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ORIGINAL ARTICLE

Outcome and molecular landscape of patients with *PIK3CA*-mutated metastatic breast cancer

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Background: α-Selective phosphatidylinositol 3-kinase (PI3K) inhibitors improve outcome in patients with *PIK3CA*-mutated, hormone receptor-positive (HR+)/Her2− metastatic breast cancer (mBC). Nevertheless, it is still unclear how to integrate this new drug family in the treatment landscape.

Patients and methods: A total of 649 patients with mBC from the SAFIR02 trial (NCT02299999), with available mutational profiles were selected for outcome analysis. *PIK3CA* mutations were prospectively determined by next-generation sequencing on metastatic samples. The mutational landscape of *PIK3CA*-mutated mBC was assessed by whole-exome sequencing ($n = 617$). Finally, the prognostic value of *PIK3CA* mutations during chemotherapy was assessed in plasma samples ($n = 44$) by next-generation sequencing and digital PCR.

Results: Some 28% (104/364) of HR+/Her2− tumors and 10% (27/255) of triple-negative breast cancer (TNBC) presented a *PIK3CA* mutation ($P < 0.001$). *PIK3CA*-mutated HR+/Her2− mBC was less sensitive to chemotherapy [adjusted odds ratio: 0.40; 95% confidence interval (0.22−0.71); $P = 0.002$], and presented a worse overall survival (OS) compared with *PIK3CA* wild-type [adjusted hazard ratio: 1.44; 95% confidence interval (1.02−2.03); $P = 0.04$]. *PIK3CA*-mutated HR+/Her2− mBC was enriched in *MAP3K1* mutations (15% versus 5%, $P = 0.0005$). In metastatic TNBC (mTNBC), the median OS in patients with *PIK3CA* mutation was 24 versus 14 months for *PIK3CA* wild-type ($P = 0.03$). We further looked at the distribution of *PIK3CA* mutation in mTNBC according to HR expression on the primary tumor. Some 6% (9/138) of patients without HR expression on the primary and 36% (14/39) of patients with HR+ on the primary presented *PIK3CA* mutation ($P < 0.001$). The level of residual *PIK3CA* mutations in plasma after one to three cycles of chemotherapy was associated with a poor OS [continuous variable, hazard ratio: 1.03, 95% confidence interval (1.01−1.05), $P = 0.007$].

Conclusion: *PIK3CA*-mutated HR+/Her2− mBC patients present a poor outcome and resistance to chemotherapy. Patients with *PIK3CA*-mutated TNBC present a better OS. This could be explained by an enrichment of *PIK3CA* mutations in luminal BC which lost HR expression in the metastatic setting.

Trial registration: SAFIR02 trial: NCT02299999.

Key words: metastatic breast cancer, PI3K inhibitors, *PIK3CA* mutation

INTRODUCTION

Phosphatidylinositol 3-kinases (PI3Ks) mediate the conversion of phosphatidylinositol 4,5-biphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). The class IA PI3Ks are heterodimers, with a catalytic subunit (p110α) and a regulatory subunit (p85α). This class of PI3Ks has a critical role in the control of various cellular processes like cell growth and proliferation, metabolism, and migration via the PI3K/AKT/mTOR pathway.[1−3] Several studies have shown that this pathway is up-regulated in up to 70% of human tumors.[4]

The *PIK3CA* gene encodes for the α-isoform of the catalytic subunit (p110α) of class IA PI3K kinase. *PIK3CA* somatic

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mutations occur in around 20%–40% of early breast cancers (eBCs) and are more frequent in hormone receptor-positive (HR+) disease.[5,6] *PIK3CA* mutations occur most frequently in three hotspots: p.E542K and p.E545K in exon 10 (corresponding to the helical domain), and p.H1047R in exon 21 (corresponding to the kinase domain).[7]

The clinical relevance of *PIK3CA* mutations has been evaluated in eBC. These studies suggested that *PIK3CA* mutations are associated with a good outcome in patients with HR+/Her2− eBC.[7–10] Although data have been extensively reported in eBC, no study has focused on the molecular characterization and clinical outcome of patients with *PIK3CA*-mutated metastatic breast cancer (mBC). There is therefore a need to better understand the characteristics of the mBC population harboring *PIK3CA* mutations. This will allow to better position PI3K inhibitors in the treatment landscape and to discover new populations for drug development.

PATIENTS AND METHODS

Patients

The population for outcome analysis included 649 patients with mBC, who received a biopsy of metastatic sites (liver 44%, lymph nodes 20%, breast 16%, skin 9%, lung 6%, pleura 1.7%, and others 3.3%) between April 2014 and March 2018 in the context of the prospective, randomized, phase II trial SAFIR02 (CT02299999), and for which the genomic profile was available. The SAFIR02 trial is described in supplementary Figure S1, available at *Annals of Oncology* online. The inclusion criteria for SAFIR02 were: patients with Her2− mBC with a metastatic lesion available for a biopsy done at inclusion, or an archival sample of metastatic lesion obtained within 1 year before (except bone metastasis only), and the presence of measurable target lesion according to RECIST v1.1. Only patients who received two or fewer lines of chemotherapy in the metastatic setting were eligible for the study. Patients with HR+/Her2− mBC must present a resistance to endocrine therapy. Patients were considered for randomization between the experimental or control arms when a stable disease or response was observed after six to eight cycles of chemotherapy (or at least after four cycles of chemotherapy if stopped for toxicity). HR and Her2 status were determined on metastasis, or on primary tumor if not available on metastasis. The cut-off for HR positivity was set at $\geq$1% immunostained cells by immunohistochemistry (IHC) according to American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) recommendations.[11] A tumor was considered Her2+ if scored 3+ by IHC, or 2+ and amplified by FISH.[12] The study was approved by Ethical Committee (Comité de Protection des Personnes Ile de France II) and all patients signed informed consent for ancillary studies.

