BIOMARKERS

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Comparison of *PIK3CA* Mutation Prevalence in Breast Cancer Across Predicted Ancestry Populations

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PURPOSE Understanding the differences in biomarker prevalence that may exist among diverse populations is invaluable to accurately forecast biomarker-driven clinical trial enrollment metrics and to advance inclusive research and health equity. This study evaluated the frequency and types of *PIK3CA* mutations (*PIK3CA*mut) detected in predicted genetic ancestry subgroups across breast cancer (BC) subtypes.

METHODS Analyses were conducted using real-world genomic data from adult patients with BC treated in an academic or community setting in the United States and whose tumor tissue was submitted for comprehensive genomic profiling.

RESULTS Of 36,151 patients with BC (median age, 58 years; 99% female), the breakdown by predicted genetic ancestry was 75% European, 14% African, 6% Central/South American, 3% East Asian, and 1% South Asian. We demonstrated that patients of African ancestry are less likely to have tumors that harbor *PIK3CA*mut compared with patients of European ancestry with estrogen receptor–positive/human epidermal growth factor receptor 2–negative (ER+/HER2–) BC (37% [949/2,593] *v* 44% [7,706/17,637]; q = 4.39E-11) and triple-negative breast cancer (8% [179/2,199] *v* 14% [991/7,072]; q = 6.07E-13). Moreover, we found that *PIK3CA*mut were predominantly composed of hotspot mutations, of which mutations at H1047 were the most prevalent across BC subtypes (35%-41% ER+/HER2– BC; 43%-61% HER2+ BC; 40%-59% triple-negative breast cancer).

CONCLUSION This analysis established that tumor *PIK3CA*mut prevalence can differ among predicted genetic ancestries across BC subtypes on the basis of the largest comprehensive genomic profiling data set of patients with cancer treated in the United States. This study highlights the need for equitable representation in research studies, which is imperative to ensuring better health outcomes for all.

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INTRODUCTION

Precision medicine clinical trials are increasingly common in drug development wherein the implementation of rational biomarker strategies to identify patients who are hypothesized as most likely to respond to treatment is a crucial component of these trials.[1] This requires accurate estimations of the target biomarker prevalence in the indication(s) of interest. Such information affects study operations, including the projection of screen fail rates to inform the total number of potential study participants needed to ensure the complete enrollment of a clinical trial in a timely manner. Estimation of biomarker prevalence is primarily on the basis of publicly available and/or proprietary genomics databases. To date, the majority of genomics databases are largely composed of sequencing data from individuals of Western European descent.[2] This lack of diversity translates into biomarker-driven patient selection strategies being designed without a clear understanding of whether these biomarkers may differ across diverse populations. This can lead to errors in study enrollment projections and, ultimately, may hinder the advancement of precision medicine for historically under-represented populations.

Although sampling biases are a topic of general concern when assessing biomarker prevalence, our analysis focuses on *PIK3CA* mutations (*PIK3CA*mut) as the biomarker of interest. The *PIK3CA* gene encodes for the p110α protein, the catalytic subunit of the phosphatidylinositol 3-kinase (PI3K) complex. p110α is a crucial cell-signaling component and is among the most frequently mutated genes in many solid tumor types, including endometrial cancer (approximately 53%), breast cancer (BC; approximately 36%), cervical cancer (approximately 26%), and head and neck squamous cell carcinoma (approximately 26%).[3-6] Within BC, *PIK3CA* is

ASSOCIATED CONTENT

Data Supplement

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

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CONTEXT

Key Objective

As both biomarker-driven precision medicine trials and calls for diversity in clinical trials become increasingly common, accurate assessment of biomarker prevalence is critical for informing study enrollment metrics. In this study, we investigated the variation in the frequency and spectrum of *PIK3CA* mutations in breast cancer (BC) across predicted genetic ancestry subgroups.

Knowledge Generated

Patients of African ancestry are less likely to have tumors that harbor *PIK3CA* mutations compared with patients of European ancestry with estrogen receptor–positive/human epidermal growth factor receptor 2–negative BC and triple-negative BC. However, across predicted genetic ancestry groups, the most frequently observed *PIK3CA* mutations were generally similar and most, but not all, are able to be identified using commercially available polymerase chain reaction–based assays.

