

Characteristics and Outcome of $AKT1^{E17K}$ -Mutant Breast Cancer Defined through AACR Project GENIE, a Clinicogenomic Registry

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ABSTRACT

AKT inhibitors have promising activity in $AKT1^{E17K}$ -mutant estrogen receptor (ER)-positive metastatic breast cancer, but the natural history of this rare genomic subtype remains unknown. Utilizing AACR Project GENIE, an international clinicogenomic data-sharing consortium, we conducted a comparative analysis of clinical outcomes of patients with matched $AKT1^{E17K}$ -mutant ($n = 153$) and $AKT1$ -wild-type ($n = 302$) metastatic breast cancer. $AKT1$ -mutant cases had similar adjusted overall survival (OS) compared with $AKT1$ -wild-type controls (median OS, 24.1 vs. 29.9, respectively; $P = 0.98$). $AKT1$ -mutant cases enjoyed longer durations on mTOR inhibitor therapy, an observation previously unrecognized in pivotal clinical trials due to the rarity of this alteration. Other baseline clinicopathologic features, as well as durations on other classes of therapy, were broadly similar. In summary, we demonstrate the feasibility of using a novel and publicly accessible clinicogenomic registry to define outcomes in a rare genomically defined cancer subtype, an approach with broad applicability to precision oncology.

SIGNIFICANCE: We delineate the natural history of a rare genomically distinct cancer, $AKT1^{E17K}$ -mutant ER-positive breast cancer, using a publicly accessible registry of real-world patient data, thereby illustrating the potential to inform drug registration through synthetic control data.

See related commentary by Castellanos and Baxi, p. 490.

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INTRODUCTION

Activation of the PI3K pathway is common in estrogen receptor-positive (ER⁺) breast cancer, with approximately 40% of cases harboring mutations in *PIK3CA*, which encodes the PI3K alpha catalytic subunit p110 α (1). Providing further clinical validation of the oncogenic role of this pathway, the selective PI3K alpha inhibitor alpelisib when added to fulvestrant prolongs progression-free survival (PFS) compared with fulvestrant alone in *PIK3CA*-mutant ER⁺ breast cancer and is now a standard of care in this indication (2). In an estimated 7% of ER⁺ breast cancers, the PI3K pathway is alternatively activated by mutation of *AKT1* (1). In these cases, *AKT1*^{E17K} predominates as the most common (~80%) alteration and constitutively activates PI3K signaling by promoting pathologic localization of AKT1 to the plasma membrane (3–5). Recent clinical studies have demonstrated promising activity of the ATP-competitive pan-AKT kinase inhibitor capivasertib (AZD5363) in *AKT1*^{E17K}-mutant metastatic breast cancer and other cancers (6–8). Importantly, however, the natural history, namely the clinicogenomic characteristics and prognostic significance of the *AKT1*^{E17K} mutation in ER⁺ breast cancer, remains unknown, leading to challenges in contextualizing available single-arm efficacy data.

The rarity of *AKT1*^{E17K} mutations in ER⁺ breast cancer necessitates the use of new approaches to define the prognostic and therapeutic significance of this biomarker. Despite expanding next-generation sequencing programs at many large centers, no single institution has a sufficient number of patients with *AKT1*^{E17K}-mutant ER⁺ breast cancer for a robust analysis. Similarly, although large commercial sequencing laboratories have amassed abundant genomic data, most do not currently possess the requisite demographic, treatment, and clinical outcome data necessary for such an analysis. Consequently, the therapeutic implications of *AKT1* mutations in ER⁺ breast cancer, including whether their presence leads to differential responses to currently approved standard treatments, remain unknown.

In 2015, the American Association for Cancer Research (AACR) launched the Project Genomics Evidence Neoplasia Information Exchange (GENIE), an international genomics registry and data-sharing consortium. The purpose of GENIE is to facilitate the sharing of clinical and genomic data through the academic and biopharma community. In its initial phase, GENIE established standards for the collation and harmonization of variant calls from clinical sequencing tests utilized across an international consortium of academic centers (9). Now in its sixth release, GENIE has made public

Table 1. Patient and disease characteristics

	AKT1-mutant, n = 153, N (%)	AKT1-wild-type, n = 302, N (%)	All, n = 455, N (%)
Age at diagnosis, median (range), years	50 (28–74)	50 (25–83)	50 (25–83)
Race			
White	124 (81%)	227 (75%)	351 (77%)
Black	9 (6%)	18 (6%)	27 (6%)
Other/unknown	20 (13%)	57 (19%)	77 (17%)
Stage at primary diagnosis			
0	0 (0%)	5 (2%)	5 (1%)
1	23 (15%)	51 (17%)	74 (16%)
2	52 (34%)	82 (27%)	134 (30%)
3	37 (24%)	70 (23%)	107 (24%)
4	26 (17%)	77 (26%)	103 (23%)
Unknown	15 (10%)	17 (6%)	32 (7%)
Histologic subtype			
Ductal	106 (69%)	218 (72%)	324 (71%)
Lobular	28 (18%)	52 (17%)	80 (18%)
Other	19 (12%)	32 (11%)	51 (11%)
Overall tumor grade			
1	7 (5%)	20 (7%)	27 (6%)
2	66 (43%)	118 (39%)	184 (40%)
3	43 (28%)	121 (40%)	164 (36%)
Unknown	37 (24%)	43 (14%)	80 (18%)
HER2 status			
Negative	140 (92%)	273 (90%)	413 (91%)
Positive	3 (2%)	6 (2%)	9 (2%)
Unknown	10 (7%)	23 (8%)	33 (7%)
Locoregional recurrence ^a	26 (21%)	48 (21%)	74 (21%)

Abbreviation: HER2, human epidermal growth factor receptor 2.