In addition, mutational profiles of *PIK3CA*-mutated mBC were analyzed. In order to address this, we assessed mutations in a set of 617 patients with mBC. In these patients, a sample of mBC had been profiled by whole-exome sequencing. Patient population, methods of sequencing, and mutation calls have been previously described.[13] A total of 297 of these 617 patients were included in SAFIR02.

Determination of PIK3CA mutations in the SAFIR02 trial

Patients across France had access to molecular testing in five regional molecular cancer genetics platforms labeled by UNICANCER for the SAFIR02 trial. *PIK3CA* mutations were detected prospectively by next-generation sequencing (NGS). *PIK3CA* mutations were defined as hotspot mutations on exons 2, 5, 10, 14, or 21. Isolation of DNA from frozen core biopsies was carried out using the AllPrep DNA/RNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA was quantified using Qubit 2.0 Fluorometer (Quant-iT™ dsDNA BR Assay Kit; Thermo Fisher Scientific, Les Ulis, France), according to the manufacturer's instructions. Frozen samples were analyzed with a custom panel targeting 63 cancer-related genes covered by 1194 amplicons. A quantity of 10 ng of DNA was used to perform the initial PCR step (17 cycles). Amplicons were then partially digested using FuPA enzyme to get rid of extremities corresponding to primer sequences. The digested product was ligated with adapters and barcodes, then amplified and purified. The adaptors contain specific indexes (barcodes) different for each sample so that a library from different samples can be mixed together before sequencing. Quality and quantity assessment of DNA libraries was achieved using the Qubit 2.0 Fluorometer and/or the BioAnalyzer profiling (Agilent Technologies, Palo Alto, CA). After equimolar pooling of libraries, the final solution was sequenced by a MiSeq (Illumina, Evry, France) or an Ion Torrent PGM or an Ion S5 System (Thermo Fisher Scientific), depending on the different regional molecular cancer genetics platforms.

A depth of coverage of $>$100 reads was required for variant calling, with thresholds of 5% for the alternate allele for calling of known single nucleotide variants/mutations (with Cosmic ID) and 10% for known indels (with Cosmic ID). Raw reads were aligned on the reference human genome hg19, and the variants were annotated using ANNOVAR and the following databases: COSMIC68, dbSNP137, 1000 genomes, ESP6500, and RefGene annotations. Only non-synonymous variants not observed in $>$0.1% of the population (1000 genomes and ESP6500) are identified as somatic mutations. All somatic mutations were annotated, sorted, and interpreted by an expert molecular biologist according to available databases (Cosmic, The Cancer Genome Atlas).

Determination of PIK3CA mutations on circulating tumor DNA

We quantified the presence of *PIK3CA* mutations on circulating DNA of 44 patients from SAFIR02 who received one to three cycles of chemotherapy. DNA was extracted from 1–7 ml of EDTA plasma obtained after a double centrifugation as previously described.[14] Extraction was carried out using a Maxwell® RSC ccfDNA Plasma Kit according to manufacturer's recommendation (Promega, Charbonnières-les-Bains,

France). Determination of *PIK3CA* mutational status was carried out, on the one hand, using an NGS approach based Oncomine™ Pan-Cancer Cell-Free Assay, and conducted according to the manufacturer's instructions using the Ion Chef device and S5 sequencer (Thermo Fisher Scientific, Darmstadt, Germany). On the other hand, analyses were carried out by Crystal™ Digital™ PCR with the Naica digital PCR (ddPCR) system (Stilla Technologies, Villejuif, France). Primers and probes were designed for the detection of *PIK3CA* (NM_006218.3) hotspot mutations p.E542K (c.1624G>A), p.E545K (c.1633G>A), p.H1047R/L (c.3140A>G & c.3140A>T), and experiments were carried out as previously described.[14]

Determination of programmed death-ligand 1 expression

Programmed death-ligand 1 (PDL1) expression was determined in a population of 115 patients with metastatic triple-negative breast cancer (mTNBC) included in SAFIR02 and for which *PIK3CA* status was available. PDL1 expression was assessed by IHC using the SP142 antibody. The antibody was diluted in 0.05 M Tris buffered saline, 0.01 M EDTA, 0.05% Brij-35 with 0.3% carrier protein and 0.05% sodium azide, a preservative. Specific antibody concentration was approximately 7 μg/ml. PDL1 expression was assessed on tumor-infiltrating immune cells as a percentage of tumor area [<1% (PDL1-negative) and ≥1% (PDL1-positive)].

Statistical analyses

Data were summarized according to frequency and percentage for qualitative variables, and by median and range (minimum—maximum) for quantitative variables. Comparisons between groups were assessed using the chi-square or Fisher's exact test for qualitative variables and the Kruskal—Wallis test for quantitative variables. A multivariable analysis based on a logistic regression model was carried out to evaluate the impact of *PIK3CA* mutation on response to chemotherapy (response or stable disease versus progression or death) adjusted for other parameters. The response to chemotherapy was assessed at the time of randomization in SAFIR02, after patients had received six to eight cycles of induction chemotherapy (at the discretion of the investigator), except for patients who stopped at cycle 4 or before because of progression or toxicity. The evaluation was assessed locally by an investigator. Overall survival (OS) was measured as the time from inclusion to death, and was estimated using the Kaplan—Meier method with 95% confidence interval (CI). Patients alive were censored at their last follow-up. Univariable and multivariable analyses were carried out using the log-rank test and Cox proportional hazards model, respectively. All statistical tests were two-sided and a P value <0.05 was considered statistically significant. All statistical analyses were carried out using STATA 13 software.