Relevance

This study highlights the need to systematically assess biomarker prevalence in historically under-represented populations to increase confidence in the generalizability and translatability of clinical trial outcomes to the population at large.

mutated in approximately 40% of hormone receptor-positive/human epidermal growth factor receptor 2–negative (HR+/HER2–) BC, approximately 30% of HER2+ BC, and approximately 15% of triple-negative breast cancers (TNBCs). Furthermore, p110α is necessary for proper embryologic morphogenesis[7,8] and *PIK3CA*mut drive noncancerous overgrowth syndromes including CLOVES (congenital lipomatous [fatty] overgrowth, vascular malformations, epidermal nevi and scoliosis/skeletal/spinal anomalies), PROS (*PIK3CA*-related overgrowth spectrum), and other vascular malformations.[9] Mutations in *PIK3CA* lead to activation of the PI3K signaling pathway, a linchpin in the regulation of cell growth, proliferation, and survival, and its dysregulation has been characterized across a number of tumor types.[10-12] Preclinical studies have demonstrated that hyperactivation of the PI3K signaling pathway is a resistance mechanism to endocrine therapy,[13] and a possible resistance mechanism to HER2-targeted therapies[14-17] and chemotherapy.[18,19] To date, alpelisib (BYL719) is the only US Food and Drug Administration (FDA)–approved p110α inhibitor for use with fulvestrant in patients with HR+/HER2– *PIK3CA*mut advanced or metastatic BC.[20] Both alpelisib and another p110α-targeting inhibitor, inavolisib (GDC-0077),[21] are being evaluated in biomarker-driven phase III clinical trials in HR+/HER2– BC (ClinicalTrials.gov identifiers: NCT03439046, NCT05038735, NCT04191499), HER2+ BC (ClinicalTrials.gov identifiers: NCT04208178, NCT05063786), and TNBC (ClinicalTrials.gov identifier: NCT04251533). It is important to understand whether differences in biomarker prevalence exist across racial/ethnic populations and/or geographical regions to accurately forecast biomarker-driven clinical trial enrollment metrics, and to strive toward achieving diverse enrollment in clinical trials.

In this study, we used one of the largest real-world databases of patient tumors profiled with comprehensive genomic profiling (CGP) to evaluate whether the prevalence of *PIK3CA*mut in the major BC subtypes differs across predicted genetic ancestries. We subsequently delved into the types of *PIK3CA*mut detected and assessed how they may reflect underlying diversity among BC subtypes and predicted genetic ancestries. Finally, we contextualized our findings in terms of the impact to biomarker screening for clinical trials.

METHODS

CGP of Patient BC Tissue Specimens

CGP of 39,572 formalin-fixed, paraffin-embedded breast tumor samples from patients with primary advanced or metastatic disease based in the United States was performed from 2013 to 2021 using the FoundationOne or FoundationOneCDx assay in a Clinical Laboratory Improvement Amendments–certified, College of American Pathologists (CAP)-accredited laboratory (Foundation Medicine Inc, Cambridge, MA). All sequenced samples featured ≥ 20% tumor content and yielded ≥ 50 ng extracted DNA. CGP was performed on hybrid-capture, adapter ligation–based libraries, to identify genomic alterations for ≥ 324 genes, as previously described.[22,23] Sites of care included academic and community settings. Approval for this study, including waiver of informed consent and Health Insurance Portability and Accountability Act (HIPAA) waiver of authorization, was obtained from the Western IRB (Protocol No. 20152817).

Classification of *PIK3CA*mut Tissue Specimens

*PIK3CA*mut specimens were defined as those with ≥ 1 single-nucleotide variant in the *PIK3CA* gene that is predicted to be pathogenic, defined as known or likely oncogenic significance. Statistical testing used two-sided Fisher's exact tests unless otherwise noted. Statistical significance is defined as Benjamini-Hochberg–adjusted *P* value (q-value) < .05.

Estimation of Patient-Level Genetic Ancestry

Patient genetic ancestry was inferred using > 40,000 germline single-nucleotide polymorphisms that were covered as part of the CGP, as previously described.[24] Patient-

level self-reported ancestry and/or ethnicity data were unavailable. Individuals were classified into inferred population groups using a random forest classifier trained on phase III 1000 Genomes samples, wherein principal component analysis was run on alternate allele counts and a model was trained using the top 10 principal component analysis features. The classifier was then applied to the CGP patient samples. To quantitate the fraction of ancestral mixture in each patient, ADMIXTURE was run on the 1000 Genomes samples to define five population signatures, and then ADMIXTURE was applied to the CGP samples in projection mode using these five signatures.[25,26]

Estrogen Receptor Status for Tumor Tissue Specimens

Specimen estrogen receptor (ER) status (ER-positive or ER-negative) was derived from ER immunohistochemistry per local assessment in pathology reports provided to Foundation Medicine, Inc. It is possible that institutes did not score ER status according to ASCO/CAP guidelines, defined as ≥ 1% of tumor cells staining positive for ER. If ER status was unavailable, it was computationally imputed using a machine learning approach (Data Supplement).