^aAmong the 352 non-stage 4 patients.

the genomic records from more than 72,000 samples. To more fully leverage the value of this data set, the ability to clinically annotate these samples is essential. As a proof-of-concept pilot study, GENIE conducted an international, multicenter, retrospective and matched study to describe the clinical, pathologic, and genomic features, as well as survival and duration on standard therapies, in patients with *AKT1*^{E17K}-mutant versus *AKT1*-wild-type ER⁺ metastatic breast cancer. A consistent approach to clinical data curation was harmonized across treatment sites and was operationalized using a common Research Electronic Data Capture (REDCap) database.

RESULTS

Patient and Disease Characteristics

A total of 455 patients with ER⁺ metastatic breast cancer were included in this study (Supplementary Fig. S1A); specifically, 302 *AKT1*-wild-type controls were matched to 153 *AKT1*-mutant cases by birth and sequencing year, center, and histologic subtype. The 2 patient cohorts ultimately had very similar baseline disease characteristics, including presenting age, stage, histologic subtype, grade, receptor subtype, and distribution of disease at metastatic presentation (Table 1 and

Supplementary Table S1). Patients were predominantly white and presented with stage 2 to 3 intermediate/high-grade disease. Although clinicopathologic enrichments should be interpreted with relative caution in a case-control study, we observed similar rates of lobular (18% vs. 17%) and ductal (69% vs. 72%) histologies in *AKT1*-mutant and *AKT1*-wild-type cohorts, respectively, interestingly despite prior analyses suggesting *AKT1* mutations predominate in lobular histologies (10). At the time of metastatic diagnosis, both cohorts had comparable rates of multiple disease sites involved. *AKT1*-mutant patients had a marginally higher rate of liver and lymph node metastases (33% vs. 23%, $P < 0.001$; 31% vs. 25%, $P = 0.026$, respectively).

Study groups were also broadly similar for therapy received in the metastatic setting (Table 2 and Supplementary Table S2). The *AKT1*-mutant group had a slightly higher rate of chemotherapy treatment as first-line therapy for metastatic disease (35% vs. 28%, $P = 0.145$). By comparison, the *AKT1*-wild-type group had received more lines of endocrine therapy for metastatic disease (median of 2 lines vs. 1 line, $P = 0.006$). This interesting observation may reflect a phenotypic difference in the study groups that prompted treating clinicians to preferentially use sequential endocrine-based therapy for the *AKT1*-wild-type group. Certainly the *AKT1*-wild-type group

Table 2. Therapy exposure

	AKT1-mutant, n = 153, N (%)	AKT1-wild-type, n = 302, N (%)	All, n = 455, %
Endocrine therapy ^a			
Tamoxifen	96 (63%)	184 (61%)	280 (62%)
Aromatase inhibitor	127 (83%)	266 (88%)	393 (86%)
Fulvestrant	79 (52%)	162 (54%)	241 (53%)
Endocrine therapy sensitivity ^b	106 (69%)	236 (78%)	342 (75%)
Chemotherapy	137 (90%)	269 (89%)	406 (89%)
Endocrine therapies for metastatic disease, median (range) ^c	1 (0-6)	2 (0-9)	2 (0-9)
Chemotherapies for metastatic disease, median (range)	2 (0-7)	2 (0-9)	2 (0-9)
Targeted therapy			
CDK4/6 inhibitor	45 (29%)	87 (29%)	132 (29%)
mTOR inhibitor	49 (32%)	97 (32%)	146 (32%)
AKT inhibitor ^{c,d}	23 (15%)	4 (1%)	27 (6%)
Therapeutic clinical trial	52 (34%)	95 (32%)	147 (32%)

^aAny endocrine therapy in adjuvant or metastatic setting.

^b≥24 months of adjuvant endocrine therapy or ≥6 months treatment duration on any metastatic endocrine therapy.

^cP < 0.05.

^dNCT01226316 was a first-in-human, phase I, multipart study of the AKT inhibitor capivasertib (AZD5363) in advanced solid tumors. This study had many cohorts including individual molecularly selected cohorts for *PIK3CA*-, *AKT1*-, and *PTEN*-mutant tumors. Four *AKT1*-wild-type patients were treated on NCT01226316: 1 *PIK3CA*-mutant and 2 *PTEN*-mutant breast cancers and 1 other patient for reasons unknown.

demonstrated a marginally higher rate of endocrine therapy sensitivity overall (78% vs. 69%, $P = 0.202$). Approximately 30% overall had prior treatment with an mTOR inhibitor, CDK4/6 inhibitor, or an investigational agent on a clinical trial. Expectedly, more *AKT1*-mutant than *AKT1*-wild-type patients received an AKT inhibitor as part of an international basket clinical trial (NCT01226316; 15% vs. 1%, respectively; $P < 0.001$).

The distribution of sequencing time relative to first metastatic diagnosis was 13.4 months (−5.23 to 197.04) in the overall study population, without a significant difference observed between the study groups [9.51 months (−2.04 to 297.04) in *AKT1*-mutant cases versus 14.62 months (−5.23 to 207.73) in *AKT1*-wild-type controls, $P = 0.171$]. Patients were identified by a variety of different sequencing platforms, most commonly using hybridization capture-based (50%–60%) followed by amplicon-based next-generation sequencing (Supplementary Tables S3A and S3B).

