Original Study

Aspirin Treatment Effect and Association with PIK3CA Mutation in Breast Cancer: A Biomarker Analysis

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Abstract

Aspirin's treatment effect in breast cancer (BRCA) is not clear. We retrospectively studied 1227 patients with BRCA who used aspirin or did not. We found regular high-dose (325 mg) aspirin use after diagnosis may confer better treatment benefit. Also, PIK3CA may serve as a biomarker for aspirin treatment in patients with BRCA but requires further assessment in future studies.

Background: Studies suggest regular aspirin use decreases breast cancer (BRCA) risk, with high doses exerting an "anti-cancer" effect. Despite reports suggesting aspirin's protective role in BRCA, no findings on aspirin dose association(s) with treatment outcomes have been reported, nor have any molecular subtype associations by which aspirin influences outcomes been elucidated. To interrogate aspirin's effect and determine which populations may benefit from its use, we retrospectively explored data from 1227 patients with BRCA. In this population, 32 used high-dose aspirin (325 mg), 121 used low-dose aspirin (81 mg), and 1074 used no aspirin before and/or after diagnosis. **Patients and Methods:** Several association tests were performed to examine the correlations of clinical variables and PIK3CA mutations from 45 patients with BRCA who used 81 mg of aspirin daily. Kaplan-Meyer survival curves and the log-rank test were utilized to compare survival outcome differences for aspirin dose, usage history, and PIK3CA mutation status. Cox proportional hazards models were used to compute the multivariate hazard ratio (HR) for death. **Results:** Patients who regularly used high-dose aspirin (325 mg) had better survival outcomes than those who used low-dose aspirin (81 mg) (HR, 0.094; 95% confidence interval [CI], 0.014-0.62; $P = .014$). Patients who used aspirin post-diagnosis only achieved significant benefits in overall survival (HR, 0.082; 95% CI, 0.023-0.3; $P = 1.39E-04$). Also, a subgroup of patients in the low-dose, long-term aspirin group with a PIK3CA mutation showed a small beneficial effect (HR, 0.37; 95% CI, 0.04-3.25; $P = .37$). **Conclusion:** High-dose aspirin after diagnosis may confer BRCA treatment benefits. Future studies should assess the comprehensive mechanism of aspirin for the PIK3CA mutant subgroup in a large study.

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Introduction

Multiple studies suggest that using aspirin regularly is linked to reducing the risk of cancer development,[1,2] metastatic adenocarcinoma,[3] and mortality for various cancer types,[4] including breast cancer (BRCA).[5-7] In fact, a meta-analysis of 33 studies involving 1,916,448 patients found a reduced BRCA risk among aspirin users.[8] Although reports suggest aspirin has a protective role in BRCA prevention,[9] additional research must determine evidence-based standards for using aspirin during treatment. First, no reports have addressed influences on aspirin dose and treatment effects. Second, differences in aspirin's effects that may arise owing to patients' history of aspirin use before and after diagnosis

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have yet to be reported. Third, the potential molecular mechanisms by which aspirin may inhibit BRCA progression and treatment response have not yet been clarified.

The PIK3CA mutation predicts a beneficial effect for nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with colorectal cancer, having pathways potentially involving platelets, inflammation, cyclooxygenase 2, hormones, or PI3 kinase.[10] Liao et al showed that regularly using aspirin 325 mg was associated with better colorectal cancer-specific survival and overall survival in patients whose tumors were PIK3CA-mutated. Similar benefits were not seen in patients whose tumors were wild-type PIK3CA.[11] Although Henry WS et al reported that aspirin suppresses cell growth in PIK3CA-mutant BRCA,[12] no current reports show the clinical benefits of aspirin in patients with BRCA with PIK3CA mutations.[13]

To address the above limitations, we analyzed the effects of aspirin dose and aspirin usage history on the survival of 1227 patients with BRCA treated at the Augusta University Cancer Center. We also examined the prognostic value of PIK3CA mutation status from formalin-fixed, paraffin-embedded (FFPE) tissues of 45 patients who had consistently used low-dose aspirin (81 mg) daily. Our purpose was to provide proof-of-concept justification for a larger study that could ultimately lead to the modification of current BRCA treatment practices.

Materials and Methods

Data Description and Study Design

The current study was performed according to a protocol approved by the Augusta University of Science and Technology Institutional Ethics Committee. A flowchart for the study design is summarized in Supplemental Figure 1 (in the online version), and the description of Tables and Figures is summarized in Supplemental Table 1 (in the online version).

A cohort of 1227 patients with BRCA, treated from 2002 to 2013 at the Augusta University Cancer Center, was included in our retrospective analysis. Patients included those who were 81-mg daily aspirin users (n = 121), 325-mg daily users (n = 32), and those reporting no aspirin usage (n = 1074). Participants were excluded if distant metastases were observed at diagnosis, if they were male patients with BRCA, had a history of other malignancies, and/or had severe deficiencies in clinical and follow-up data. All patients provided written informed consent.

Target Sequencing of PI3K in Human BRCA Panel

From our cohort of patients with BRCA who had taken 81 mg of aspirin daily, we selected FFPE tissues from 45 patients who had taken aspirin prior to diagnosis for target sequencing. Data collection, tissue sample handling, and data analysis were carried out as previously described[14]; however, gene sequencing was completed at the Augusta University Cancer Center Shared Core Facility. DNA samples were extracted from FFPE tissues and analyzed using a GeneRead DNA FFPE kit (Qiagen), and were sequenced on a Miseq sequencer (Illumina).

Statistical Analysis

To assess associations with categorical variables, either the χ^2 or Fisher's exact tests were performed. To assess associations with continuous variables, *t* tests or analysis of variance tests were performed. All *P*-values were 2-sided.

Survival outcome differences for aspirin dose and usage history effects were compared by using a log-rank test; survival curves were estimated using the Kaplan-Meier product-limit method.[15] The *P*-value from the log-rank test to compare group-difference was displayed in each survival figure respectively. To adjust for clinical confounders such as age, grade, and stage, multivariate Cox proportional hazards models[16] were used to determine the hazard ratio (HR) for death according to aspirin use. Starting points post-diagnosis were calculated from the date of the patient's first aspirin dose during treatment to either the date of the most recent follow-up contact or death.

Assessment of Aspirin Use and PIK3CA Mutations

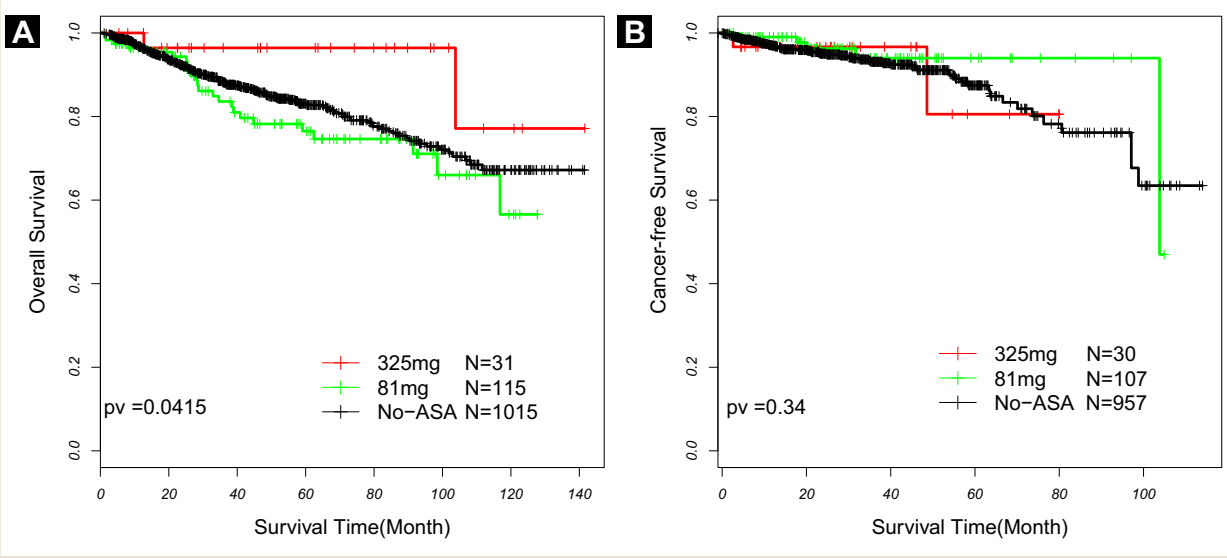
To better utilize our limited resources, we focused on PIK3CA sequencing only in patients who were taking aspirin in our cohort and compared their overall survival with patients from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort, who were not treated with aspirin. The METABRIC dataset contains around 2000 genomic/transcriptomic profiles of BRCAs with long-term clinical follow-up data.[17,18] To investigate the association between aspirin treatment and PIK3CA mutation status (mutant or wild-type) on survival, we used METABRIC data only as an external historical control (reference).