Software

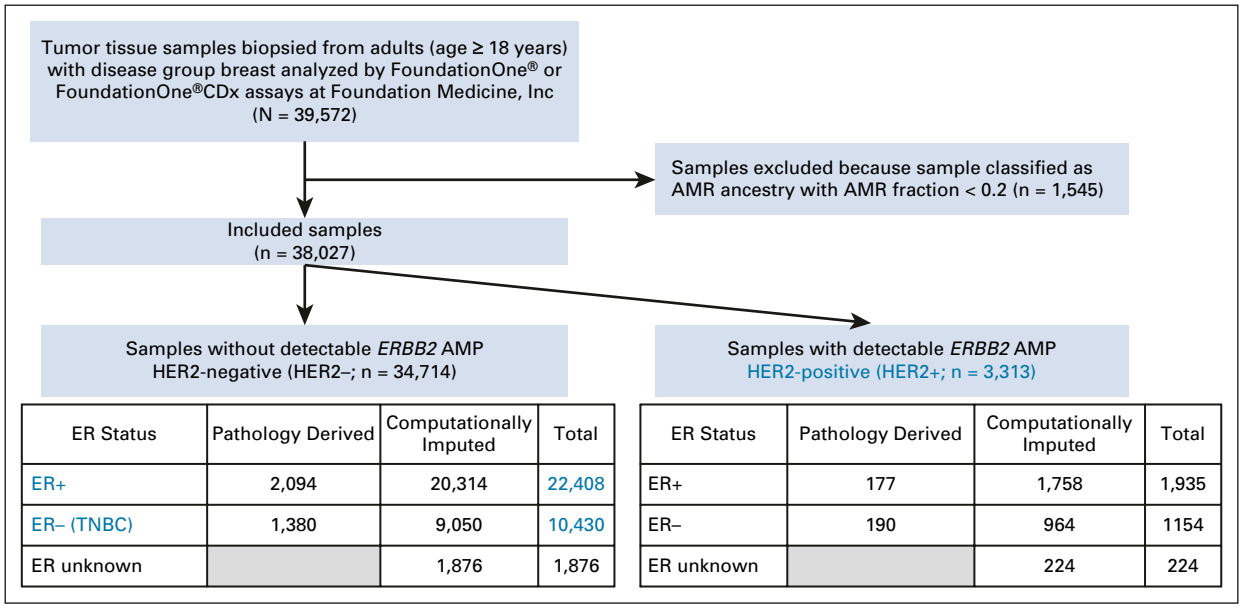
Statistics, computation, and plotting were carried out using R v3.6.1.

RESULTS

Demographics of the Study Cohort

The overall cohort consisted of samples from 39,572 individuals with BC. These samples were stratified by predicted ancestry and BC subtype, resulting in 36,151 samples that were further analyzed herein: 22,408 ER+/HER2– BC samples, 10,430 TNBC samples, and 3,313 HER2+ BC samples (Fig 1). A total of 3,421 samples were excluded from the analysis because of either low confidence of the ancestry classification for individuals of predicted Central/South American (AMR) ancestry with AMR fraction < 0.2 (n = 1,545) or indeterminate ER status for HER2– BC samples (n = 1,876). BC subtype was based on ER and HER2 status; progesterone receptor (PR) status was unavailable. As HER2-positivity is defined in this analysis by the presence of *ERBB2* amplification, it may not encompass all HER2+ samples as defined per ASCO/CAP guidelines.[27] Because the frequency of ER–/PR+ BC is rare,[28,29] a small number of these samples may be misclassified as TNBC.

The patients were predominantly female (approximately 99%) with a median age at the time of biopsy of 60 years for ER+/HER2– BC, 55 years for HER2+ BC, and 56 years for TNBC. TNBC is not hormonally driven and is more frequently observed in a younger demographic group[30] compared with HR+ BC, a disease largely diagnosed in postmenopausal women.[31] We postulate the median age of the study cohort is younger than the reported median age of BC diagnosis[32] because of a bias in the data wherein younger patients diagnosed with BC, who as a group tend to have worse outcomes,[33,34] are more likely to have their tumors genomically profiled to guide treatment decisions. Across BC subtypes, patients of European (EUR) ancestry were significantly older than patients of other ancestries (Table 1, Data Supplement). Patients were predominantly of EUR ancestry (79% in ER+/HER2– BC; 73% in HER2+ BC; 68% in TNBC), followed by African (AFR) ancestry (12% in ER+/HER2– BC;



ER Status	Pathology Derived	Computationally Imputed	Total
ER+	2,094	20,314	22,408
ER– (TNBC)	1,380	9,050	10,430
ER unknown		1,876	1,876

ER Status	Pathology Derived	Computationally Imputed	Total
ER+	177	1,758	1,935
ER–	190	964	1154
ER unknown		224	224

FIG 1. Flowchart of the analysis population. Blue-colored text denotes the breast cancer subtypes that are included in the analysis population. Details on the computational imputation of ER status are described in the Data Supplement. AFR, African; AMP, amplification; AMR, Central/South American; CDx, companion diagnostic; EAS, East Asian; ER, estrogen receptor; ERBB2, erb-b2 receptor tyrosine kinase 2; EUR, European; HER2, human epidermal growth factor receptor 2; n, sample size; SAS, South Asian; TNBC, triple-negative breast cancer.