RESULTS

A total of 649 consecutive patients included in SAFIR02 from April 2014 to March 2018 were analyzed. Three hundred and sixty-four patients had HR+/Her2− tumors, 255 mTNBC, and 10 Her2-overexpressing mBC. A total of 592 out of 649 patients (91%) were eligible to receive first line chemotherapy at the inclusion. A total of 337 out of 364 (93%) patients with HR+/Her2− mBC previously received hormonotherapy. One hundred and forty-three (22%) patients presented *PIK3CA* mutation. Six (4.2%) patients presented a *PIK3CA* amplification. All of them occurred in *PIK3CA*-mutated mBC.

Characteristics and outcome of patients with PIK3CA-mutated HR+/Her2− mBC

A total of 104 out of 364 (28%) patients presented *PIK3CA* mutation ($P < 0.001$). Some 56% (64/115) of mutations were hotspot mutations in the kinase domain (exon 21) and 38% (44/115) were hotspot mutations in the helical domain (exon 10). Some 6% (7/115) of mutations were found in other domains (such as the C2 domain, etc.). Double *PIK3CA* mutations were reported in 11 patients, and are described in supplementary Table S1, available at *Annals of Oncology* online. Characteristics of the patients according to *PIK3CA* mutations are reported in Table 1. *PIK3CA* mutations were associated with older age ($P = 0.03$) and lower tumor grade (30% versus 44% SBR3, $P = 0.02$).

Patients harboring a *PIK3CA* mutation were less sensitive to chemotherapy compared with the wild-type (WT) cohort. Some 51% of patients with *PIK3CA* mutation presented either a stable disease or objective response after induction chemotherapy, compared with 69% of patients with WT tumor ($P = 0.005$) (Figure 1A). A multivariable analysis showed that the chemoresistance associated with *PIK3CA* mutation was independent of other parameters [adjusted odds ratio for response or stable disease: 0.40, 95% CI (0.22—0.71), $P = 0.002$] (Table 2). *PIK3CA* mutations were also associated with poor survival. Median OS was 19.6 months versus 23.5 months for patients presenting a *PIK3CA* mutation or not, respectively ($P = 0.04$) (Figure 2A). This finding was confirmed in a multivariable analysis [adjusted hazard ratio: 1.44, 95% CI (1.02—2.03), $P = 0.04$] (Table 2).

We then assessed which genes were more frequently mutated in HR+/Her2− mBC with *PIK3CA* mutation ($n = 381$). In order to address this question, we selected genes to be drivers as defined by Bertucci et al.,[13] and to be mutated in more than 6% of patients. *PIK3CA*-mutated HR+/Her2− mBC presented a higher rate of *MAP3K1* mutations compared with *PIK3CA*-WT tumors (15% versus 5%, $P = 0.0005$). In contrast, *PIK3CA*-WT mBC presented an increased frequency of *GATA3* mutations (24% versus 15%, $P = 0.03$) and *AKT1* mutations (11% versus 2%, $P = 0.001$) compared with *PIK3CA*-mutated mBC (Figure 3). *MAP3K1* mutation was an independent prognostic parameter in patients with *PIK3CA*-mutated HR+/Her2− mBC [adjusted hazard ratio: 1.81, CI 95% (1.03—3.2), $P = 0.04$].

Characteristics and outcome of patients with PIK3CA-mutated mTNBC

A total of 27 out of 255 (10%) patients presented *PIK3CA* mutation ($P < 0.001$). Some 63% (17/27) of mutations were

Table 1. Patient and tumor characteristics according to *PIK3CA* mutational status

Characteristics	HR+/Her2− (*n* = 364)		*P* value	TNBC (*n* = 255)		*P* value
	PIK3CA-mutated (*n* = 104)	*PIK3CA* wild-type (*n* = 260)		*PIK3CA*-mutated (*n* = 27)	*PIK3CA* wild-type (*n* = 228)	
Age at inclusion						
Median (years)	57	54	0.06	59	51	**0.02**
≤65 years	74 (71%)	211 (81%)	**0.03**	19 (70%)	192 (84%)	0.1
>65 years	30 (29%)	49 (19%)		8 (30%)	36 (16%)	
Histopathological grade						
SBR 1	14 (14%)	18 (7%)	**0.02**	0	3 (1%)	**0.03**
SBR 2	56 (56%)	118 (49%)		11 (46%)	45 (21%)	
SBR 3	30 (30%)	107 (44%)		13 (54%)	170 (78%)	
Clinical size (largest lesion)						
Median (mm)	30	30	0.5	28	40	0.3
≤50 mm	53 (83%)	133 (79%)	0.5	10 (77%)	101 (69%)	0.7
>50 mm	11 (17%)	35 (21%)		3 (23%)	46 (31%)	
Pathological size (largest lesion)						
Median (mm)	25	25	0.9	24	25	0.5
≤50 mm	76 (90.5%)	195 (89%)	0.7	19 (90%)	154 (87%)	1
>50 mm	8 (9.5%)	24 (11%)		2 (10%)	23 (13%)	
Number of nodes involved						
Median	2	1	0.9	2	1	0.4
Histological type						
Ductal	83 (80%)	209 (80%)	0.9	22 (82%)	202 (89%)	**0.04**
Lobular	14 (13%)	36 (14%)		3 (11%)	4 (2%)	
Others	7 (7%)	15 (6%)		2 (7%)	21 (9%)	
Tumor form						
Unifocal	73 (72%)	187 (72%)	0.9	18 (69%)	183 (81%)	0.1
Multifocal	28 (28%)	72 (28%)		8 (31%)	44 (19%)	
Interval between diagnosis and metastatic disease						
Median (months)	38	41.5	0.7	24	15	**0.04**
≤60 months	67 (65%)	172 (66%)	0.8	19 (70%)	200 (90%)	**0.01**
>60 months	36 (35%)	87 (34%)		8 (30%)	22 (10%)	
Interval between metastatic disease and inclusion						
Median (months)	6.5	3	0.2	1	1.4	0.7
≤24 months	82 (80%)	211 (81%)	0.8	26 (96%)	216 (98%)	0.5
>24 months	20 (20%)	48 (19%)		1 (4%)	5 (2%)	
Metastatic sites						
Liver	81 (79%)	185 (71%)	0.1	14 (52%)	85 (38%)	0.1
Bone	72 (70%)	176 (68%)	0.6	12 (44%)	79 (36%)	0.3
Lung	22 (21%)	67 (26%)	0.3	6 (22%)	100 (45%)	**0.02**
Pleura	9 (9%)	37 (14%)	0.1	2 (7%)	32 (14%)	0.5
Previous hormonotherapy						
Yes	102 (98%)	235 (90%)	**0.01**	14 (52%)	27 (12%)	**<0.0001**
No	2 (2%)	25 (10%)		13 (48%)	195 (88%)	
Metastatic setting	52 (50%)	108 (41.5%)	0.1	4 (15%)	8 (4%)	**0.02**
Previous chemotherapy in any setting						
Yes	96 (92%)	248 (95%)	0.2	23 (85%)	216 (95%)	0.07
No	8 (8%)	12 (5%)		4 (15%)	12 (5%)	
Setting of chemotherapy						
Neoadjuvant	22 (21%)	69 (26.5%)	0.3	10 (37%)	103 (45%)	0.4
Adjuvant	48 (47%)	137 (53%)	0.2	13 (48%)	99 (43%)	0.6
1st line at screening	91 (87.5%)	234 (90%)	0.4	25 (93%)	213 (93%)	0.6
2nd line at screening	13 (12.5%)	26 (10%)		2 (7%)	15 (7%)	

