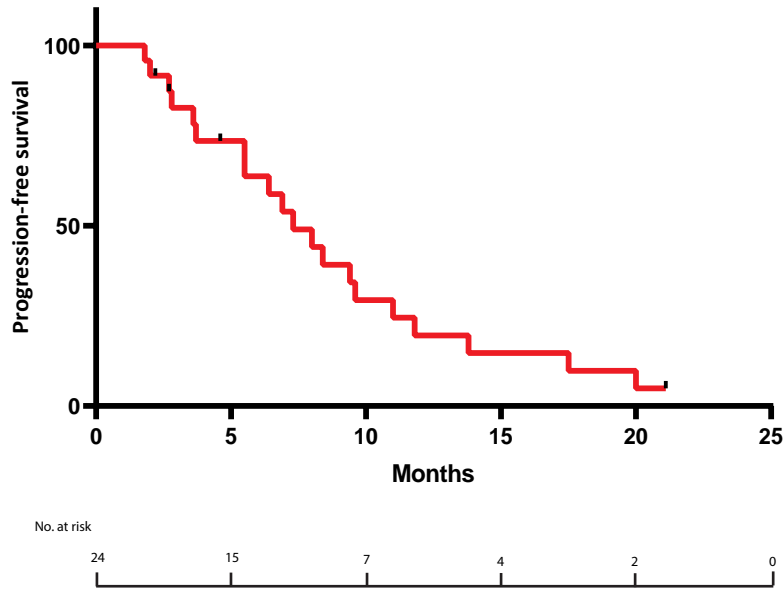


Fig. 2

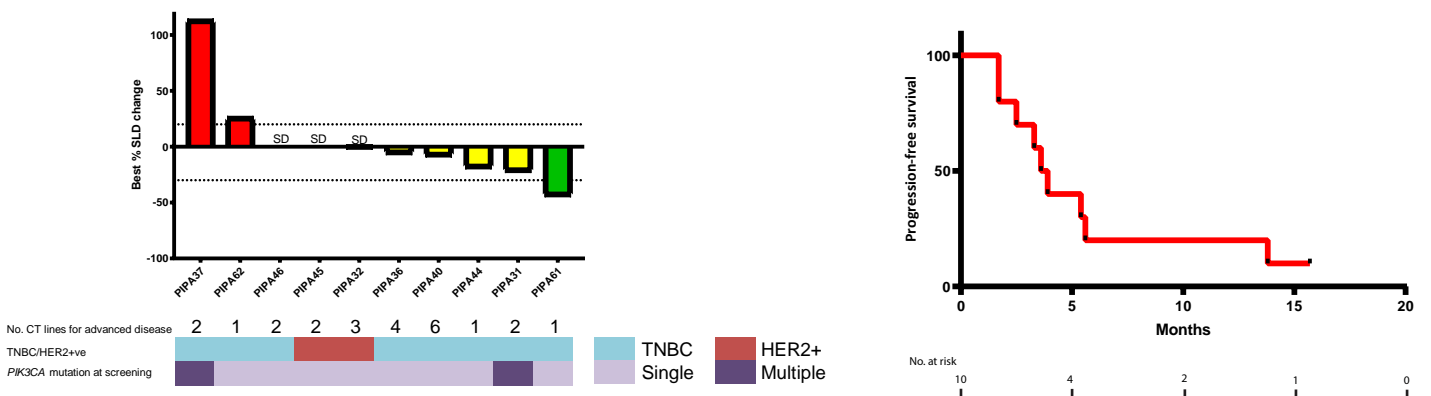
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B