TABLE 1. Demographics of the Study Cohort

Characteristic	ER+/HER2– BC (n = 22,408)	HER2+ BC (n = 3,313)	TNBC (n = 10,430)
Sex, No. (%)			
Female	22,073 (98.5)	3,284 (99.1)	10,410 (99.8)
Male	332 (1.5)	29 (0.9)	16 (0.2)
Unknown	3 (0.01)	0 (0.0)	4 (0.04)
Age, years, median (IQR); q-value[a]			
All	60 (51-68)	55 (46-64)	56 (46-65)
EUR	61 (53-69)	56 (48-65)	57 (48-67)
AFR	57 (47-65); q = 7.1E-59	54 (45-62); q = 7.3E-04	54 (46-62); q = 4.8E-21
AMR	54 (45-62); q = 6.5E-72	49.5 (42-59); q = 1.0E-11	48 (41-58); q = 7.5E-43
EAS	58 (49-66); q = 1.9E-12	53 (45.75-62); q = 1.5E-02	52 (43-61); q = 4.8E-09
SAS	54 (45-64); q = 7.0E-14	51 (41.5-58); q = 3.4E-03	49 (42-59.75); q = 3.7E-08
Predicted ancestry, No. (%)			
EUR	17,637 (78.7)	2,420 (73.1)	7,072 (67.8)
AFR	2,593 (11.6)	444 (13.4)	2,199 (21.1)
AMR	1,171 (5.2)	250 (7.6)	719 (6.9)
EAS	753 (3.4)	148 (4.5)	302 (2.9)
SAS	254 (1.1)	51 (1.5)	138 (1.3)

Abbreviations: AFR, African; AMR, Central/South American; BC, breast cancer; EAS, East Asian; ER, estrogen receptor; EUR, European; HER2, human epidermal growth factor receptor 2; IQR, interquartile range; n, sample size; SAS, South Asian; TNBC, triple-negative breast cancer.

[a]Differences in age of patients of non-EUR ancestry compared with patients of predicted EUR ancestry were evaluated using pairwise Wilcoxon rank sum tests wherein statistical significance is defined as Benjamini-Hochberg–adjusted *P* value (q-value) < .05.

13% in HER2+ BC; 21% in TNBC); patients of South Asian (SAS) ancestry comprised the lowest percentage (1% in ER+/HER2– BC; 2% in HER2+ BC; 1% in TNBC; Table 1). The samples were largely from late-stage tumors per analysis of tumor stage and site of tumor biopsy (Table 2) and obtained from a mix of local tumor sites and metastatic lesions (Data Supplement). No other notable demographic differences were observed in the analysis population.

*PIK3CA*mut Prevalence Across Predicted Ancestries

We next evaluated the prevalence of *PIK3CA*mut across the predicted ancestries within each BC subtype. As this study aimed to evaluate the impact of biomarker prevalence data on precision medicine clinical trial design, analysis of *PIK3CA*mut was limited to pathogenic single-nucleotide variants. This biomarker definition broadly encompasses the clinically relevant *PIK3CA*mut included in the biomarker eligibility criteria for clinical trials investigating PI3K inhibitors.[20,35] *PIK3CA* indels, amplifications, and rearrangement events are incompletely characterized.

In ER+/HER2– BC, *PIK3CA*mut were identified in 44% (n = 7,706/17,637) of patients of EUR ancestry, 37% (n = 949/2,593) of patients of AFR ancestry, 40% (n = 472/1,171) of patients of AMR ancestry, 43% (n = 323/753) of patients of East Asian (EAS) ancestry, and 48% (n = 123/254) of patients of SAS ancestry. *PIK3CA*mut were detected significantly less frequently in patients of AFR (q = 4.39E-11) and AMR (q = 0.0417) ancestries compared with patients of EUR ancestry. No difference in *PIK3CA*mut prevalence was observed in patients of EAS (q = 0.680) and SAS (q = 0.171) ancestries compared with patients of EUR ancestry (Fig 2A).

In HER2+ BC, *PIK3CA*mut were identified in 34% (n = 829/2,420) of patients of EUR ancestry, 30% (n = 135/444) of patients of AFR ancestry, 31% (n = 77/250) of patients of AMR ancestry, 45% (n = 67/148) of patients of EAS ancestry, and 51% (n = 26/51) of patients of SAS ancestry. *PIK3CA*mut were detected significantly more frequently in patients of EAS (q = 0.0182) and SAS (q = 0.0334) ancestries compared with patients of EUR ancestry. No difference in *PIK3CA*mut prevalence was observed in patients of AFR (q = 0.168) and AMR (q = 0.320) ancestries compared with patients of EUR ancestry (Fig 2C).