Overall Survival

The median duration of follow-up among survivors was 35.4 months (range, 0.2–232.8 months). Approximately 60% of the sequencing tests utilized to identify patients for this study were obtained within 2 years following metastatic diagnosis (Fig. 1A and Table 3). To account for this selection bias, overall survival (OS) estimates were analyzed using left truncation methodology, in which patients enter the risk set based on time of sequencing. After this adjustment, the difference in median OS between the *AKT1*-mutant and *AKT1*-wild-type cohorts was not statistically significant (24.1 vs. 29.9 months, respectively; HR 1.0; 95% CI, 0.74–1.36; $P = 0.98$; Fig. 1B). The uncorrected median OS in the *AKT1*-mutant and *AKT1*-wild-type cohorts,

estimated using the Kaplan–Meier method and ignoring bias, was statistically similar at 64.6 versus 52.3 months, respectively (HR, 1.16; 95% CI, 0.86–1.57; $P = 0.331$; Fig. 1C and Table 3).

Treatment Outcomes

Treatment outcomes across lines and classes of therapy were also analyzed (Table 3). Given the challenges of retrospectively determining date of progression, duration of treatment (DOT) was selected as a surrogate of benefit received. The DOT for first-line endocrine-containing therapy, and for fulvestrant specifically, was longer in the *AKT1*-mutant compared with the *AKT1*-wild-type group, although this difference did not reach statistical significance. DOT for endocrine therapy and chemotherapy was similar for second-line treatment of metastatic disease. In total, 131 patients (44 *AKT1*-mutant and 87 *AKT1*-wild-type) received CDK4/6 inhibitor-containing therapy in any line of therapy, with a median DOT of 5.3 months in both groups. Interestingly, the median DOT for mTOR inhibitor (everolimus)-containing therapy in any line of therapy (49 *AKT1*-mutant and 97 *AKT1*-wild-type) was longer in the *AKT1*-mutant cohort (7.8 vs. 4.5 months, $P = 0.032$). A similar trend was observed for fulvestrant-containing therapy when evaluated in any line of therapy, although the absolute numerical difference here was smaller (5.8 vs. 4.8 months; $P = 0.045$).

Genomic Comparison

Broader genomic data on 428 (126 *AKT1*-mutant and 302 *AKT1*-wild-type) of the total 455 patients were available for analysis (reasons for exclusion are detailed in Supplementary Fig. S1B). To evaluate whether the presence of *AKT1* mutations was associated with other oncogenic alterations, the