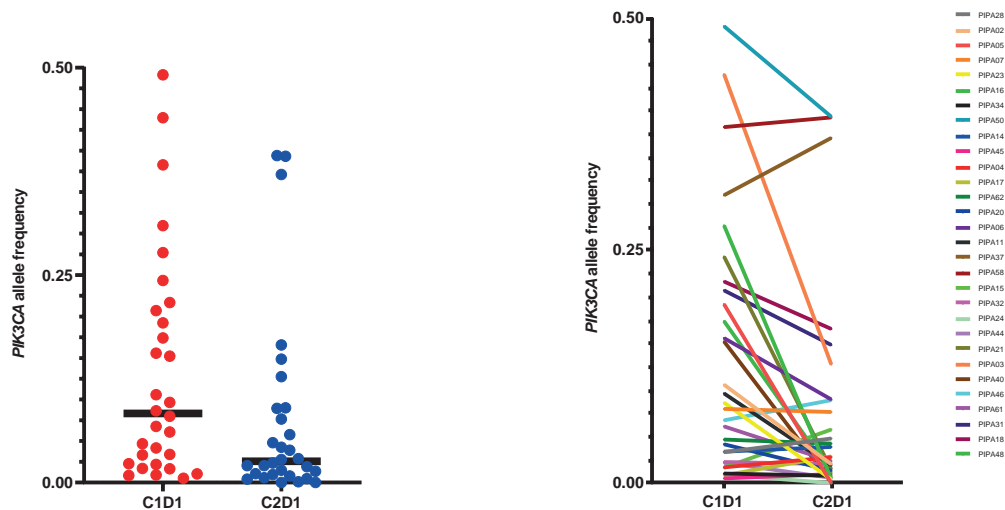


C

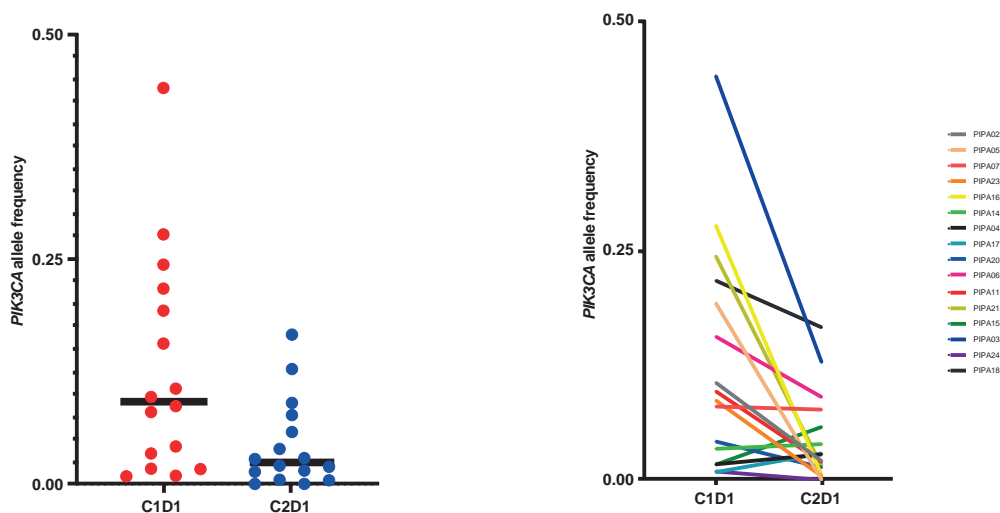




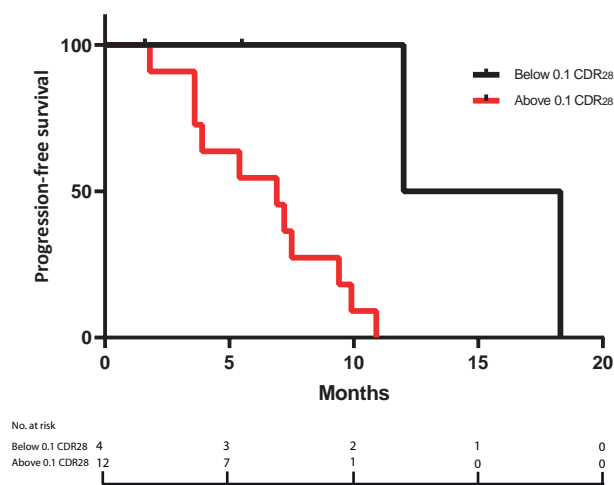
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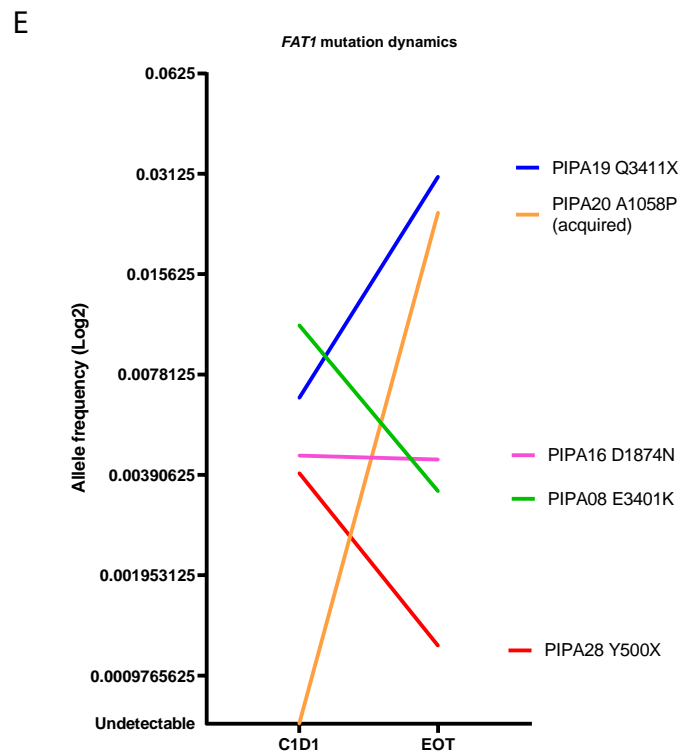