Bold values are statistically significant.

hotspot mutations in the kinase domain (exon 21) and 30% (8/27) were hotspot mutations in the helical domain (exon 10). Seven percent (2/27) of mutations were in other domains. There were no patients with double *PIK3CA* mutations.

Characteristics of patients according to *PIK3CA* mutation are reported in Table 1. *PIK3CA* mutations were significantly associated with an older age ($P = 0.02$), SBR2 tumor grade ($P = 0.03$), lobular subtype ($P = 0.04$), and previous hormonotherapy ($P < 0.0001$). Since *PIK3CA* mutations are traditionally associated with the luminal subtype, we looked at the distribution of HR expression on primary tumor according to *PIK3CA* mutational status on metastatic sample (available for 179 cases). We observed that 61% (14/23) of patients with *PIK3CA*-mutated mTNBC were HR+/Her2− on the primary tumor versus 16% (25/156) of patients with *PIK3CA*-WT mTNBC ($P < 0.001$) (Figure 4A). No difference in chemosensitivity between the *PIK3CA*-mutated and WT cohort was observed in mTNBC (Figure 1B). Patients with *PIK3CA*-mutated mTNBC presented a better OS compared with *PIK3CA*-WT mTNBC (24 versus 14 months, $P = 0.03$) (Figure 2B).

Since anti-PDL1 agents are being developed in mTNBC, we looked at the distribution of PDL1 expression according to *PIK3CA* mutations. Out of 115 patients with mTNBC tested, 37 (32%) expressed PDL1. There was no difference of

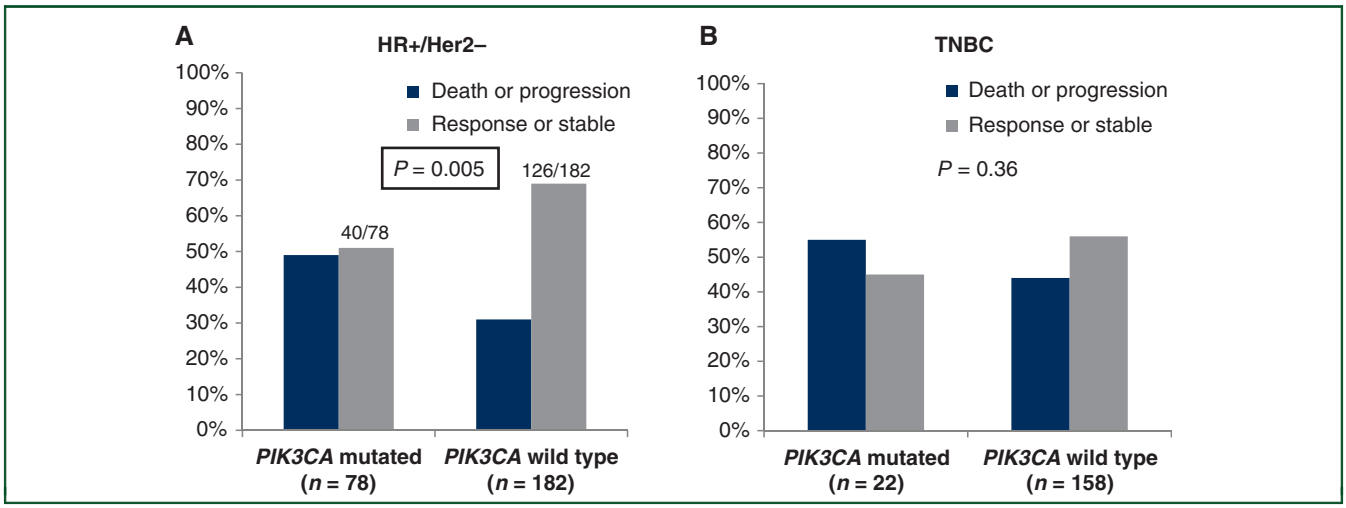

Figure 1. **(A) Response rate to chemotherapy of HR+/Her2− metastatic breast cancer (mBC) according to *PIK3CA* mutational status. (B) Response rate to chemotherapy of metastatic triple-negative breast cancer (mTNBC) according to *PIK3CA* mutational status.**

PDL1 expression between *PIK3CA*-mutant and WT mTNBC as reported in Figure 4B.