In TNBC, *PIK3CA*mut were identified in 14% (n = 991/7,702) of patients of EUR ancestry, 8% (n = 179/2,199) of patients of AFR ancestry, 10% (n = 72/719) of patients of AMR ancestry, 21% (n = 64/302) of patients of EAS ancestry, and 20% (n = 27/138) of patients of SAS ancestry. Compared with the patients of EUR ancestry, *PIK3CA*mut were detected significantly less frequently in patients of AFR (q = 6.07E-13) and AMR (q = 7.50E-03) ancestries, and more frequently in patients of EAS (q = 4.00E-03) ancestry. No difference in *PIK3CA*mut prevalence was observed in patients of SAS (q = 0.124) ancestry compared with patients of EUR ancestry (Fig 2E).

TABLE 2. Tumor Stage Distribution of the Study Cohort

Breast Cancer Subtype (n)	Tumor Stage	EUR (n = 17,637), No. (%)	AFR (n = 2,593), No. (%)	AMR (n = 1,171), No. (%)	EAS (n = 753), No. (%)	SAS (n = 254), No. (%)
ER+/HER2– BC (n = 22,408)	0	22 (0.1)	11 (0.4)	0 (0.0)	1 (0.1)	2 (0.8)
	I	248 (1.4)	21 (0.8)	12 (1.0)	15 (2.0)	9 (3.5)
	II	613 (3.5)	93 (3.6)	40 (3.4)	24 (3.2)	12 (4.7)
	III	636 (3.6)	88 (3.4)	49 (4.2)	34 (4.5)	12 (4.7)
	IV	8,011 (45.4)	1,145 (44.2)	512 (43.7)	351 (46.6)	105 (41.3)
	Unknown	8,107 (46.0)	1,235 (47.6)	558 (47.7)	328 (43.6)	114 (44.9)
		EUR (n = 2,420)	**AFR (n = 444)**	**AMR (n = 250)**	**EAS (n = 148)**	**SAS (n = 51)**
HER2+ BC (n = 3,313)	0	1 (0.04)	3 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
	I	28 (1.2)	4 (0.9)	4 (1.6)	0 (0.0)	0 (0.0)
	II	56 (2.3)	11 (2.5)	7 (2.8)	3 (2.0)	1 (2.0)
	III	86 (3.6)	21 (4.7)	12 (4.8)	11 (7.4)	2 (3.9)
	IV	855 (35.3)	164 (36.9)	99 (39.6)	53 (35.8)	20 (39.2)
	Unknown	1,394 (57.6)	241 (54.3)	128 (51.2)	81 (54.7)	28 (54.9)
		EUR (n = 7,072)	**AFR (n = 2,199)**	**AMR (n = 719)**	**EAS (n = 302)**	**SAS (n = 138)**
TNBC (n = 10,430)	0	11 (0.2)	6 (0.3)	2 (0.3)	1 (0.3)	0 (0.0)
	I	91 (1.3)	18 (0.8)	5 (0.7)	8 (2.6)	1 (0.7)
	II	335 (4.7)	86 (3.9)	40 (5.6)	11 (3.6)	5 (3.6)
	III	465 (6.6)	166 (7.5)	76 (10.6)	17 (5.6)	14 (10.1)
	IV	2,378 (33.6)	786 (35.7)	236 (32.8)	91 (30.1)	48 (34.8)
	Unknown	3,792 (53.6)	1,137 (51.7)	360 (50.1)	174 (57.6)	70 (50.7)

NOTE. Tumor stage refers to the tumor stage information reported by the physician on the test requisition form. Of note, the reported tumor stage has not been verified by an independent review of the pathology reports. Consequently, the reported tumor stage may not correspond to the actual stage of the tumor sample if, for example, an archival tumor sample was submitted for CGP from a patient with late-stage BC.

Abbreviations: AFR, African; AMR, Central/South American; BC, breast cancer; CGP, comprehensive genomic profiling; EAS, East Asian; ER, estrogen receptor; EUR, European; HER2, human epidermal growth factor receptor 2; n, sample size; SAS, South Asian; TNBC, triple-negative breast cancer.

Our analysis demonstrates that tumor *PIK3CA*mut prevalence may differ among predicted ancestries across all BC subtypes evaluated. We observed similar findings when the analysis was limited to samples with pathology report–derived ER status. Specifically, *PIK3CA*mut were detected significantly less frequently in patients of AFR ancestry compared with patients of EUR ancestry in ER+/HER2– BC (29% [n = 71/247] *v* 39% [n = 630/1,619]; q = 2.37E-03) and TNBC (10% [n = 23/238] *v* 22% [n = 222/996]; q = 5.01E-06). Interestingly, *PIK3CA*mut were detected more frequently in patients of SAS ancestry compared with patients of EUR ancestry in ER+/HER2– BC (68% [n = 17/25] *v* 39% [n = 630/1,619]; q = 6.03E-03). However, this finding is based on a small sample size of ER+/HER2– patients of SAS ancestry (Data Supplement).

