Clinical Cancer Research

A Phase Ib Study of Alpelisib (BYL719), a PI3Kα-Specific Inhibitor, with Letrozole in ER$^+$/HER2$^-$ Metastatic Breast Cancer

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Abstract

Purpose: Alpelisib, a selective oral inhibitor of the class I PI3K catalytic subunit p110α, has shown synergistic antitumor activity with endocrine therapy against ER$^+$/*PIK3CA*-mutated breast cancer cells. This phase Ib study evaluated alpelisib plus letrozole's safety, tolerability, and preliminary activity in patients with metastatic ER$^+$ breast cancer refractory to endocrine therapy.

Experimental Design: Twenty-six patients received letrozole and alpelisib daily. Outcomes were assessed by standard solid-tumor phase I methods. Tumor blocks were collected for DNA extraction and next-generation sequencing.

Results: Alpelisib's maximum-tolerated dose (MTD) in combination with letrozole was 300 mg/d. Common drug-related adverse events included hyperglycemia, nausea, fatigue, diarrhea, and rash with dose-limiting toxicity occurring at 350 mg/d of alpelisib. The clinical benefit rate (lack of progression ≥6 months) was 35% (44% in patients with *PIK3CA*-mutated and 20% in *PIK3CA* wild-type tumors; 95% CI, 17%–56%), including five objective responses. Of eight patients remaining on treatment ≥12 months, six had tumors with a *PIK3CA* mutation. Among evaluable tumors, those with *FGFR1/2* amplification and *KRAS* and *TP53* mutations did not derive clinical benefit. Overexpression of *FGFR1* in ER$^+$/*PIK3CA* mutant breast cancer cells attenuated the response to alpelisib *in vitro*.

Conclusions: The combination of letrozole and alpelisib was safe, with reversible toxicities. Clinical activity was observed independently of *PIK3CA* mutation status, although clinical benefit was seen in a higher proportion of patients with *PIK3CA*-mutated tumors. Phase II and III trials of alpelisib and endocrine therapy in patients with ER$^+$ breast cancer are ongoing. *Clin Cancer Res; 23(1); 26–34. ©2016 AACR.*

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Introduction

The PI3K pathway is the most frequently altered pathway in cancer, including mutation and/or amplification of the genes encoding the PI3K catalytic subunits p110α (*PIK3CA*) and p110β (*PIK3CB*), the PI3K regulatory subunit p85α (*PIK3R1*), AKT1-3, and the phosphatidylinositol-3,4,5 trisphosphate (PIP_3) phosphatases *PTEN* and *INPP4B*, among others. *PIK3CA* mutations induce a transformed phenotype, including growth factor- and anchorage-independent growth, resistance to anoikis, and drug resistance (1–4). About 40% of ER$^+$ breast cancers harbor *PIK3CA* mutations (5–7).

We and others have shown that aberrant activation of PI3K signaling is associated resistance to endocrine therapy (8). PI3K signaling has been shown to promote estrogen-independent growth of ER$^+$ breast cancer cells (9, 10), and this growth is inhibited by the addition of PI3K inhibitors to antiestrogens (11). Additionally, inhibition of PI3K prevents the emergence of hormone-independent cells, which suggests that early intervention with antiestrogens and PI3K inhibitors could limit escape from endocrine therapy.

Drugs targeting multiple levels of the PI3K network have been developed (12, 13). Alpelisib (BYL719; Novartis Pharma AG) is an oral inhibitor that selectively targets p110α (14, 15). A phase I study of alpelisib, alone and in combination with fulvestrant, declared its maximum tolerated dose (MTD) as 400 mg/d (16).

The primary objective of this phase Ib trial (NCT01791478) was to determine the safety and tolerability of letrozole, an aromatase inhibitor, in combination with alpelisib in patients with ER$^+$/HER2$^-$ metastatic breast cancer refractory to endocrine therapies. Secondary objectives included antitumor activity and correlation of clinical outcome with presence of *PIK3CA* mutations in tumor specimens. Preliminary data from other ongoing clinical trials of alpelisib with letrozole (NCT01872260) show no evidence of pharmacokinetic (PK) drug–drug interactions (17), therefore, no PK analysis was planned for this trial.

Patients and Methods

Patient population

The patient population included postmenopausal patients with histologically confirmed ER$^+$/HER2$^-$ metastatic breast

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Translational Relevance

The combination of the PI3Kα-specific inhibitor alpelisib and the aromatase inhibitor letrozole is safe and clinically active in patients with ER$^+$/*PIK3CA*-mutant breast cancer refractory to primary endocrine therapy. This result suggests a novel molecularly targeted strategy that may abrogate or delay the emergence of antiestrogen resistance in hormone-dependent breast cancer.

cancer refractory to at least one line of endocrine therapy in the metastatic setting, or diagnosed with metastatic breast cancer during or within 1 year of adjuvant endocrine therapy; evaluable disease as defined by Response Evaluation Criteria In Solid Tumors (RECIST); age ≥18 years; life expectancy ≥6 months; ECOG performance status ≤1; adequate bone marrow, hepatic, and renal function; and fasting plasma glucose levels ≤140 mg/dL (7.8 mmol/L). A tumor specimen (primary or metastatic) from archival material or a fresh biopsy was required for study enrollment. Key exclusion criteria were CYP3A4 modifier drug treatment ≤2 weeks before starting alpelisib, clinically manifested diabetes mellitus, and prior treatment with PI3K inhibitors. Prior treatment with everolimus was allowed.

Approval was obtained from the ethics committees (IRB #101057, Vanderbilt University) at the participating institutions and regulatory authorities. All patients gave informed consent. The study followed the Declaration of Helsinki and Good Clinical Practice guidelines.