To reduce selection bias for the current cohort, we used the propensity score matching method,[19] matching patients with an aspirin treatment to the control group at a 1:2 ratio. We performed nearest neighbor matching via R package MatchIt[20] to model the probability of each patient receiving the aspirin treatment, and then used predicted "probabilities" to balance the sample for clinical treatment confounders. The "probability" or "propensity" scores of each patient were estimated by logistic regression and adjusted by clinical variables, including age, race, histology, grade, stage, estrogen receptor (ER)/progesterone receptor (PR)/human epidermal growth factor receptor 2 (HER2) status, and treatment. The use of a linear combination of covariates to obtain a single score attempts to mimic randomization by balancing a group of patients who used aspirin with patients who did not. To test the main hypothesis on the prognostic effect of aspirin use in the presence of PIK3CA mutation status, we interpreted results cautiously, given the nature of subgroup analyses.

Results

Comparisons between low and high-dose aspirin were made using a mixture of patients whose first aspirin consumption was both before and after BRCA diagnosis (Figure 1). Then, a more in-depth comparison between aspirin use before and after BRCA diagnosis versus after diagnosis only was conducted, using a mixture of patients taking both 325-mg and 81-mg aspirin (Figure 2). Furthermore, the comparisons between PIK3CA mutate and wild-type status for non-aspirin and 81-mg (low-dose aspirin) treatment groups were performed in the patients with hormone receptor-positive (HR^+)/HER2-negative ($HER2^-$) subtype (Figure 3). Additional comparisons include overall survival analyses of low-dose (81 mg) aspirin use after diagnosis versus non-aspirin use (see Supplemental Figure 2A in the online version) and aspirin use after diagnosis versus long-term (see Supplemental Figure 2B in the

Figure 1 Aspirin Use Dose-effect Comparisons. Aspirin Use Dose Effect Comparison by Log-rank Test for Overall Survival ($P = .0415$) (A) and Cancer-free Survival ($P = .34$) (B). Patients Who Used High-dose Aspirin Daily Showed a Better Overall Survival than Those Who Used Low-dose Aspirin Daily. Cancer-free Survival did Not Show Statistical Significance, But It May be Owing to Short Follow-Up Time. These Dose Effect Comparisons Are From a Mixture of Patients Whose First Aspirin Consumption was Both before and after Diagnosis



Abbreviation: ASA = aspirin.

Figure 2 Survival Using Aspirin After Breast Cancer Diagnosis Versus Before and After Diagnosis. Comparison by Log-rank Test for Patients Who Used Aspirin Before and After Breast Cancer Diagnosis Versus Patients Who Used Aspirin Only After Diagnosis: Overall Survival ($P = .0176$) (A) and Cancer-free Survival ($P = .272$) (B). Results Show Patients Who Used Aspirin Long-term Had a Poor Overall Survival and Cancer-free Survival. These Aspirin Use History Comparisons Are From a Mixture of Patients Who Took High- (325 mg) and Low- (81 mg) Dose Aspirin

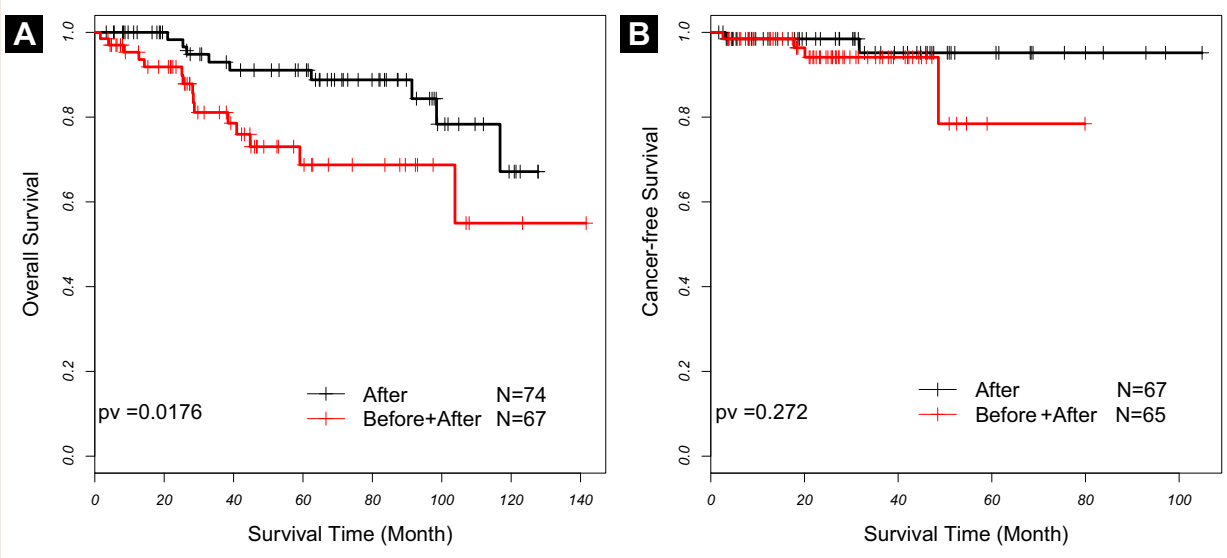
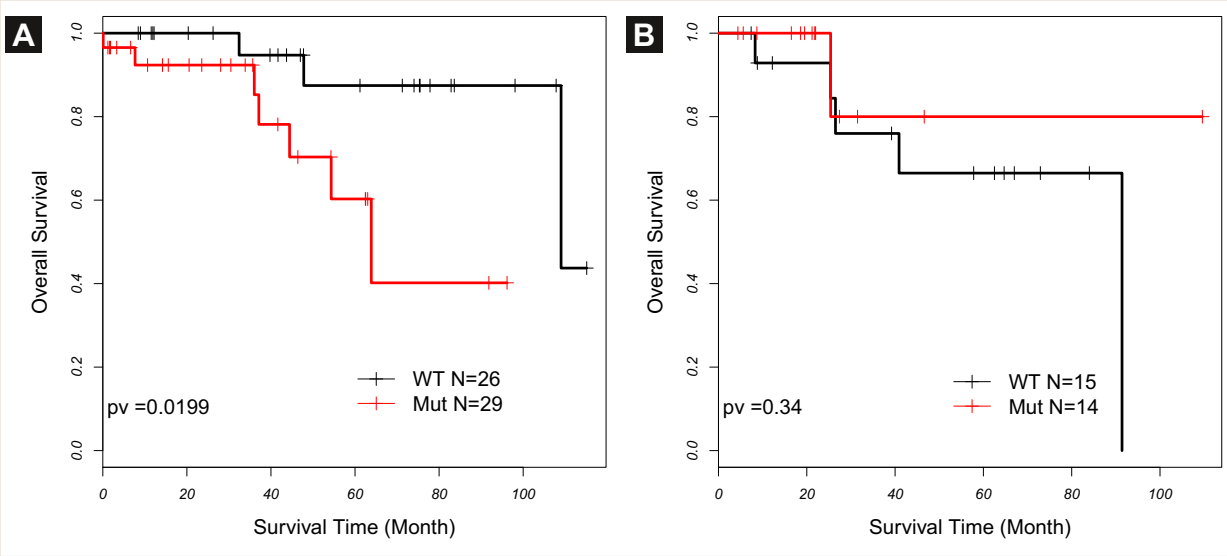