Spectrum of *PIK3CA*mut in BC

We next evaluated the spectrum of *PIK3CA*mut detected. Activating mutations in *PIK3CA* occur largely in exons 9 and 20, which encode the helical and kinase domains, respectively, of p110α.[12] Samples with *PIK3CA*mut were classified as harboring a single hotspot mutation (ie, occurring at H1047, E542, or E545), a single nonhotspot mutation (Data Supplement), or multiple mutations (ie, tumor harbors ≥ 2 *PIK3CA*-mut; Data Supplement). Across all subgroups evaluated, hotspot *PIK3CA*mut were most predominant; mutations at H1047 (35%-41% ER+/HER2– BC; 43%-61% HER2+ BC; 40%-59% TNBC) were the most frequent and mutations at E542 (7%-13% ER+/HER2– BC; 4%-12% HER2+ BC; 8%-12% TNBC) were the least frequent (Figs 2B, 2D and 2F) among these hotspot mutations. Multiple *PIK3CA*mut were more frequently observed in ER+/HER2– BCs (14%-21%) compared with HER2+ BCs (4%-8%) and TNBCs (0%-13%) across all predicted ancestries. Within BC subtypes, no significant difference was observed in the proportional distribution of *PIK3CA*mut across predicted ancestries (q-value > 0.05; Figs 2B, 2D and 2F).

DISCUSSION

Our findings demonstrated that tumor *PIK3CA*mut prevalence differs among predicted ancestries in BC. We have shown that ER+/HER2– BC and TNBC tumors from patients of AFR and AMR ancestries are less likely to harbor *PIK3CA*mut compared with patients of EUR ancestry.

