Tumor PIK3CA genotype and prognosis in early-stage breast cancer:

Zardavas, Dimitrios; Te Marvelde, Luc; Milne, Roger L.; Fumagalli, Debora; Fountzilas, George; Kotoula, Vassiliki; Razis, Evangelia; Papaxoinis, George; Joensuu, Heikki; Moynahan, Mary Ellen; Hennessy, Bryan T.; Bieche, Ivan; Saal, Lao H.; Stal, Olle; Iacopetta, Barry; Jensen, Jeanette Dupont; O'Toole, Sandra; Lopez-Knowles, Elena; Barabaraeschi, Mattia; Noguchi, Shinzaburo

DOI:

[10.1200/JCO.2017.74.8301](https://doi.org/10.1200/JCO.2017.74.8301)

License:

Other (please specify with Rights Statement)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Zardavas, D, Te Marvelde, L, Milne, RL, Fumagalli, D, Fountzilas, G, Kotoula, V, Razis, E, Papaxoinis, G, Joensuu, H, Moynahan, ME, Hennessy, BT, Bieche, I, Saal, LH, Stal, O, Iacopetta, B, Jensen, JD, O'Toole, S, Lopez-Knowles, E, Barabaraeschi, M, Noguchi, S, Azim, HA, Lerma, E, Bachelot, T, Wang, Q, Perez-Tenorio, G, Can De Velde, CJH, Rea, D, Sabine, V, Bartlett, JMS, Sotiriou, C, Michiels, S & Loi, S 2018, 'Tumor PIK3CA genotype and prognosis in early-stage breast cancer: A pooled analysis of individual patient data', *Journal of Clinical Oncology*, vol. 36, no. 10, pp. 981-990. <https://doi.org/10.1200/JCO.2017.74.8301>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

© 2018 by American Society of Clinical Oncology

Published in Journal of Clinical Oncology on 01/04/2018

DOI: 10.1200/JCO.2017.74.8301

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Tumor *PIK3CA* Genotype and Prognosis in Early-Stage Breast Cancer: A Pooled Analysis of Individual Patient Data

Dimitrios Zardavas, Luc te Marvelde, Roger L. Milne, Debora Fumagalli, George Fountzilas, Vassiliki Kotoula, Evangelia Razis, George Papaxoinis, Heikki Joensuu, Mary Ellen Moynahan, Bryan T. Hennessy, Ivan Bieche, Lao H. Saal, Olle Stal, Barry Iacopetta, Jeanette Dupont Jensen, Sandra O'Toole, Elena Lopez-Knowles, Mattia Barbaraeschi, Shinzaburo Noguchi, Hatem A. Azim Jr, Enrique Lerma, Thomas Bachelot, Qing Wang, Gizeh Perez-Tenorio, Cornelis J.H. van de Velde, Daniel W. Rea, Vicky Sabine, John M.S. Bartlett, Christos Sotiriou, Stefan Michiels, and Sherene Loi

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on February 22, 2018.

Corresponding author: Sherene Loi, MD, PhD, Peter MacCallum Cancer Centre, University of Melbourne, 305 Grattan St, Melbourne 3000, Victoria, Australia; e-mail: sherene.loi@petermac.org.

© 2018 by American Society of Clinical Oncology

0732-183X/18/3610w-981w/\$20.00

ABSTRACT

Purpose

Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations are frequently observed in primary breast cancer. We evaluated their prognostic relevance by performing a pooled analysis of individual patient data.

Patients and Methods

Associations between *PIK3CA* status and clinicopathologic characteristics were tested by applying Cox regression models adjusted for age, tumor size, nodes, grade, estrogen receptor (ER) status, human epidermal growth factor receptor 2 (HER2) status, treatment, and study. Invasive disease-free survival (IDFS) was the primary end point; distant disease-free survival (DDFS) and overall survival (OS) were also assessed, overall and by breast cancer subtypes.

Results

Data from 10,319 patients from 19 studies were included (median OS follow-up, 6.9 years); 1,787 patients (17%) received chemotherapy, 4,036 (39%) received endocrine monotherapy, 3,583 (35%) received both, and 913 (9%) received none or their treatment was unknown. *PIK3CA* mutations occurred in 32% of patients, with significant associations with ER positivity, increasing age, lower grade, and smaller size (all $P < .001$). Prevalence of *PIK3CA* mutations was 18%, 22%, and 37% in the ER-negative/HER2-negative, HER2-positive, and ER-positive/HER2-negative subtypes, respectively. In univariable analysis, *PIK3CA* mutations were associated with better IDFS (HR, 0.77; 95% CI, 0.71 to 0.84; $P < .001$), with evidence for a stronger effect in the first years of follow-up (0 to 5 years: HR, 0.73; 95% CI, 0.66 to 0.81; $P < .001$; 5 to 10 years: HR, 0.82; 95% CI, 0.68 to 0.99; $P = .037$); > 10 years: (HR, 1.15; 95% CI, 0.84 to 1.58; $P = .38$; P heterogeneity = .02). In multivariable analysis, *PIK3CA* genotype remained significant for improved IDFS ($P = .043$), but not for the DDFS and OS end points.

Conclusion

In this large pooled analysis, *PIK3CA* mutations were significantly associated with a better IDFS, DDFS, and OS, but had a lesser prognostic effect after adjustment for other prognostic factors.