Figure 3 Overall Survival Comparison for PIK3CA Mutation Status in HR$^+$/HER2$^-$ BRCA Subtype. Overall Survival Comparison for HR$^+$/HER2$^-$ Patients With PIK3CA-Mutated and Wild-type Tumor in: Non-Aspirin Treatment Group (P = .0199) (A) and 81 mg Daily Aspirin Treatment Group (P = .34) (B) by Log-rank test



Abbreviations: HER2$^-$ = Human epidermal growth factor receptor 2-negative; HR$^+$ = hormone receptor-positive; MUT = mutated; WT = wild-type.

online version) respectively, as well as overall survival by PIK3CA mutation status regardless of BRCA subtypes (see Supplemental Figure 3 in the online version).

Patient Characteristics

Baseline characteristics were obtained for all 1227 patients with BRCA enrolled in the Augusta University Cancer Center, including both aspirin users and non-aspirin users. Supplemental Table 2 (in the online version) displays this information. Table 1 provides baseline characteristics for the 45 patients who used 81 mg of aspirin, including their PIK3CA mutation status. Among 45 patients, 3 of them were lost to follow-up and removed from survival analysis (see Supplemental Figure 4 in the online version). At an overall median follow-up of 27 months (interquartile range, 11.5-55 months) and a cancer-free median follow-up of 21 months (interquartile range, 8.8-35.1 months), patients with a mutated-PIK3CA had fewer than 5 deaths. Therefore, we also used the Fisher's exact test to examine associations between PIK3CA mutation statuses with survival outcomes, as log-rank test or Cox regression analysis may not yield a robust statistical analysis for small sample sizes.

Characteristics of 90 matched non-aspirin users from the METABRIC[17] cohort, along with their tumor PIK3CA mutation data (presence or absence), are summarized in Supplemental Table 3 (in the online version). The standardized absolute mean differences for the majority of covariates are less than 0.1 after matching in the METABRIC, which means the covariates are balanced for comparison in 2 cohorts (see Supplemental Figure 5 in the online version). The proportion of mutated-PIK3CA tumors in our study cohort was similar to that reported for the METABRIC cohort for both aspirin and non-aspirin users. As shown in Table 1, our cohort's proportion of mutated-PIK3CA tumors was 44.4% (20 of 45 patients) among patients who used aspirin; it was 43.2% (37 of 90 patients) for METABRIC cohort patients who did not use aspirin.

Associations of Aspirin Use and Survival According to Different Daily Doses

Survival analysis for patients with BRCA enrolled in our study confirmed previously published findings, showing a beneficial effect with the daily use of 325 mg of aspirin. Figure 1 shows the overall survival (Figure 1A) and cancer-free survival (Figure 1B) for patients using or not using aspirin. Our results revealed that high-dose aspirin usage (325 mg daily) led to marginally improved benefits than did daily, low-dose 81 mg of aspirin (HR, 0.26; 95% CI, 0.06-1.10; P = .066) in overall survival. Findings from a multivariate Cox regression model showed a near marginal trend toward significance (HR, 0.30; 95% CI, 0.06-1.61; P = .16), after adjusting for confounders such as age, race, tumor stage, histology, grade, ER/PR/HER2 status, and treatment types. Consistent with observations of an aspirin dose effect in the treatment of colorectal cancer,[21,22] this trend suggests that a high daily dose of aspirin can exert an anti-cancer effect in combination with standard BRCA treatment regimes. Of note, cancer-free survival did not show statistical significance in our evaluation (P = .34, log-rank test). This may be owing to short follow-up time, as the Kaplan-Meier curve trend suggests a potential protective role for aspirin use in BRCA.

Table 1 Baseline Characteristics of 45 Patients With Breast Cancer with 81-mg Daily Use of Aspirin by PIK3CA Mutation Status

	PIK3CA Mutant (n = 20), n (%)	PIK3CA WT (n = 25), n (%)	Odds Ratio (95% CI)	*P* Value
Mean age, y (SD)	64.15 (11.63)	65.88 (12.75)		
Race				.77
White	10 (50)	14 (56)	0.79 (0.20-2.99)	
Black	10 (50)	11 (44)		
IHC Subtypes				.18
$HR^+/HER2^-$	18 (90)	16 (64)		
$HR^+/HER2^+$	1 (5)	3 (12)		
$ER^-, PR^-, HER2^+$	1 (5)	2 (8)		
$ER^-, PR^-, HER2^-$ (TNBC)	0	4 (16)		
Grade				.99
1	6 (30)	7 (28)		
2	6 (30)	7 (28)		
3	8 (40)	10 (40)		
Tumor Stage				.22
T1	7 (35)	8 (32)		
T2	8 (40)	14 (56)		
T3	2 (10)	3 (12)		
T4	3 (15)	0 (0)		
Histology Type				.62
IDC	14 (70)	19 (76)		
ILC	1 (5)	3 (12)		
MC	2 (10)	1 (4)		
Mixed	3 (15)	2 (8)		
Treatment Types, Yes				
Chemotherapy	7 (35)	11 (44)	1.44 (0.37-5.89)	.76
Radiotherapy	9 (45)	5 (20)	0.31 (0.06-1.36)	.11
Hormonal therapy	14 (70)	13 (52)	0.47 (0.11-1.86)	.36
Aspirin Before/After Diagnosis			0.43 (0.11-1.65)	.23
After	7 (35)	14 (56)		
Before and after	13 (65)	11 (44)		
Median survival time, mos	19.5	40.9		
Vital Status			0.53 (0.099-2.51)	.5
Death	4 (20)	8 (32)		
Alive	16 (80)	17 (68)		
Median cancer-free time, mos	20.4	27.4		
Cancer Status			5.54 (0.49-294.7)	.16
Yes	4 (20)	1 (4)		
No	16 (80)	23 (96)		

The Fisher's exact or analysis of variance tests were used for group difference comparisons.
Abbreviations: CI = confidence interval; ER = estrogen receptor; IDC = invasive ductal carcinoma; IHC = immunohistochemistry; ILC = invasive lobular carcinoma; MC = medullary carcinoma; PR = progesterone receptor; TNBC = triple-negative breast cancer; WT = wild-type.