Characteristics and outcome of patients according to the residual level of PIK3CA mutation on circulating DNA during chemotherapy

We then assessed the outcome of patients according to the level of cell-free circulating *PIK3CA* mutations, determined by both NGS and ddPCR, in the plasma obtained after one (66%), two (20%), and three cycles of chemotherapy (14%). A total of 44 patients included in SAFIR02 and presenting a *PIK3CA* mutation on the metastatic samples were included. Patient characteristics are reported in supplementary Table S2, available at *Annals of Oncology* online. *PIK3CA* mutations were detected in the plasma of 26 (59%) patients. The level of *PIK3CA* mutations detected in plasma by NGS was associated with a poor outcome [continuous variable, hazard ratio: 1.03, 95% CI (1.01−1.05), $P = 0.007$]. The median OS for patients with a minor allele frequency (MAF) $\geq$ median was

Table 2. **Univariable and multivariable analysis of response to chemotherapy and overall survival in hormone receptor−positive (HR+)/Her2− population**

	Response to chemotherapy						**Overall survival**					
	Univariable analysis			Multivariable analysis			Univariable analysis			Multivariable analysis		
	Death or progression ($n = 94$)	Response or stable ($n = 166$)	*P* value	Odds ratio	Confidence interval (95%)	*P* value	Median (months)	Confidence interval (95%)	*P* value	Hazard ratio	Confidence interval (95%)	*P* value
PIK3CA mutation												
Yes	38 (40%)	40 (24%)	**0.005**	0.40	0.22−0.71	**0.002**	19.6	14.5−23.8	**0.04**	1.44	1.02−2.03	**0.04**
No	56 (60%)	126 (76%)		1	–		23.5	20.0−28.5		1	–	
Previous chemotherapy (neoadjuvant or adjuvant)												
Yes	76 (81%)	107 (64.5%)	**0.005**	0.36	0.19−0.69	**0.002**						
No	18 (19%)	59 (35.5%)		1	–							
Line of chemotherapy at screening												
1st line	76 (81%)	151 (91%)	**0.01**	1	–	**0.016**	22	20.0−25.7	**0.4**			
2nd line	18 (19%)	15 (9%)		0.39	0.18−0.84		18.4	11.8−28.9				
Number of metastatic sites												
≤2	42 (45%)	81 (49%)	**0.5**				23.8	20.8−NR	**0.01**	1	–	**0.03**
>2	52 (55%)	85 (51%)					19.4	15.1−24.0		1.44	1.04−1.98	
Interval between advanced disease and inclusion												
<12 months	60 (64%)	111 (68%)	**0.4**				21.1	16.9−24.3	**0.005**	1	–	**0.3**
12−24 months	18 (19%)	22 (13%)					20.1	12.7−23.5		1.24	0.82−1.87	
>24 months	16 (17%)	31 (19%)					30.1	20.7−NR		0.55	0.34−0.88	**0.01**
Age at inclusion												
≤65 years	69 (73%)	134 (81%)	**0.1**				22	19.7−25.5	**0.5**			
>65 years	25 (27%)	32 (19%)					20	14.9−28.5				
Previous chemotherapy in any setting												
Yes	90 (96%)	151 (91%)	**0.1**				21.4	19.4−24.7	**0.4**			
No	4 (4%)	15 (9%)					22.3	19.6−NR				
Previous hormonotherapy												
Yes	90 (96%)	158 (95%)	**1**				22.3	19.9−25.5	**0.2**			
No	4 (4%)	8 (5%)					16.9	9.4−NR				

NR, not reached.
Bold values are statistically significant.