J Clin Oncol 36:981-990. © 2018 by American Society of Clinical Oncology

INTRODUCTION

Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations affect the gene corresponding to the alpha isoform of the catalytic subunit (p110) of the class IA kinase and has been extensively studied for its role in human carcinogenesis.¹ These mutations have been reported in a variety of human cancers, including colorectal, endometrial, and ovarian cancer, among others.²⁻⁴ In primary breast cancer

(BC), *PIK3CA* mutations are frequent, with the highest frequency among the hormone receptor-positive tumors.⁵⁻⁷ Approximately 80% of mutations cluster in hot spots located within the helical domain and the kinase domain. The functional consequences of these hot-spot mutations have been studied extensively at the pre-clinical level, indicating that they are potent mediators of oncogenesis through AKT activation and evasion of apoptosis, as well as induction of an invasive and migratory phenotype.⁸⁻¹¹ In addition, *PIK3CA* mutations have been associated at

ASSOCIATED CONTENT

Data Supplement
DOI: <https://doi.org/10.1200/JCO.2017.74.8301>

DOI: <https://doi.org/10.1200/JCO.2017.74.8301>

the preclinical and clinical level with resistance to paclitaxel, trastuzumab, and endocrine treatment.^{9,12-15}

On the basis of the high frequency of *PIK3CA* mutations in BC, as well as the preclinical data supporting their multifaceted oncogenic functions, several studies have assessed their clinical relevance for patients with early-stage disease.¹⁶ In particular, their prognostic relevance has been evaluated with conflicting results.¹⁵ Of note, many of these studies were conducted among heterogeneous patient populations in terms of BC subtypes and treatments received, with some notable exceptions.^{17,18} Therefore, in this study, we pooled individual patient data from these previous studies of patients with early-stage BC to robustly evaluate the prognostic associations of these commonly occurring aberrations (and hence their potential relevance for clinical decision making) overall by mutation gene location as well as interactions by BC subtype.

PATIENTS AND METHODS

Types of Studies and Search Strategies

Potentially eligible studies were retrieved through an electronic search on PubMed/MEDLINE using the MeSH terms “breast neoplasm” and “*PIK3CA* protein, human.” The literature search was conducted independently by two investigators (D.Z. and D.F.) in January 2013. Identified studies were eligible for this pooled analysis of individual patient data if they met the following requirements: (1) studies conducted in patients with early-stage BC assessing the *PIK3CA* genotype in primary breast tumor, (2) studies comparing clinical outcomes in association with the *PIK3CA* genotype, and (3) studies published in the English language.

There were no restrictions for inclusion in our study in terms of number of patients included, duration of follow-up, prospective versus retrospective nature of the study, patients' age, menopausal status, BC subtype, or treatment modalities applied. In addition, no restrictions were applied in terms of *PIK3CA* mutational status assessment method. However, studies conducted in the neoadjuvant setting associating the *PIK3CA* genotype with pathologic complete response rate without reporting results of further clinical outcome were excluded from this pooled analysis. Cross-referencing from relevant studies was performed to confirm retrieval of all potentially eligible studies. In terms of study eligibility, final decisions were taken on consensus between the two investigators who performed the research.

The investigators of the eligible trials were contacted and requested to provide individual patient data on (1) baseline characteristics, including patients' demographics and clinicopathologic characteristics; (2) *PIK3CA* genotype and method used; (3) type of (neo)-adjuvant treatment received; (4) clinical outcome, including type and time of event that occurred; and (5) survival status. Individual patient data were used for all analyses, rather than combining results as in the usual types of meta-analyses (Data Supplement; Fig 1).¹⁷⁻³¹

Statistical Analysis

The primary objective of this study was to assess the potential impact of *PIK3CA* mutations on invasive disease-free survival (IDFS), and secondary objectives were to assess the prognostic impact in terms of distant disease-free survival (DDFS) and overall survival (OS).

IDFS was defined as the time from diagnosis until local, regional, or distant recurrence; contralateral BC; second primary malignancy; or death.³² IDFS analysis time was censored at the last date the patient was known to be alive and recurrence free. DDFS included only distant recurrence and death as events.³² DDFS analysis time was censored at the date last known to be alive and distant recurrence free. OS included only death as the event; patients were censored at the date last known to be alive.

Differences in patient and tumor characteristics by *PIK3CA* mutation status were assessed using χ^2 tests for categorical variables, the Cochran-Armitage trend test for ordinal variables, and the Wilcoxon rank sum test for continuous variables. Cox proportional hazard models were used to assess associations

Fig 1. PRISMA study flow chart.

between *PIK3CA* mutation status and IDFS, DDFS, and OS. Hazard ratios (HRs) and 95% CIs were estimated from univariable and multivariable models. Multivariable models included age (fitted as cubic splines because the effect of age on prognosis is U-shaped), stratified on tumor size (T1/T2/T3/T4), positive nodes (yes/no), local histologic grade (1 to 2/3 to 4), estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status (positive/negative), and treatment (chemotherapy/endocrine therapy/both). Variables for which the proportional hazards assumption was violated were included as strata rather than covariables. The global test of proportional hazards was not violated for any of the results presented below, unless stated otherwise. We used time intervals defined by the Early Breast Cancer Trialists' Collaborative Group to subdivide the time scale (0 to 5, 5 to 10, and > 10 years).³³ Exploratory objectives were to explore interactions between *PIK3CA* genotype and clinical outcome according to BC subtype (defined using ER and HER2 status), age, and the possible impact of *PIK3CA* genotypes on the timing of recurrence (early v late). Median follow-up time was calculated using the reverse Kaplan-Meier method. All data analyses were conducted in R, version 3.1.2.