Treatment Effect Comparison of Aspirin Use Before and After Diagnosis

Figure 2A shows that patients who used both high and low-dose aspirin before and after cancer diagnosis experienced a significantly poor overall survival, in comparison to those who used aspirin post-diagnosis (HR, 2.66; 95% CI, 1.15-6.14; $P = .02$). Cancer-free survival shows a similar trend in Figure 2B, although the *P*-value is not statistically significant (HR, 2.54; 95% CI, 0.45-14.3; $P = .27$). After multivariate adjustment for clinical variables, including age, race, grade, stage, histology, ER/PR/HER2 status, and treatment types, the HR for overall survival of patients using aspirin before and after their BRCA diagnosis, as compared with those who used aspirin only afterward, was 12.2 with a 95% CI of 3.37-54.8 and a *P*-value = 1.39E-04. Multivariate analysis also showed that high-dose aspirin use is significantly associated with better overall survival than low-dose (HR, 0.094; 95% CI, 0.014-0.62; $P =$

Table 2 Multivariate Cox Regression Analysis Adjusted for Other Clinical Variables

	No. Patients	Hazard Ratio (95% CI)	*P* Value
Aspirin Before/After Diagnosis			
After	74	Reference	
Before and after	74	12.2 (3.37-54.8)	1.39E-04***
Aspirin Dose, mg			
81	121	Reference	
325	32	0.095 (0.014-0.62)	.014*
Age, y	153	1.10 (1.03-1.19)	5.91E-03**
Race			
White	72	Reference	.236
Black	80	1.93 (0.65-5.09)	
Grade			
1	42	Reference	
2	40	0.25 (0.06-0.81)	.0506
3	63	0.17 (0.03-0.76)	.0378*
4	8	6.81E-06 (2.13E-62-2.18E+51)	.858
Stage			
T0	28	Reference	
T1	55	4.26E+03 (1.97E-45-9.18E+51)	.883
T2	43	4.47E+03 (2.074E-45-9.65E+51)	.882
T3	23	7.58E+03 (3.51E-45-1.64E+52)	.875
T4	4	1.27E+06 (5.68E-43-2.85E+54)	.805
ER			
Negative	37	Reference	
Positive	116	8.46E-02 (6.44E-03-1.11)	.060
PR			
Negative	41	Reference	
Positive	112	0.66 (6.55E-02-6.55)	.719
HER2			
Negative	131	Reference	
Positive	22	1.20 (0.26-5.54)	.819
Chemotherapy			
No	99	Reference	
Yes	54	0.21 (3.42-122.4)	9.42E-04**
Radiotherapy			
No	80	Reference	
Yes	73	0.47 (0.14-1.54)	.210
Hormonal Therapy			
No	74	Reference	
Yes	79	6.78 (0.97-47.3)	.053

Results show that patients will not benefit (HR, 12.2; $P = 1.39E-04$) from aspirin prescription during the cancer treatment if they used aspirin before.
Abbreviation: CI = confidence interval.
*$P \leq .05$, **$P \leq .01$, ***$P \leq .001$.

.014). Also, there is no interaction between aspirin dose and use history as shown in Supplemental Tables 4A and 4B (in the online version). Table 2 displays a full description of this analysis, confirming previous findings that high-dose aspirin provides a better treatment effect.

In addition, multivariate Cox regression analyses was performed to compare the survival difference between aspirin use before and after diagnosis in the $HR^+/HER2^-$ subtype group adjusted by other confounders including age, race, grade, stage, histology, and treatment types. We also found a poor prognosis for patients continuously using aspirin before and after diagnosis (HR, 12.9; 95% CI, 1.97-84.3; $P = 7.6E-03$) when compared with patients using aspirin only after diagnosis in the $HR^+/HER2^-$ subtype. Our study suggests there may be a beneficial effect of aspirin use after BRCA diagnosis; this interpretation will require validation in future studies.

To further explore the treatment benefit observed for patients who took low-dose aspirin only after diagnosis, we compared this group of patients with those who never took aspirin. Using a 1:2 ratio, we conducted a propensity score matching for treated and untreated patients. The final group of matched patients included 62 patients treated with an 81-mg daily aspirin dose compared with 124 patients receiving no aspirin treatments. Survival analyses indicated a trend for better survival among patients using aspirin versus those who did not (HR, 0.646; 95% CI, 0.30-1.40; $P = .267$) (see Supplemental Figure 2A in the online version). Of interest, survival analyses also indicated that low-dose aspirin use after diagnosis carries a significantly better prognosis (HR, 0.317; 95% CI, 0.13-0.78; $P = .012$) than continuous use before and after diagnosis, as shown in Supplemental Figure 2B (in the online version).

For 12 of the 32 patients taking high-dose (325 mg) aspirin after diagnosis, no detrimental effect was observed ($P = .26$; Fisher's exact test) as shown in Supplemental Table 4C (in the online version). Statistical power is limited in this study, owing to the small sample size for the 325-mg group and the relatively short follow-up time (31.5 month median follow-up time) post-diagnosis.

Aspirin Use and Survival According to PIK3CA Mutation Status

No significant difference in overall survival was observed for patients treated with low-dose aspirin in the mutant PIK3CA and wild-type PIK3CA groups as a whole, as shown in Supplemental Figure 3 (in the online version). However, Figure 3A shows that patients with the $HR^+/HER2^-$ subtype with PIK3CA mutation were associated with poor overall survival in the aspirin non-use group (HR, 5.48; 95% CI, 1.11-26.95; $P = .037$). Either estrogen receptor-positive (ER^+) or progesterone receptor-positive (PR^+) tumors are defined as HR^+, which are responsive to hormonal therapy. Figure 3B shows the overall survival for $HR^+/HER2^-$ patients with a PIK3CA mutation was slightly improved for the aspirin use group, though not significantly, in comparison to the PIK3CA wild-type group (HR, 0.37; 95% CI, 0.04-3.25; $P = .37$).

Discussion

Aspirin is among the most widely used NSAIDs for pain treatment,[23] fever, and inflammation, and is used primarily to prevent heart disease and stroke. Recent epidemiologic studies indicated that regular high- or low-dose aspirin use is associated with a reduced risk for various cancers, including colon, breast, and other cancers.[4,6,21,22,24] Studies have suggested that aspirin may mediate PIK3CA mutation activities in patients with colon cancer; however, its effects are unknown in patients with BRCA.

An in vitro study shows that aspirin suppresses growth in PIK3CA-mutant BRCA by activating AMPK while inhibiting mTORC1 signaling.[12] Neither aspirin's potential benefit for patients with a PIK3CA mutation nor the daily dosage needed to maximize treatment effects has been reported for patients with BRCA. Our study sought to identify populations with BRCA that may benefit from aspirin use at optimal doses and molecular interactions, to improve the overall survival of patients.

In patients with a PIK3CA mutation taking 81 mg of aspirin, we observed no improvements that could provide useful prognostication in this sampled population of patients with BRCA. However, we observed a slight association between low-dose aspirin and improved survival among patients with the $HR^+/HER2^-$ subtype with PIK3CA-mutated tumors. Our results, which confirm recently reported findings that low-dose aspirin is only beneficial for the $HR^+/HER2^-$ subpopulations,[24] suggest that this benefit accrues within a molecular subtype level.

Likely, aspirin's anti-tumor effect through PIK3CA-related gene activation is useful in differing molecular subgroups, such as ER, PR, and HER2 status.[25,26] Confirmation of these findings is evidenced in previous studies, which indicate that the PIK3CA—mutation-related gene signature can predict the usefulness of adding everolimus to letrozole in ER^+ BRCA treatments.[27] Also, PIK3CA mutation accelerates HER2-driven transgenic mammary tumors, is associated with trastuzumab-resistant BRCA, and links to worse survival.[28-30] All these studies further confirmed our conclusion that aspirin benefits the hormonal responsive group with PIK3CA mutations, but not for the $HER2^+$ group.