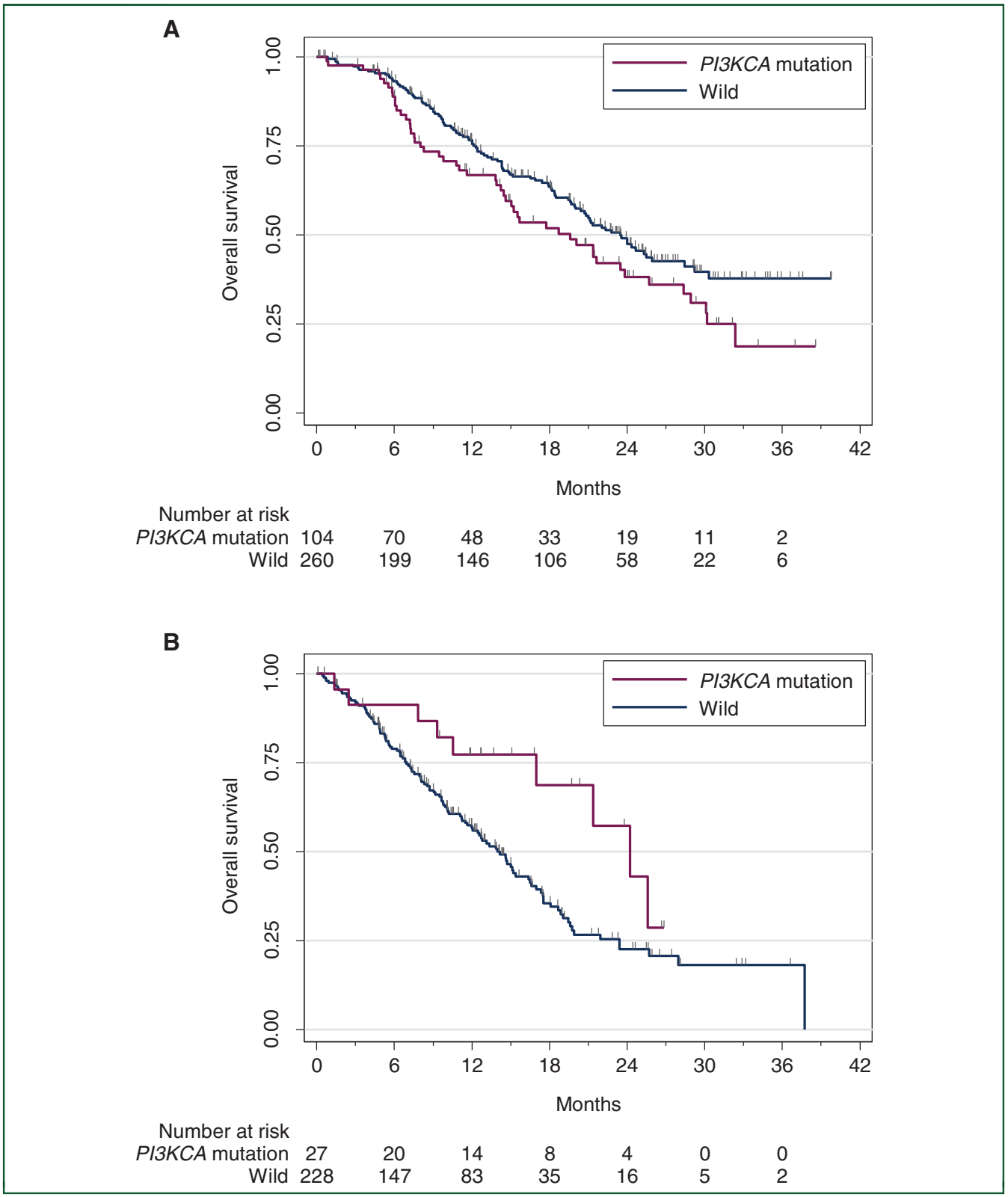

Figure 2. Kaplan–Meier curves for (A) overall survival in HR+/Her2− metastatic breast cancer (mBC) according to *PIK3CA* mutational status; (B) overall survival (OS) in metastatic triple-negative breast cancer (mTNBC) according to *PIK3CA* mutational status.
CI, confidence interval; HR, hazard ratio.

14 months versus 26 months for patients with an MAF < median (P <0.0001), and 6 months versus 25.6 months for patients with an MAF ≥ or <5, respectively (P < 0.0001) (Figure 5).

DISCUSSION

In the present study, 22% of the overall population and 28% of patients with HR+/Her2− mBC presented a *PIK3CA* mutation, similar to eBC.[15,16] As reported by Juric et al.,[17]

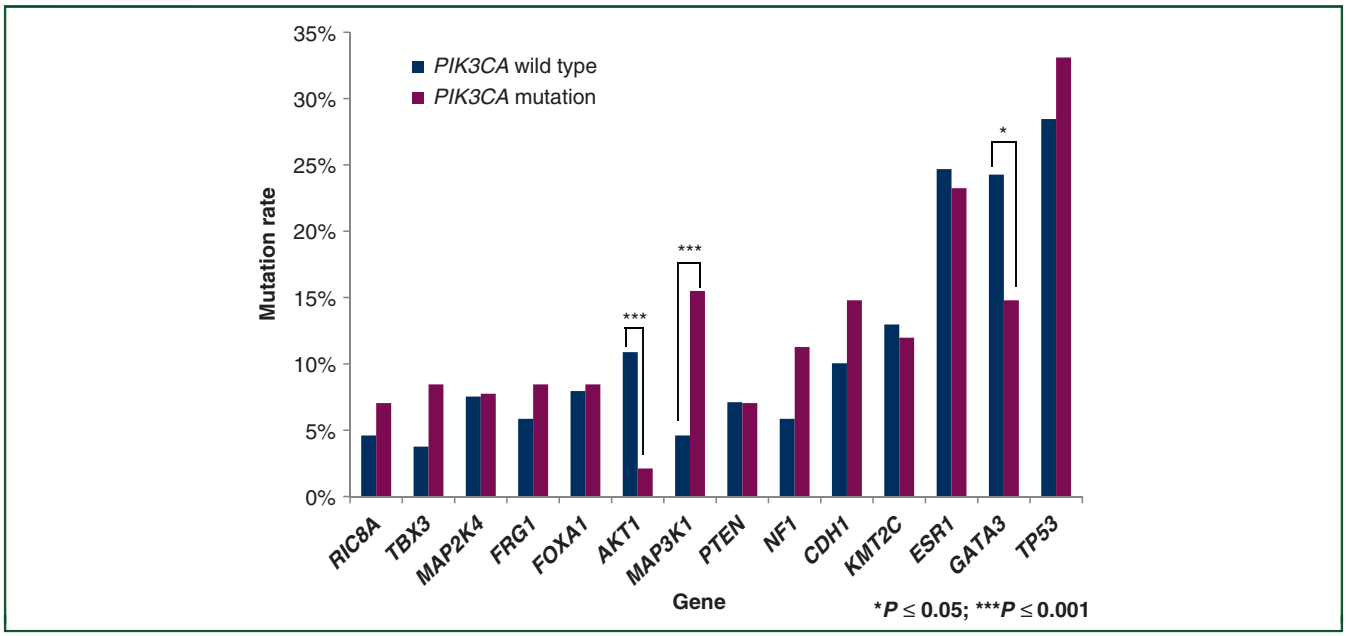

Figure 3. Analysis of 14 drivers according to *PIK3CA* mutational status in HR+/Her2− metastatic breast cancer (mBC) by whole-exome sequencing.

the rate of *PIK3CA*-WT eBC that switched to *PIK3CA* mutations is very rare, and that could explain a stable incidence between early and late stage BC. The field of *PIK3CA* mutations has recently regained interest with the publication of the SOLAR1 trial showing a clinically relevant improvement in progression-free survival with an α-selective PI3K inhibitor.[18] Our study suggests that patients with HR+/Her2− mBC and *PIK3CA* mutation present a resistance to chemotherapy and a worse outcome, and that this population represents an unmet medical need. These findings are consistent with studies showing that the activation of the PI3K/AKT pathway could mediate chemoresistance in breast cancer.[19–21] PIK3CA-mutated eBC presented a decreased rate of pathologic complete response to chemotherapy ± anti-Her2 therapy (23.0% versus 38.8% for *PIK3CA*-WT, $P < 0.0001$).[15] These data would suggest that the better positioning of PI3K inhibitors might be before the first line of chemotherapy. As suggested in preclinical studies, there is also a strong rationale to test these compounds in combination with chemotherapy.[22,23]