Study design

This phase Ib, multicenter, open-label study enrolled subjects using a standard 3+3 dose escalation design. All patients received letrozole 2.5 mg/d; alpelisib was initiated at 300 mg/d, 25% below the single-agent MTD. Both medications were administered daily on a 28-day cycle. In case of adverse events requiring dose adjustments, alpelisib was reduced to 250 mg/d. Intrapatient dose reductions were allowed after the initial 4 weeks of treatment. Patients were treated until disease progression, unacceptable toxicity, or withdrawal of consent.

Dose-limiting toxicities (DLTs) were defined as Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 grade ≥3 toxicities. Exceptions were any grade ≥2 toxicity necessitating treatment interruption for more than 21 consecutive days, and non-CTCAE grade 2 hyperglycemia not resolved to grade 0 within 14 consecutive days of initiation of oral antidiabetic medications (metformin). Grade ≥3 anemia was not considered a DLT unless judged to be a hemolytic process secondary to study drug. Grade ≥3 lymphopenia was not considered a DLT unless clinically significant.

The MTD was defined as the highest dose of alpelisib in combination with letrozole not causing DLT in more than 33% of patients in the first treatment cycle. Twenty or more evaluable patients had to be treated before declaration of the MTD, with ≥6 evaluable patients treated at the MTD for one cycle. Criteria for evaluability were ≥21 days on alpelisib in cycle 1 or early discontinuation due to a DLT. The recommended phase II dose was defined as the highest dose at or below the MTD at which ≥75% of the patients could tolerate therapy for a minimum of 8 weeks without development of grade ≥2 hyperglycemia for more than 14 consecutive days despite initiation of oral antidiabetic medications; and grade ≥3 rash, grade ≥2 nausea, vomiting or diarrhea, and grade ≥2 rash, all for more than 14 consecutive days of optimal medical treatment.

Safety and radiographic assessments

Clinical and laboratory assessments were conducted at baseline and weekly during cycle 1; on days 1 and 15 of cycle 2; and on day 1 of subsequent cycles. Safety assessments included serial electrocardiograms and fasting plasma glucose. Radiographic responses were assessed every 2 months using RECIST version 1.1. Best response analysis was conducted in any patient that surpassed 8 weeks of treatment, therefore having at least one set of radiographic assessments. Clinical benefit rate was defined as any patient that did not have disease progression on their radiographic assessments for a set time frame (i.e., ≥ 6 or 12 months). Patients that discontinued study treatment due to toxicity prior to their first radiographic assessment were considered nonevaluable.

Mutation analyses

DNA was extracted using DNEasy or QiaAMP DNA tissue kits from formalin-fixed paraffin-embedded (FFPE) archival tumor sections or snap-frozen biopsies of metastases, respectively. Tumor cellularity was assessed by an expert breast pathologist (MES); specimens with ≥20% tumor nuclei were considered evaluable. In the few cases with paucicellular samples (<20% tumor cells), multiple sections were macro-dissected to achieve ≥20% tumor cellularity.

DNA was initially subjected to SNaPShot (18) analysis of 18 mutations in *PIK3CA*, *PTEN*, and *AKT1*, including the common hotspot mutations in exons 9 and 20 of *PIK3CA*, as previously described (19). SNaPShot is based on multiplex PCR, primer extension with fluorescently tagged dideoxy-nucleotides and capillary electrophoresis; it is a fast, high-throughput, multiplex profiling method based on the Applied Biosystems SNaPshot platform.

Subsequently, next-generation sequencing (NGS) was performed using the MSK-IMPACT (Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets; ref. 20), a hybridization capture-based assay for targeted deep sequencing of all exons and selected introns of 341 key cancer genes in DNA from FFPE tumor sections. Custom DNA probes targeting exons and selected introns of 341 genes were synthesized using the NimbleGen SeqCap EZ library custom oligo system and biotinylated to allow for sequence enrichment by capture using streptavidin-conjugated beads. Pooled libraries containing captured DNA fragments were subsequently sequenced on the Illumina HiSeq 2500 system (rapid-run mode) as 2 × 100-bp paired-end reads.

FGFR1 fluorescence *in situ* hybridization (FISH)

Three- to 4-μm tissue sections were mounted on sialinized slides and hybridized overnight with the ZytoLight SPEC FGFR1/CEN 8 Dual-Color Probe (ZytoVision). Briefly, deparaffinization, pretreatment, then the slides were denatured in the presence of 10-μL probe for 6 minutes at 73°C and hybridized at 37°C overnight in StatSpin (Thermobrite, Abbott Molecular, Inc.). Posthybridization saline sodium citrate washes were performed at 72°C, and the slides were next stained with DAPI before analysis. Slides were analyzed with Reflected light

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fluorescent microscope (Olympus BX60) at 100× for hotspots. Representative images of tumor cells were captured using Cytovision software. Thirty tumor cell nuclei from hotspots or random areas were individually evaluated with the 100× by counting green *FGFR1* and orange centromer 8 (CEN8) signals. The average of *FGFR1* copy number and the *FGFR1*/CEN8 was calculated. Cases were considered as *FGFR1* positive ('amplified') under one of the following conditions:

a. The *FGFR1*/CEN8 ratio is ≥2.0.
b. The average number of *FGFR1* signals per tumor cell nucleus is ≥6.
c. The percentage of tumor cells containing ≥15 *FGFR1* signals or large clusters is ≥10%.
d. The percentage of tumor cells containing ≥5 *FGFR1* signals is ≥50%, with (a–c) representing a high-level and (d) a low-level amplification (21).

Cell lines

MCF7 and CAMA-1 cells were obtained from ATCC and were cultured in DMEM supplemented with 10% FBS.