Additionally, the current study's results show that patients benefit more from high-dose (325 mg daily) than low-dose (81 mg daily) aspirin, irrespective of aspirin use or non-use before diagnosis in treating breast cancer. This indicates that any beneficial aspirin effects are dose-dependent and corroborate with findings on aspirin's dose dependence effects.[21,31] In tandem with those studies, our findings strongly suggest that aspirin's effects on activating the signaling pathway require higher drug concentrations. Furthermore, our results show that patients who only use aspirin post-diagnosis may confer a better treatment response than patients who used aspirin pre- and post-diagnosis. Li et al have a similar observation in colorectal cancer.[32] Potential explanations include mechanisms of aspirin's protective roles, which may differ before and after cancer, through activation of various pathways in cancer initiation and metastasis.[3] Aspirin may prevent cancer metastasis by inhibiting epithelial-mesenchymal-transition (EMT) in circulating tumor cells.[32] Direct platelet-tumor cell contacts may activate tumor growth factor-β/SMAD and nuclear factor-κB (NFκB) pathways in cancer cells, resulting in the acquisition of EMT phenotype. This markedly reduces inhibition of cytotoxic T-lymphocytes-mediated tumor cell lysis, leading to metastasis.[33,34] The protective role of aspirin for patients who used aspirin before tumor initiation may stem from the inhibition of cyclooxygenase, involved in prostaglandin synthesis and modulating inflammatory response. Owing to immune system changes in the tumor microenvironment, we speculate the inflammatory response for patients who used aspirin long-term is not as sensitive as those who never used aspirin before chemotherapy or hormonal treatments. Moreover, the adverse effects of aspirin toxicity, such as gastrointestinal tract bleeding, should be considered for patients using aspirin long-term. The current study suggests that aspirin

will have an optimal benefit as a chemo-preventative agent after diagnosis.

A lack of specificity with respect to patients' duration of aspirin use (including intervals of aspirin use to address other conditions) prior to diagnosis poses a limitation to this study. Comprehensive studies with large sample sizes and regarding molecular mechanisms related to aspirin are currently being planned. Study limitations also include a narrow collection of molecular data measurements during cohort enrollment. For example, the limited number of tissue samples from patients using 325 mg of aspirin in our study precluded our ability to explore the potential influence of molecular mechanisms for beneficial findings in this subgroup. Thus, future studies should seek to decipher interactions between aspirin doses and PIK3CA regulatory mechanisms for particular subtypes in the sufficiently powered, randomized clinical trials. However, a sufficient understanding of any potentially beneficial effects in BRCA treatment using aspirin in combination with other therapy cannot be achieved with any lone biomarker assessments.[35-37] Moreover, as complicated molecular interaction mechanisms are likely involved in BRCA development and responses to various treatment regimes, further biological validation and more in-depth data mining are necessary to better characterize aspirin's role in supporting better cancer outcomes.

Conclusions

Our study provides evidence that a beneficial effect accrues by utilizing high-dose aspirin in combination with BRCA treatment regimes. Our findings show improved overall survival with 325-mg daily aspirin use, in comparison to an 81-mg aspirin dose taken continuously before and after BRCA diagnosis. However, long-term aspirin users appear to have a poorer prognosis than non-aspirin users. We found an improved, though not significant, overall survival for $HR^+/HER2^-$ BRCA patients with PIK3CA mutations when treated daily with 81 mg of aspirin. Significant activations of the PIK3CA signaling pathway in $HR^+/HER2^-$ subtypes require further exploration from larger sample size. Our findings suggest that further exploration of aspirin use within the same cohort for patients with PIK3CA-mutant BRCA should be conducted in future studies.

Clinical Practice Points

- Many studies reported aspirin's protective role in BRCA. However, aspirin's dose effect in BRCA treatment, patients' history of aspirin uses, and potential molecular targeted by aspirin have not been clarified.
- In this project, we found that using 325-mg high-dose aspirin after diagnosis is associated with better overall survival in comparison with 81-mg low-dose aspirin. We also found an improved overall survival for patients with $HR^+/HER2^-$ BRCA with PIK3CA mutations, which suggested the potential role of aspirin in PIK3CA signaling pathway.
- The current study suggested aspirin will have an optimal benefit as a chemo-preventative agent in BRCA treatment. Future studies with larger sample size should be planned to confirm these findings.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental tables and figures accompanying this article can be found in the online version at https://doi.org/10.1016/j.clbc.2019.05.004.

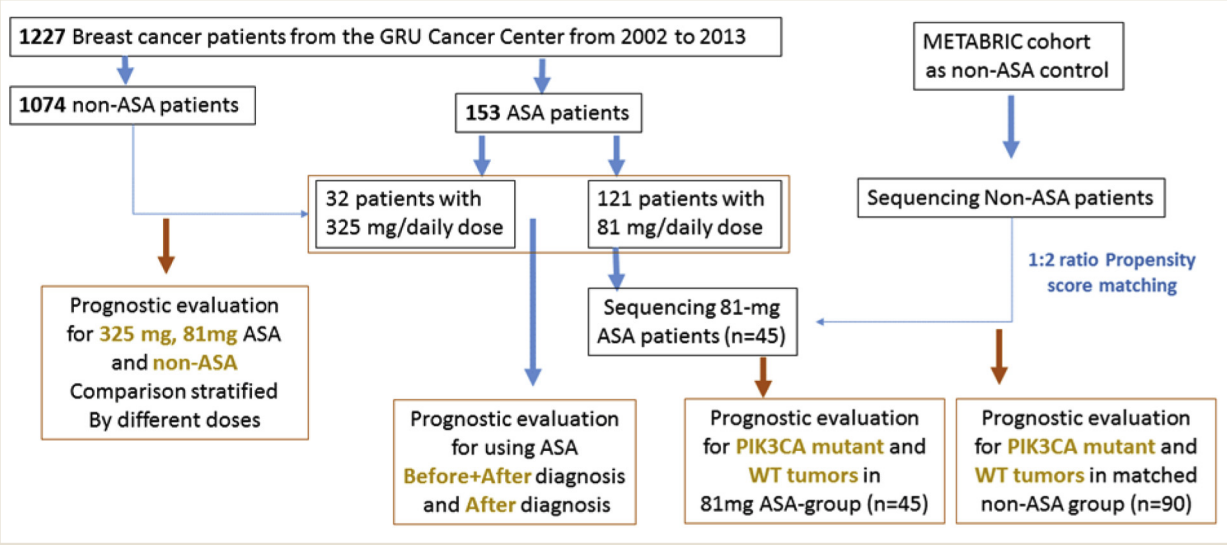
References

1. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* 2012; 379:1591-601.
2. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. *Nat Rev Clin Oncol* 2012; 9:259-67.
3. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol* 2012; 13:518-27.
4. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. *Epidemiology* 1994; 5:138-46.
5. Chen WY, Holmes MD. Role of aspirin in breast cancer survival. *Curr Oncol Rep* 2017; 19:48.
6. Holmes MD, Chen WY, Li L, Hertzmark E, Spiegelman D, Hankinson SE. Aspirin intake and survival after breast cancer. *J Clin Oncol* 2010; 28:1467-72.
7. Fraser D, Sullivan F, Thompson A, McCowan C. Aspirin use and survival after the diagnosis of breast cancer: a population-based cohort study. *Br J Cancer* 2014; 111:623-7.
8. Luo T, Yan H-M, He P, Luo Y, Yang Y-F, Zheng H. Aspirin use and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat* 2012; 131:581-7.
9. Lazzeroni M, Petrera M, Marra D, DeCensi A. Aspirin and breast cancer prevention. *Curr Breast Cancer Rep* 2013; 5:202-7.
10. Domingo E, Church DN, Sieber O, et al. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. *J Clin Oncol* 2013; 31:4297-305.
11. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med* 2012; 367:1596-606.
12. Henry WS, Laszewski T, Tsang T, et al. Aspirin suppresses growth in PI3Kmutant breast cancer by activating AMPK and inhibiting mTORC1 signaling. *Cancer Res* 2017; 77:790-801.
13. Khuder S, Mutgi A. Breast cancer and NSAID use: a meta-analysis. *Br J Cancer* 2001; 84:1188-92.
14. Tang S-C, Shaheta N, Chernenko G, Khalifa M, Wang X. Expression of BAG-1 in invasive breast carcinomas. *J Clin Oncol* 1999; 17:1710-9.
15. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; 53:457-81.
16. Collett D. *Modelling Survival Data in Medical Research*. New York: Chapman and Hall/CRC; 2015.
17. Pereira B, Chin S-F, Rueda OM, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nat Commun* 2016; 7:11479.
18. Curtis C, Shah SP, Chin S-F, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012; 486:346-52.
19. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behav Res* 2011; 46:399-424.
20. Ho DE, Imai K, King G, Stuart EA. MatchIt: nonparametric preprocessing for parametric causal inference. *J Statistic Software* 2011; 42:1-28.
21. Friis S, Sørensen HT, McLaughlin J, Johnsen SP, Blot W, Olsen J. A population based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin. *Br J Cancer* 2003; 88:684-8.
22. Flossmann E, Rothwell PM, British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet* 2007; 369:1603-13.
23. Goel S, Krop IE. PIK3CA mutations in HER2-positive breast cancer: an ongoing conundrum. *Ann Oncol* 2016; 27:1368-72.
24. Clarke CA, Canchola AJ, Moy LM, et al. Regular and low-dose aspirin, other nonsteroidal anti-inflammatory medications and prospective risk of HER2defined breast cancer: the California Teachers Study. *Breast Cancer Res* 2017; 19:52.
25. Loi S, Haibe-Kains B, Majjaj 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 U S A* 2010; 107:10208-13.