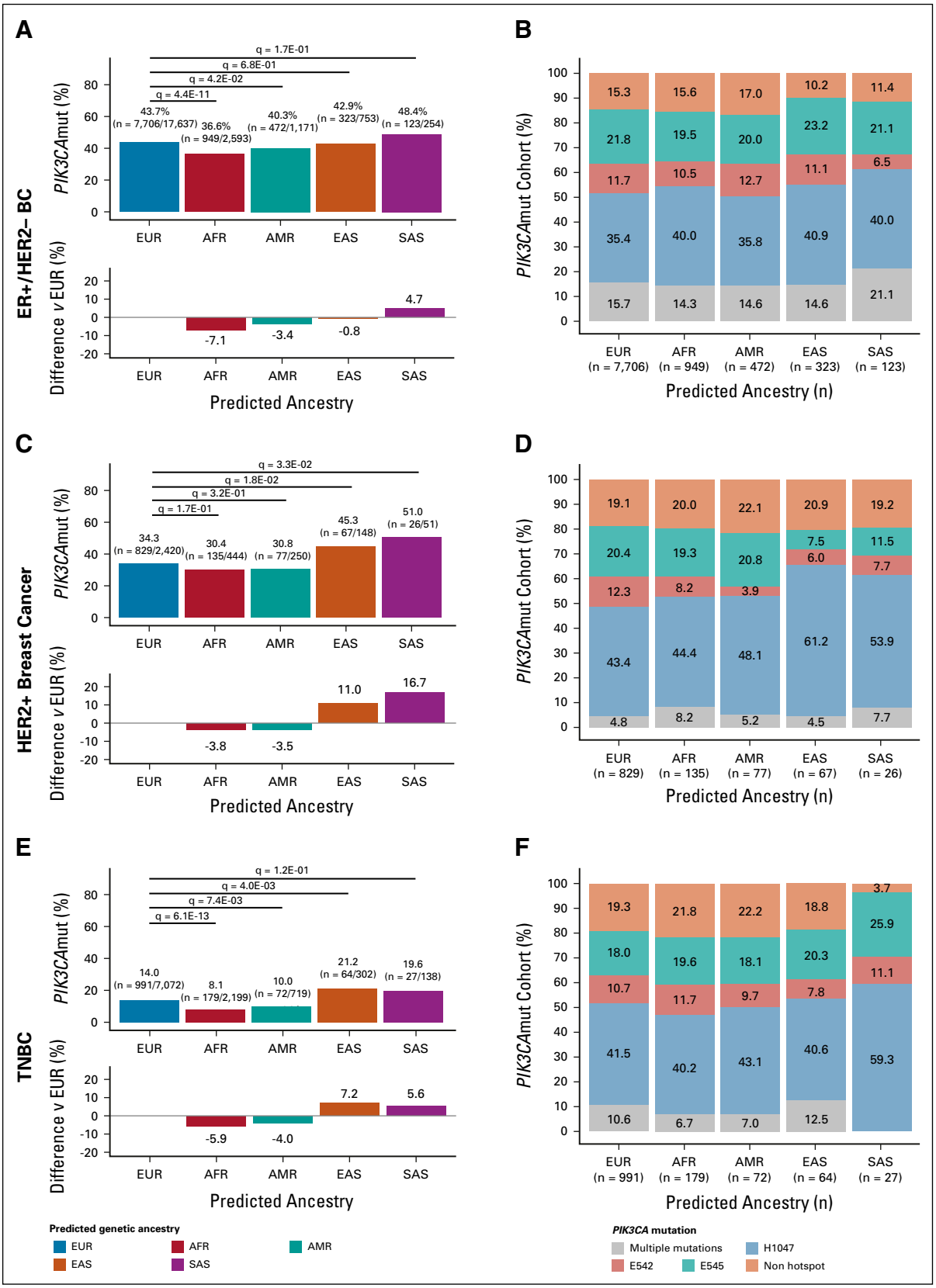


FIG 2. *PIK3CA*mut landscape across BC subtypes by predicted ancestries: (A) *PIK3CA*mut prevalence and (B) composition of *PIK3CA*mut tumors of ER+/HER2– BC; (C) *PIK3CA*mut prevalence and (D) composition of *PIK3CA*mut tumors of HER2+ BC; and (E) *PIK3CA*mut prevalence and (F) composition of *PIK3CA*mut tumors of TNBC. (A, C, E) Bar plots illustrate *PIK3CA*mut frequency within each predicted (continued on following page)

FIG 2. (Continued). ancestry subgroup (top) and the difference in *PIKCA*mut prevalence relative to patients of EUR ancestry (bottom). Differences in *PIK3CA*mut prevalence were evaluated using Fisher's exact test wherein statistical significance is defined as Benjamini-Hochberg–adjusted *P* value (q-value) < .05. (B, D, F) Percent stacked bar charts illustrate the relative frequency of the types of *PIK3CA*mut among *PIK3CA*mut samples within each predicted ancestry subgroup. Samples that harbor *PIK3CA*mut were classified as harboring either a single hotspot mutation (ie, occurring at H1047, E542, or E545), a single nonhotspot mutation, or multiple mutations (ie, tumor harbors ≥ 2 *PIK3CA*mut). BC, breast cancer; ER, estrogen receptor; TNBC, triple-negative breast cancer.

Additionally, compared with patients of EUR ancestry, patients of EAS ancestry with HER2+ BC and TNBC, as well as patients of SAS ancestry with HER2+ BC, were more likely to have tumors that harbor *PIK3CA*mut (Figs 2A, 2C and 2E). The ancestry-specific tumor *PIK3CA*mut prevalences observed across BC subtypes is supported by the findings from studies that sampled substantially fewer participants in Africa,[36-39] Latin America,[40-44] China or Korea,[45-52] and South Asian countries.[53-57] Inconsistencies between *PIK3CA*mut prevalences identified in our study and those reported in the literature were observed when sample sizes were small and when differences existed in the clinical characteristics of the populations evaluated and/or assay methodologies. It is as yet unknown whether statistically significant differences in *PIK3CA*mut prevalence across the predicted ancestries translate into clinically meaningful differences in study enrollment metrics.

Furthermore, our analysis of the types of *PIK3CA*mut identified in BC revealed several similarities across all subgroups evaluated. Overall, within BC subtypes, the proportional distribution of *PIK3CA*mut did not differ among predicted ancestries (Figs 2B, 2D and 2F). The spectrum of *PIK3CA*mut was predominantly composed of hotspot mutations, of which mutations at H1047 were the most prevalent. These observations suggest that the overall types of *PIK3CA*mut that arise in BC tumors are not different between patients of different predicted ancestries. From a clinical trial perspective, these data suggest that no ancestry subgroup would be at an innate disadvantage toward meeting the biomarker definition for inclusion in a PI3K-inhibitor clinical trial. Single nonhotspot mutations comprised a small but sizable proportion of the identified mutations (10%-17% ER+/HER2– BC; 19%-22% HER2+ BC; 4%-22% TNBC; Figs 2B, 2D and 2F; Data Supplement). Although some nonhotspot mutations are included on commercial testing panels, the clinical utility of nonhotspot *PIK3CA*mut may vary depending on the drug and indication under investigation. Although resources[58] exist to support clinical interpretation of somatic variants, studies evaluating their functional consequences may be lacking because of the rarity of some mutations. Therefore, it is unsurprising that commercial polymerase chain reaction–based *PIK3CA*mut assays were developed to detect *PIK3CA* hotspot mutations. Consequently, the most frequent *PIK3CA*mut detected across predicted ancestries can be identified in academic and community clinical settings. However, CGP offers the opportunity to identify a broader spectrum of mutations and more complex mutation patterns (eg, double *PIK3CA*mut), thus enabling the execution of clinical trials with compound or expanded biomarker inclusion criteria or even the opportunity for referral to other clinical trials on the basis of a single test result. Unfortunately, populations without access to high-quality health care may have limited or no access to CGP that would afford them greater opportunities to participate in such trials.