RESULTS

Patient Characteristics

Data on 10,319 patients with known *PIK3CA* genotype originating from 19 studies were available (Fig 1; Data

Table 1. Summary of Patient and Disease Characteristics According to *PIK3CA* Mutation Type

Characteristic	PIK3CA Mutation Status		P	All Patients
	Wild Type	Mutant		
Age (continuous; years)			< .001	
Mean (SD)	58.4 (12.5)	61.0 (11.6)		59.2 (12.3)
Median (range)	59 (18-95)	61 (21-96)		59.7 (18-96)
Interquartile range	50-67.3	53-69.1		51-68
Unknown	27	12		39
Age (categorical; years)			< .001	
≤ 50	1,789 (75.1)	594 (24.9)		2,383 (23.2)
> 50	5,222 (66.1)	2,675 (33.9)		7,897 (76.8)
Unknown	27	12		39
Menopausal status			< .001*	
Premenopausal	1,174 (76.5)	361 (23.5)		1,535 (19.0)
Perimenopausal	7 (63.6)	4 (36.4)		11 (0.1)
Postmenopausal	4,230 (65.0)	2,282 (35.0)		6,512 (80.8)
Unknown	1,627	634		2,261
Histology			< .001	
Ductal	4,727 (69.1)	2,111 (30.9)		6,838 (82.2)
Ductal-lobular	137 (57.3)	102 (42.7)		239 (2.9)
Lobular	585 (61.5)	366 (38.5)		951 (11.4)
Other	244 (82.7)	51 (17.3)		295 (3.5)
Unknown	1,345	651		1,996
Local histologic grade (ordered)			< .001†	
Poorly differentiated/undifferentiated	2,752 (76.2)	861 (23.8)		3,613 (41.1)
Moderately differentiated	2,722 (64.0)	1,528 (36.0)		4,250 (48.3)
Well differentiated	487 (52.3)	445 (47.7)		932 (10.6)
Unknown	1,077	447		1,524
Central histologic grade (ordered)			.001†	
Poorly differentiated/undifferentiated	128 (75.7)	41 (24.3)		169 (47.1)
Moderately differentiated	108 (69.7)	47 (30.3)		155 (43.2)
Well differentiated	14 (40.0)	21 (60.0)		35 (9.7)
Unknown	6,788	3,172		9,960
ER status			< .001	
Negative	1,348 (81.7)	301 (18.3)		1,649 (16.1)
Positive	5,627 (65.5)	2,959 (34.5)		8,586 (83.9)
Unknown	63	21		84
PR status			< .001	
Negative	2,329 (76.0)	736 (24.0)		3,065 (33.2)
Positive	3,970 (64.4)	2,196 (35.6)		6,166 (66.8)
Unknown	739	349		1,088
HER2 status			< .001	
Negative	5,120 (65.9)	2,648 (34.1)		7,768 (79.7)
Positive	1,540 (77.7)	441 (22.3)		1,981 (20.3)
Unknown	378	192		570
Subtype			< .001	
HER2 negative/ER negative	790 (82.5)	167 (17.5)		957 (9.8)
HER2 negative/ER positive	4,313 (63.5)	2,475 (36.5)		6,788 (69.8)
HER2 positive	1,540 (77.7)	441 (22.3)		1,981 (20.4)
Unknown	395	198		593
Tumor size (mm)			.001	
Mean (SD)	26.3 (16.1)	25.00 (14.3)		25.90 (15.5)
Median (range)	23 (0-250)	22 (0-180)		22 (0-250)
Interquartile range	16-30	15-30		16-30
Unknown	622 (71.6%)	247 (28.4%)		869
Tumor size (ordered)			< .001†	
T0	1 (16.7)	5 (83.3)		6 (0.1)
T1	2,861 (65.4)	1,511 (34.6)		4,372 (43.2)
T2	3,510 (69.8)	1,516 (30.2)		5,026 (49.7)
T3	423 (74.3)	146 (25.7)		569 (5.6)
T4	92 (63.0)	54 (37.0)		146 (1.4)
Unknown	151	49		200

NOTE. Data are represented as No. (%) unless otherwise noted.

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor; SD, standard deviation.

*Test included premenopausal and postmenopausal only.

†Test for trend.

Table 2. Association of *PIK3CA* Mutations With IDFS, DDFS, and OS

Univariable	PIK3CA Hazard Ratio (95% CI) (MT relative to WT)			
	IDFS	DDFS	OS	
All data	0.77 (0.71 to 0.84); $P < .001$ * (n = 9,863; 2,433 events)	0.79 (0.72 to 0.86); $P < .001$ * (n = 9,697; 2,139 events)	0.90 (0.82 to 0.99); $P = .027$ * (n = 9,578; 2,094 events)	
0-5 years after diagnosis	0.73 (0.66 to 0.81); $P < .001$ (n = 9,857; 1,749 events)	0.74 (0.66 to 0.83); $P < .001$ (n = 9,695; 1,517 events)	0.89 (0.79 to 1.00); $P = .057$ (n = 9,576; 1,285 events)	
5-10 years after diagnosis	0.82 (0.68 to 0.99); $P = .039$ (n = 6,797; 520 events)	0.82 (0.67 to 1.00); $P = .045$ (n = 6,881; 471 events)	0.93 (0.79 to 1.10); $P = .393$ (n = 7,010; 644 events)	
> 10 years after diagnosis	1.15 (0.84 to 1.58); $P = .380$ (n = 1,045; 160 events)	1.18 (0.85 to 1.63); $P = .323$ (n = 1,032; events = 151)	0.87 (0.62 to 1.20); $P = .383$ (n = 927; 165 events)	
Multivariable†	IDFS	DDFS	OS	
All data	0.88 (0.78 to 1.00); $P = .043$ (n = 6,120; 1,417 events)	0.88 (0.77 to 1.00); $P = .054$ (n = 5,919; 1,245 events)	0.98 (0.86 to 1.12); $P = .799$ (n = 5,730; 1,184 events)	
0-5 years after diagnosis	0.87 (0.75 to 1.00); $P = .048$ (n = 6,120; 1,020 events)	0.85 (0.73 to 0.99); $P = .042$ (n = 5,919; 891 events)	0.99 (0.83 to 1.16); $P = .869$ (n = 5,730; 718 events)	
5-10 years after diagnosis	0.88 (0.68 to 1.13); $P = .324$ (n = 4,300; 307 events)	0.92 (0.70 to 1.21); $P = .548$ (n = 4,328; 268 events)	0.99 (0.79 to 1.24); $P = .911$ (n = 4,354; 384 events)	
> 10 years after diagnosis	1.10 (0.64 to 1.88); $P = .740$ (n = 691; 90 events)	1.04 (0.60 to 1.81); $P = .886$ (n = 684; 86 events)	0.91 (0.49 to 1.68); $P = .764$ (n = 585; 82 events)	

Abbreviations: DDFS, distant disease-free survival; IDFS, invasive disease-free survival; MT, mutated; OS, overall survival; WT, wild type.