Lentivirus transduction

FGFR1- and GFP-expressing lentiviruses were generated by cotransfecting 4-μg proviral pLX302-FGFR1 or pLX302-GFP plasmids (Open BioSystems), 3 μg psPAX2 (plasmid encoding *gag, pol, rev*, and *Tat* genes) and 1 μg pMDG2 envelope plasmid (Sigma Aldrich) into 293FT cells using Lipofectamine 2000 (Thermo Fisher). 293FT cells were fed with growth media 24 hours after transfection; virus-containing supernatants were harvested 48 and 72 hours after transfection, diluted 1:4 and applied to target cells with 8 μg/mL polybrene (Sigma Aldrich). Target cells were selected with 1 μg/mL puromycin.

Immunodetection

Cells were plated in phenol red-free IMEM + 10% dextran-charcoal–treated FBS and treated for 6 hours with drugs or vehicle. Stimulation with FGF2 (Sigma Aldrich) was performed 20 minutes before the lysis. Lysates were generated by removing media from cells, washing monolayers with cold PBS and lysis with RIPA: 50 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 1% Nonidet P-40, 0.5% Deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 50 mmol/L NaF, 1 mmol/L NaVO4, and 1× protease inhibitor mixture (Roche). Lysates were clarified by centrifugation at 15,000 × *g* for 15 minutes. Protein concentration was determined by BCA assay (Thermo scientific). For immunoblot analysis, equal amounts of protein/lane were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and probed with the following antibodies: phospho-Akt (Ser473), total-AKT, phospho-p44/42 MAPK (Erk1/2), total p44/p42 MAPK (Erk1/2), and actin (Cell Signaling Technologies); FGFR1 antibody was obtained from Abcam. Immunoreactive proteins were visualized by enhanced chemiluminescence (Pierce).

Clonogenic assays

For longer-term growth assays, CAMA-1 cells were seeded into 6-well plates in estrogen free-media (IMEM + 10% dextran-charcoal–treated FBS) plus 5 ng/mL FGF2 and treated with vehicle, alpelisib 2 μmol/L, lucitanib 2 μmol/L (provided by Clovis Oncology), and the combination. Media and inhibitors were replenished every 3 days, and cells were grown for 2 weeks until the untreated wells achieved 80% confluence. Cells were fixed and stained in 20% methanol with 0.5% crystal violet and washed with water. Dried plates were imaged on a flatbed scanner. Afterward, the crystal violet was solubilized with 20% acid acetic and quantitated by spectrophotometric detection at 490 nm using a plate reader (GloMax-Multi Detection System, Promega).

Cell viability

MCF-7 cells were plated in 96-well plates (3,000 cells per well) in a volume of 100 μL of phenol red-free IMEM + 10% dextran-charcoal–treated FBS. Twenty-four hours later, DMSO (vehicle) or alpelisib (0.05–4 μmol/L) ± lucitanib 1 μmol/L, all in the presence of 5 ng/mL FGF2 were added to the wells. After 72 hours, cell proliferation was measured using the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Percentage inhibition was calculated relative to median signal from DMSO-treated wells.

Results

Study population

Twenty-six patients were enrolled from April 2013 to February 2014 (Table 1). Overall, 85% of the patients had bone metastases and approximately 30% had visceral metastases. All patients not presenting with *de novo* metastatic disease had previously been exposed to an aromatase inhibitor in the adjuvant setting. In the metastatic setting, 85% had been exposed to at least one line of endocrine therapy, 69% to an aromatase inhibitor, and 31% to chemotherapy. Evaluable tumor samples were obtained in all 26 patients; 74% of these were FFPE blocks from the primary tumor.

Dose escalation and MTD

Alpelisib dose was started at 300 mg/d. DLT (grade 3 rash) occurred in two patients at the 350 mg/d dose. These rashes eventually resolved with medical intervention (high-dose antihistamines, topical, and/or oral corticosteroids) and drug interruption. One patient was able to restart at 300 mg/d. The alpelisib MTD was set at 300 mg/d; a total of 20 patients were treated at this dose (escalation and expansion phases). Less than 25% of

Table 1. Patient characteristics

Characteristics	*N* = 26
Median age (range)	53 (31–72)
Median time since original diagnosis (months, range)	34 (0–99)
Metastatic lesions	
Bone	22 (85%)
Visceral	
Liver	7 (27%)
Lung	6 (23%)
Pleura/peritoneum	2 (8%)
Other	
Lymph nodes	6 (23%)
Ovary	1 (4%)
Prior therapies in the metastatic setting[a]	
Median number of prior therapies (range)	2 (1–4)
Endocrine therapy	
Any	22 (85%)
Aromatase inhibitor	18 (69%)
Chemotherapy	8 (31%)

[a]All patients (except for the ones presenting with *de novo* metastatic disease) had exposure to an aromatase inhibitor in the adjuvant setting.

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patients required alpelisib interruption or dose reduction during the first 8 weeks of treatment.

Safety findings

Adverse events in this trial were similar to reports from the phase I single-agent alpelisib trial (16) and are summarized in Table 2. The most common side effects were gastrointestinal disorders (73%), hyperglycemia (62%), fatigue (54%), and rash (42%); all of these were dose dependent. Grade 3 adverse events associated with alpelisib were uncommon and generally cumulative (such as hyperglycemia and gastrointestinal disturbances), except for maculopapular rash, which tended to appear within the first 2 weeks of treatment. Of note, rash was less frequent in incidence and severity in patients that had been on antihistamines (i.e., due to seasonal allergies) prior to trial enrollment. Rashes (of any grade) responded well to high-dose antihistamines (twice-daily dosing), obviating the need for corticosteroids in the majority of patients developing this adverse event.