Patients with *PIK3CA* mutations presented a higher frequency of *MAP3K1* mutations. These mutations are recurrent drivers in eBC, and are involved in the activation of the MEK pathway. Our data are consistent with other

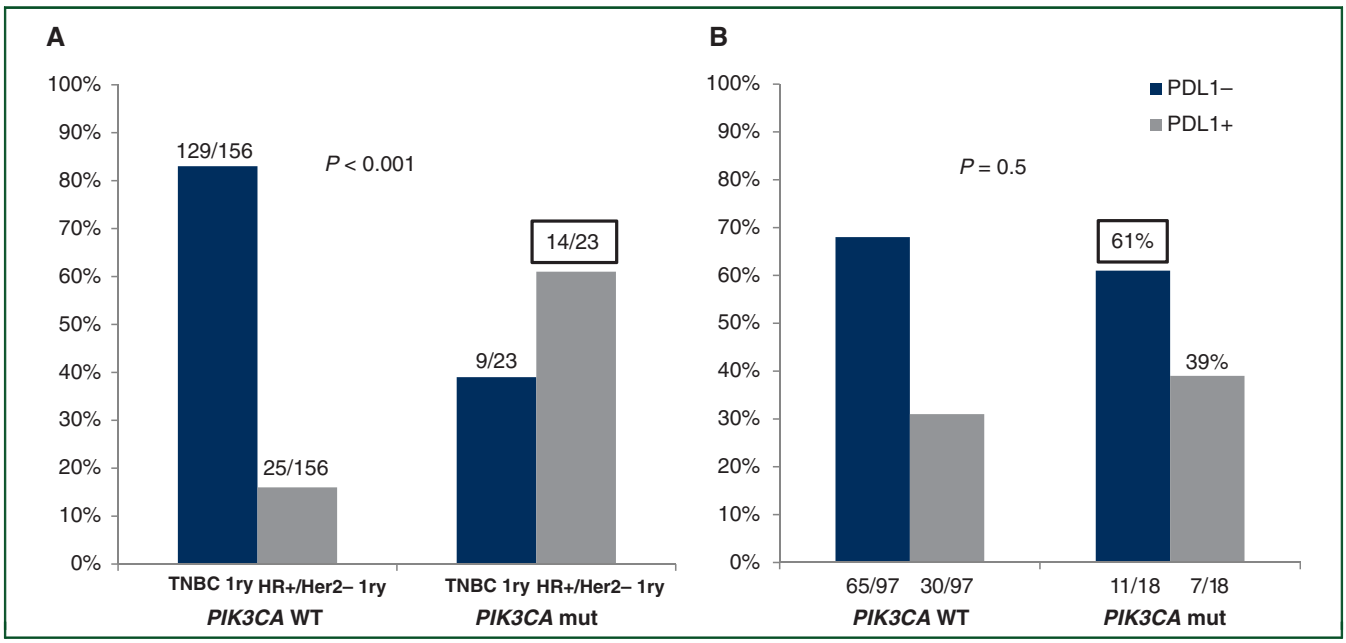

Figure 4. (A) Distribution of HR expression on primary tumor according to *PIK3CA* mutational status in metastatic triple-negative breast cancer (mTNBC). (B) Distribution of programmed death-ligand 1 (PDL1) expression according to *PIK3CA* mutation in metastatic triple-negative breast cancer (mTNBC). 1ry, Primary.