*Proportional hazard assumption violated (IDFS: $P < .001$; DDFS: $P < .001$; OS: $P = .27$).

†Adjusted for age (fitted as cubic spline) and stratified on tumor size (T1/T2/T3/T4), positive nodes (yes/no), local grade (poorly differentiated or undifferentiated/moderately differentiated/well differentiated), estrogen receptor (positive/negative), human epidermal growth factor receptor 2 (positive/negative), and treatment (chemotherapy/hormone therapy/both).

Fig 2. Unadjusted effect of *PIK3CA* genotype on invasive disease-free survival (IDFS), distant disease-free survival (DDFS), and overall survival (OS) by study and for all pooled data by data set. HR, hazard ratio.

Supplement). Overall, the median age at diagnosis was 60 years (range, 18 to 95), and the median follow-up time was 6.9 years (range, 2 days to 21.5 years); 1,787 patients (17%) received

chemotherapy, 4,036 (39%) received endocrine treatment, 3,583 (35%) received both, and 895 (9%) received none or the treatment was unknown.

Fig 3. Kaplan-Meier curves according to *PIK3CA* genotype for (A) invasive disease-free survival, (B) distant disease-free survival, and (C) overall survival using all pooled data. MT, mutated; WT, wild type.

Associations Between *PIK3CA* Mutations With Clinicopathologic Variables

Summary results from the comparison of baseline characteristics and *PIK3CA* mutation status are listed in Table 1. *PIK3CA* mutations were identified in the tumors of 3,281 patients (32%); 1,705 (52%) of these were in the kinase domain and 1,263 (39%) were in the helical domain. *PIK3CA* mutations were more common in older patients, ER-positive tumors, and lower-grade and smaller tumors. The frequency of *PIK3CA* mutations also differed significantly by BC subtypes defined by combined ER and HER2

status (HER2-negative/ER-negative [also known as triple-negative breast cancer], 18%; HER2-negative/ER-positive [also known as luminal], 37%; HER2-positive, 22%; $P < .001$).

Associations Between *PIK3CA* Mutations and Prognosis

In the univariable analysis, *PIK3CA* mutations were significantly associated with better IDFS (HR, 0.77; 95% CI, 0.71 to 0.84; $P < .001$; Table 2). Figure 2 shows the estimated HRs and 95% CIs for each of the studies individually and for all data combined for IDFS, DDFS, and OS. No significant heterogeneity was found

Fig 4. Kaplan-Meier curves for (A) invasive and (B) distant disease-free survival and (C) according to *PIK3CA* genotype and age category. MT, mutated; WT, wild type.

between studies in the univariable effect of *PIK3CA* mutation status on IDFS (*PIK3CA* genotype by study interaction; $P = .12$). For both DDFS and OS, significant heterogeneity was found between studies ($P = .004$ and $P = .009$, respectively). Evidence of departure from the proportional hazards assumption was observed ($P < .001$), implying that the effect of *PIK3CA* genotype on IDFS changed over time. Patients with *PIK3CA* mutant tumors had better IDFS during the first 10 years after diagnosis (0 to 5 years: HR, 0.73; 95% CI, 0.66 to 0.81; $P < .001$; 5 to 10 years: HR, 0.82; 95% CI, 0.68 to 0.99; $P = .037$) but not after 10 years (HR, 1.15; 95% CI, 0.84 to 1.58; $P = .38$; P heterogeneity = .02; Table 2; Fig 3).

There was similar evidence of a nonproportional hazard of relapse over time for DDFS ($P < .001$), although not for OS ($P = .27$). Of note, the nonproportionality was significant only when all BC subtypes were combined (Data Supplement).

After adjusting for age, tumor size, nodal status, local grade, ER status, HER2 status, and treatment, *PIK3CA* status remained significant for IDFS (HR, 0.88; 95% CI, 0.78 to 1.00; $P = .043$) but not for DDFS ($P = .054$) and OS ($P = .8$; Table 2).

The effects of *PIK3CA* genotype on the IDFS and DDFS by BC subtype can be found in the Data Supplement. Notably, there were no significant interactions observed between *PIK3CA* mutation status, BC subtype, and prognosis with the exception of HER2

disease and OS (IDFS: $P = .16$; DDFS: $P = .39$; OS: $P = .04$; Data Supplement) where *PIK3CA* mutations were associated with a worse OS. Mutation location (helical v kinase domain) also did not seem to significantly affect prognosis (IDFS: $P = .74$; DDFS: $P = .92$; OS $P = .65$; Data Supplement). In an exploratory analysis, a significant interaction between *PIK3CA* status and continuous age at diagnosis was observed for IDFS and OS but not for DDFS (IDFS: $P = .032$; DDFS: $P = .20$; OS: $P < .001$; shown in Figure 4 using categorized age at 50 years) where younger patients with a mutation had better survival.

DISCUSSION

The potential prognostic relevance of *PIK3CA* mutations in early-stage BC has thus far been unclear. The larger data sets have reported *PIK3CA* mutations as a favorable aberration, associated with a better clinical outcome, seemingly somewhat at odds with the notion of *PIK3CA* being considered an oncogenic driver.¹⁸ Our study, pooling data from 19 cohorts reaching a total of 10,319 patients, confirms this finding in the univariable analyses. *PIK3CA* mutations were found to be associated with improved IDFS rates (HR, 0.77; $P < .001$) in the univariable analysis, but this effect was less strong in the multivariable model because of its association with favorable clinicopathologic characteristics, namely, older age, ER positivity, lower grade, and smaller tumor size. Overall, our data did not reveal a consistent difference in its prognostic effect according to BC subtype, with the exception of HER2-positive disease and the end point of OS.