Radiographic assessments

Twenty-three of 26 patients (88%) were evaluable for best response, three of 26 patients discontinued treatment due to toxicity prior to their first radiographic assessment (Table 3). Five patients (19%) achieved a partial response; four of these patients had ER$^+$/*PIK3CA*-mutated breast cancer. Clinical benefit rate (lack of disease progression $\geq$6 months) was seen in nine patients (35%; 95% CI, 17%–56%), and eight patients (31%; 95% CI, 14%–52%) continued to have clinical benefit for more than 12 months. Of those eight patients, six had ER$^+$/*PIK3CA*-mutant breast cancer, and all had previous exposure to an aromatase inhibitor in the metastatic setting (Fig. 1). Five patients are still on study for more than 12 months (data cutoff, June 2015).

Mutation analyses

Seventy-five percent (61%) of analyzed tumors were FFPE sections from the original breast cancer diagnosis (median time since original diagnosis 34 months; range, 0–99). We initially screened for *PIK3CA* mutations with SNaPShot (ref. 18; on all 26 tumors' DNA). We then followed with targeted capture NGS (23 tumors were evaluable for this analysis) in order to identify somatic alterations associated with clinical benefit or lack thereof. In all cases, findings by SNaPShot were confirmed by NGS. NGS analysis showed fewer genomic alterations in tumors from patients with clinical benefit compared with those without clinical benefit (41 vs. 79 genomic alterations; not shown). Most common alterations (>10%) were *PI3KCA*, *TP53*, *GATA3*, and *BRCA1/2* mutations, *MCL1*, *FGFR1*, *CCND1*, *FGF3/4/19*, and *CCND1* amplifications, and deletions/truncations in *PTEN* (Fig. 2A). The majority of *PIK3CA* mutations were in exons 9 and 20 (E545K, E542K, and Q546K, seven tumors; H1047R alone in five tumors). Interestingly, four of five (80%) patients with tumors with *PIK3CA*^{H1047R} had durable clinical responses, whereas only two of seven (28%) tumors with mutations in exon 9 exhibited a clinical response in excess of 6 months. One of these tumors had a mutation in the Ras-binding domain (I273V) in addition to E545K. A tumor with a novel deletion in the C2 domain of *PIK3CA* (P447_L455del) exhibited a solid partial response in liver metastases (Fig. 1).

Seventeen of 23 patients (74%) with NGS data were evaluable for clinical benefit $\geq$6 months; three patients discontinued study treatment due to toxicity prior to their first radiographic assessment; three patients discontinued study treatment due to toxicity prior to their second radiographic assessment and were therefore considered nonevaluable for the NGS data/outcome analysis. Seven out of 17 patients (41%) had durable responses to the combination (Fig. 2A). Five of seven (71%) patients with clinical benefit had a *PIK3CA*-mutant tumor, in contrast to five of 10 (50%) patients without clinical benefit (Fig. 2B). Two patients with tumors harboring both *PIK3CA* and a known activating *ESR1* mutation (Y537S and D538G, respectively) in their primary tumor exhibited stable disease or partial response. Interestingly, among patients evaluable for response, all those with cancers with *FGFR1* and *FGFR2* amplification, and *KRAS* and *TP53* mutations progressed on treatment. *FGFR1* amplification detected by NGS was confirmed by FISH analysis (Fig. 2C).

FGFR1 overexpression blocks the effect of alpelisib of ER$^+$/*PIK3CA* mutant cells

To explore whether overexpression of *FGFR1* is causal to resistance to alpelisib, we first examined the effect of alpelisib

Table 2. Most common adverse events by dose

Toxicity category/toxicity (CTCAE v.4)	300 mg/d (*N* = 20) *n* (%) of subjects		350 mg/d (*N* = 6)	
	Total	Grade 3	Total	Grade 3
Gastrointestinal disorders				
Nausea	12 (60%)		4 (67%)	1 (17%)
Diarrhea	16 (80%)	2 (10%)	3 (50%)	
Mucositis (oral)	2 (10%)		1 (17%)	
Vomiting	5 (25%)		2 (33%)	
Dysgeusia	4 (20%)		3 (50%)	
General disorders				
Fatigue	9 (45%)		5 (83%)	
Investigations				
Transaminase elevation	4 (20%)	1 (5%)	1 (17%)	
Metabolism and nutrition disorders				
Hyperglycemia	11 (55%)	2 (10%)	5 (83%)	2 (33%)
Anorexia	5 (25%)		3 (50%)	
Skin and subcutaneous tissue disorders				
Rash maculopapular	9 (45%)		2 (33%)	2 (33%)

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Table 3. Clinical activity[a] by *PIK3CA* mutation status

	PIK3CA mutation status		
	Mutated	Wild-type	Total
Clinical Activity[a]	**16**	**10**	**26**
Partial response	4 (25%)	1 (10%)	5 (19%)
Stable disease	6 (38%)	6 (60%)	12 (46%)
Disease progression	4 (25%)	2 (20%)	6 (23%)
Clinical benefit (No disease progression)			
≥ 6 months	7 (44%)	2 (20%)	9 (35%)
≥ 12 months	6 (38%)	2 (20%)	8 (31%)
Nonevaluable	2 (13%)	1 (10%)	3 (12%)
Still on study	3 (19%)	2 (20%)	5 (19%)

[a]Objective response was assessed by RECIST on the first post-baseline target lesion.

in the ER$^+$ CAMA-1 breast cancer cells, which harbor *FGFR1* amplification. FISH analysis of these cells showed a *FGFR1*/CEN8 ratio of 2.2, consistent with *FGFR1* gene amplification (Fig. 3A). Growth of CAMA-1 cells in estrogen-free medium supplemented with FGF2 was completely insensitive to alpelisib, but inhibited by the FGFR1-3 tyrosine kinase inhibitor (TKI), lucitanib (Fig. 3B). Treatment with alpelisib inhibited p-AKT but not p-ERK, whereas lucitanib inhibited p-FRS2 and p-ERK but not p-AKT (Fig. 3C). We then stably transduced ER$^+$/*PIK3CA*-mutant MCF7 cells with a *FGFR1* expression vector. Compared with MCF7^{GFP} control cells, *FGFR1*-overexpressing cells exhibited slightly higher levels of p-AKT and p-ERK (Fig. 3D). Similar to the results with CAMA-1 cells, treatment with alpelisib inhibited p-AKT but not p-ERK, whereas the addition of lucitanib mainly inhibited p-ERK but not p-AKT levels. Finally, MCF7^{GFP} cells grown as monolayers had an IC_{50} to alpelisib <1 μmol/L, whereas this was at least 10-fold higher in MCF7^{FGFR1} cells. The addition of lucitanib resensitized MCF7^{FGFR1} cells to the PI3Kα inhibitor (Fig. 3E). Altogether, these data suggest a causal association between *FGFR1* overexpression and activation of ERK with relative resistance to alpelisib.