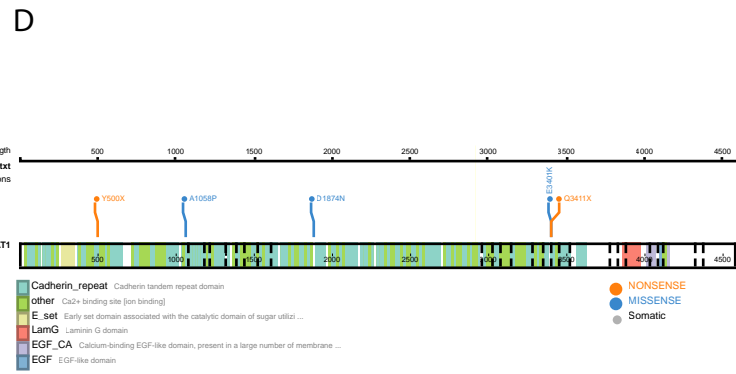
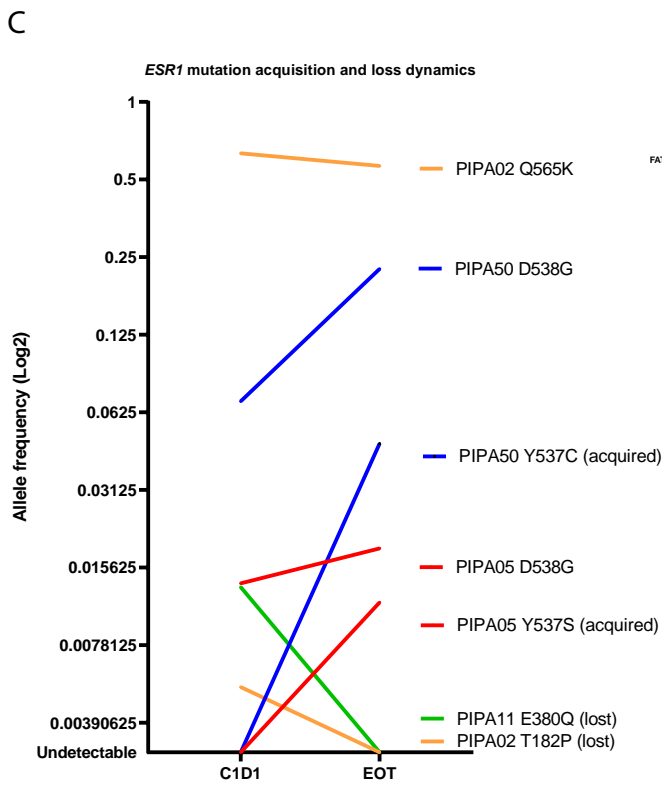
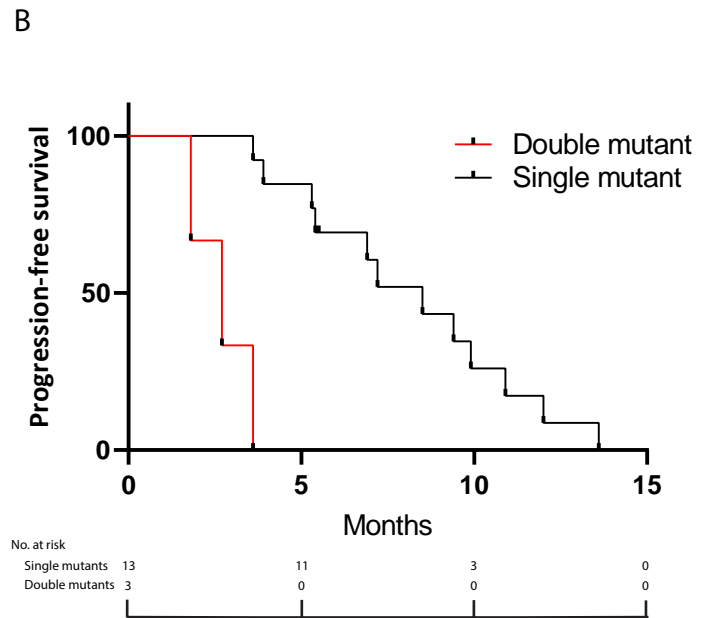
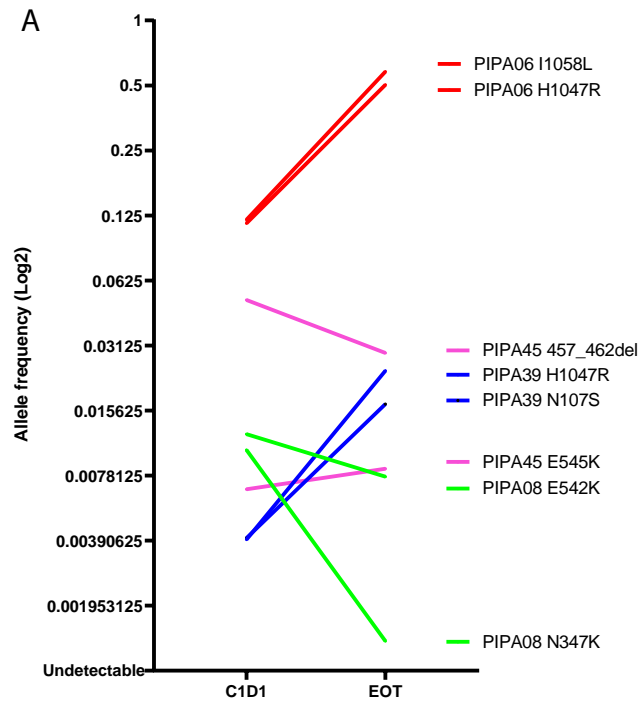


B



C







# CANCER DISCOVERY

## Triplet therapy with palbociclib, taselisib and fulvestrant in PIK3CA mutant breast cancer and doublet palbociclib and taselisib in pathway mutant solid cancers.

Javier Pascual, Joline S.J. Lim, Iain R J Macpherson, et al.

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