Preclinical evidence indicates possible biologic differences between *PIK3CA* mutations affecting the helical or the kinase domain.^{34,35} In particular, mutations of the helical domain have been associated with a more aggressive phenotype.³⁶ In our pooled analysis, including 1,263 and 1,705 patients with *PIK3CA* mutations in the helical and kinase domain, respectively, we found no significant differences in their prognostic impact, similar to studies previously reported.^{18,23}

Associations with ER positivity remain intriguing. Tikoo et al³⁷ reported an increase in the luminal progenitor population in their *PIK3CA* knock-in mouse model, suggesting that *PIK3CA* mutation was important in BC initiation.³⁷ This is supported by the observation that *PIK3CA* mutations exist at high frequency in DCIS.³⁸ Loi et al³⁹ reported decreased mTORC1 signaling as well as upregulation of ER-related genes at the gene expression level in *PIK3CA* mutant ER-positive primary BCs. These data suggest that *PIK3CA* mutations could drive oncogenesis through ER signaling.³⁹ Alternatively, *PIK3CA* mutations have been associated with the induction of senescence in BC, with similar findings reported in other tumor histologies.^{40,41} Hence, despite being a known driver mutation, *PIK3CA* mutations seem to contribute to a favorable clinicopathologic phenotype and behave less aggressively than BCs with driver gene amplifications. These observations should be distinguished from reports that PI3K pathway activation per se is associated with endocrine therapy resistance.⁴²

Of note, we confirm a previous observation that the positive prognostic relevance of *PIK3CA* mutations is nonproportional, that is, the strongest effect is in the first 5 years and decreases over

time. The explanation for this remains unclear.¹⁷ However, we note that the proportion of patients with over 10 years of follow-up is $< 10\%$ and critically does not exclude (lower bound of HR, 0.84) better IDFS; therefore, the departure from proportional hazards could be due to either a reduction in benefit over time or a loss of this effect, or bias in patients with longer follow-up. Taking into account the natural history of BC and the latency of the disease, with relapses occurring even after considerable time after the primary diagnosis, the median follow-up of patients in our pooled analysis (6.9 years) should be taken into consideration when interpreting the results. We also observed a significant interaction between age and *PIK3CA* mutation status. It would be valuable to further validate this finding in adjuvant endocrine studies of premenopausal women.⁴³

Our study has significant strengths, namely, that it is the largest to date performed in patients with early-stage BC using large data sets derived from prospective randomized clinical trials with the use of individual patient-level data. Of note, the size of this data set enabled us to assess the prognostic relevance of *PIK3CA* mutations across all subtypes of BC, and the use of prospective clinical trial data sets may overcome some biases inherent in retrospective institutional series. We acknowledge the limitations: (1) significant heterogeneity among data sets; (2) the treatments administered did not follow a set protocol; in particular, some of the patients with HER2-positive BC did not receive (neo)adjuvant trastuzumab-based treatment, and some of the treatments administered differed from current standards (Data Supplement); (3) only articles published in English were included; (4) only published studies were included (ie, possible bias toward positive results); and (5) heterogeneity in terms of methods of assessment of *PIK3CA* mutation status.

In summary, our results indicate that *PIK3CA* somatic mutations are associated with a significantly better clinical outcome in the univariable but to a lesser extent in the multivariable analysis in early-stage BC. Next-generation sequencing studies have reported that *PIK3CA* mutations often coexist with other genetic alterations.⁴⁴ Integration of coexistent genetic alterations and, potentially, plasma analyses and other markers of PI3K pathway activation will better refine prognostic assessments of *PIK3CA* mutant early-stage BC patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Dimitrios Zardavas, Debora Fumagalli, Sherene Loi

Financial support: Sherene Loi

Administrative support: Christos Sotiriou, Sherene Loi

Provision of study materials or patients: George Papaxoinis, Heikki Joensuu, Olle Stal, Jeanette Dupont Jensen, Gizem Perez-Tenorio, Christos Sotiriou, Sherene Loi

Collection and assembly of data: Dimitrios Zardavas, Luc te Marvelde, Debora Fumagalli, George Fountzilas, Vassiliki Kotoula, Evangelia Razis, George Papaxoinis, Mary Ellen Moynahan, Bryan T. Hennessy, Ivan Bieche, Lao H. Saal, Barry Iacopetta, Jeanette Dupont Jensen, Sandra O'Toole, Elena Lopez-Knowles, Mattia Barbareschi, Enrique Lerma, Thomas Bachelot, Vicky Sabine, John M.S. Bartlett, Stefan Michiels, Sherene Loi

Data analysis and interpretation: Dimitrios Zardavas, Luc te Marvelde, Roger L. Milne, Debora Fumagalli, Heikki Joensuu, Mary Ellen

Moynahan, Ivan Bieche, Lao H. Saal, Olle Stal, Barry Iacopetta, Sandra O'Toole, Shinzaburo Noguchi, Hatem A. Azim Jr, Thomas Bachelot, Qing Wang, Gizeh Perez-Tenorio, Cornelis J.H. van de Velde, Daniel W. Rea, John M.S. Bartlett, Christos Sotiriou, Stefan Michiels, Sherene Loi