Discussion

The initial rationale for the development of isozyme-specific antagonists was to allow anti-p110α, anti-p110β, and anti-p110δ agents to be delivered at maximal target-inhibitory doses while potentially avoiding the side effects of pan-PI3K inhibitors. Specific inhibitors of p110α, such as alpelisib and MLN1117, and p110β-sparing inhibitors (e.g., taselisib, GDC-0032) are being

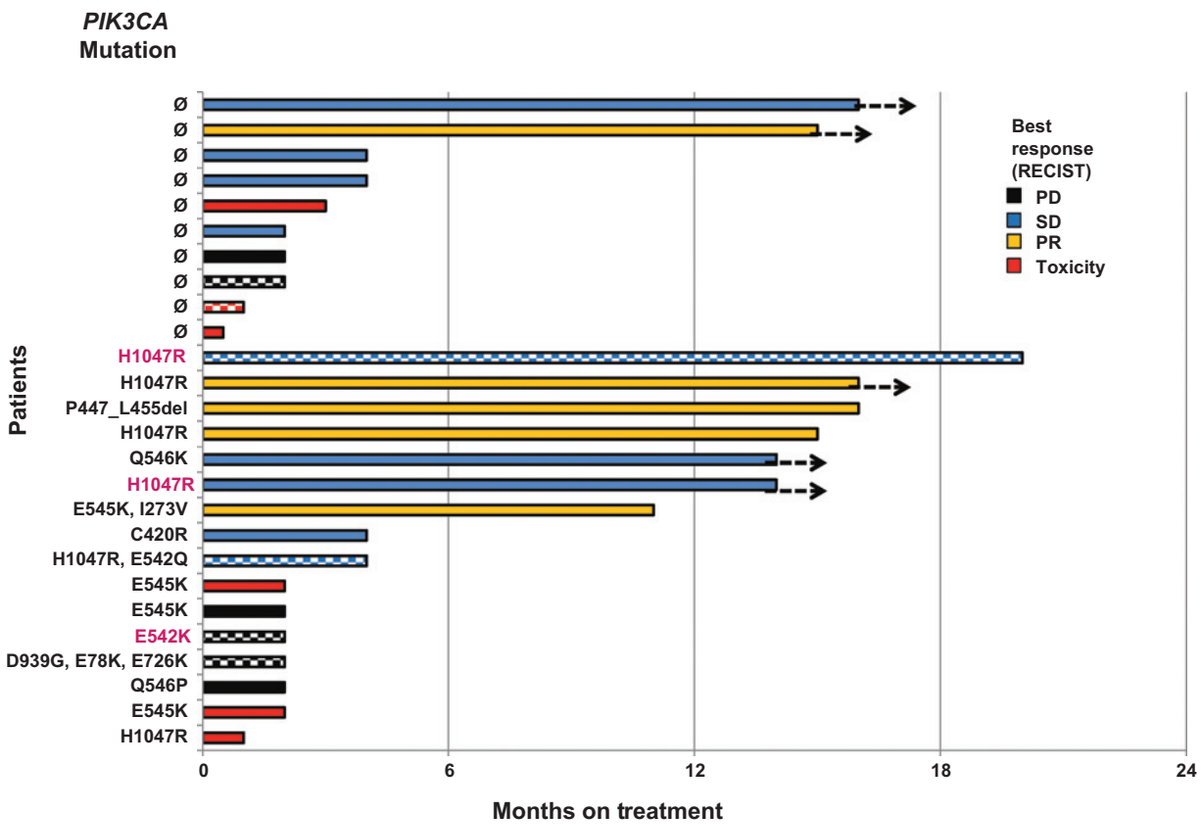

Figure 1.
Treatment duration (months) with corresponding radiologic response and tumor *PIK3CA* mutation status. Best response by RECIST v1.1 is depicted in yellow (partial response), blue (stable disease), and black (progression of disease) shading. Red shading indicates discontinuation of treatment due to toxicity or withdrawal. Textured bars reflect patients treated with 350 mg/d of alpelisib; solid bars reflect the ones on 300 mg/d. Arrows indicate patients that are still on study. *PIK3CA* mutations by NGS (in black) or SNaPShot (in pink) are shown on the vertical axis; Ø represents absence of *PIK3CA* mutation. Mutations on exons 9 and 20 (hotspots) were the most prevalent ones; exon 20 (H1047R) mutations appear to be associated with more clinical durable responses, in contrast to exon 9 mutations (E545K).