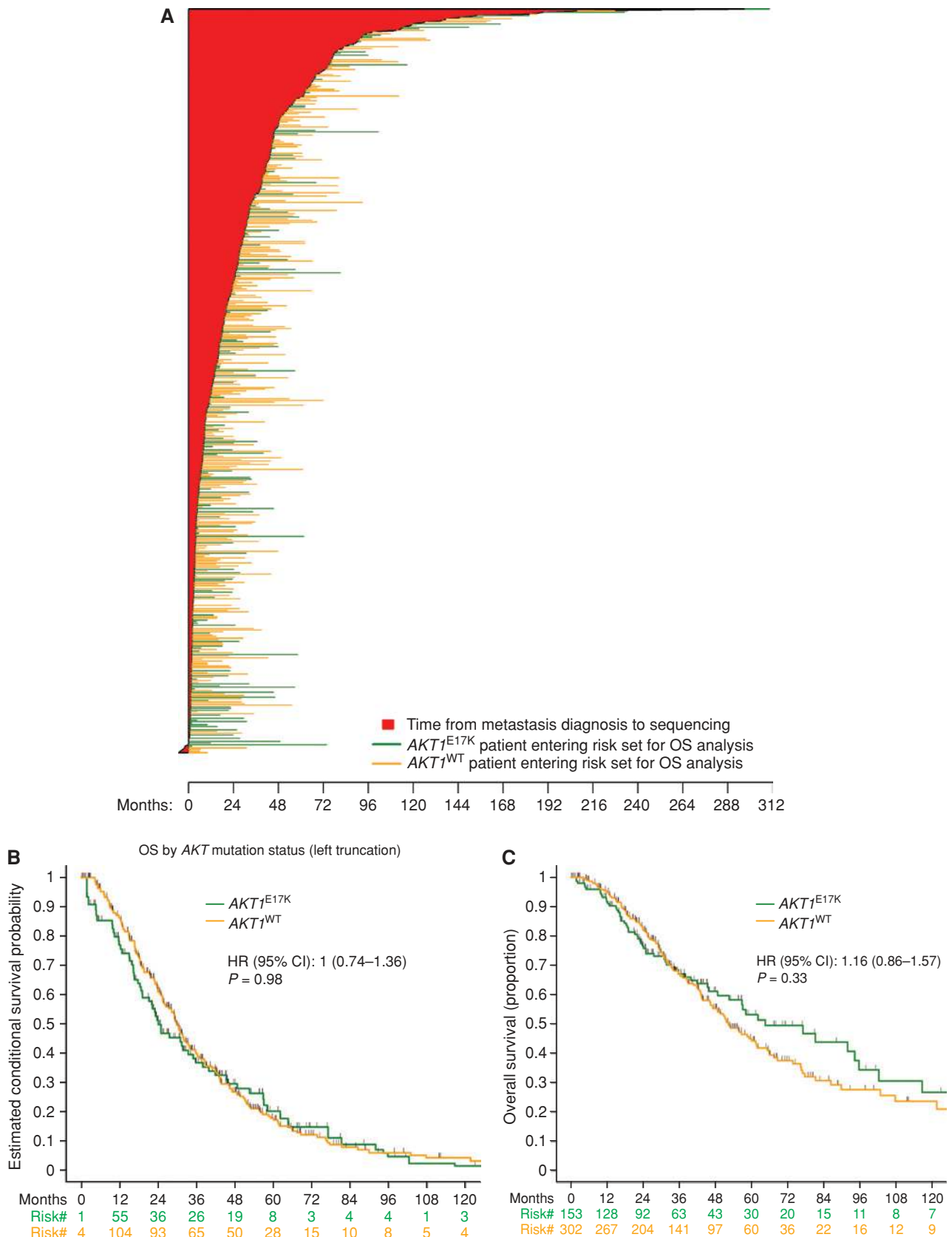


Figure 1. OS by AKT1 mutation status. **A**, Time from metastatic diagnosis to death or last follow-up, as well as tumor sequencing (5 patients underwent tumor sequencing before their metastatic diagnosis). **B**, OS using left truncation. **C**, OS without left truncation. WT, wild-type.

Table 3. OS and treatment outcomes

	AKT1-mutant, n = 153, N (%)	AKT1-wild-type, n = 302, N (%)	P
Unadjusted OS			
Median, months (95% CI)	64.6 (48.8-95.7)	52.3 (46.4-61.6)	0.33
5-year survival rate, % (95% CI)	53 (43-63)	45 (38-52)	
Adjusted OS, ^a months (95% CI)			
Median, months (95% CI)	24.1 (11.4-35)	29.9 (25.4-33.8)	0.97
5-year survival rate, % (95% CI)	20 (11-31)	18 (12-24)	
First line, ^b median DOT, months (95% CI)			
Endocrine therapy (n = 289)	13 (8.2-15.3)	9.4 (7.9-11)	
Fulvestrant (n = 65)	13.2 (3.6-16.2)	9.3 (6-12.9)	
Chemotherapy (n = 135)	3.9 (3-4.6)	4.1 (3.1-4.7)	
Second line, ^b median DOT, months (95% CI)			
Endocrine therapy (n = 219)	6 (5-9)	5.3 (4.1-7.3)	
Fulvestrant (n = 84)	4 (3-8.4)	4.6 (3.7-5.5)	
Chemotherapy (n = 137)	4.2 (2.5-5)	4.1 (3.2-4.8)	
Received in any treatment line after metastatic diagnosis, median DOT, months (95% CI)			
CDK4/6 inhibitor (n = 131)	5.3 (4-6.2)	5.3 (3.4-8.1)	
mTOR inhibitor (n = 145) ^c	7.8 (4.3-9)	4.5 (3.5-5.3)	
Fulvestrant (n = 237) ^c	5.8 (4-8.4)	4.8 (4-5.8)	

Abbreviation: DOT, duration on treatment.

^aApplying left truncation technique; see Methods for details.

^bRefers only to treatment given after metastatic diagnosis.

^cP < 0.05.

total number of variants was compared across cohorts, with no significant difference observed (Fig. 2A). There were a total of 198 patients (64 AKT1-mutant and 134 AKT1-wild-type) with available copy-number alteration (CNA) data. We observed that the fraction of genome altered was significantly lower in AKT1-mutant tumors compared with AKT1-wild-type tumors (median, 19.4% vs. 24.3%, respectively; $P = 0.01$; Fig. 2B). To better understand the basis of this apparent difference, we compared the genome-wide CNA frequencies of AKT1-mutant versus AKT1-wild-type tumors. We found that chromosome 8q, containing the known *MYC* proto-oncogene, was more frequently affected by copy-number gain in AKT1-wild-type compared with AKT1-mutant tumors. Of interest, a locus including *AKT1*, at the very end of chromosome 14 (14q32.33), was more frequently gained in AKT1-mutant versus AKT1-wild-type tumors. We found that other focal regions containing known oncogenes, including *PIK3CA*, *FGFR1*, and *GATA3*, were also more frequently gained in AKT1-wild-type tumors. Finally, two regions containing known tumor suppressors (*ARID1B* and *PTEN*) were more frequently lost in AKT1-wild-type tumors compared with AKT1-mutant tumors (Fig. 2C). To investigate differences at the gene level, we identified 72 recurrent oncogenic alterations, including 39 mutated genes, 23 amplified genes, and 10 genes with homozygous deletion (Supplementary Table S4). We found that *PIK3CA* and *PTEN* mutations were more frequent in AKT1-wild-type patients (39.4% vs. 5.6%, $FDR = 2.5 \times 10^{-12}$ and 6.3% vs. 0%, $FDR = 0.054$, respectively; Fig. 2D and Supplementary Table S4). Notably, only 7.1% of AKT1-mutant tumors had co-occurring alterations in the

PI3K pathway (Supplementary Fig. S2). Next, we interrogated the alteration frequency of 9 canonical oncogenic signaling pathways: cell cycle, Hippo, MYC, Notch, NRF2, RTK-RAS, TGF β signaling, p53, and β -catenin/WNT. The cell-cycle and RTK-RAS pathways were significantly more frequently altered in AKT1-wild-type patients compared with AKT1-mutant patients ($FDR < 0.05$, Fig. 2E).