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

REFERENCES

- Engelman JA, Luo J, Cantley LC: The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 7:606-619, 2006
- Velho S, Oliveira C, Ferreira A, et al: The prevalence of *PIK3CA* mutations in gastric and colon cancer. *Eur J Cancer* 41:1649-1654, 2005
- Krakstad C, Birkeland E, Seidel D, et al: High-throughput mutation profiling of primary and metastatic endometrial cancers identifies *KRAS*, *FGFR2* and *PIK3CA* to be frequently mutated. *PLoS One* 7:e52795, 2012
- Kuo K-T, Mao T-L, Jones S, et al: Frequent activating mutations of *PIK3CA* in ovarian clear cell carcinoma. *Am J Pathol* 174:1597-1601, 2009
- Banerji S, Cibulskis K, Rangel-Escareno C, et al: Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature* 486:405-409, 2012
- Koboldt DC, Fulton RS, McLellan MD, et al: Comprehensive molecular portraits of human breast tumors. *Nature* 490:61-70, 2012
- Stephens PJ, Tarpey PS, Davies H, et al: The landscape of cancer genes and mutational processes in breast cancer. <http://www.nature.com/doifinder/10.1038/nature11017>
- Samuels Y, Diaz LA Jr, Schmidt-Kittler O, et al: Mutant *PIK3CA* promotes cell growth and invasion of human cancer cells. *Cancer Cell* 7:561-573, 2005
- Isakoff SJ, Engelman JA, Irie HY, et al: Breast cancer-associated *PIK3CA* mutations are oncogenic in mammary epithelial cells. *Cancer Res* 65:10992-11000, 2005
- Zhao JJ, Liu Z, Wang L, et al: The oncogenic properties of mutant p110alpha and p110beta phosphatidylinositol 3-kinases in human mammary epithelial cells. *Proc Natl Acad Sci USA* 102:18443-18448, 2005
- Kang S, Bader AG, Vogt PK: Phosphatidylinositol 3-kinase mutations identified in human cancer are oncogenic. *Proc Natl Acad Sci USA* 102:802-807, 2005
- Berns K, Horlings HM, Hennessy BT, et al: A functional genetic approach identifies the *PI3K* pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 12:395-402, 2007
- Kataoka Y, Mukohara T, Shimada H, et al: Association between gain-of-function mutations in *PIK3CA* and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. *Ann Oncol* 21:255-262, 2010
- Massarweh S, Osborne CK, Creighton CJ, et al: Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function. *Cancer Res* 68:826-833, 2008
- Zardavas D, Fumagalli D, Loi S: Phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin pathway inhibition: A breakthrough in the management of luminal (ER+/HER2-) breast cancers? *Curr Opin Oncol* 24:623-634, 2012
- Zardavas D, Phillips WA, Loi S: *PIK3CA* mutations in breast cancer: Reconciling findings from preclinical and clinical data. *Breast Cancer Res* 16:201, 2014
- Loi S, Michiels S, Lambrechts D, et al: Somatic mutation profiling and associations with prognosis and trastuzumab benefit in early breast cancer. *J Natl Cancer Inst* 105:960-967, 2013
- Sabine VS, Crozier C, Brookes CL, et al: Mutational analysis of *PI3K/AKT* signaling pathway in tamoxifen exemestane adjuvant multinational pathology study. *J Clin Oncol* 32:2951-2958, 2014
- Azim HA Jr, Brohee S, Peccatori FA, et al: Biology of breast cancer during pregnancy using genomic profiling. *Endocr Relat Cancer* 21:545-554, 2014
- Boyault S, Drouet Y, Navarro C, et al: Mutational characterization of individual breast tumors: TP53 and *PI3K* pathway genes are frequently and distinctively mutated in different subtypes. *Breast Cancer Res Treat* 132:29-39, 2012
- Barbareschi M, Buttitta F, Felicioni L, et al: Different prognostic roles of mutations in the helical and kinase domains of the *PIK3CA* gene in breast carcinomas. *Clin Cancer Res* 13:6064-6069, 2007
- Sternke-Hale K, Gonzalez-Angulo AM, Lluch A, et al: An integrative genomic and proteomic analysis of *PIK3CA*, *PTEN*, and *AKT* mutations in breast cancer. *Cancer Res* 68:6084-6091, 2008
- Papaxoinis G, Kotoula V, Alexopoulou Z, et al: Significance of *PIK3CA* mutations in patients with early breast cancer treated with adjuvant chemotherapy: A Hellenic Cooperative Oncology Group (HeCOG) study. *PLoS One* 10:e0140293, 2015
- Li SY, Rong M, Griew F, et al: *PIK3CA* mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat* 96:91-95, 2006
- Jensen JD, Knoop A, Laenkholm AV, et al: *PIK3CA* mutations, *PTEN*, and *pHER2* expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab. *Ann Oncol* 23:2034-2042, 2012
- Gallardo A, Lerma E, Escuin D, et al: Increased signalling of EGFR and IGF1R, and deregulation of *PTEN/PI3K/Akt* pathway are related with trastuzumab resistance in HER2 breast carcinomas. *Br J Cancer* 106:1367-1373, 2012
- Perez-Tenorio G, Alkhori L, Olsson B, et al: *PIK3CA* mutations and *PTEN* loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. *Clin Cancer Res* 13:3577-3584, 2007
- Kalinsky K, Jacks LM, Heguy A, et al: *PIK3CA* mutation associates with improved outcome in breast cancer. *Clin Cancer Res* 15:5049-5059, 2009
- Maruyama N, Miyoshi Y, Taguchi T, et al: Clinicopathologic analysis of breast cancers with *PIK3CA* mutations in Japanese women. *Clin Cancer Res* 13:408-414, 2007
- Lopez-Knowles E, O'Toole SA, McNeil CM, et al: *PI3K* pathway activation in breast cancer is associated with the basal-like phenotype and cancer-specific mortality. *Int J Cancer* 126:1121-1131, 2010
- Saal LH, Holm K, Maurer M, et al: *PIK3CA* mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with *PTEN* loss in human breast carcinoma. *Cancer Res* 65:2554-2559, 2005
- Hudis CA, Barlow WE, Costantino JP, et al: Proposal for standardized definitions for efficacy end points in adjuvant breast cancer trials: the STEEP system. *J Clin Oncol* 25:2127-2132, 2007
- Dowsett M, Cuzick J, Ingle J, et al: Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. *J Clin Oncol* 28:509-518, 2010
- Zhao L, Vogt PK: Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci USA* 105:2652-2657, 2008
- Chaussade C, Cho K, Mawson C, et al: Functional differences between two classes of oncogenic mutation in the *PIK3CA* gene. *Biochem Biophys Res Commun* 381:577-581, 2009
- Pang H, Flinn R, Patsialou A, et al: Differential enhancement of breast cancer cell motility and metastasis by helical and kinase domain mutations of class IA phosphoinositide 3-kinase. *Cancer Res* 69:8868-8876, 2009
- Tikoo A, Roh V, Montgomery KG, et al: Physiological levels of *Pik3ca*(H1047R) mutation in the mouse mammary gland results in ductal hyperplasia and formation of ERα-positive tumors. *PLoS One* 7:e36924, 2012
- Miron A, Varadi M, Carrasco D, et al: *PIK3CA* mutations in situ and invasive breast carcinomas. *Cancer Res* 70:5674-5678, 2010
- Loi S, Haibe-Kains B, Majaj S, et al: *PIK3CA* mutations associated with gene signature of low mTORC1 signaling and better outcomes in estrogen receptor-positive breast cancer. *Proc Natl Acad Sci USA* 107:10208-10213, 2010
- Zardavas D, Fumagalli D, Borwn D, et al: Abstract P2-11-02: Understanding the biology and prognosis of *PIK3CA* gene mutations in primary breast cancer using gene expression profiling: A pooled analysis. *Cancer Res* 73, 2013 (24 suppl; abstr P2-11-02)
- Eser S, Reiff N, Messer M, et al: Selective requirement of *PI3K/PDK1* signaling for *Kras* oncogene-driven pancreatic cell plasticity and cancer. *Cancer Cell* 23:406-420, 2013
- Miller TW, Balko JM, Arteaga CL: Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. *J Clin Oncol* 29:4452-4461, 2011
- Pagani O, Regan MM, Walley BA, et al: Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. *N Engl J Med* 371:107-118, 2014
- Loi S, Luen S, Savas P, et al: Abstract S1-10: Clinical implications of somatic mutations in postmenopausal early-stage estrogen receptor (ER)-positive, HER2-negative breast cancer (BC): Results from the BIG 1-98 study. *Cancer Res* 77, 2017 (4 suppl; abstr S1-10)
- Cizkova M, Susini A, Vacher S, et al: *PIK3CA* mutation impact on survival in breast cancer patients and in ERα, PR and ERBB2-based subgroups. *Breast Cancer Res* 14:R28, 2012