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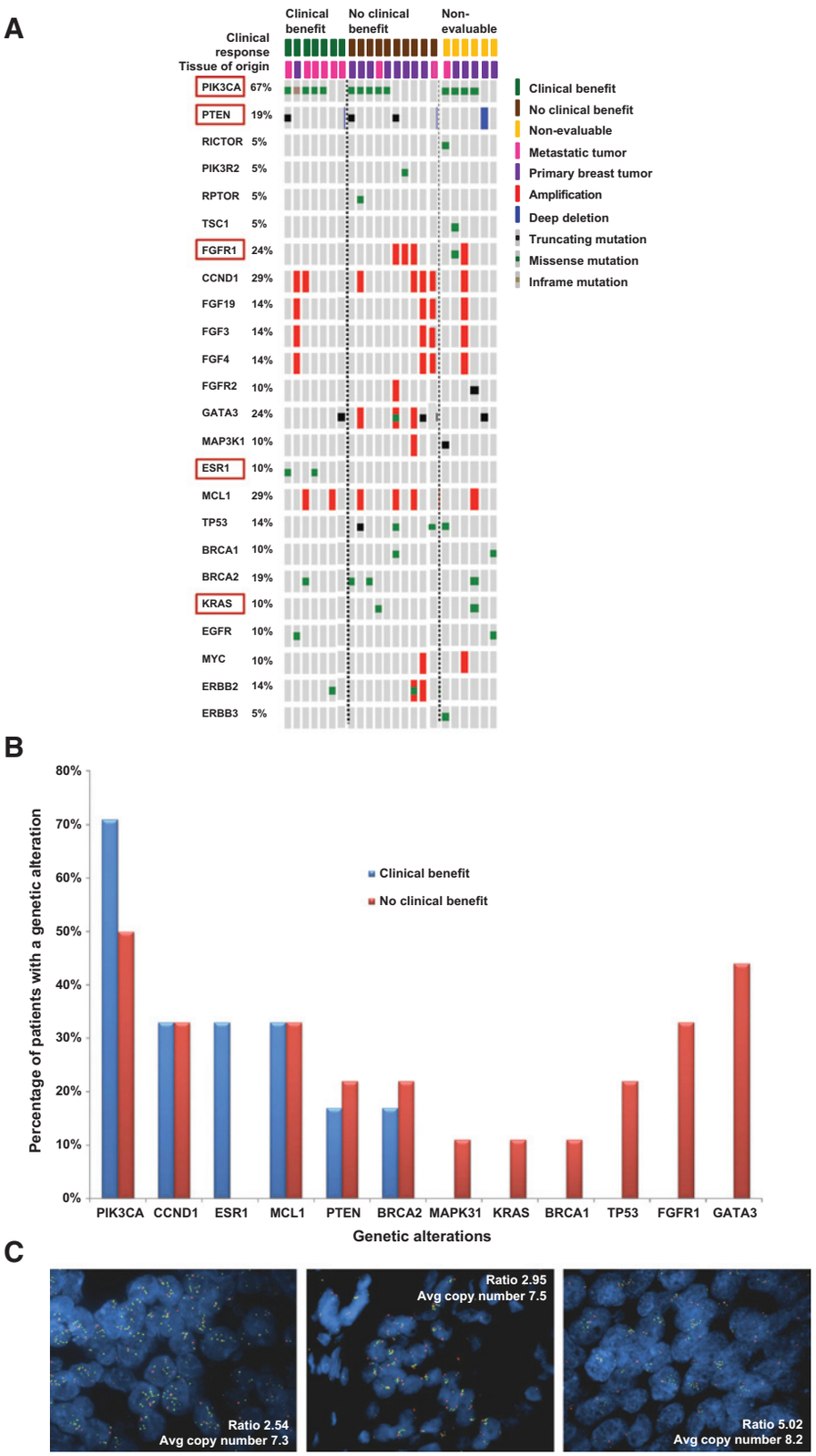

Figure 2.
NGS analysis of DNA from tumors of patients evaluable for clinical response. **A,** Of 341 cancer-associated genes, the somatic alterations (amplifications, red; deletions, blue; truncating, black; inframe, brown; and missense, green mutations) most commonly seen (i.e., >5% cases) are depicted here. Clinical benefit was defined as lack of progression for at least 6 months on letrozole/alpelisib. A total of 23 cases with adequate tissue for DNA extraction are depicted. Tissue origin in 9 of 23 (39%) tumors was a metastatic biopsy (pink), whereas 14 of 23 (61%) of tumors were from the primary diagnostic tumor specimen (purple). Tumors from patients with clinical benefit are depicted in green (top), and tumors from patients without clinical benefit are depicted in maroon (top). Patients that discontinued trial early (i.e., < 6 months) due to toxicity are depicted in gold as nonevaluable (top). A higher proportion of *PIK3CA* mutations were seen in patients with clinical benefit, and overall less genomic alterations were seen when compared with patients without clinical benefit. Two of the patients with clinical benefit were found to have both *PIK3CA* and *ESR1* mutations in their tumor. *FGFR1/2* amplification and mutation in *TP53, BRCA1*, and *KRAS* were found in only cancers from patients who did not benefit from treatment. *PTEN* mutations were found in both groups. **B,** Of patients with tumors harboring the genetic alterations depicted in the X axis, the blue bars indicate the proportion of patients with clinical benefit; red bars indicate the proportion of patients without clinical benefit. The graph reveals that most patients with *PIK3CA*- and *ESR1*-mutated tumors had clinical benefit, in contrast to patients with alterations in *KRAS, TP53*, or *FGFR1*, none of which had clinical benefit. **C,** *FGFR1* amplification was confirmed by FISH analysis in the three evaluable patients with *FGFR1* amplification detected by NGS.