26. 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* 2005; 65:2554-9.
27. Loi S, Michiels S, Baselga J, et al. PIK3CA genotype and a PIK3CA mutationrelated gene signature and response to everolimus and letrozole in estrogen receptor positive breast cancer. *PLoS One* 2013; 8:e53292.
28. Chandarlapaty S, Sakr RA, Giri D, et al. Frequent mutational activation of the PI3K-AKT pathway in trastuzumab-resistant breast cancer. *Clin Cancer Res* 2012; 18:6784-91.
29. Cizkova M, Dujaric M, Lehmann-Che J, et al. Outcome impact of PIK3CA mutations in HER2-positive breast cancer patients treated with trastuzumab. *Br J Cancer* 2013; 108:1807-9.
30. Hanker AB, Pfefferle AD, Balko JM, et al. Mutant PIK3CA accelerates HER2-driven transgenic mammary tumors and induces resistance to combinations of anti-HER2 therapies. *Proc Natl Acad Sci U S A* 2013; 110:14372-7.
31. Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Wu K, Fuchs CS. Aspirin dose and duration of use and risk of colorectal cancer in men. *Gastroenterology* 2008; 134:21-8.
32. Li P, Wu H, Zhang H, et al. Aspirin use after diagnosis but not prediagnosis improves established colorectal cancer survival: a meta-analysis. *Gut* 2015; 64: 1419-25.
33. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. *Cancer Cell* 2011; 20:576-90.
34. Akalay I, Janji B, Hasmim M, et al. Epithelial-to-mesenchymal transition and autophagy induction in breast carcinoma promote escape from T cell-mediated lysis. *Cancer Res* 2013; 73:2418-27.
35. 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* 2012; 14:R28.
36. Kalinsky K, Jacks LM, Heguy A, et al. PIK3CA mutation associates with improved outcome in breast cancer. *Clin Cancer Res* 2009; 15:5049-59.
37. Li SY, Rong M, Grieu F, Iacopetta B. PIK3CA mutations in breast cancer are associated with poor outcome. *Breast Cancer Res Treat* 2006; 96:91-5.

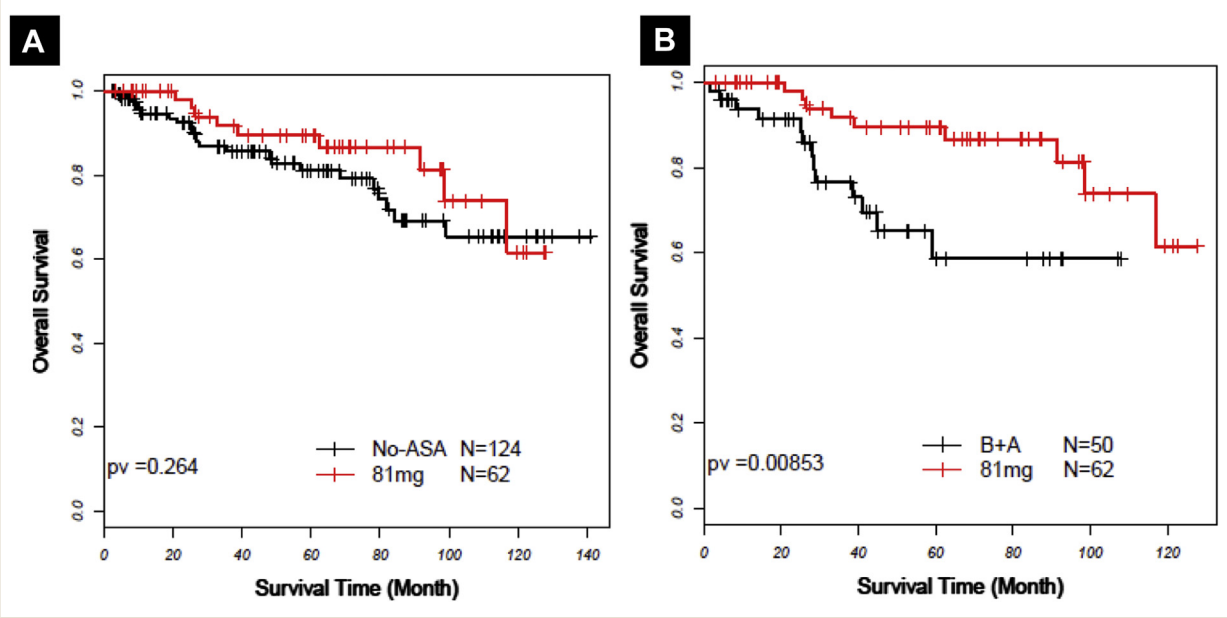
Supplemental Data

Supplemental Figure 1 Project Design Overview



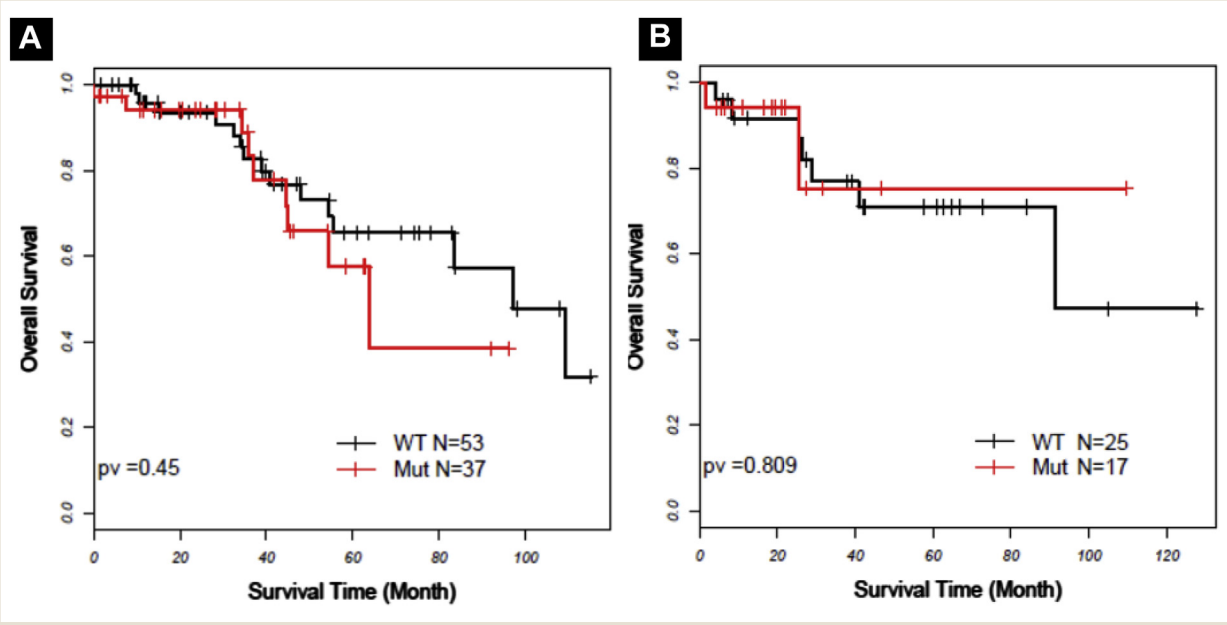
Abbreviations: ASA = aspirin; METABRIC = Molecular Taxonomy of Breast Cancer International Consortium; WT = wild-type.