Intrigued by the AKT1-mutant cohort's comparative benefit from mTOR inhibitor therapy, we went on to evaluate further genomic determinants of mTOR inhibitor benefit by looking at the impact of coincident PI3K pathway mutation/activation (PI3KPW_MT) in 137 patients (41 AKT1-mutant; 96 AKT1-wild-type) with comprehensive genomic data available and who had received an mTOR inhibitor. Interestingly, we found that in a small number of patients (7/41, 17%) with a "double hit" within the pathway, namely, an AKT1 mutation along with another PI3K pathway alteration, median mTOR inhibitor DOT was longest at 13 months (3.2-NA/infinity; Supplementary Table S5 and Supplementary Fig. S3). This thought-provoking observation, although only hypothesis-generating at this point given the small sample size, does echo one recently observed with PI3K inhibitors, where patients with double mutations in the same allele of *PIK3CA* resulting in hyperactivation of PI3K signaling and increased oncogenicity had enhanced sensitivity to therapy targeting PI3K (11). In our analysis, the presence of an AKT1 mutation remained important, however, as patients with PI3K pathway activation in the absence of a coincident AKT1 mutation seemed to fare the same on an mTOR inhibitor as patients without any hit in the pathway.

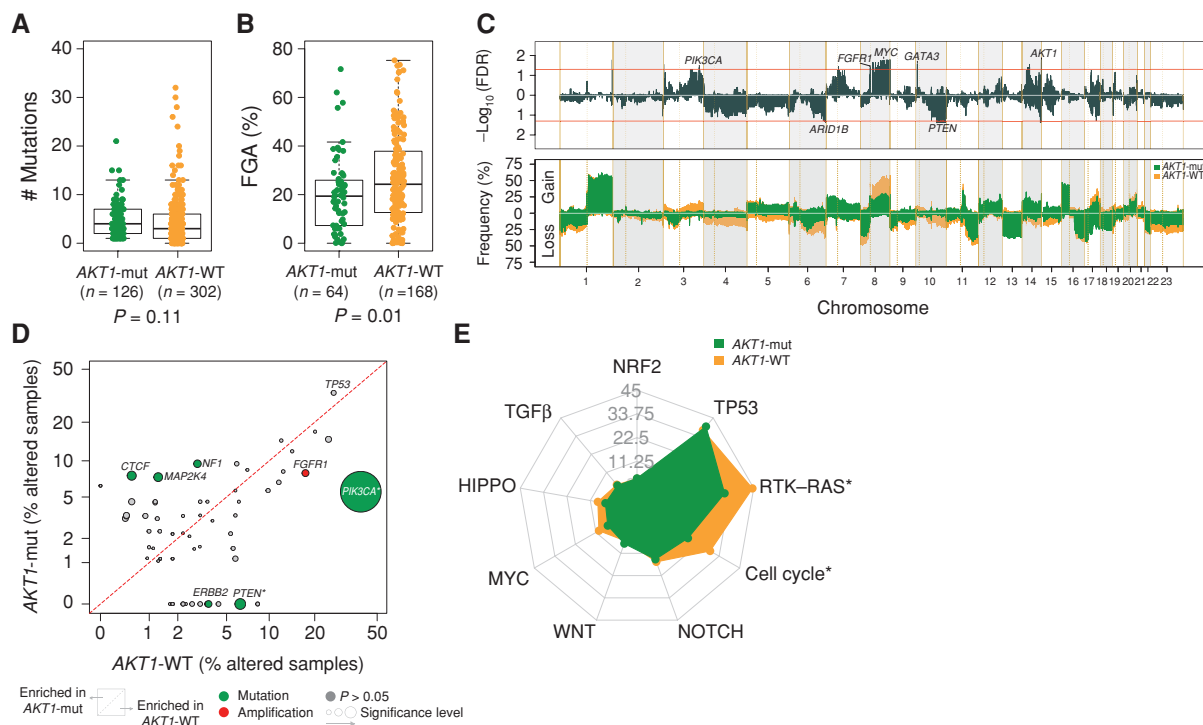


Figure 2. Genomic landscape of breast cancer according to *AKT1* mutations. Comparison of (A) mutation number, (B) fraction of genome altered (FGA), and (C) CNA frequencies in *AKT1*-mutant (mut; green) versus *AKT1*-wild-type (WT; orange). FDR derived from the Fisher exact test. The red lines indicate significance FDR < 0.05. D, Comparison of the alteration frequency for 72 genomic alterations in *AKT1*-mut versus *AKT1*-WT tumors. *, FDR < 0.1. E, Radar plots showing the percentage of patients with alterations in the corresponding canonical oncogenic signaling pathways according to *AKT1* mutation. Statistically significant pathways associated with *AKT1* mutation are indicated with an asterisk. *, FDR < 0.05.

DISCUSSION

Leveraging the AACR Project GENIE framework, we assembled and deeply clinically annotated a large, multi-institutional, international cohort of patients with *AKT1*-mutant ER⁺ metastatic breast cancer and matched controls. In doing so, we found that the initial presentation, outcome to standard therapy, and OS were broadly similar between the groups, with a few notable exceptions. For example, we found that *AKT1*-mutant patients had longer DOT when treated with mTOR inhibitors. Interestingly, limited enrollment of *AKT1*-mutant patients into the BOLERO-2 trial (a phase III, randomized trial that showed improved PFS with the addition of everolimus to exemestane in 724 patients with ER⁺ metastatic breast cancer) precluded analysis of this biomarker within the context of this practice-changing study, demonstrating how even previous pivotal data sets can be underpowered to evaluate the impact of rare genomic alterations (12). Collectively, these data provide important context to the efficacy data generated with AKT inhibitors in *AKT1*-mutant breast cancer while also demonstrating the unique power of an academic-led data-sharing consortium to address emerging needs in drug development.