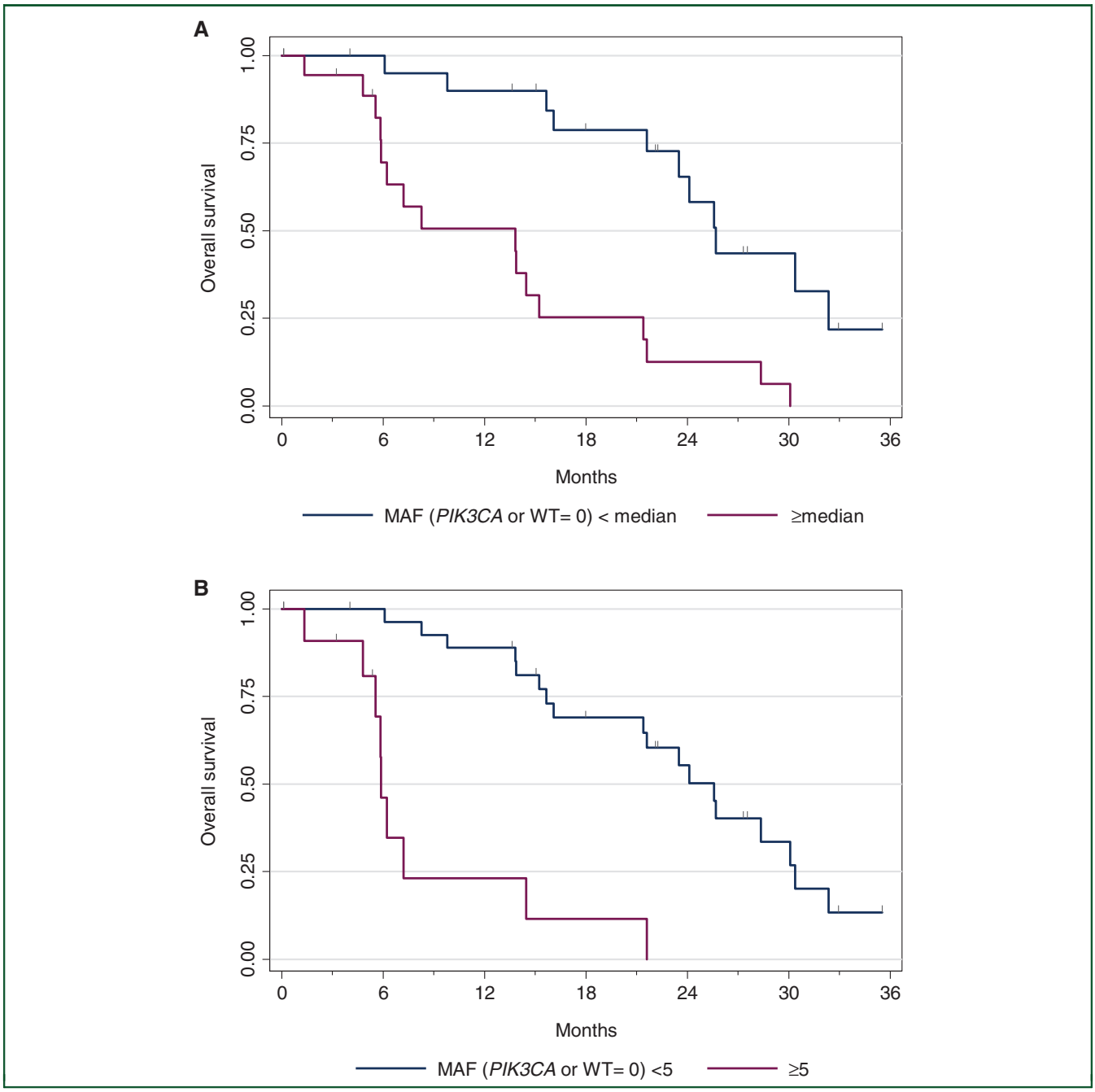

Figure 5. Kaplan–Meier curves for overall survival (OS) according to the residual level of *PIK3CA* mutation in plasma during chemotherapy.
MAF, minor allele frequency.

analyses reporting that *MAP3K1* mutations are found in approximately 11% of *PIK3CA*-mutated breast cancers. Avivar-Valderas et al.[24] described that *MAP3K1* loss of function, in the context of *PIK3CA* mutation, mediates resistance to α-selective PI3K inhibitors by activating IRS1. Whether *MAP3K1* mutations mediate resistance to PI3K inhibitors in patients remains to be evaluated. This finding could lead to the development of the combination of PI3K and MEK inhibitors.[25]

In addition, we observed that *PIK3CA* mutations are mutually exclusive with *AKT1* mutations. This is consistent with previous studies in breast cancer.[6] Lefebvre et al.[26] have suggested that a subset of *PIK3CA* mutations could be associated with the APOBEC mutational signature. Further works are needed to better define if *PIK3CA* mutations on exon 9 could be the consequence of APOBEC activation. Interestingly, 11 (8%) patients with *PIK3CA* hot spot mutations also presented another alteration on the same gene. Six (4%) patients presented a *PIK3CA* amplification and nine (6%) patients a mutation outside hot spot domains.

In patients with early TNBC, *PIK3CA* mutations have been associated with expression of the androgen receptor and apocrine subtype, and are inversely correlated with the activation of the immune system and *PTEN* alterations.[27,28]

Interestingly, PI3K/AKT/PTEN pathway alteration is described in 25%—40% of patients with mTNBC, supporting the current development of AKT inhibitors in these tumors.[29,30] In the metastatic setting, our study indicates that 61% of *PIK3CA* mutations were detected in patients whose primary tumor expressed HR. Since *PIK3CA*-mutated luminal BC presents sensitivity to PI3K inhibitors, there is a strong rationale to develop PI3K inhibitors in this setting. In addition, these data suggest that further trials that will test PI3K inhibitors in mTNBC will have to stratify patients based on HR expression on the primary tumor. Since preliminary studies have shown that anti-PDL1 agents provide benefit in patients with mTNBC,[31] we further evaluated whether *PIK3CA* mutation correlated with PDL1 expression. We could not find any association between PDL1 expression and *PIK3CA* mutations, suggesting that there is a population in which PI3K inhibitors could be developed independently from anti-PDL1 agents.

The strengths of our analysis include the sample sizes and the prospective design of the study. One of the weaknesses of our study is the exclusion of patients with bone-only disease.

In summary, our study highlights that patients with *PIK3CA*-mutated HR+/Her2− mBC present an unmet medical need where new drugs are needed. In addition, there is a need to investigate the predictive value of *MAP3K1* mutations and *PIK3CA* co-alterations for the sensitivity to PI3K inhibitors. In mTNBC, our study suggests that there is an opportunity to develop PI3K inhibitors, especially in patients whose primary tumors express HR.

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CLT reports participation in advisory boards from Amgen, GSK, Astra Zeneca, Nanobiotix, MSD, BMS, Merck Serono, Roche. MPS reports courses for laboratory Servier. JCS reports that he has been a full-time employee of AstraZeneca since September 2017. Over the past 5 years he has received consultancy fees from AstraZeneca, Astex, Clovis, GSK, GamaMabs, Lilly, MSD, Mission Therapeutics, Merus, Pfizer, PharmaMar, Pierre Fabre, Roche/Genentech, Sanofi, Servier, Symphogen, and Takeda. Also he is a shareholder of AstraZeneca and Gritstone. MC reports honoraria from Novartis. FD reports honoraria from Novartis. TB reports grants from Novartis, Astrazeneca, Pfizer; personal fees from Roche, Novartis, Astrazeneca, Pfizer, SeattleGenetics; and non-financial support from Roche, Novartis, Astrazeneca, Pfizer. FA reports research grants from Novartis, speaker, and advisory boards compensated to the hospital. Outside the submitted work grants from Pfizer, Lilly, Sanofi, Roche, Astrazeneca, Daiichi. All remaining authors have declared no conflicts of interest.

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