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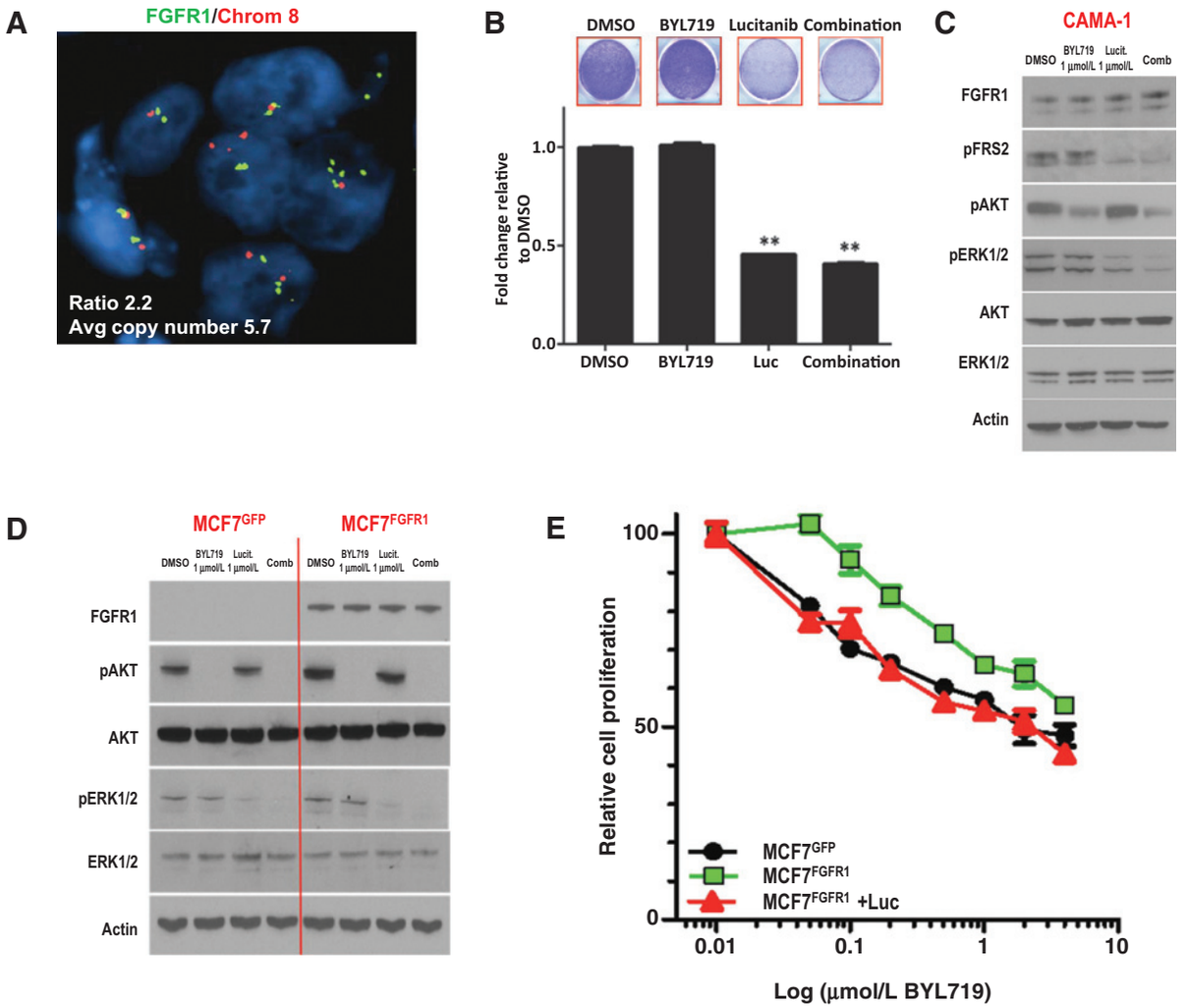

Figure 3.

FGFR1 overexpression attenuates the antiproliferative action of alpelisib. **A,** *FGFR1* amplification was confirmed by FISH analysis in the CAMA-1 ER$^+$ breast cancer cells as described in Materials and Methods. **B,** CAMA-1 cells in estrogen-free media (IMEM + 10% dextran-charcoal–treated FBS) with 5 ng/mL FGF2 were treated with 2 μmol/L alpelisib, 2 μmol/L lucitanib, or the combination. Representative images and quantification of integrated intensity are shown (**, $P < 0.01$ vs. control, *t* test). Growth of CAMA-1 cells was completely insensitive to alpelisib but inhibited by lucitanib. **C,** Immunoblot analysis with the indicated antibodies of lysates from CAMA-1 cells treated for 6 hours with vehicle, alpelisib, lucitanib and the combination of both drugs in estrogen-free media supplemented with 5 ng/mL FGF2. Alpelisib inhibits p-AKT but not p-ERK, whereas lucitanib inhibits p-ERK but not p-AKT. **D,** Immunoblot analysis with the indicated antibodies of lysates of MCF7^{GFP} and MCF7^{FGFR1} cells that were treated for 6 hours with vehicle, alpelisib, lucitanib, and the combination in estrogen-free media with 5 ng/mL FGF2. Alpelisib inhibits p-AKT but not p-ERK, whereas lucitanib inhibits p-ERK but not p-AKT. **E,** MCF7^{GFP} and MCF7^{FGFR1} cells were plated in estrogen-free media and FGF2 and treated with alpelisib alone or in combination with lucitanib for 72 hours. Cell proliferation was assessed by CellTiter-Glo (Promega) assay; each data point represents the mean ± SD of triplicate wells. MCF7^{GFP} cells (black) grown as monolayers had an IC_{50} to alpelisib <1 μmol/L, whereas this was at least 10-fold higher in MCF7^{FGFR1} cells (green). Addition of lucitanib resensitized MCF7^{FGFR1} cells to alpelisib (red).

developed with a focus on *PIK3CA*-mutant tumors. The results from this study provide evidence that alpelisib plus letrozole is safe, tolerable, and active in postmenopausal patients with ER$^+$/HER2$^-$ metastatic breast cancer refractory to endocrine therapy. The MTD and recommended dose for phase II trials of alpelisib in combination with letrozole was defined as 300 mg/d. This finding was in contrast to the alpelisib single-agent MTD, which is 400 mg/d (16).