This analysis has several important limitations. Although we found similar outcomes and survival regardless of *AKT1* mutational status, we recognize that this analysis does not definitely exclude the possibility that such differences may

exist. Although the study cohorts were ultimately well balanced for baseline disease characteristics, we lacked data on ECOG performance and comorbid states, variables that could potentially affect outcome, and therefore we cannot rule out imbalances in these factors among the study groups. Additionally, our chosen study endpoints are limited by the nature of retrospective electronic health records (EHR) interrogation in that, first, survival data can often lag in recency at institutions and, second, although we used duration on therapy as a surrogate for PFS, therapy may have been discontinued for reasons other than cancer progression, such as toxicity. Finally, there is an inherent potential bias associated with patient selection solely from GENIE consortium institutions with access to broad tumor sequencing, testing that at the time was not yet broadly available or standard of care for breast cancer management across the world.

Our experience also demonstrates some of the potential challenges associated with the use of real-world data sets assembled on the basis of prospectively obtained clinical sequencing. Specifically, a meaningful proportion of patients were sequenced long after their metastatic diagnosis. Nonetheless, this delay did balance between cohorts, suggesting that it may not have affected our ability to detect differences in outcome between groups. However, this source of ascertainment bias has an impact on survival estimates, as clearly demonstrated by different median OS estimates obtained by different statistical techniques. Conversely, evolution in

standards of care, such as the recent approval of alpelisib for *PIK3CA*-mutant breast cancer (2), is likely to diminish this source of bias over time, as more patients with metastatic breast cancer undergo tumor sequencing earlier in their metastatic disease course. Appreciation of these and other related issues will be important as we continue to expand our use of real-world evidence to aid in clinical and regulatory decision-making. Indeed, analyses of real-world data sets have already managed to clearly replicate therapeutic associations previously described in prospective randomized clinical trials (13).

Precision medicine in oncology is now largely focused on targeting increasingly rare genomically defined subpopulations (14). By necessity, interventions in these orphan populations are often single-arm, noncomparative studies (15). When this approach yields dramatic efficacy, the need to rigorously define the natural history of these orphan patient populations to existing standards may be viewed as less urgent. However, when the effect size is somewhat more modest but still promising, as is arguably the case with AKT inhibition in *AKT1*-mutant cancers, the successful advancement of novel therapies in these populations will increasingly rely on our ability to define how this population is likely to have fared with existing standards. Thus, providing a (synthetic) control group can be of value in comparative interpretation of these nonrandomized studies. In these situations, the ability to efficiently and seamlessly generate high-quality linked genomic and phenomic data will be increasingly important if opportunities to benefit patients are not to be lost. This approach has been specifically endorsed by global health authorities. One recent demonstration is the approval of palbociclib for male breast cancer using real-world data (16). Finally, like many consortium approaches, data-sharing initiatives such as these require the understanding and collaboration of industry partners and academic investigators alike, recognizing that the eligibility restrictions of traditional clinical trial platforms are unlikely to rigorously answer questions about the clinicogenomic implications of rare genomic events such as *AKT1*.

In conclusion, we demonstrate that AACR Project GENIE can be used to successfully define the natural history of rare genomic subsets such as *AKT1*-mutant breast cancer. Although as a genomic biomarker *AKT1*^{E17K} did not appear to have obvious prognostic implications, it did nonetheless appear predictive of benefit to mTOR inhibitor therapy. Linkage of genomic, therapeutic, and phenomic data enables the discovery of predictive biomarkers with clinical utility in therapy selection. Harmonization of standard ontologies and use of a common curation platform enable inter-institutional collaborations, thereby accelerating the potential for discovery of actionable findings from molecular tumor profiles and more broadly permitting an understanding of the clinical phenotypes associated with alterations in the cancer genome.

METHODS

Patients

Eligible patients had radiologically confirmed distant metastatic (*de novo* or relapsed), hormone receptor–positive, human epidermal growth factor receptor 2 (HER2)–negative breast cancer and had undergone tumor sequencing at one of the AACR GENIE consor-

tium institutions. This consortium included 6 international academic medical centers with large-scale tumor-sequencing initiatives.

Study Design

This was a multicenter, retrospective, and observational matched study of patients with *AKT1*^{E17K}-mutant (*AKT1*-mutant) and *AKT1*^{E17K}-wild-type (*AKT1*-wild-type) ER⁺ metastatic breast cancer, conducted between November 2016 and November 2017.

After Institutional Review Board approval was obtained at each member institution, genomic data linked to core patient-level and specimen-level clinical data were submitted to SAGE Bionetworks on all patients undergoing tumor sequencing at these institutions, through a secure web-based platform (Synapse), as previously described (9). Core clinical data attributes submitted included sex, race, ethnicity, birth year, age at sequencing, primary cancer diagnosis (using the OncoTree cancer type ontology), and sample type (primary/metastatic).