Supplemental Figure 2 Survival Analyses Comparisons for Low-dose (81 mg) Aspirin. A, The Overall Survival Comparison for Patients With 81-Mg Daily Use After Diagnosis Versus Patients Who Never Took Aspirin Before. B, The Overall Survival Comparison for Patients With 81-Mg Daily Use After Diagnosis Versus Patients Who Used 81 mg of Aspirin Long-term. Patients With Missing Survival Information Were Excluded for Analysis



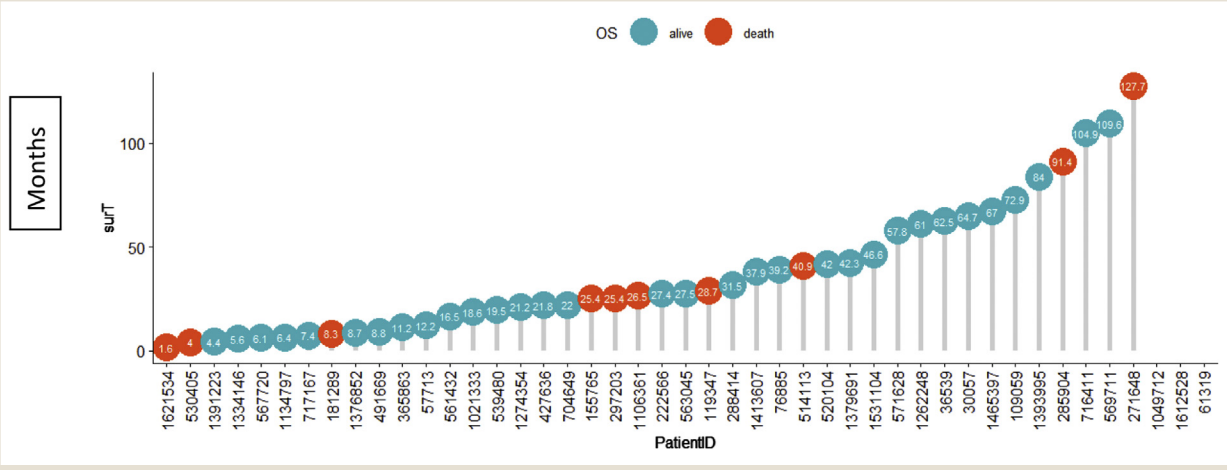
Abbreviations: ASA = aspirin; B+A = before and after diagnosis.

Supplemental Figure 3 Overall Survival Stratified by PIK3CA Mutation Status. Overall Survival Comparison Stratified by PIK3CA Mutation Status in Aspirin Non-use and 81-Mg Regular Group: Aspirin Non-Use Group (n = 90) (A); Aspirin 81 mg Regular Use Group (n = 42, After Removing 3 Cases With Missing Survival Time) (B)



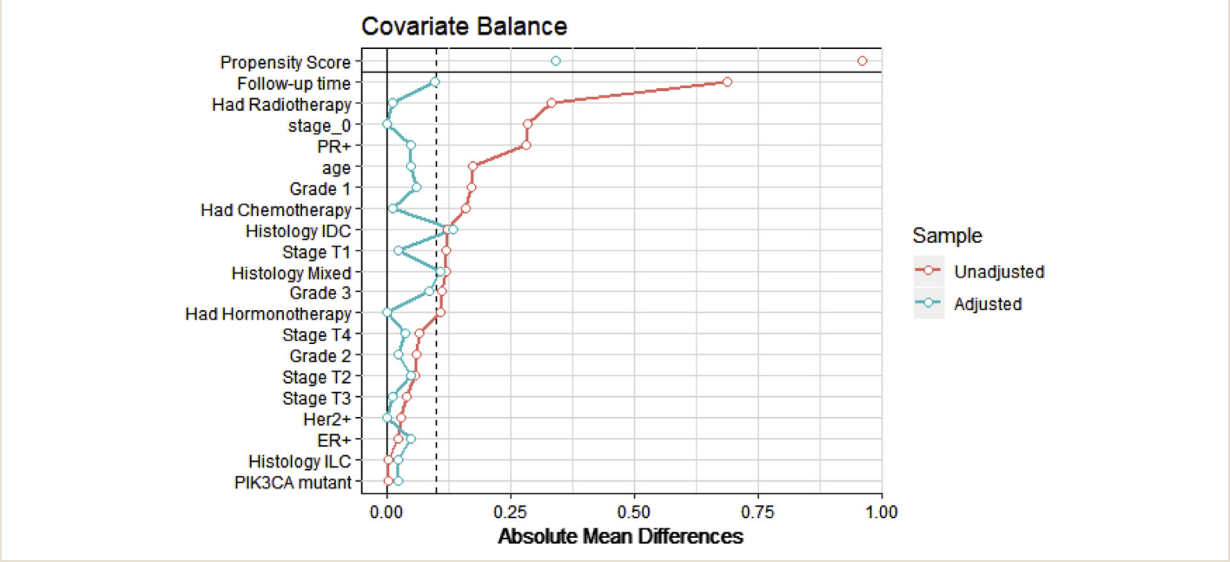
Abbreviations: MUT = mutant; WT = wild-type.

Supplemental Figure 4 Survival Status Over Time (Median Follow-Up Time, 27 Months)



Abbreviation: OS = overall survival.

Supplemental Figure 5 **Absolute Standardized Differences Before and After Propensity Score Matching for the Dataset From Our Cohort and METABRIC. This Plot Shows That Balance Was Improved on Almost All Variables After Adjustment, Bringing the Majority of Covariates Below the Threshold of 0.1 for Standardized Absolute Mean Differences, Which Indicted Small Differences Between the Aspirin Using (Our Cohort) and Without Using (METABRIC Cohort) Groups in the Matched Samples**



Abbreviations: ER^+ = estrogen receptor-positive; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; $HER2^+$ = human epidermal growth factor receptor 2-positive; METABRIC = Molecular Taxonomy of Breast Cancer International Consortium; PR^+ = progesterone receptor-positive.