Most common side effects (hyperglycemia, rash, nausea, diarrhea, and fatigue) were of similar frequency to those seen in the phase I study of single-agent alpelisib (16). However, the rate of severe (grade 3) maculopapular rash was lower, most likely due to a reduced alpelisib accumulation with the 300 mg/d dose, and/or the unintended prophylactic use of antihistaminics (i.e., due to seasonal allergies) prior to trial enrollment. In view of the urticarial-like nature of the rash, we would endorse the prophylactic use of antihistaminics in order to minimize the incidence and severity of the alpelisib-associated rash. Consistent with the hyperglycemia seen with pan-PI3K inhibitors (22–24), more than 50% of patients developed mild to moderate hyperglycemia, an on-target toxicity common to all PI3K inhibitors, especially the ones with more sustained inhibition of p110α. Severe

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hyperglycemia was uncommon, with none of the patients requiring administration of insulin; all of them were successfully managed with oral hypoglycemic agents such as metformin. Of note, transaminitis and gastrointestinal side effects were less frequent and severe than those observed in trials with pan-PI3K inhibitors (22–24).

The combination of letrozole and alpelisib showed sustained clinical activity: 35% of patients remained on treatment $\geq$6 months and 31% remained on treatment $\geq$12 months. The clinical benefit rate $\geq$6 and $\geq$12 months seen in patients with ER$^+$/*PIK3CA*-mutant metastatic breast cancer was numerically higher than in patients with wild-type *PIK3CA* tumors. Additionally, four of the five partial responses seen were in patients with a *PIK3CA*-mutant tumor. The significance of these findings is uncertain due to the small number of patients, but suggests that p110α-specific inhibitors may have greater activity against breast cancers of this genotype. The preliminary observation that the most frequent mutation in *PIK3CA*, H1047R, appears to be associated with higher clinical benefit from the p110α inhibitor compared with mutations in the helical domain further increases the enthusiasm for the development of *PIK3CA* mutant–specific inhibitors. Interestingly, a novel deletion in the C2 domain of *PIK3CA* (P447_L455del) was associated with a partial response. This domain is in contact with the iSH2 domain of p85, the regulatory subunit of PI3K (25). This deletion would be predicted to disrupt the interaction of p110α with p85, thus de-repressing p110α from p85-mediated inhibition. Confirmation of this hypothesis will require additional investigation beyond this report, but the partial response observed in this patient suggests that this mutation is oncogenic and, thus, may confer tumor dependence on PI3K function.

Several genomic alterations were detected in addition to *PIK3CA* mutations, and a higher proportion of those were seen in the group of patients without clinical benefit. *FGFR1* and *FGFR2* amplifications and mutations in *TP53*, *BRCA1/2*, and *KRAS* were found only in tumors from patients that progressed on therapy. Tumors with aberrant activation of the RAS/RAF/MEK/ERK pathway, such as *KRAS*-mutant cancers, do not respond to PI3K pathway inhibitors (26) and would not be expected to respond to alpelisib. Of note, the predominant pathway activated downstream of FGFRs has been shown to be ERK both in developmental models and in cancer cells (27, 28). Amplification of *FGFR1* occurs in approximately 10% of breast cancers, predominantly in ER$^+$ cancers where it has been associated with resistance to tamoxifen (29). Thus, to investigate whether an excess of *FGFR1* is also associated with a lower response to PI3K inhibitors, we engineered ER$^+$/*PIK3CA*-mutant cells with *FGFR1* overexpression. In these cells, the IC_{50} to alpelisib was increased >10-fold compared with cells without *FGFR1* amplification, suggesting a causal association between *FGFR1* overexpression and drug resistance. Treatment with the FGFR TKI lucitanib completely restored the antitumor effect of alpelisib while inhibiting ERK but not AKT. Similar results were obtained with ER$^+$/*FGFR1*-amplified CAMA-1 breast cancer cells, which were resistant to alpelisib but sensitive to lucitanib. These results are consistent with the lack of clinical benefit observed in the *FGFR*-amplified tumors in the trial herein and suggest a causal association between *FGFR* overexpression and both aberrant activation of *ERK* and resistance to PI3K inhibitors.

Two of the patients who did not have clinical benefit had amplification of *ERBB2 (HER2)* in a metastatic biopsy, consistent with the notion that this genetic alteration confers resistance to endocrine therapy (30, 31). Of note, the primary tumors in these two patients were not found to have *HER2* amplification by FISH. Interestingly, two patients with clinical benefit were found to have *ESR1* mutations in addition to *PIK3CA* mutations in metastatic biopsies. These *ESR1* mutations (Y537S and D538G) are in the ligand-binding domain of ERα and have previously been shown to exhibit estrogen-independent transcriptional activity (32–34). They are rarely found in primary untreated tumors and are usually detected in metastases in patients who progress after prolonged endocrine therapy. The clinical benefit seen in these patients suggests the possibility of an interaction between mutant *PIK3CA* and ERα that requires further study.

The results of this study demonstrate that the combination of the p110α-specific inhibitor alpelisib with letrozole is safe, tolerable, and active in patients with endocrine therapy–resistant ER$^+$ advanced breast cancer, particularly those that also harbor an activating mutation in *PIK3CA*. Several ongoing clinical trials are now exploring the use of alpelisib and endocrine therapy in patients with both ER$^+$/*PIK3CA*-mutant or wild-type breast cancer in the neoadjuvant and metastatic settings.

Disclosure of Potential Conflicts of Interest

I.A. Mayer, D. Juric, and L.C. Cantley are consultant/advisory board members for Novartis. J.M. Balko is the co-inventor of a patent using MHC-II expression for prediction of response to anti-PD-L1 inhibitors, which is owned by Vanderbilt University. No potential conflicts of interest were disclosed by the other authors.

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