Finally, we hypothesize that the differences observed in the prevalence of tumors that harbor multiple *PIK3CA*mut across BC subtypes highlight the molecular diversity of BC. ER+/HER2– breast tumors are more likely to harbor multiple *PIK3CA*mut than other BC subtypes (14%-21% of ER+/HER2– BCs *v* 4%-8% of HER2+ BCs and 0%-13% of TNBCs; Figs 2B, 2D and 2F; Data Supplement). The prevalence of multiple *PIK3CA*mut across solid tumors is uncommon, occurring in < 1% of cancers.[59] In preclinical studies, multiple *PIK3CA*mut *in cis* led to robust activation of the PI3K pathway, along with enhanced cell proliferation and tumor growth compared with single *PIK3CA*mut.[60] The lower prevalence of multiple *PIK3CA*mut observed in HER2+ BCs and TNBCs relative to ER+/HER2– BCs may indicate decreased reliance on PI3K signaling mediated by *PIK3CA*mut, and dependence on alternative mechanisms to dysregulate PI3K signaling. For example, the HER2-PI3K axis is involved in regulation of cell proliferation, survival, and growth,[61,62] and overexpressed HER2 (often because of amplified *ERBB2*) activates PI3K signaling.[61,63,64] Likewise, genomic loss of *PTEN*, detected in 15% TNBCs,[4] contributes to dysregulation of PI3K signaling.[65,66] Nevertheless, preclinical studies are required to elucidate whether the breadth of genomic alterations capable of activating PI3K signaling are able to perturb downstream signaling effectors at comparable measures.

In summary, this study leverages one of the largest available genomic databases to enable detailed subanalyses of *PIK3CA*mut prevalence across predicted ancestries (ie, EUR, AFR, AMR, EAS, and SAS) and BC subtypes (ie, ER+/HER2–, HER2+, and TNBC); this information may inform study enrollment metrics for biomarker-based clinical trials and encourage similar analyses for biomarkers relevant to other cancer types. Nevertheless, our study has limitations. First, the data are confined to samples from patients in the United States, lack information about generational status of the patients (ie, length of familial residence in the United States), and represent a population with access to a level of health care that offered next-generation sequencing (NGS)-based genomic testing, all factors that may affect the generalizability of the findings. In the absence of publicly available somatic mutation data sets with a sizable number of

patients from diverse backgrounds residing outside North America, it is challenging to know whether the genetic ancestry classifications are a suitable proxy for the world's diverse populations as historically stratified on the basis of geographical location in clinical trial analyses. Moreover, a subset of ER statuses was imputed (Data Supplement), with a classification accuracy of 83% (95% CI, 76 to 89) on an independent validation cohort (Data Supplement). While of consideration, similar findings were observed when the analysis was restricted to samples whose ER status was solely pathology report–derived (Data Supplement). Finally, the analysis may be underpowered because of the small sample size of some non-EUR ancestry cohorts.

Overall, this study sought to better understand potential differences in biomarker prevalence among diverse populations to improve the design of biomarker-driven clinical trials. Currently, enrollment forecasting for multicountry/multiregion clinical trials rarely account for variations in biomarker prevalence, which may directly affect study operations and lead to a lack of diversity in clinical trials. These issues are underscored by the > 400 FDA-approved drugs with biomarker(s) in their label.[67] Such concerns may, in part, be mitigated by efforts such as the NIH Revitalization Act of 1993, the FDA's Drug Trials Snapshots Program, and the FDA's recent push for clinical trial sponsors to include diversity plans in clinical trial applications.[68,69] We hope this study encourages the scientific community to improve efforts to collect specimens from diverse populations irrespective of race, ethnicity, and socioeconomic status, perform inclusivity analyses as part of routine demographics analyses, and consider incorporation of molecular diagnostic testing into routine care. Collectively, these efforts would increase representation of real-world patient populations in genomics databases, which may enable more equitable representation in research studies and advance personalized care for all patients with cancer.

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DATA SHARING STATEMENT

Because of HIPAA requirements, Foundation Medicine, Inc (FMI) is not consented to share individualized patient genomic data, which contains potentially identifying or sensitive information. FMI is committed to collaborative data analysis and has well-established mechanisms by which investigators can query their core genomic database of > 400,000 deidentified sequenced cancer specimens to obtain aggregated data sets.

EQUAL CONTRIBUTION

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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