For the purpose of this study, all *AKT1*-mutant cases were first identified at each center. *AKT1*-wild-type controls were then matched in a 2:1 ratio to *AKT1*-mutant cases from within each center. The control population was selected from SAGE data provided by each center, using the Tier 1A variables linked to each patient's genomic record, specifically: year of birth, year of tumor sequencing, and OncoTree code (histology). The control selection process applied frequency matching of birth year (10-year range) and sequencing year (4-year range) and breast cancer OncoTree code (histologic breast cancer subtype), where available. In order to be eligible for complete data curation in this study, both cases and controls first required confirmation of ER⁺, HER2⁻, and metastatic state. If patients were found to be ineligible at this point, no further data were entered. Controls found to be ineligible at this step were replaced by the same selection process until a final complete eligible data set of 2:1, cases:controls, was available for complete data curation at each site.

Detailed clinical annotation was then performed on the identified study population at each center using available EHRs, with study data collected and managed using REDCap electronic data capture tools (17, 18). REDCap is a secure, web-based software platform designed to support data capture for research studies, providing (i) an intuitive interface for validated data capture; (ii) audit trails for tracking data manipulation and export procedures; (iii) automated export procedures for seamless data downloads to common statistical packages; and (iv) procedures for data integration and interoperability with external sources.

In efforts to harmonize data collection across study sites, a data collection guide (Appendix file 1) with directives on EHR interpretation and data entry and data dictionary (Appendix file 2) was created, data abstractor training was provided, and a common REDCap data curation tool (Appendix file 3) was designed and shared across each of the 6 study centers. Data fields in the REDCap database captured information about baseline patient attributes, baseline tumor attributes including stage, histology at primary diagnosis, and metastatic disease characteristics, and included all therapeutic exposures to antineoplastic therapy with start and stop dates.

Information on standard-of-care biomarkers (ER, PR, HER2) was collected at 3 points in the database: the initial eligibility section and the primary and metastatic diagnosis sections, where “positive” for each biomarker was defined as: ER: >1% (IHC); PR: >1% (IHC); HER2: 3+ (IHC)/>2 (FISH ratio). For the 336 patients who had a biopsy at metastatic diagnosis, data were collected on receptor discordance compared with the primary tumor (Supplementary Table S1).

The EHRs (medications lists and provider notes) were interrogated for details on anticancer therapies received and were recorded in 3 different sections according to disease state (primary, locoregionally recurrent, and distant metastatic diagnoses) with each single drug entered separately, even if it was administered as part of a regimen. When drugs formed part of a combination therapy regimen, this

was specifically captured/tagged as such within the database and considered, at the time of analysis, as one line of therapy. If the exact/complete start/stop date of a therapy was not known, guidance was provided to estimate the date, using known data components.

Survival data were derived from institutional EHR data, which in some centers were linked to the National Death Index. Patient records were curated from date of diagnosis to death and were censored at their last known follow-up at the study site, if alive.

An independent data quality review/monitoring plan (Appendix file 4) was used at each site, whereby source data verification (SDV) was required on all study patients for core eligibility data (ER, HER2, *AKT1*, and metastatic status and tumor histology). Complete SDV was then additionally required on all first patients entered per data abstractor at each site and on a minimum of 10% of patients at each site chosen at random. All data queries were resolved internally with local principal investigators (PI) and, where required, escalated to the lead PI for review. Documentation relating to data-quality reviews was tracked and kept on file at the local site.

Statistical Considerations

The data cutoff for this report was August 2018, when the database was locked to further data updates. Patients were excluded from the final analysis data set if insufficient core clinical data were available. Analyses were specified in a statistical analysis plan. The number of lines of endocrine therapy/chemotherapy was determined by considering only each specific class of therapy and counting each new agent received by the patient as a line of therapy in that class. Endocrine therapy received as part of a combination regimen with, for example, a CDK4/6 or mTOR inhibitor or ovarian function suppression was considered as one line of therapy. Descriptive statistics, including medians and ranges, were calculated. All statistical analyses and all plots were performed and generated using R 3.5.1. Associations between variables and *AKT1* status were analyzed using the Wilcoxon rank-sum test for continuous variables and χ^2 test/Fisher exact test (for count <5) for categorical variables. The primary endpoint, OS, was calculated from the date of (radiologically determined) distant metastatic diagnosis to the date of death. Patients still alive were censored at last follow-up date. To account for the method of sample selection (selection bias), i.e., the fact that patients must have survived long enough to undergo tumor sequencing, Kaplan-Meier method with left truncation was used to estimate the median survival and 5-year survival rate. The Cox proportional hazards model with left truncation at the date of the sequencing report was applied to obtain the *P* value (19). Additionally, all *AKT1*-mutant patients enrolled on the international clinical trial (NCT01226316) with the AKT inhibitor AZD5363 were censored at the time of starting this therapy. The secondary endpoint, DOT, was analyzed as a time-to-event variable and was calculated from the start to stop date of therapy or death, with patients remaining on therapy censored at the last follow-up date. In the DOT analyses, we did not censor patients at AZD5363 start date (i.e., they were considered events). DOT was analyzed using the Kaplan-Meier method, and comparisons between the various groups were obtained using the log-rank test. For the DOT analyses, the definition of an event was met by the therapy stop date or if this date was missing in a patient who died within 3 months of starting this therapy. Patients with a missing therapy stop date who died beyond 3 months of starting therapy were censored at 3 months from therapy start. Similarly, patients with a missing therapy stop date who were lost to follow-up were censored at 3 months from therapy start or the patient's last known follow-up date, whichever came first. All statistical endpoints were compared across the *AKT1*-mutant and *AKT1*-wild-type patient cohorts. Reported *P* values were two-tailed, and differences were considered significant when the *P* was less than 0.05.