Affiliations

Dimitrios Zardavas and **Debora Fumagalli**, Breast International Group; **Christos Sotiriou**, Université Libre de Bruxelles, Brussels, Belgium; **Luc te Marvelde** and **Roger L. Milne**, Cancer Council; **Roger L. Milne** and **Sherene Loi**, University of Melbourne, Melbourne; **Barry Iacopetta**, University of Western Australia, Western Australia; **Sandra O'Toole** and **Elena Lopez-Knowles**, Garvan Institute of Medical Research, Darlinghurst, Australia; **George Fountzilas** and **Vassiliki Kotoula**, Hellenic Foundation for Cancer Research/Aristotle University of Thessaloniki, Thessaloniki; **Evangelia Razis**, Hygeia Hospital; **George Papaxoinis**, Hippokration Hospital, Athens, Greece; **Heikki Joensuu**, Helsinki University Hospital and University of Helsinki, Helsinki, Finland; **Mary Ellen Moynahan**, Memorial Sloan Kettering Cancer Center, New York, NY; **Bryan T. Hennessy**, Beaumont Hospital and Royal College of Surgeons, Dublin, Ireland; **Ivan Bieche**, Curie Institut, Paris; **Thomas Bachelot**, Centre de Recherche en Cancérologie de Lyon, Lyon; **Stefan Michiels**, Gustave Roussy and Inserm, Univ. Paris-Sud, Univ. Paris-Saclay, Villejuif, France; **Lao H. Saal**, Lund University, Lund; **Olle Stal**, **Qing Wang**, and **Gizeh Perez-Tenorio**, Linköping University, Linköping, Sweden; **Jeanette Dupont Jensen**, University of Southern Denmark, on behalf of the Danish Breast Cancer Cooperative Group, Odense, Denmark; **Elena Lopez-Knowles**, Royal Marsden NHS Trust and Institute of Cancer Research, London; **Daniel W. Rea**, University of Birmingham, Birmingham, United Kingdom; **Mattia Barbraeschi**, Santa Chiara Hospital, Trento, Italy; **Shinzaburo Noguchi**, Osaka University, Osaka Japan; **Hatem A. Azim Jr**, American University of Beirut (AUB), Beirut, Lebanon; **Enrique Lerma**, Universitat Autònoma de Barcelona, Barcelona, Spain; **Cornelis J.H. van de Velde**, Leiden University Medical Center, Leiden, the Netherlands; **Vicky Sabine**, University of Guelph, Guelph; **John M.S. Bartlett**, Ontario Institute for Cancer Research, Toronto, Ontario, Canada.

Support

S.L. is supported by the Cancer Council Victoria John Colebatch Fellowship; National Health and Medical Research Council of Australia; National Breast Cancer Foundation, Australia; and Breast Cancer Research Foundation, New York. C.S. is supported by the Fonds de la Recherche Scientifique and Breast Cancer Research Foundation, New York. J.M.S.B. is supported by the Ontario Institute for Cancer Research. Research at the Ontario Institute for Cancer Research is supported by the Government of Ontario. M.B. is supported by the Trentino Biomolecular Oncologic Network (Trebionet) project, founded by the Fondazione Cassa di Risparmio di Trento e Rovereto, Trento, Italy. L.H.S. is supported by the Swedish Research Council and the Swedish Cancer Society.