Supplemental Table 1 Summary of Tables and Figures

Summary of Results	
Patient cohorts	Supplemental Table 2. Patient characteristics for all 1227 patients in the study
	Table 1. Patient characteristics for 45 patients with PIK3CA mutation status
Study design	Supplemental Figure 1. Project design overview
Prognostic evaluation for dosage effect comparison	Figure 1. Overall survival and cancer-free survival comparison for different daily aspirin dosage: 325 mg, 81 mg, no aspirin
Prognostic evaluation for aspirin used history comparison	Figure 2. Overall survival and cancer-free survival comparison for patients who used aspirin before and after diagnosis versus patients who only used aspirin after diagnosis
	Supplemental Figure 2A. Overall survival comparison for 81-mg daily aspirin use versus non-use after the breast cancer diagnosis Supplemental Figure 2B. Overall survival comparison for 81-mg aspirin use after diagnosis versus long-term (before and after diagnosis) use
	Table 2. Multivariate Cox regression analysis for patients who used aspirin before and after diagnosis with patients who only used aspirin after diagnosis
Prognostic evaluation for PIK3CA mutation status comparison	Figure 3. Overall survival and cancer-free survival comparison of PIK3CA mutation status for 45 patients treated by 81-mg daily dosage
	Supplemental Figure 3. Patients who used 81 mg of aspirin and non-aspirin users overall survival comparison stratified by PIK3CA mutation status
	Supplemental Table 3. Patient characteristics for matched non-ASA patients with PIK3CA mutation status from METABRIC dataset
Survival status over time	Supplemental Figure 4. Survival status over time of de-identified patient data
Likelihood ratio test between aspirin dose-effect and history	Supplemental Table 4A**.** Likelihood ratio test for model comparison. Model 1 included the major effect of dose and aspirin use history; Model 2 included the major effect and the dose × history interaction term. Results showed no difference between the 2 models.
2 + 2 table for patient groups	Supplemental Table 4B. 2-by-2 table for the number of patients in each of the two sets of groups. Group A represents patients taking aspirin after diagnosis. Group B + A represents patients taking aspirin before and after diagnosis. Supplemental Table 4C. 2-by-2 table for the survival status of patients in the 325-mg aspirin group
Covariate balance plot after matching	Supplemental Figure 5. Summary plot of covariate balance before and after propensity score matching for our cohort and METABRIC cohort

Abbreviation: METABRIC = Molecular Taxonomy of Breast Cancer International Consortium.

Supplemental Table 2 Patient Characteristics of Augusta University Cancer Center Study Cohorts from 2002 to 2013

Our Cohort	325 mg ASA (n = 32), n (%)	81 mg ASA (n = 121), n (%)	Non-ASA (n = 1074), n (%)
Age, y	59.34 (10.32)	63.54 (11.61)	58.46 (12.40)
Race			
White	12 (38)	60 (50)	604 (56)
Black	20 (63)	60 (50)	454 (42)
Asian	0 (0)	1 (1)	16 (1)
IHC Subtypes			
$HR^+/HER2^-$	22 (68.8)	87 (71.9)	714 (66.5)
$HR^+/HER2^+$	4 (12.5)	8 (6.6)	93 (8.7)
ER^-, PR^-, $HER2^+$	1 (3.1)	9 (7.4)	90 (8.4)
ER^-, PR^-, $HER2^-$ (TNBC)	5 (15.6)	17 (14)	177 (16.5)
Grade			
1	10 (31)	32 (26)	226 (21)
2	7 (22)	33 (27)	347 (32)
3	14 (44)	49 (40)	450 (42)
4	1 (3)	7 (6)	51 (5)
Tumor Stage			
T0	7 (22)	21 (17)	176 (16)
T1	14 (44)	41 (34)	382 (36)
T2	8 (25)	35 (29)	296 (28)
T3	3 (9)	20 (17)	143 (13)
T4	0 (0)	4 (3)	77 (7)
Histology Type			
IDC	4 (13)	12 (10)	107 (10)
DCIS	20 (63)	80 (66)	702 (65)
ILC	1 (3)	8 (7)	85 (8)
MC	2 (6)	3 (2)	16 (1)
Mixed	5 (16)	18 (15)	164 (15)
Treatment Types, Yes			
Chemotherapy	13 (41)	41 (34)	461 (43)
Radiotherapy	19 (59)	54 (45)	755 (70)
Hormonal therapy	14 (44)	65 (54)	575 (54)
Surgery	31 (97)	115 (95)	986 (92)
Median survival time, mos	60.84 (39.53)	50.61 (35.74)	42.44 (33.52)
Vital status			
Death	3 (9)	28 (23)	174 (16)
Alive	29 (91)	93 (77)	900 (84)
Median cancer time, mo	31.36 (21.96)	31.44 (23.16)	29.50 (22.63)
Cancer status	3 (10)	12 (10)	133 (13)

Our sampled population included 1227 patients with breast cancer. Characteristics of patients include 121 patients using 81 mg of aspirin daily and 32 patients using 325 mg daily.
Abbreviations: ASA = aspirin; CI = confidence interval; DCIS = ductal carcinoma in situ; ER = estrogen receptor; IDC = invasive ductal carcinoma; IHC = immunohistochemistry; ILC = invasive lobular carcinoma; MC = medullary carcinoma; PR = progesterone receptor; TNBC = triple-negative breast cancer.

Supplemental Table 3 Summary of METABRIC Cohort Patients' Characteristics

	PIK3CA Mutant (n = 37), n (%)	PIK3CA WT (n = 53), n (%)
Mean age, y	66.24 (15.73)	60.81 (14.68)
IHC Subtypes		
$HR^+/HER2^-$	29 (78.4)	26 (49.1)
$HR^+/HER2^+$	3 (8.1)	7 (13.2)
ER^-, PR^-, $HER2^+$	2 (5.4)	10 (18.9)
ER^-, PR^-, $HER2^-$ (TNBC)	3 (8.1)	10 (18.9)
Grade		
1	12 (32)	5 (9)
2	9 (24)	14 (26)
3	16 (43)	34 (64)
Tumor Stage		
T1	6 (16)	14 (26)
T2	25 (68)	29 (55)
T3	4 (11)	8 (15)
T4	2 (5)	2 (4)
Histology Type		
IDC	34 (92)	46 (87)
ILC	2 (5)	6 (11)
Mixed	1 (3)	1 (2)
Treatment Types, Yes		
Chemotherapy	11 (30)	23 (43)
Radiotherapy	3 (8)	8 (15)
Hormonal therapy	20 (54)	25 (47)
Median survival time, mos	33.9	39.7
Vital Status		
Death	9 (24)	15 (28)
Alive	28 (76)	38 (72)

Summary of aspirin non-use in matched groups from the METABRIC cohort, according to PIK3CA mutation status.
Abbreviations: ER = estrogen receptor; IDC = invasive ductal carcinoma; IHC = immunohistochemistry; ILC = invasive lobular carcinoma; METABRIC = Molecular Taxonomy of Breast Cancer International Consortium; PR = progesterone receptor; TNBC = triple-negative breast cancer; WT = wild-type.

Supplemental Table 4A Likelihood Ratio Test for Model Comparison

Model Description	Degrees of Freedom	Log Likelihood	χ^2	*P* Value (>χ^2)
Model 1 Dose + history	2	−96.151		
Model 2 Dose + history + dose × history	3	−95.718	0.8664	.352

There is no interaction between aspirin dose effect and aspirin use history ($P = .352$) in Model 2, with a dose × history interaction term compared with Model 1. It means no difference for the 2 models with or without the interaction term for aspirin dose effect (high vs. low dose) and aspirin-use history (before and after diagnosis).

Supplemental Table 4B 2 by 2 Table for Patient Groups

Aspirin Dose	Group A	Group B + A
81 mg	62	56
325 mg	12	18

A 2-by-2 table for the number of patients in each of the 2 sets of groups.
Group A represents patients taking 81-mg and 325-mg daily doses of aspirin after diagnosis.
Group B + A represents patients taking 81-mg and 325-mg daily doses of aspirin before and after diagnosis.
There are no significant differences between the groups of patients in the 2 sets based on the Fisher's exact test, with a *P*-value of .31.

Supplemental Table 4C 2 by 2 Table for Vital Status in 325-mg Aspirin Group

Survival Status for 325-mg Aspirin Group		
Vital Status	**Group A**	**Group B + A**
Death	0	3
Alive	12	15

No detrimental effect was observed ($P = .26$).
Statistical power is limited in this study, owing to the small sample size.
Group A represents patients taking 81-mg and 325-mg daily doses of aspirin after diagnosis.
Group B + A represents patients taking 81-mg and 325-mg daily doses of aspirin before and after diagnosis.