Notably, the power in this study is limited by the rarity of this alteration and the small event rate (deaths = 205) occurring in the

study population during the observed study period. Nonetheless, all *AKT1*-mutant breast cancers identified at each consortium institution between 1990 and 2017 were included and the *AKT1*-wild-type control group was then doubled to decrease the variance in that group.

Genomic Analyses

OncoKB was used to define oncogenic driver variants (20). For the alteration level analysis, recurrent oncogenic alterations were defined as oncogenic as per OncoKB annotation and present in at least 1% in the whole cohort. Segmented copy-number data were processed using CNtools package v1.4. The canonical oncogenic pathway level alterations were computed using the curated pathway templates described by Sanchez-Vega and colleagues (21). All genomic analyses were performed using Rv3.5.2 (<https://www.R-project.org>) and Bioconductor version 3.4.

Data Availability

All patient-level clinical outcome and genomic data are available on cBioPortal.org (http://genie.cbioportal.org/study?id=brca_akt1_genie_2019).

Disclosure of Potential Conflicts of Interest

L.M. Smyth is consultant for Roche Genentech, Pfizer, and Novartis, reports receiving commercial research grants from AstraZeneca, Puma Biotechnology Inc., and Roche Genentech, and has received other remuneration from AstraZeneca, Roche Genentech, Pfizer, and Puma Biotechnology Inc. M. Arnedos reports receiving commercial research support from Lilly and has received honoraria from the speakers bureaus of Novartis, AstraZeneca, and Seattle Genetics. C.M. Micheel is a consultant at Roche and reports receiving a commercial research grant from GenomOncology. S.M. Sweeney is director of the AACR Project GENIE Coordinating Center at the AACR and reports receiving commercial research grants from Amgen, Inc., Analysis Group, Novartis, AstraZeneca UK Limited, Bayer Healthcare Pharmaceuticals, Inc., Boehringer Ingelheim, Bristol-Myers Squibb Company, Genentech, Janssen Pharmaceuticals, Inc., H3 Biomedicine, and Merck Sharp & Dohme Corp. J. Lee reports receiving commercial research grants from Amgen, Inc., Bristol-Myers Squibb Company, Janssen Pharmaceuticals, Inc., Analysis Group, Merck Sharp & Dohme Corp., AstraZeneca UK Limited, Genentech, Novartis, Bayer Healthcare Pharmaceuticals, Inc., H3 Biomedicine, Puma Biotechnology, and Boehringer Ingelheim. S. Sheffler-Collins is a project manager at the AACR and reports receiving commercial research grants from Amgen Inc., Bristol-Myers Squibb Company, Janssen Pharmaceuticals, Inc., Analysis Group, Merck Sharp & Dohme Corp., AstraZeneca UK Limited, Genentech, Novartis, Bayer Healthcare Pharmaceuticals, Inc., H3 Biomedicine, Puma Biotechnology, and Boehringer Ingelheim. B.H. Park is an SAB member/consultant for Loxo Oncology and Celcuity and a consultant for Jackson Laboratories, Casdin Capital, H3 Biomedicine, Roche, and AstraZeneca, reports receiving commercial research grants from Foundation Medicine, Pfizer, and AbbVie, has ownership interest (including patents) in Loxo Oncology and Celcuity, and is a consultant/advisory board member for Tempus. C.L. Sawyers is on the board of directors of Novartis, is a scientific advisory board member for Agios, Nextech, Foghorn, Arsenal, Beigene, Blueprint, KSO, PMV, Oric, Petra, Housey, and Column Group, has ownership interest (including patents) as inventor of enzalutamide and apalutamide, and is a science trustee for Cold Spring Harbor Laboratories. F. André reports receiving commercial research grants (to institution) from AstraZeneca, Novartis, Pfizer, Lilly, and Roche. F. Meric-Bernstam is Chair at MD Anderson Cancer Center, reports receiving a commercial research grant from AstraZeneca, and has received honoraria from the speakers bureaus of Novartis and Genentech. P.L. Bedard reports

receiving commercial research grants from AstraZeneca, Novartis, PTC Therapeutics, Signalchem Life Sciences, Servier, Sanofi, Lilly, BMS, GSK, Seattle Genetics, Pfizer, Merck, Genentech/Roche, Mersana, and Nektar and is a consultant/advisory board member for BMS and Sanofi. A. Iasonos is a consultant for Intellegentica and is a consultant/advisory board member for Mylan and Brightpath. D. Schrag is Editor of JAMA and has received honoraria from the speakers bureau of Pfizer. D.M. Hyman is a scientific advisory board member for Lilly and Fount and a consultant for Chugai, Boehringer Ingelheim, AstraZeneca, Pfizer, Bayer, and Genentech, and reports receiving commercial research grants from Bayer, AstraZeneca, and Puma Biotechnology. The AACR Project GENIE Consortium reports receiving commercial research grants from Amgen, Inc., Bristol-Myers Squibb Company, Merck Sharp & Dohme Corp., AstraZeneca UK Limited, Genentech, Novartis, H3 Biomedicine, Boehringer Ingelheim, Janssen Pharmaceuticals, Inc., and Analysis Group. No potential conflicts of interest were disclosed by the other authors.

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