Cancer.Net Mobile App for Patients

Cancer.Net's award-winning app is the mobile companion for patients to stay informed about cancer and to organize important personal data often needed for visits to physicians. It includes interactive tools to help patients get answers to important questions, track side effects, and manage medications. Patients using Spanish language—enabled devices can also access the tools and information in Spanish. Direct your patients to cancer.net/app to download the Cancer.Net mobile app.

ASCO Cancer.Net

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Tumor *PIK3CA* Genotype and Prognosis in Early-Stage Breast Cancer: A Pooled Analysis of Individual Patient Data

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/jco/site/ifc.

Dimitrios Zardavas

No relationship to disclose

Luc te Marvelde

No relationship to disclose

Roger L. Milne

No relationship to disclose

Debora Fumagalli

No relationship to disclose

George Fountzilas

Stock or Other Ownership: Ariad Pharmaceuticals (I)

Honoraria: AstraZeneca

Consulting or Advisory Role: Pfizer, Sanofi, Roche

Vassiliki Kotoula

No relationship to disclose

Evangelia Razis

Honoraria: Roche, AstraZeneca, GlaxoSmithKline, Amgen, Novartis, Zeincro, Merck, Pfizer, Bristol-Myers Squibb

Consulting or Advisory Role: Roche, Amgen, AstraZeneca, Janssen-Cilag, Astellas Pharma, Novartis, Bristol-Myers Squibb, Merck, Pfizer, Zeincro

Research Funding: Sanofi, Roche/Genentech, Novartis, AstraZeneca, Celldex, Parexel International, Demo Pharmaceutical

Travel, Accommodations, Expenses: Genesis Pharmaceuticals, LEO Pharma, Pfizer, Roche, GlaxoSmithKline, Sanofi, Amgen, Bristol-Myers Squibb, Genekor, Eisai, Merck, Pierre Fabre, Novartis

George Papaxoinis

No relationship to disclose

Heikki Joensuu

Stock or Other Ownership: Orion Pharma, Sartar Therapeutics, Faron Pharmaceuticals

Honoraria: Orion Pharma, Ariad Pharmaceuticals, Orion Pharma

Consulting or Advisory Role: Blueprint Medicines, Ariad Pharmaceuticals, Orion Pharma

Mary Ellen Moynahan

Consulting or Advisory Role: Novartis

Research Funding: Novartis (Inst)

Bryan T. Hennessy

No relationship to disclose

Ivan Bieche

No relationship to disclose

Lao H. Saal

Employment: SAGA Diagnostics

Leadership: SAGA Diagnostics

Stock or Other Ownership: SAGA Diagnostics

Patents, Royalties, Other Intellectual Property: Patent filed for methods related to ultrasensitive quantification of nucleotide sequence variants

Olle Stal

No relationship to disclose

Barry Iacopetta

No relationship to disclose

Jeanette Dupont Jensen

No relationship to disclose

Sandra O'Toole

Employment: Australian Clinical Laboratories

Honoraria: Astra Zeneca, Pfizer, Merck Serono

Elena Lopez-Knowles

No relationship to disclose

Mattia Barbaraeschi

No relationship to disclose

Shinzaburo Noguchi

Honoraria: AstraZeneca, Taiho Pharmaceutical, Chugai Pharma, Nippon Kayaku, Pfizer, Takeda, Daichi-Sankyo, Sysmex, Novartis

Consulting or Advisory Role: AstraZeneca, Taiho, Novartis

Research Funding: Chugai Pharma, Nippon Kayaku, Pfizer, Takeda, Daichi-Sankyo, Sysmex, Novartis, AstraZeneca

Patents, Royalties, Other Intellectual Property: Patent for Curebest 95GC-breast

Hatem A. Azim Jr

Employment: Innate Pharma

Honoraria: GlaxoSmithKline, Novartis, Roche, Genentech

Speakers' Bureau: GlaxoSmithKline

Research Funding: Amgen

Enrique Lerma

No relationship to disclose

Thomas Bachelot

Consulting or Advisory Role: Roche, Novartis, AstraZeneca, Pfizer

Research Funding: Roche (Inst), Novartis (Inst), AstraZeneca (Inst)

Travel, Accommodations, Expenses: Roche, Novartis, AstraZeneca

Qing Wang

No relationship to disclose

Gizeh Perez-Tenorio

No relationship to disclose

Cornelis J.H. van de Velde

No relationship to disclose

Daniel W. Rea

No relationship to disclose

Vicky Sabine

No relationship to disclose

John M.S. Bartlett

Honoraria: Oncology Education

Consulting or Advisory Role: Insight Genetics, BioNTech, bioTheragnostics

Research Funding: Thermo Fisher Scientific

Patents, Royalties, Other Intellectual Property: Five pending patents: methods and devices for predicting anthracycline treatment efficacy; systems, devices, and methods for constructing and using a biomarker; histone gene module predicts anthracycline benefit; 95-gene signature of residual risk after endocrine treatment; immune gene signature predicts anthracycline benefit (Inst)

Christos Sotiriou

Consulting or Advisory Role: Puma Biotechnology (Inst), Seattle Genetics (Inst), Ipsen (Inst)

Patents, Royalties, Other Intellectual Property: PI3K pathway gene signature granted by the European and US patent offices (Inst)

Stefan Michiels

Consulting or Advisory Role: IDDI, Hexal, Johnson & Johnson, Ipsen, Gentical, Mabxience, Steba, Roche

Patents, Royalties, Other Intellectual Property: Patent EP163058381

Sherene Loi

Research Funding: Roche/Genentech (Inst), Pfizer (Inst), Novartis (Inst), Merck (Inst), Puma Biotechnology (Inst), Bristol-Myers Squibb (Inst)

Patents, Royalties, Other Intellectual Property: PI3K pathway gene signature granted by the European and US patent offices (Inst)